Antimicrobial Resistance in Urinary Tract Infections caused by *Escherichia coli*

Submitted by

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Statement of Authorship and Sources

This thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma.

No parts of this thesis have been submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgement in the main text of the thesis.

All research procedures reported in the thesis received the approval of the relevant Human Research Ethics Committee (Appendix F).

I declare that Chapters 1, 2, 3, 6 and 7 of the final thesis draft were externally edited for conventions of grammar, spelling and punctuation by Merran Laver, Eyeline Editing. Copyediting of the research thesis was done in accordance with the requirements of the *Guidelines on the Preparation and Presentation of a Research or Professional Doctoral Thesis for Examination* (18 February 2015).

I declare that I have received financial assistance from a number of organisations to support my research program. Organisations that have provided financial assistance are listed below.

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Abstract

Introduction

Urinary tract infections (UTI) are one of the most common bacterial infections in hospital and community settings requiring antimicrobial treatment. *Escherichia coli* (*E. coli*), a bacterium frequently implicated in UTI, is becoming increasingly resistant to antimicrobials. Antimicrobial resistance (AMR) reduces the effectiveness of antimicrobial agents, leading to difficulty in treatment of patients, with the potential to prolong the duration of illness and increase mortality in patients. To date in Australia, there is a paucity of data comparing resistance patterns over time for hospital- and community-acquired *E. coli* UTI with no published data on incidence and risk of urinary *E. coli* resistance in Australia. Ciprofloxacin, a high priority critically important antimicrobial, is not recommended for empirical therapy of UTI yet resistance to this antimicrobial agent is increasing. There are no systematic reviews of studies investigating ciprofloxacin resistance in hospital- and community-acquired *E. coli* UTI. Therefore, the research program sought to address these knowledge gaps in three separate but interrelated studies. The research described in this thesis is the first of its kind in Australia.

Aims

The overall research program aim was to contribute to the body of knowledge about AMR in *E. coli* UTI. The individual study aims were to: (1) systematically review the literature and conduct a meta-analysis of international observational studies published in the last ten years, investigating ciprofloxacin resistance in community-acquired and hospital-acquired *E. coli* UTI; (2) evaluate AMR temporal trends and compare the prevalence of AMR in hospital-acquired and community-acquired *E. coli* UTI at the Canberra Hospital over a five-year period and also evaluate trends and seasonal variation in antimicrobial use at the Canberra Hospital over a five-year period; and (3) evaluate the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of Australian Capital Territory (ACT) residents over a five-year period. Each aim was addressed as a separate study.

Methods

First, observational studies published between 2004 and 2014 were identified and systematically reviewed in study one. DerSimonian-Laird random-effects meta-analysis of studies was undertaken and pooled estimates of ciprofloxacin resistance were evaluated. Second, a large regional microbiology laboratory dataset was retrospectively reviewed to address the second and third study aims. For study two, a laboratory-based cross sectional study of *E. coli* UTI from patients attending Canberra Hospital was undertaken and time series analysis was performed to illustrate resistance trends at the hospital. Time series analysis was also performed on supplementary antimicrobial use data to evaluate trends and seasonal variation in antimicrobial use at the hospital. Finally, study three used a laboratory-based retrospective cohort design to determine the incidence and patient risk factors for single drug-, multidrug-, extensively drug- and pandrug-resistant *E. coli* UTI in a cohort of ACT residents. Studies two and three also addressed methodological issues with appropriately calculating prevalence and incidence of resistance respectively, using microbiological laboratory data.

Results

For study one, the systematic review and meta-analysis identified that ciprofloxacin resistance was significantly higher ($P<0.0001$) in hospital-acquired UTI (pooled resistance 38%; 95% confidence interval (CI) 36–41%) compared with community-acquired UTI (27%; 95% CI 24–31%). A significant rise in resistance over time was observed for studies reporting on community-acquired *E. coli* UTI ($n=47$, $rs = 0.4313$, $P = 0.003$). Resistance significantly varied by region and country and was higher in developing countries compared to developed countries.

The cross sectional study of laboratory-based data undertaken in study two identified overall five-year resistance was high for ampicillin and trimethoprim. Resistance to amoxycillin-clavulanate, cefazolin, gentamicin and piperacillin-tazobactam was statistically significantly higher in hospital- compared to community-acquired UTI. Statistically significant increases in resistance over the five years were noted for amoxycillin-clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin, cefazolin, gentamicin, ceftriaxone and trimethoprim-sulphamethoxazole. The study also provided evidence of the impact of

denominators selected for calculating resistance prevalence on resistance. Analysis of supplementary antimicrobial use data showed a decrease in overall hospital antimicrobial use over the five-year period but increased use of newer broad spectrum antimicrobials. Seasonal use of ceftriaxone at the hospital was identified.

For study three, the laboratory-based retrospective cohort study showed incidence of resistance was high for ampicillin, trimethoprim and cefazolin. Although no possible pandrug-resistant *E. coli* UTI was identified, there was a relatively low incidence of multidrug- and possible extensively drug-resistant *E. coli* UTI. Multivariate logistic regression analyses indicated that female sex and age over 38 years was statistically significantly associated with single drug- and multidrug-resistance. A previously unrecognised Australian patient group that may be at high risk of developing an antimicrobial resistant *E. coli* UTI, specifically those receiving care at after-hours general practice health services, was identified. The study also provided new knowledge on an appropriate method for clearly identifying incident cases of resistant *E. coli* UTI using microbiological laboratory data.

Discussion

These findings have implications both nationally and internationally and have strengthened the evidence base for AMR through research consistent with the World Health Assembly's global action plan on AMR. Despite all that is being done internationally through the World Health Organization and United Nations, as well as nationally through the recently developed Antimicrobial Use and Resistance in Australia Surveillance System, it is surprising to see rising resistance specifically in *E. coli*, the pathogen evaluated in this research program. Rising resistance might indicate that antimicrobials are still being misused, overused and inappropriately prescribed. There is more to be done in curtailing rising resistance. In addition, presence of multidrug- and possible extensively drug-resistant *E. coli*, although low in the ACT, emphasises the need for continued surveillance of AMR in *E. coli* UTI to inform development and implementation of effective interventions to reduce resistant *E. coli* UTI. It is important to note that as this thesis focused on microbiological laboratory data, which is only one aspect of what informs therapeutic management of *E. coli* UTI, the thesis could only inform some aspects of decision-making about prescribing.

Conclusions

Based on the research findings, policy recommendations include: linkage of microbiological laboratory and clinical databases; compulsory completion of minimum patient data on microbiological laboratory request forms; development and implementation of policy limiting use of ciprofloxacin as an empirical agent for UTI management. Recommendations for clinical practice include: ongoing education of general practitioners who consult at after-hours clinics on judicious use of antimicrobials; and education of healthcare staff on the importance of adequately completing laboratory request forms. Potential areas for future research include: prospective studies of patient-based AMR data and linking these data to antimicrobial use data; and examining the association between policy regulation on antimicrobial use and AMR. Recommendations for research methodology include: uptake of the new methodological approach for clearly identifying incident cases of resistant *E. coli* UTI; and further evaluation of the impact of denominators selected for calculating resistance prevalence. Recommendations for reporting of research include: establishment of an expert panel to develop a standardised classification system for infections based on the setting of acquisition to allow consistency in reporting as well as comparison of data; and improved compliance with reporting guidelines during journal submission processes. The findings will be provided to local health authorities with the aim of implementing the recommendations and informing clinical practice.

Abbreviations

acNAPS	Aged Care National Antimicrobial Prescribing Survey
ACSQHC	Australian Commission on Safety and Quality in Health Care
ACT	Australian Capital Territory
AGAR	Australian Group on Antimicrobial Resistance
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
AURA	Antimicrobial Use and Resistance in Australia
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
DDD	Defined Daily Dose
DRG	Diagnosis Related Group
E. coli	*Escherichia coli*
ESBL	Extended Spectrum Beta Lactamase
EUCAST	European Union Committee for Antimicrobial Susceptibility Testing
GP	General Practice
HAI	Healthcare-associated Infection
IDSA	Infectious Diseases Society of America
MDR	Multidrug-resistant
MeSH	Medical Subject Heading
MIC	Minimum Inhibitory Concentration

NAPS	National Antimicrobial Prescribing Survey
NATA	National Association of Testing Authorities
NAUSP	National Antimicrobial Utilisation Surveillance Program
NOS	Newcastle-Ottawa Scale
OBD	Occupied Bed Days
PDR	Pandrug-resistant
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
SEIFA	Socio-Economic Indexes for Areas
SES	Socioeconomic Status
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
UK	United Kingdom
US	United States
UTI	Urinary Tract Infection
WHO	World Health Organization
XDR	Extensively drug-resistant

Glossary

Antimicrobial	A drug or other substance that prevents the growth of microbes or pathogens such as bacteria, fungi, parasites or viruses (adapted from Sefton, 2002)
Antimicrobial resistance	The ability of a microbe to resist the effect of an antimicrobial agent (Australian Commission on Safety and Quality in Health Care, 2013a)
Community-acquired urinary tract infection	Positive *E. coli* urine culture obtained within the first 48 hours of admission (including cultures from non-admissions such as outpatient clinics)
Extensively drug-resistance	"Non-susceptibility to at least one agent in all but two or fewer antimicrobial categories" (Magiorakos et al., 2012, p. 277)
Incidence	The number of new (incident) cases of a condition (e.g., a disease) in people at risk of developing the condition during a specified time period (Buttner & Muller, 2011; Porta, 2014)
Hospital-acquired urinary tract infection	Positive *E. coli* urine culture obtained more than 48 hours after admission and within 48 hours of discharge
Isolate	"An organism identified in pure form in a microbial culture" (Venes, 2013, p. 1299)
Microbe	"A unicellular or small multicellular organism including bacteria, fungi, viruses and protozoa" (Venes, 2013, p. 1510)
Multidrug-resistance	"Non-susceptibility to at least one agent in three or more antimicrobial categories" (Magiorakos et al., 2012, p. 277)

Pandrug-resistance	"Non-susceptibility to all agents in all antimicrobial categories" (Magiorakos et al., 2012, p. 277)
Pathogen	"A microorganism capable of producing a disease" (Venes, 2013, p. 1752)
Prevalence	The proportion of people with a disease either at a specified point in time (point prevalence) or during a particular period of time (period prevalence) (Buttner & Muller, 2011)
Surveillance	"Systematic and continuous collection, analysis, and interpretation of data, closely integrated with the timely and coherent dissemination of the results and assessment to those who have the right to know so that action can be taken" (Porta, 2014, p. 239)
Urinary tract infection	Positive laboratory confirmation of a quantitative culture of urine sample containing *E. coli* of greater than or equal to 10^7 colony forming unit per litre of urine

Chapter 1: Introduction

1.1 Overview

Antimicrobial resistance (AMR) is a major global public health concern. In response to the threat of AMR in Australia, the first national AMR strategy was released in June 2015 (Australian Government, 2015). *Escherichia coli* (*E. coli*), a Gram-negative bacterium, is identified both in Australia and globally as a priority organism for targeted AMR surveillance in human health because of its impact in hospital and community settings. This bacterium is frequently implicated in urinary tract infections (UTI), which are one of the most common bacterial infections in hospital and community settings. To date, there is no published research exploring the issue of AMR in urinary *E. coli* infections in Australia and there is a need to address the current research gaps, hence this research program.

The aim of chapter one is to introduce the thesis subject of AMR in UTI caused by *E. coli*, provide some context to the thesis topic, outline the overall research program and individual study aims and highlight the significance of the research program. This chapter will begin with a synopsis of the thesis subject. A brief description of Australia and its health structure will be outlined. An overview of the history and classification of antimicrobial agents will be provided, including a discussion on antimicrobial use and development of AMR as well as the mechanisms of AMR. The evidence base for the importance of AMR surveillance will also be discussed. A brief description of the current status of AMR surveillance in Australia is provided, highlighting the key areas for development.

Chapter one also contains an outline of the research program, which briefly describes the three studies which make up the research program, the significance as well as the importance of the research program. The study aims, research questions and hypotheses are also detailed within this chapter. The chapter will conclude with an outline of the thesis structure, which provides brief information on subsequent chapters.

1.2 Background of the author

Working as a medical doctor in a developing country, I was faced daily with challenges on how to provide adequate care to patients using the limited resources available. It quickly became clear to me as a clinician that even in the presence of adequate resources, such as revolutionary medical equipment and novel drugs, it was imperative to ensure effective infection prevention and control strategies were being applied in order to control and prevent further spread of infection. Effective infection prevention and control strategies were also important to improving outcomes for the patients. My interest in infection prevention and control led to my undertaking a Master's degree in Public Health and Tropical Medicine, from which I developed a keen interest in the field of research, culminating in accepting a position as a research associate at the Australian Catholic University. In this position, I took a lead role in the strategic planning and management of a project focused on reducing healthcare associated UTI in Australian acute care hospitals and aged care facilities. Particularly, working in this position provided me with insight into the issues related to managing UTI and raised key questions on how best to address the rising levels of resistance in drugs commonly used to treat UTI, as well as ensuring reliable and valid analysis of microbiology laboratory data on urine samples.

1.3 Introduction to the thesis subject

Urinary tract infections are one of the most frequently occurring bacterial infections in both hospital and community settings (Foxman, 2003; Hooton, 2012; Mazzulli, 2012). They are also the most common bacterial infections acquired by women (Stamm & Norrby, 2001). Urinary tract infections occur in men, but less commonly than in women (Foxman, 2003). Women are more predisposed to UTI because bacteria easily enter the bladder via the shorter female urethra (Foxman, 2003; Hooton, 2000). Although UTI can be caused by a wide range of bacterial and some fungal pathogens, a Gram-negative bacterium known as *E. coli* is the predominant pathogen isolated in patients (Nicolle, 2013; Ronald, 2002). Health implications of UTI include approximately 2.4 days of restricted activity and 1.2 work days lost (Foxman, 2002; Nicolle, 2008). Complicated infections (e.g. paraurethral or renal abscesses) may occur more frequently in patients with underlying health conditions, such as diabetes, leading to increased morbidity and frequency of hospitalisation (Foxman, 2002; Nicolle, 2005). Furthermore, UTI have the potential to spread to the bloodstream, causing

bacteremic UTI associated with increased mortality (Al-Hasan, Eckel-Passow, & Baddour, 2010).

Urinary tract infections are treated with antimicrobial agents, most commonly antibiotics as the majority of UTI are caused by bacteria (Mazzulli, 2012). Approximately one in three women will have had at least one UTI episode requiring treatment with an antimicrobial agent by 24 years of age (Foxman, 2003). In most cases, antimicrobials are prescribed empirically while awaiting results of the urine sample sent for laboratory culture (Vellinga, Cormican, Hanahoe, Bennett, & Murphy, 2011). Empirical treatment refers to the use of antimicrobials when treatment must be commenced before the culture susceptibility results are available, the clinical situation is not serious enough to warrant taking cultures, or appropriate material for culture cannot be obtained (Antibiotic Expert Groups, 2014). The rationale for the empirical approach is based on the predictable causative agents for UTI, the predictable susceptibilities of these pathogens to commonly used antimicrobials, the identification of a UTI based on clinical presentation and the likely positive response to short-course antimicrobial treatment (Stamm & Norrby, 2001). Despite the advantages of this approach, which include reduced laboratory testing costs and patient visits, a disadvantage may be the inappropriate use of antimicrobials as well as treatment failures resulting in recurrence of infection (Stamm & Norrby, 2001). Furthermore, the choice of empirical therapy for UTI is based on the local susceptibility patterns of common causative pathogens, which can change over time (Teoh et al., 2013). When suboptimal treatment is provided, pathogens causing UTI may develop resistance to antimicrobials (Trautner, 2010). Antimicrobial resistance leads to decreased efficacy of antimicrobial agents, making the treatment of patients difficult, expensive or in some instances impossible when resistance to multiple agents develop. Antimicrobial resistance may also prolong the duration of illness and increase mortality in patients (World Health Organization, 2014). Empirical therapy is still regarded as the best approach for UTI treatment (Little et al., 2010; Olson & Haith, 2012), so monitoring of resistance patterns is essential to ensure appropriate treatment for patients.

During the past two decades, there have been significant increases in the resistance patterns of bacteria to commonly used antimicrobials (Blaettler et al., 2009; Kronvall, 2010; Levy & Marshall, 2004; Linhares, Raposo, Rodrigues, & Almeida, 2013; Maraki, Mantadakis,

Michailidis, & Samonis, 2013; Tadesse et al., 2012). This is especially important for UTI because it is usually treated empirically. The changes in resistance patterns need to be taken into consideration when deciding on the most appropriate antimicrobial (Ronald, 2002). In addition, most infections can be classified based on the setting in which they are acquired (World Health Organization, 2002); that is, healthcare setting such as a hospital, or community setting (e.g. non-healthcare facility or patient's home). These settings are not isolated from each other with opportunity for transfer of resistant pathogens between settings (Cohen, 1992). For example, hospital-acquired infections may not produce any clinical symptoms until the patient has been discharged home. Likewise, patients with community-acquired infections may receive treatment in healthcare facilities (World Health Organization, 2002). The issue of antimicrobial resistant *E. coli* UTI involves both hospital and community settings, hence describing AMR in UTI should take into account the setting of infection acquisition.

The problem of AMR was initially addressed with the development of new antimicrobials. In recent years, the rate at which pharmaceutical companies discover new antimicrobial agents against resistant pathogens has been declining (Moellering, 2006). Pharmaceutical companies tend to pursue more profitable treatments such as development of drugs for treatment of chronic disease conditions, such as hypertension and cancer (Australian Commission on Safety and Quality in Health Care, 2013a; Power, 2006). Further, as pharmaceutical companies strive to survive in a commercial environment, it is difficult to justify the costs for research and development required for new antimicrobials. This is because evidence shows that resistance to a new antimicrobial is likely to develop in the near future, thereby rendering the new drug less marketable (Australian Commission on Safety and Quality in Health Care, 2013a). In addition, new antimicrobials are likely to be restricted to reduce their use, thereby leading to decreased revenue and discouraging commercial companies from investing in new antimicrobial development (Power, 2006). Hence, reliance solely on the development of new antimicrobials to address the issue of AMR is impractical and other strategies must be investigated (Australian Commission on Safety and Quality in Health Care, 2013a). Therefore, in the era of increasing AMR (Schito et al., 2009), it is necessary to undertake continued research in this area to ensure patients are effectively treated, resulting in good clinical outcomes. Routinely collected microbiology laboratory data serves as an importance data source for research evaluating AMR levels in

pathogens such as *E. coli* (Cornaglia et al., 2004). However, as routinely collected data are not primarily collected for the purposes of research, they pose some methodological challenges to the analyses of AMR data.

This research program evaluates AMR in *E. coli* UTI, with the aim of providing knowledge about the resistance patterns of *E. coli*, the pathogen most frequently implicated in UTI. This research program provides research-based insight into resistance in hospital- and community-acquired *E. coli* UTI worldwide using data from published studies and also provides knowledge of resistant *E. coli* UTI in the Australian Capital Territory (ACT) using data from a regional microbiology laboratory. The research program highlights the methodological challenges with evaluating microbiological laboratory AMR data. The overall results from this research program have the potential to inform policy and clinical practice relating to AMR in *E. coli* UTI. The results provide global AMR data, including regional data which will specifically contribute to a national dataset, thereby serving as a baseline for monitoring successive interventions. The study findings will guide clinicians in their treatment decisions for UTI both internationally and nationally and contribute methodologically to analysis of future AMR data.

1.3.1 Australia and its healthcare system

Australia is the sixth largest country in the world by land mass with a land area of approximately 7.7 million km^2 (Australian Government, 2016a). Its land mass is estimated to be 32 times greater than the United Kingdom (UK) and almost as great as the United States (US) (Australian Government, 2016a). As of 31 December 2015, Australia's estimated resident population was 23.9 million people (Australian Bureau of Statistics, 2016) so it is a large country with a low population density. Australia has six states and two mainland territories.

The World Health Organization (WHO) describes a health system as inclusion of all activities, whose principal aim is the promotion, restoration and maintenance of health (World Health Organization, 2016). The Australian healthcare system is multifaceted, consisting of public and private healthcare providers, settings and patients (Australian Institute of Health and Welfare, 2014). The health system comprises public health, primary health, emergency, hospital-based, rehabilitation and palliative services (Australian Institute of Health and

Welfare, 2014). Local, state, territory and Australian governments provide public health services. Some private health service providers include medical practices and private hospitals (Australian Institute of Health and Welfare, 2014). About 70% of hospital care is provided by public hospitals (Australian Commission on Safety and Quality in Health Care, 2016).

The healthcare system in Australia is primarily funded by federal, state and territory governments, with 42.4% of the total health expenditure contributed by the Australian federal government during 2011 and 2012 (Australian Institute of Health and Welfare, 2014). The state and territory governments contributed 27.3% with the remaining contributions made by patients, private health insurers and accident compensation schemes (Australian Institute of Health and Welfare, 2014). The funding contribution by the Australian government includes the Medicare scheme. Medicare provides free or subsidised healthcare to all Australians and also subsidises a number of prescription medicines including antibiotics through the Pharmaceutical Benefits Scheme (Australian Commission on Safety and Quality in Health Care, 2016; Australian Institute of Health and Welfare, 2014).

In Australia, the primary healthcare system is a person's first point of contact with the healthcare system (Australian Institute of Health and Welfare, 2014). This healthcare system includes services provided by a range of clinical and allied health professionals such as general medical and dental practitioners and physiotherapists (Australian Institute of Health and Welfare, 2014). Patients with UTI typically present to general medical practitioners. General practitioner waiting times vary across Australia with the proportion of people waiting longer than they felt acceptable for a general practitioner appointment decreasing over the last two years from 23% in 2013-14 to 19% in 2015-16 (Australian Bureau of Statistics, 2016). Given the complex mix of public and private funding of the Australian healthcare system, payment for general practitioner consultation and diagnostic testing may be covered through the Medicare scheme for eligible Australian permanent residents or may incur out-of-pocket expenses depending on the clinical service provided and type of testing required (Australian Government, 2017). Urine sample examination, which includes examination for cell count, culture, colony count and antimicrobial susceptibility testing, is covered under Medicare item number 69333 (Medicare Australia, 2016). Payment for this

item is eligible for a Medicare benefit or rebate under the Medicare scheme (Australian Government, 2017).

In the primary healthcare system, general practitioners initially evaluate patients presenting with UTI based on clinical history and physical examination. A urine dipstick is performed at the time of consultation to check for nitrites and/or leukocyte esterases which may indicate the presence of UTI (Jarvis, Chan, & Gottlieb, 2014). General practitioners are guided by the Australian Therapeutic Guidelines for management of patients with UTI. The guidelines recommend that antimicrobial treatment can be commenced empirically in symptomatic patients (Antibiotic Expert Groups, 2014). In non-pregnant women who are suspected to have uncomplicated UTI, urine cultures and susceptibility testing are not mandatory. However, urine samples for cultures and susceptibility testing should be obtained prior to commencement of empirical antimicrobials in pregnant women, men, aged care residents, patients who have recently taken antimicrobials or failed treatment, patients with recurrent UTI and those who have travelled internationally within the past six months (Antibiotic Expert Groups, 2014).

1.3.2 Study setting

Australia's capital city, Canberra, is located in the Australian Capital Territory (ACT), one of the mainland territories (Australian Government, 2016b). The estimated population of the ACT as of December 2015 was 393,000 (Australian Bureau of Statistics, 2016). Two of the three studies contributing to this research program utilised data belonging to the residents of the ACT, which were processed at the public regional microbiology laboratory, ACT Pathology. Australian Capital Territory Pathology is located within the Canberra Hospital (ACT Government, 2015a). This 600 bed hospital is the largest publicly-funded tertiary hospital in the ACT providing acute and specialist care services to people in the region (ACT Government, 2015b). Australian Capital Territory Pathology provides specialist pathology services to all inpatients of public hospitals in the ACT as well as people attending public hospital emergency departments and some specialist outpatient clinics (ACT Government, 2015a). ACT Pathology also services an estimated 13% of patients (Medicare Australia, 2016) attending private hospitals, general practice clinics and nursing homes in the community. Urine samples from patients attending the Canberra Hospital are processed at the laboratory and these data were included as part of the research

program. The rationale for the selection of the ACT, including ACT Pathology as the data collection site, as opposed to another state or territory and laboratory is because of the geographic isolation of the ACT, with almost all healthcare for ACT residents provided within the jurisdiction (Kennedy, Roberts, & Collignon, 2008). Also, the wide reach of ACT Pathology, with a dataset that includes data on ACT residents in both public and private hospital settings and also in the community, made it a very suitable site to obtain data for use as part of the research program.

Recent data show that the median age of 35.1 years for ACT residents was slightly lower than the overall Australian median age of 37.4 years and the second lowest in comparison to other Australian states and territories (Australian Bureau of Statistics, 2015). In 2015, the ACT male to female sex ratio of 98.5 to 100 was similar to that for the whole of Australia (Australian Bureau of Statistics, 2015). There are no available jurisdictional data on the incidence and prevalence of *E. coli* UTI in Australia; however published data show that *E. coli* is the most common cause of bloodstream infection in Canberra with UTI identified as the most frequent focus of infection (Kennedy et al., 2008). The seven-day case fatality rate due to *E. coli* bloodstream infection in Canberra has been estimated to be 5% (Kennedy et al., 2008).

1.3.3 History and classification of antimicrobial agents

The development of antibiotics has been a major advancement in patient care. Their discovery is considered one of the most remarkable health-related events in the history of medicine (Aminov, 2010; Davies & Davies, 2010). For over 60 years, antibiotics have been regarded as the solution to curing hospital- and community-acquired infections (World Health Organization, 2014). The word 'antibiotic' was first used as a noun in 1941 by Dr Selman Waksman to describe compounds produced by microorganisms which prevent the growth of other microorganisms (Waksman, 1973). However, as this description only applied to naturally occurring compounds and with the development of synthesized antibiotics over the years, the description of an antibiotic has expanded to include a broader category known as antimicrobials (Mishra & Agrawal, 2012). An antimicrobial is a drug or substance that prevents the growth of microbes or pathogens such as bacteria, fungi, parasites or viruses (Sefton, 2002). The words antibiotic and antimicrobial are often used

interchangeably in the literature (Leekha, Terrell, & Edson, 2011). For the purpose of this research program, the word antimicrobial is used.

In 1910, the first antimicrobial agent, salvarsan, was synthesized by Ehrlich and Hata for the treatment of syphilis (Davies & Davies, 2010). This was followed with the discovery of sulfonamides by Domagk in 1935. In 1928, Alexander Fleming discovered penicillin, which was eventually introduced for use in the 1940s (Davies & Davies, 2010; Fleming, 1929). With the discovery of these three antimicrobials, over the next two decades came the development of other antimicrobials in a period commonly referred to as the golden era of antimicrobial discovery, which is when most of the antimicrobials used today were discovered (Aminov, 2010; Davies & Davies, 2010). Subsequent antimicrobials were developed either from evaluating naturally occurring compounds or by chemically modifying previously discovered antimicrobials (Powers, 2004). Given the number of antimicrobials in existence from the 1940s to early 1960s, clinicians were presented with a wide variety of treatment options for their patients (Powers, 2004).

Antimicrobials can be broadly classified based on their chemical structure. The major antimicrobial classes are penicillins, cephalosporins, fluoroquinolones, aminoglycosides, polypeptides or glycopeptides, tetracyclines, macrolides, chloramphenicol, ansamycins, lincosamides, trimethoprim, fosfomycin, carbapenems and 5-nitroimidazoles (Bryskier, 2005; Mishra & Agrawal, 2012). The penicillins, cephalosporins and carbapenems are all members of the beta-lactam family (Bryskier, 2005). Antimicrobials can also be classified, based on their spectrum of activity against pathogens, into broad or narrow spectrum (van Saene, Fairclough, & Petros, 1998). Broad spectrum antimicrobials are effective against a broad range of Gram-negative and Gram-positive bacteria (Bryskier, 2005; Mishra & Agrawal, 2012). Examples of broad spectrum antimicrobials include tetracyclines, chloramphenicol and some cephalosporins and fluoroquinolones (Aldred, Kerns, & Osheroff, 2014; Bryskier, 2005; Mishra & Agrawal, 2012). Narrow spectrum antimicrobials are only active against a specific group of pathogens. For example, glycopeptides are only effective against Gram positive bacteria (Mishra & Agrawal, 2012).

The discovery and subsequent use of antimicrobials led to a dramatic reduction in morbidity and mortality due to infectious diseases in comparison to the pre-antibiotic era (Powers,

2004). For example, the use of sulfonamides decreased the mortality due to acute meningococcal meningitis to 10%, compared to 70–99% in the pre-antibiotic era (Powers, 2004). However, the use of these 'miracle' drugs has been accompanied by the emergence of pathogens resistant to antimicrobials (Davies & Davies, 2010). Previously effective antimicrobials against certain pathogens are now no longer effective, which poses significant threats to public health and the possibility of return to the pre-antibiotic era if urgent action is not taken (Cohen, 1992; Davies & Davies, 2010). The next section describes the effect of antimicrobial use on the development of resistance.

1.3.4 Antimicrobial use and development of AMR

Overuse or misuse of antimicrobials may pose an important clinical challenge known as antimicrobial resistance (AMR) (Fishman, 2006). Antimicrobial resistance is the ability of a microbe to resist the effect of an antimicrobial agent (Australian Commission on Safety and Quality in Health Care, 2013a). This occurs when an antimicrobial agent is unable to perform its function as a result of a change in the microbe rendering the antimicrobial agent clinically ineffective (Australian Commission on Safety and Quality in Health Care, 2013a). As described in the previous section with the use of the words antibiotic and antimicrobial, the terms antibiotic resistance and antimicrobial resistance are also used synonymously. Antibiotic resistance, strictly speaking, refers to the development of resistant bacteria strains or the ability of bacteria to develop resistance to antibiotics (Tenover, 2006) as opposed to the inclusion of viruses, parasites and fungi. This research program will focus specifically on antibiotic resistance to bacteria, namely *E. coli*, although using the term AMR. The term AMR was chosen as it is more widely used (Robinson et al., 2016).

Development of AMR is a naturally occurring process for microbes but is accelerated by the selective pressure resulting from the misuse and overuse of antimicrobials both in humans and animals (World Health Organization, 2014). Alexander Fleming, who discovered the antibiotic known as penicillin, raised concerns while giving his Nobel Prize speech in 1945 that bacterial pathogens could develop resistance to antimicrobial agents (World Health Organization, 2014). Not surprisingly, with the development of each new antimicrobial agent there has been detection of resistance to the agent following subsequent use (Davies & Davies, 2010; Levy & Marshall, 2004). Antimicrobial resistant bacteria were first identified in hospital settings in the 1930s but subsequently appeared in the community as well (Levy

& Marshall, 2004). The overuse and misuse of antimicrobial agents will continue to provide selective pressures with further development and spread of AMR (Cohen, 1992).

Antimicrobials have now become widely used and also misused in both human populations and food-producing animals (World Health Organization, 2014). The increase in use is further driving resistance as the greater the number of antimicrobials used, the higher the chances that antimicrobial resistant pathogens will succeed in the fight for survival (Center for Disease Dynamics, Economics and Policy, 2015a). There is evidence globally (Center for Disease Dynamics, Economics and Policy, 2015b; World Health Organization, 2014) showing an increase in resistance of urinary *E. coli* isolates to commonly prescribed antimicrobials. There is also strong evidence to support the association between antimicrobial use and this development of resistance in *E. coli* UTI (Bergman et al., 2009; Goossens, Ferech, Vander Stichele, & Elseviers, 2005). A critique of the evidence is provided in the literature review chapter (chapter two). The evidence highlights the need for continued monitoring of AMR patterns in *E. coli* UTI, a common infection in both hospital and community settings. This research program aims to contribute to the body of knowledge about AMR patterns in *E. coli* UTI and use the findings to make recommendations for clinical practice, future research as well as development of policies targeted towards control of AMR and antimicrobial use.

1.3.5 Antimicrobial resistance and One Health

The One Health concept is described as "a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for people, animals and the environment" (Gibbs, 2014, p. 86). It is now acknowledged that the issue of AMR is a One Health issue recognising the connection between human, animal and environmental health (Australian Government, 2015; Robinson et al., 2016). Transmission of urinary *E. coli* isolates to humans has been identified to occur through environmental exposures including food, animal and travel (Nicolle, 2013; Robinson et al., 2016). There is evidence to show widespread dissemination of antimicrobial resistant urinary *E. coli* clones both within Australia and globally (Johnson et al., 2009). Also, a population-based surveillance study in Canada reported a significantly increased risk of isolation of urinary *E. coli* isolates with overseas travel, particularly to India, the Middle East and Africa (Laupland, Church, Vidakovich, Mucenski, & Pitout, 2008). Furthermore, the use of antimicrobials in animals as well as subsequent deposition of antimicrobial residues in the environment is

also driving the spread of AMR in general (Center for Disease Dynamics, Economics and Policy, 2015a). The increased demand for food-producing animals due to worldwide population growth is leading to higher use of antimicrobials in the agricultural sector, also favouring the development of resistance (Center for Disease Dynamics, Economics and Policy, 2015a). Some of the antimicrobials used in agriculture and aquaculture end up in the environment, adding to the burden of AMR in both animals and humans (Center for Disease Dynamics, Economics and Policy, 2015a; Daghrir & Drogui, 2013). A One Health approach to surveillance of antimicrobial resistant *E. coli* infections is therefore important, with collaboration from the medical, veterinary and agricultural sectors (Australian Government, 2015). Although the effect of environmental exposures as well as antimicrobial use in food animals is important in addressing the issue of AMR, this topic is not central to the thesis and will not be covered further.

1.3.6 Mechanisms of resistance to antimicrobial agents

The mechanisms of AMR are multifaceted. Resistance can either be intrinsic or acquired. Intrinsic resistance refers to the naturally occurring resistance of bacteria to antimicrobials (Sefton, 2002). Some bacterial species have intrinsic resistance to one or more antimicrobial classes. When this occurs, the strains of that bacterial species are also resistant to all agents in those antimicrobial groups (Tenover, 2006). In acquired resistance, bacteria that were initially susceptible to an antimicrobial agent become resistant, multiply and spread under the selective pressure, following the use of the antimicrobial agent (Tenover, 2006). Acquired resistance can either be genetic or biochemical (Sefton, 2002). Genetic resistance can occur either from mutation or from acquiring resistant genes from other bacterial species. Acquisition of resistant genes may occur through genetic mechanisms such as conjugation, transduction or transformation (Tenover, 2006). Conjugation occurs either when resistance genes that contain plasmid, a type of genetic material, are transferred from Gram-negative bacteria to other bacteria or during the process of mating between Gram-positive bacteria. In transduction, transfer of resistance genes between bacteria occurs via bacterial viruses, also known as bacteriophage. During transformation, resistant bacteria undergo cell lysis with release of their DNA, which is acquired and incorporated into the DNA fragments of susceptible bacteria (Tenover, 2006). These processes of exchange of genetic material that encode resistance between bacteria are the main mechanisms by

which many bacteria have developed resistance to multiple antimicrobial agents (Tenover, 2006).

Possible mechanisms for acquired biochemical resistance by bacteria include production of drug inactivating enzymes, decreased cell permeability, modification of an existing target and acquisition of a target by-pass system (Sefton, 2002). For example, bacteria may modify or change the existing target, which is described as the specific location or site the antimicrobial drug is designed to attach to on the bacterium (Tenover, 2006). They may also produce enzymes that destroy or inactivate the antimicrobial agent before it has an effect. Bacteria may also alter a protein channel on their cell wall or outer membrane, preventing the antimicrobial drug from entering the bacterial cell wall. Finally, they may use what are known as efflux pumps to expel the antimicrobial agent from the bacterial cell, thereby by-passing its target site without it having an effect on the bacteria (Tenover, 2006).

Acquired AMR mechanisms in Enterobacteriaceae such as *E. coli* include production of enzymes known as β-lactamases (Paterson, 2006a). These include extended-spectrum β-lactamases (ESBLs) such as: 'active on CefoTaXime, first isolated in Munich' (CTX-M) enzymes; AmpC β-lactamases; and carbapenemases. Other resistance mechanisms exhibited by Gram-negative bacteria are alterations to target enzymes and plasmid-mediated resistance (Paterson, 2006a). Resistance to specific antimicrobials such as fluoroquinolones occurs by alterations to the chromosomal gene leading to changes in the target mechanism or by alterations to the cytoplasmic membrane efflux protein gene, resulting in modifications to the permeation mechanism (Dalhoff, 2012a). The development and spread of resistance in Enterobacteriaceae, including *E. coli,* threatens to create species that will become resistant to all currently available antimicrobials (Paterson, 2006a).

1.3.7 The importance of surveillance of antimicrobial resistant *E. coli* UTI

Surveillance can be defined as "systematic and continuous collection, analysis, and interpretation of data, closely integrated with the timely and coherent dissemination of the results and assessment to those who have the right to know so that action can be taken" (Porta, 2014, p. 239). Surveillance of AMR can therefore be described as the collection, analysis and interpretation of data on antimicrobial resistant bacteria, including antimicrobial use. Evidence shows that judicious use of antimicrobials may reduce AMR,

hence surveillance of AMR should be undertaken in conjunction with data collection on antimicrobial use (World Health Organization, 2002). Antimicrobial resistance surveillance is the foundation for evaluating the burden of resistance and for providing information for action in support of strategies developed at the local, national, regional and global levels (World Health Organization, 2015a). The main aim of undertaking AMR surveillance is to detect changes in resistance of organisms to antimicrobial agents and inform clinicians, policy makers and the general public about such changes as soon as possible (Bax et al., 2001). Surveillance is vital to understanding AMR, as the collection of reliable data can help guide actions aimed at prevention and control of AMR spread and also evaluate the outcomes of interventions directed at tackling the problem (World Health Organization, 2014). Surveillance is also essential in monitoring the public health impact of AMR and antimicrobial use. Furthermore, dissemination of information obtained from AMR surveillance studies can educate the public and consumers about rational antimicrobial use (Bax et al., 2001).

Given the frequency of occurrence of UTI both in hospital and community settings, with an increasing trend in resistance to urinary *E. coli* globally (World Health Organization, 2014), it is essential to undertake resistance surveillance of this bacterium. The Infectious Diseases Society of America also recommends undertaking surveillance of *E. coli* and other UTI causing pathogens to monitor changes in AMR (Warren et al., 1999). Antimicrobial resistance has been identified as a predictor of treatment failure, especially in patients with hospital-acquired UTI (Koningstein et al., 2014), further emphasising the need for continued monitoring of resistance patterns in *E. coli* UTI. Surveillance of resistance in urinary *E. coli* isolates is important in understanding the extent and significance of the problem. Global surveillance is vital as evidence shows that resistance genes are able to cross international borders (Bax et al., 2001). National level surveillance is also essential as the data can inform policy decisions such as updates to antibiotic guidelines, as well as identify priority areas for public health action; for example, regulatory measures for antimicrobial use (Shaban, Cruickshank, & Christiansen, 2013). As most UTIs are treated empirically with treatment based on the local susceptibility patterns of the common causative bacteria, surveillance of resistant *E. coli* at the local level is important. Data obtained from local surveillance are most highly beneficial for clinicians who require these data to guide empirical therapy, because resistance problems vary based on the hospital type and patient case mix (Bax et

al., 2001). For example, published studies have shown that urinary *E. coli* resistance to trimethoprim-sulphamethoxazole can vary considerably by geographic region (Gupta, Sahm, Mayfield, & Stamm, 2001; Sahm, Thornsberry, Mayfield, Jones, & Karlowsky, 2001) emphasising the importance of local level surveillance.

A major advancement in AMR surveillance is the increasing use of routine susceptibility data from the microbiology laboratory (Cornaglia et al., 2004). These data represent an inexpensive and easily accessible source for AMR information. Data can be electronically downloaded directly from the microbiology laboratory database and can be linked to population denominators (Cornaglia et al., 2004). Furthermore, WHO recognises the importance of laboratory-based surveillance in the control of AMR (World Health Organization, 2001). Urine cultures represent the most common type of culture processed by the microbiology laboratory, accounting for 24% to 40% of submitted cultures to the laboratory, with 80% of these cultures sent from outpatient settings (Wilson & Gaido, 2004). Therefore analysis of this large and widely accepted laboratory-based data source provides an opportunity for AMR surveillance in urinary *E. coli* isolates.

To successfully address AMR, it is important to monitor resistance prevalence and incidence. Local surveillance of resistance will provide current information on resistance prevalence and incidence (Levy & Marshall, 2004). Specifically, treatment of patients with antimicrobials should be tailored to the local resistance levels. This research program provides an in-depth analysis of routine susceptibility data obtained from a territory-level microbiology laboratory and makes available information on changes in urinary *E. coli* resistance, thereby helping in the development of policies aimed at control of AMR and providing recommendations for antimicrobial use in regards to UTI.

1.3.8 Current status of AMR surveillance in Australia

In June 2015, Australia released its first national AMR strategy in response to the increasing problem of AMR, with the goal of minimising the emergence and spread of AMR and ensuring the continued availability of antimicrobials which are effective (Australian Government, 2015). Prior to the release of the strategy, AMR supervision in Australia was by the Antimicrobial Resistance Standing Committee which was formed in 2012 (Shaban et al., 2013). This committee recommended: improvement in the current systems of data

gathering; reporting on antimicrobial use and AMR patterns; and the establishment of a national coordinating centre (Shaban et al., 2013), which are all included in the current strategy. The seven objectives as they appear in the new national AMR strategy (Australian Government, 2015, p.5) are:

1) Increase awareness and understanding of antimicrobial resistance, its implications, and actions to combat it through effective communication, education and training.
2) Implement effective antimicrobial stewardship practices across human health and animal care settings to ensure the appropriate and judicious prescribing, dispensing and administering of antimicrobials.
3) Develop nationally coordinated One Health surveillance of antimicrobial resistance and antimicrobial usage.
4) Improve infection prevention and control measures across human health and animal care settings to help prevent infections and the spread of antimicrobial resistance.
5) Agree a national research agenda and promote investment in the discovery and development of new products and approaches to prevent, detect and contain antimicrobial resistance.
6) Strengthen international partnerships and collaboration on regional and global efforts to respond to antimicrobial resistance.
7) Establish and support clear governance arrangements at the local, jurisdictional, national and international levels to ensure leadership, engagement and accountability for actions to combat antimicrobial resistance (Australian Government, 2015, p.5).

The Australian Commission on Safety and Quality in Health Care (ACSQHC) has the role of implementing Australia's national surveillance of resistance and antimicrobial use in human health (Australian Government, 2015). In June 2016, the ACSQHC released the first national report on antimicrobial use and resistance in human health with data obtained using the new Antimicrobial Use and Resistance in Australia (AURA) Surveillance System (Australian Commission on Safety and Quality in Health Care, 2016). The surveillance system brings together existing initiatives to allow integrated analysis and national reporting of AMR and antimicrobial use data (Australian Commission on Safety and Quality in Health Care, 2016). The AURA report integrates data from public and private facilities, as well as the community, and the findings will be used to inform clinical and public health decision making as well as

monitor and evaluate the effectiveness of interventions (Australian Commission on Safety and Quality in Health Care, 2016).

Although high-quality and coordinated surveillance of AMR and antimicrobial use in both hospital and community settings, identified in objective three of the national strategy, is a key priority to tackling the worldwide issue of AMR, surveillance alone cannot solve the issue. The other six objectives in the national strategy are also important to minimising further resistance development and spread. Although they are not the focus of this research program, these objectives are essential.

To ensure a targeted approach to surveillance of AMR, it is important to focus on priority organisms (Australian Government, 2015). *Escherichia coli* is one of the seven bacteria of international concern identified by the WHO (World Health Organization, 2014). This bacterium is also listed as a priority organism for targeted surveillance in human health in the Australian national resistance strategy because of its impact in both hospital and community settings (Australian Government, 2015), and is therefore the bacterium evaluated in this research program.

A well conducted research study aimed to provide AMR data specifically in urinary *E. coli* at the territory level will help address some of the current gaps, including those outlined in the national strategy, hence this research program. The findings of this research program will contribute to future national surveillance reports, further strengthening the quality of AMR reporting.

1.4 The doctoral research program

This doctoral research program comprises three separate but interrelated studies focused on providing knowledge about AMR in urinary *E. coli* infections. In the first study, a systematic review and meta-analysis of observational studies published in the last ten years, investigating ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTI was undertaken. This provided a broad context of the issues around AMR in *E. coli* UTI with the opportunity for me to address some of the issues using my own dataset in the subsequent two studies. The second study described the AMR temporal trends of *E. coli* UTI over five years, from January 2009 to December 2013, at the Canberra Hospital (an Australian tertiary

level hospital) and compared the prevalence of resistance between hospital- and community-acquired *E. coli* UTI. The second study also evaluated trends and seasonal variation in antimicrobial use at the Canberra Hospital. The third study evaluated the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of ACT residents over the same five-year period.

The three studies utilised three different datasets. Study one utilised data from published literature. Study two and study three utilised data from a territory microbiology laboratory, specifically ACT Pathology. Study two also utilised supplementary data on antimicrobial use for the Canberra Hospital. There is no similar published research in Australia.

1.4.1 Significance of the research

These three studies are both significant and timely given the recent release of Australia's first AMR strategy (Australian Government, 2015), with this research program aligning with objective 3 of the national strategy, which is the development of nationally coordinated One Health surveillance of AMR and antimicrobial use. Further, the global action plan on AMR was recently endorsed at the May 2015 World Health Assembly calling all countries to implement national strategies within the next two years (World Health Organization, 2015a). Five objectives were outlined at the Assembly, one of which is to strengthen the knowledge and evidence base of AMR through surveillance and research. This research program aligns with that objective. The academic community has been identified as having an important role to play in generating knowledge on AMR incidence and prevalence, which can be translated into practice (World Health Organization, 2015a).

The rationale for undertaking this research program is, first, to contribute internationally to the evidence base of AMR by providing data on global estimates of resistance to commonly used antimicrobials in the treatment of *E. coli* UTI. Data from countries worldwide may justify reconsidering the empirical use of ciprofloxacin in countries where it is recommended as first choice for UTI treatment and making recommendations for strategies to counteract the development of further resistance. The second major significance of this research program is the potential for use of the study outcomes to inform decisions on the treatment of patients with UTI based on local resistance patterns. Antibiotics prescribed for UTI are based on national treatment guidelines but local resistance patterns should be used to

refine therapy due to the regional variability of AMR (Gupta, Sahm, et al., 2001; Schito et al., 2009). One of the five essential strategies for antimicrobial stewardship in hospitals, according to the ACSQHC, includes modifying the local prescribing guidelines according to local organisms and susceptibility patterns (McKenzie, Rawlins, & Del Mar, 2013). Third, the findings also have the potential to guide therapeutic recommendations for UTI based on site of acquisition. Although the effects of AMR, such as increased risks of complications and longer hospital stay, are mainly felt in healthcare facilities, the greatest use of antimicrobials occurs in the community (Coxeter, Looke, Hoffmann, Lowe, & Del Mar, 2013). Comparing resistance in hospital- and community-acquired UTI may provide satisfactory data to make both AMR control policy and therapeutic recommendations for UTI based on site of acquisition. The fourth major significance of this research program is the potential to provide information on patient risk factors associated with resistant *E. coli* UTI and use this information to target specific patient groups that are at risk of developing resistant *E. coli* UTI, thereby preventing further development of resistance. Fifth, the research program will contribute to improving the quality of analysis of microbiological laboratory data which are not collected primarily for research purposes, and proffer approaches to addressing some of the methodological challenges in the synthesis of these data.

Resistance to frequently prescribed antimicrobials used for treating UTI has adverse health consequences, with a higher risk of patient morbidity and mortality, further highlighting the need for research in this area. Antimicrobial resistance data is useful in understanding resistance trends, determining best treatment options for patients, informing health policy, identifying important areas for interventions, and monitoring the effect of interventions to contain further spread of resistance (World Health Organization, 2012). In summary, the outcomes of these studies will provide information on global and local resistance trends which will serve as data for action towards development and implementation of AMR control policies; identify areas for interventions; contribute methodologically to synthesis of resistance data from the microbiological laboratory, and also inform the treatment guidelines for UTI both internationally and nationally. As the focus of the thesis is on microbiological laboratory data, which is only one aspect of what informs therapeutic management of *E. coli* UTI, the thesis will only inform some aspects of decision-making about prescribing.

1.4.2 Study aims

The overall research program aim is to contribute to the body of knowledge about AMR in *E. coli* UTI. The individual study aims are to:

1) Systematically review the literature and conduct a meta-analysis of observational studies published in the last ten years, investigating ciprofloxacin resistance in community-acquired and hospital-acquired *E. coli* UTI.
2) (a) Evaluate AMR temporal trends and compare the prevalence of AMR in hospital-acquired and community-acquired *E. coli* UTI at the Canberra Hospital over a five-year period.

   (b) Evaluate trends and seasonal variation in antimicrobial use at the Canberra Hospital over a five-year period.
3) Evaluate the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of ACT residents over a five-year period.

Each aim will be addressed in a separate study.

1.4.3 Study one – Systematic review of ciprofloxacin resistance in *E. coli* UTI

Background:

Ciprofloxacin is the most commonly prescribed fluoroquinolone for UTI and during the last ten years urinary *E. coli* resistance to ciprofloxacin has increased (Mcquiston, Rosborg, Sternhagen, Llor, & Bjerrum, 2013). The fluoroquinolone class of antimicrobials is also listed as one of the highest priority critically important class of antimicrobial agents because they are used in the treatment of more serious infections, for example, septicaemia. Therefore, resistance to fluoroquinolones can have significant clinical implications. Systematic and accurate global data about the prevalence of ciprofloxacin resistance in hospital- and community-acquired *E. coli* UTI are absent. These data may guide effective empirical therapy of UTI internationally and also make available information to assist with control of resistant bacteria. In addition, the pooled data can provide a baseline for future interventions to be measured. A systematic review of the literature also has the potential to identify gaps in published AMR studies which can be addressed in the other two research studies.

Research question:

1) For studies published from 1st January 2004 to 31st December 2014, what is the prevalence of ciprofloxacin resistance in community-acquired *E. coli* UTI compared to hospital-acquired infections?

Research hypotheses:

1) It was hypothesised that at an international level there would be a higher prevalence of ciprofloxacin resistance in hospital-acquired *E. coli* UTI than community-acquired infections.
2) It was hypothesised that ciprofloxacin resistance would be higher in developing countries compared to developed countries.

1.4.4 Study two – Prevalence of antimicrobial resistance in *E. coli* UTI

Background:

Urinary *E. coli* isolates are becoming increasingly resistant to current antimicrobials, hence research examining resistance patterns is paramount to inform effective treatment regimens and improve clinical outcomes of patients. No Australian data that directly compare resistance patterns over time for hospital-acquired and community-acquired UTI were located after an extensive literature search. Comparing resistance prevalence in hospital- and community-acquired UTI may provide data to make therapeutic recommendations for UTI based on site of acquisition. Despite the evidence to support the association between antimicrobial use and development of resistance, to my knowledge there are no published studies demonstrating the association between antimicrobial use and AMR in *E. coli* UTI in Australia, hence additional data on antimicrobial use for Canberra Hospital were obtained. The antimicrobial use data were found to be for all infections, not only UTI, and could not be linked to the AMR data. Hence the analysis of supplementary antimicrobial use data is included separately and provides results of the trends and seasonal variation in antimicrobial use at the Canberra Hospital.

Research questions:

1) For the period January 2009 to December 2013, does the prevalence of AMR differ for hospital-acquired *E. coli* UTI when compared with community-acquired UTI at the Canberra Hospital?

2) For the period January 2009 to December 2013, what is the AMR trend of *E. coli* UTI at the Canberra Hospital?
3) For the period January 2009 to December 2013, what is the trend and seasonal variation in antimicrobial use at the Canberra Hospital?

Research hypotheses:

1) It was hypothesised that at the Canberra Hospital there would be a higher prevalence of AMR in hospital-acquired *E. coli* UTI than community-acquired *E. coli* UTI.
2) It was hypothesised that at the Canberra Hospital there would be an increasing trend in resistance prevalence over the five-year period.
3) It was hypothesised that at the Canberra Hospital there would be an increasing trend in antimicrobial use over the five-year period.

1.4.5 Study three – Incidence of antimicrobial resistance in *E. coli* UTI

Background:

In Australia, the Australian Group on Antimicrobial Resistance (AGAR) reports only on prevalence without providing any estimates of incidence. There are no available Australian data to provide information on the incidence of antimicrobial resistant UTI over time. Prevalence data provide information on disease burden, that is, how common antimicrobial resistant UTI is. Conversely, incidence data provide information on the risk of disease occurrence, which can help identify specific patient groups that are at risk of developing antimicrobial resistant *E. coli* UTI. Obtaining both incidence and prevalence data are important because they provide information about the health status of a population and contribute to disease management decisions.

Research questions:

1) For the period January 2009 to December 2013, what is the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of ACT residents?

Research hypotheses:

1) It was hypothesised that there would be an increasing incidence of resistance in a cohort of ACT residents with *E. coli* UTI over the five-year period.

1.5 Thesis structure

This thesis is divided into seven chapters. It is a thesis by publication and contains three manuscripts (two peer reviewed publications in Q1 ranking (Scimago, 2016) journals – 2015 Impact Factors for publications one and two are 2.690 and 3.234 respectively). A third manuscript is being revised for resubmission to the Medical Journal of Australia (Q1 ranking journal, 2015 Impact Factor of 3.369) based on feedback received from the Journal. Appendix A1 provides a list of publications included in the thesis. For ease of reading, manuscripts are inserted in a form consistent with non-published sections and there is a single reference list at the end of the thesis. The tables and figures are numbered consecutively in each chapter to also allow for ease of reading. The thesis is solely my work. Given that this is a thesis by publication, for the published papers the contribution of each author is clearly articulated (Appendix A2). Excluding the published papers, reference to myself is in the first person singular everywhere else in the thesis. Published manuscripts are provided as appendices in the format they appear online (Appendices B and C).

Chapter two comprises an extensive literature review, which begins with a summary of the epidemiology, aetiology, types and treatment of UTI. International and national literature is used to describe in detail the issue of AMR with its consequent health and financial implications, especially in regards to *E. coli* UTI. This chapter also explores the complex nature of AMR given the multiple factors that may propagate resistance development in *E. coli* UTI, with a particular emphasis on antimicrobial use. Evidence to support this is obtained from a broad range of peer reviewed and grey literature. The final section of this chapter highlights the gaps in knowledge and the key areas to be addressed in the research program.

Chapter three presents the methodology of the three studies. While this chapter provides a comprehensive discussion of the methods used in undertaking the research, a brief presentation of the methods is also provided in each of the manuscripts given that it is a thesis by publication. Hence, there may be some repetition of the methods in the thesis. Chapter three includes a discussion of the methodological considerations taken into account during the conduct of the studies and provides details of the data collection processes, data analysis and ethical conduct.

The fourth chapter comprises the results of study one as a published systematic review and meta-analysis of observational studies investigating ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTI. The review has been published (Fasugba, Gardner, Mitchell, & Mnatzaganian, 2015) and is incorporated into this thesis in its published version (section 4.2 and Appendix B).

Chapter five provides the findings of the second study, which compares the prevalence of resistance in community- and hospital-acquired *E. coli* UTI over five years at the Canberra Hospital. Study two also describes the AMR temporal trends and seasonal variation of *E. coli* UTI over the same period. Study two has been published (Fasugba et al., 2016) and is incorporated into the thesis in its published version (section 5.2 and Appendix C). This chapter also reports on the analysis of supplementary antimicrobial use data over the study period at the Canberra Hospital.

The sixth chapter details the results of study three, which investigates the incidence and risk of resistance to *E. coli* UTI in a cohort of ACT residents. The study also provides information of the incidences of multidrug-resistant, extensively drug-resistant and pandrug-resistant *E. coli* UTI. The manuscript reporting the findings of study three was finalised, submitted to and reviewed by the Medical Journal of Australia while the thesis was under examination. The journal review suggested that the manuscript be revised as a short report focusing on the incidence findings. Given the brevity of a short report, the detailed, previously submitted version of the manuscript has been included in the thesis. A short report focusing on the incidence findings is being prepared for resubmission to the Medical Journal of Australia.

Chapter seven presents the discussion, which synthesises the overall results, links together the three studies and highlights their contribution to knowledge in regards to controlling antimicrobial resistant urinary *E. coli* infections. The strengths and limitations of the research are provided. The clinical and policy implications of the research are stated. This chapter concludes by providing a summary of recommendations for future research.

1.6 Summary

This chapter introduced the thesis topic of AMR in *E. coli* UTI. To adequately control AMR levels, it is necessary to monitor AMR prevalence and incidence and provide timely data to influence action. Surveillance of AMR is essential to understanding resistance trends, developing therapeutic guidelines and assessing the success of interventions. Collection, analysis and evaluation of AMR data in urinary *E. coli* isolates will help to better understand the problem of AMR in *E. coli*, support ongoing activities at the international and local level and inform activities aimed at controlling resistance in this pathogen. This will be the focus of the three studies in this research program. The findings will provide information that can be applied at an international level on ciprofloxacin resistance in *E. coli* UTI in various world regions and provide baseline data for future interventions to be measured. These studies also have the potential to inform decisions on the treatment of patients with UTI based on local resistance patterns and may influence therapy for UTI based on site of acquisition.

The next chapter presents a detailed review of the literature on AMR in *E. coli* UTI with evidence of the gaps in knowledge as they relate to Australia.

Chapter 2: Literature review

2.1 Overview

In this chapter a review of the international and national published and grey literature about the thesis subject, AMR in *E. coli* UTI, is provided with a critique of the quality of analysis and reporting in the existing literature. The evidence from the literature is used to situate the thesis topic within the broader context of AMR. In order to contain the thesis subject, the topic of 'One Health and AMR', which was briefly discussed in the introduction chapter, is not considered in the literature review. The specific objectives of the literature review are to: (1) describe the epidemiology, health and economic implications, aetiology, types and treatment of UTI; (2) quantify antimicrobial use in humans both globally and in Australia; (3) discuss the health and economic implications of AMR in general and specifically in antimicrobial resistant *E. coli* UTI as well as describe global and Australian AMR patterns and trends in *E. coli* infections; (4) demonstrate the potential link between antimicrobial use and resistance in humans; (5) highlight the importance of the microbiology laboratory in AMR surveillance; and (6) identify the gaps in evidence as related to global and Australian surveillance of antimicrobial resistant *E. coli* UTI.

2.2 Search strategy

Three broad searches of the literature were performed. First, the electronic bibliographic databases – EMBASE, MEDLINE, PubMed and CINAHL – were searched using the following combination of terms: 'antimicrobial resistance'; 'antibiotic resistance'; '*Escherichia coli*'; 'urinary tract infections'; 'antimicrobials'; 'antimicrobial use', and 'surveillance'. Searches were undertaken for words in the title, keywords or abstract. Medical Subject Heading (MeSH) terms were also used: '*Escherichia coli*' [MeSH]; 'Drug Resistance' [MeSH]; 'Urinary Tract Infections' [MeSH]. Second, another search of the electronic bibliographic databases was conducted to obtain information specific to Australia, hence the addition of the search term 'Australia' was used in conjunction with the other aforementioned search terms. Third, where there was a lack of information on a specific topic in the peer reviewed literature, information was sourced from grey literature by searching websites of government organisations and health agencies; in particular, a paucity of published literature on AMR in Australia was identified. Websites of the ACSQHC, AGAR, territory and state governments were searched for relevant information. In addition, where seminal international reports

from notable organisations like the WHO were cited in the peer reviewed literature, these grey literature were also sourced to obtain important information relevant to the literature review.

As a result of variation in the use of the terms 'antimicrobial' and 'antibiotic', search strategies utilised both terms. The reference lists of papers retrieved were screened to locate additional papers. There were no time limits applied to the search strategy, as preliminary searches identified that limiting the search period to the last ten to fifteen years excluded relevant studies which have contributed significantly to describing the epidemiology of UTI, and also studies that have contributed to gaining a better understanding of the changes in AMR patterns over time, their impact and association with antimicrobial use. Search results were limited to studies published in the English language and studies involving humans. To focus the discussion in this chapter and address the specific objectives of the literature review, I therefore included only papers: describing the epidemiology, health and economic implications, aetiology, types and treatment of UTI; quantifying antimicrobial use in humans both globally and in Australia; discussing the health and economic implications of AMR in *E. coli* UTI; describing global and Australian AMR patterns and trends in *E. coli* infections; demonstrating the potential link between antimicrobial use and resistance in humans; and discussing the importance of the microbiology laboratory in AMR surveillance.

In total, 28,991 peer reviewed papers were retrieved from the combined electronic database searches. After the exclusion of duplicates, 7,102 papers were remaining. Initial review of the titles and abstracts of these papers was undertaken. All papers deemed irrelevant to the objectives of the literature review were excluded. For example, studies describing diagnostic testing of UTI and modelling of resistance mechanisms and virulence genes in *E. coli* isolates were not included. In total, 6,484 papers were excluded with 618 papers remaining. Further review of the remaining 618 papers was undertaken and studies that described AMR in non-*E. coli* isolates (such as *Klebsiella pneumoniae*) and non-urinary *E. coli* isolates (such as intestinal or bloodstream isolates) were excluded. These types of studies were excluded to ensure the review was focused on describing resistance in *E. coli* UTI. The final number of peer reviewed papers included in the literature review was 85. Searches of government organisation and international health agency websites for grey

literature retrieved 19 relevant papers for inclusion in the literature review. Hence, the final number of papers included in the literature review was 104.

2.3 Urinary tract infections

This section first describes the epidemiology of UTI, providing incidence and prevalence data on international and national estimates of UTI. The risk factors as well as population groups most affected by UTI are highlighted. Second, the health and economic implications of UTI are discussed, highlighting the impact of UTI on quality of life as well as the adverse health outcomes resulting from this infection. The economic burden associated with UTI is also stated. Third, the section also provides an overview of the aetiology and types of UTI. The final part of this section discusses the treatment of UTI and the current international and national therapeutic recommendations are outlined.

2.3.1 Epidemiology

Urinary tract infections are one of the most common bacterial infections affecting people in hospitals as well as in the community (Laupland, Ross, Pitout, Church, & Gregson, 2007). Data from the combined National Ambulatory Health Care Surveys in the US for 2009–2010 showed that UTI accounted for approximately 9.8 million visits to ambulatory care settings such as primary care, outpatient and emergency departments (Centers for Disease Control and Prevention & National Center for Health Statistics, 2015). This may be an underestimation in the US as the data are not based on all ambulatory care visits. In the UK, prevalence of UTI was estimated as 6.0% from 2008 to 2010, although this is based on data from children aged five years and below attending general practice clinics (O'Brien, Edwards, Hood, & Butler, 2013). Urinary tract infection was said to be the third most common healthcare-associated infection (HAI) (19% of all HAIs) in the 2011–2012 point prevalence survey of HAIs in Europe (European Centre for Disease Prevention and Control, 2013). In Beijing, China, UTI was noted to be the second most frequently identified HAI in 2014, accounting for 15% of all HAIs (Liu, Wu, Cai, & Zhou, 2016). A limitation of most of these studies is that the majority of national data have either estimated UTI prevalence or incidence in specific subpopulations such as females or in particular settings; that is, healthcare-associated UTI, further highlighting the difficulty with global estimation of UTI.

In Australia, there are no available national overall incidence and prevalence data on UTI. As it is not a notifiable disease, estimates are difficult to determine. Despite this limitation, estimates of UTI incidence in Australia were obtained from studies identified from the literature search. Results from Western Australia showed the average monthly incidence of *Staphylococcus saprophyticus* UTI was 152 per 1000 UTI in 1993 (Schneider & Riley, 1996). Another study conducted in New South Wales in children below 15 years showed an increase from 0.5 to 0.9 per 1000 children in the age standardised annual incidence of UTI between 1981 and 1994 (Craig, Irwig, Knight, & Roy, 1997). These studies were conducted over two decades ago. Data were obtained from a single private pathology laboratory in the first study and a specific age group from one state in the second study, thereby limiting generalisability of these findings to the Australian population more widely. A recent study from 82 hospitals and 17 aged care facilities in Australia reported a point prevalence of 1.4% and 1.5% respectively for healthcare-associated UTI (Mitchell, Fasugba, Beckingham, Bennett, & Gardner, 2016). An incidence of 1.73% was reported from eight hospitals during a four-year period also for healthcare-associated UTI (Mitchell, Ferguson, Anderson, Sear, & Barnett, 2016). The prevalence or incidence of community-acquired UTI was not investigated in these studies.

Females are more predisposed to UTI as bacteria easily enter the bladder via the short urethra with reported incidence rates being higher than males. For example, in men below 50 years of age, the incidence of UTI has been approximated to be 0.0005–0.0008 per person-year (Seminerio, Aggarwal, & Sweetser, 2011) compared with an incidence of 0.5–0.7 per person-year in young women (Hooton et al., 1996). Although the latter study was prospective, with over 96% of participants followed up, these results are not generalisable to all young women given the strict inclusion criteria. The inclusion criteria included women who were about to begin using a new contraceptive method or women who started contraception within six weeks of being enrolled into the study and had not used this contraceptive method in the last three months. Urinary tract infections not only affect young women and men but also the very young and old, demonstrating the presence of this infection across all age groups. In fact, UTI is considered high on the list of differential diagnosis for an infant presenting with fever in the first few months of life. A prevalence of 9% was reported in infants less than 60 days old in the US. The authors acknowledge that their findings may not be generalisable to other clinical settings because study participants

were patients evaluated only in the emergency department (Zorc et al., 2005). Recent findings from a prospective study of 598 elderly women aged 65–80 years reported a prevalence of about 17% (n=99) (Marques et al., 2012). In this study, no information was provided on the follow-up rate with the potential for underestimation of UTI prevalence if a large proportion of participants were lost to follow-up.

The major risk factors for UTI include the use of spermicidal agents, frequent sexual intercourse, a prior UTI episode and having a first-degree female family member with a history of UTI (Hooton, 2012). Particular groups of people who have a higher risk of developing a UTI include diabetics, pregnant women, the elderly, people with multiple sclerosis, those with underlying urologic anomalies, as well as immune compromised patients such as those with human immunodeficiency virus (HIV) and cancer (Foxman & Brown, 2003).

Although an accurate estimate of the worldwide incidence or prevalence of UTI is difficult to determine, evidence shows that UTI is an infection which commonly affect females and males, including children and the elderly.

2.3.2 Health and economic implications of UTI

Urinary tract infections pose significant health and economic implications to society. They are a cause of morbidity in the community and also in the hospital (Rogers & Peterson, 2011). Urinary tract infections impact considerably on the quality of life of those affected. An investigation of the impact of UTI on the health-related quality of life of female nurses in Taiwan showed that symptoms of UTI, such as urinary frequency and urgency, negatively impacted on the quality of life of the participants, especially in relation to their physical health (Liao et al., 2009). Recurrent episodes of infection also occur. Recurrent infection may be either re-infection, caused by a new infecting organism, or relapsing infection, caused by the same organism present before therapy. Relapse may occur either because the infecting organism was not completely eradicated from the genitourinary tract by antimicrobial therapy or because of re-infection by a persistent colonizing strain in the gut reservoir (Nicolle, 2002). In a cohort study of 113 women enrolled at the University of Michigan in the US, 27% (n=30) experienced a recurrent infection within six months of the first infection (Foxman, 1990). Determination of recurrence was based on review of medical

records which may not have sufficiently documented information on UTI, thereby underestimating the recurrence rates.

Significant adverse health outcomes may occur, especially in people who have a higher risk of developing a UTI, such as pregnant women and immunocompromised patients. For example, there is a greater risk of development of pyelonephritis and pre-eclampsia in pregnant women (Matuszkiewicz-Rowińska, Małyszko, & Wieliczko, 2015). Immunocompromised patients, for example patients undergoing organ transplant and those with HIV, have a higher rate of bacteremia which occurs when UTI spread to the bloodstream (Tolkoff-Rubin & Rubin, 1997). A retrospective study of UTI patients presenting to an emergency department in Israel in 2004 reported the presence of bacteremia in 15% of patients with a UTI (Bahagon, Raveh, Schlesinger, Rudensky, & Yinnon, 2007). In patients presenting with bacteremic UTI, the 30-day all-cause mortality rate can be as high as 25% (Hounsom, Grayson, & Melzer, 2011). This may be an underestimation as patients with septicaemia who did not have a blood culture taken were excluded from the study (Hounsom et al., 2011). Factors such as age of patient and presence of underlying medical conditions influence the progression to mortality in patients with bacteremic UTI. If antimicrobial treatment is delayed, this may negatively affect the patient's outcome (Foxman, 2002; Hounsom et al., 2011; Van Nieuwkoop et al., 2010). When UTI is not associated with mortality, patients may require additional stay in hospital of up to four days, which places a significant economic burden on the health system (Mitchell, Ferguson, et al., 2016).

The economic burden of UTI is substantial, primarily due to the frequency of occurrence of UTI (Foxman, 2002). A case control study of UTI in female college students found that each episode of UTI resulted, on average, in 6.1 days of symptoms, 2.4 restricted activity days, 1.2 work or class days lost including 0.4 bed days (Foxman & Frerichs, 1985). Although this study was conducted over thirty years ago, it was identified from the literature search as the study primarily cited, representing seminal work undertaken in this area with no other studies providing such data to date. Given the study population, the findings may not be generalisable to all people. Estimation of costs is based on physician visits, antimicrobial therapy, laboratory diagnosis, hospitalisation as well as non-medical costs attributed to work days lost and morbidity (Foxman, 2002). In the US, it is estimated that over $1 billion is

expended for community-acquired UTI and $451 million for healthcare-associated UTI respectively (Hsueh et al., 2011; Jacobsen, Stickler, Mobley, & Shirtliff, 2008). In Italy, the mean yearly cost per patient for the diagnosis and treatment of UTI was estimated to be €229, with antimicrobial therapy identified as contributing the most to the total cost (Ciani, Grassi, & Tarricone, 2013). This cost estimate was deemed to be conservative given the model assumption that consumption of healthcare resources was constant over the study period as well as the exclusion of indirect costs including loss of productivity (Ciani et al., 2013). Annual estimates from Ireland are approximately €19.2 million at the national level and this is said to cover general practice consultations, antimicrobial therapy and laboratory costs (Callan et al., 2014). The cost estimates for UTI reported in this section should be interpreted in relation to the varying population sizes for the countries mentioned. In Australia, it is estimated that approximately 380,600 extra bed-days are used in public hospitals each year by patients acquiring a UTI in hospital (Mitchell, Ferguson, et al., 2016).

The health and economic implications of a potentially preventable disease such as UTI are considerable, which demands further investigation. Especially of importance is the use of antimicrobials for the treatment of this common infection, with an increase in the risk of patients developing antimicrobial resistance. This research program, which evaluates AMR in UTI, has the potential to inform policy making that may improve health and economic outcomes for patients and the health system as a whole.

2.3.3 Aetiology and types of urinary tract infections

Over 80% of UTIs are caused by *E. coli* (Nicolle, 2008; Rogers & Peterson, 2011). This Gram-negative bacterium belongs to the Enterobacteriaceae family, which comprises Gram-negative bacteria responsible for important hospital- and community-acquired infections (Australian Group on Antimicrobial Resistance, 2011). The remaining 20% are caused by other bacteria in the Enterobacteriaceae family such as *Klebsiella*, *Proteus* and *Enterobacter* species as well as other pathogens, which include *Staphylococcus saprophyticus*, *Enterococcus* species, Group B streptococcus and *Pseudomonas aeruginosa* (Mazzulli, 2012; Ronald, 2002).

Urinary tract infections can be classified based on location of UTI acquisition and provision of healthcare services, into either community-acquired UTI or the broad category of

healthcare-associated UTI, which includes hospital-acquired infections. Classification based on location of infection acquisition is discussed in detail in the methods chapter as this is an important methodological consideration. Subsequent paragraphs in this section will focus specifically on the types of UTI based on anatomical and clinical classification.

Urinary tract infections can be classified anatomically depending on the part of the urinary tract affected. Infection of the lower urinary tract affecting the bladder is referred to as cystitis. Upper urinary tract infection involving the renal tissue is referred to as pyelonephritis (Flores-Mireles, Walker, Caparon, & Hultgren, 2015; Kumar, Dave, Wolf, & Lerma, 2015; Lichtenberger & Hooton, 2008). Cystitis and pyelonephritis can be further classified clinically into uncomplicated or complicated UTI (Kumar et al., 2015), as discussed in the next paragraph.

The clinical grouping of uncomplicated and complicated UTI depends on the host condition (Nielubowicz & Mobley, 2010). Uncomplicated UTI affects otherwise healthy individuals presenting, for example, as uncomplicated cystitis and uncomplicated pyelonephritis (Nicolle, 2008). Patients with uncomplicated UTI have no evidence of structural abnormalities of the urinary tract (Flores-Mireles et al., 2015; Hooton, 2012). Complicated UTI affects people with a structurally and functionally abnormal urinary tract or those with an underlying medical or surgical health issue (Lichtenberger & Hooton, 2008; Neal & Durwood, 2008). Complicated UTI is associated with factors that have an effect on the urinary tract or host defense such as urinary obstruction, pregnancy, diabetes mellitus and immunosuppression (Flores-Mireles et al., 2015). Complicated UTI could present as acute pyelonephritis with intrarenal, perirenal or pararenal abscess and septicaemia (Neal & Durwood, 2008; Nielubowicz & Mobley, 2010).

2.3.4 Treatment of urinary tract infections

Antibiotics are considered the standard treatment for bacterial UTI. They aim to eliminate the causative organisms and provide symptom relief to patients (Jancel & Dudas, 2002; Stuck et al., 2012). Urinary tract infections account for about 15% of outpatient antibiotic prescriptions in the US (Stuck et al., 2012). In Australia, there is no information on the proportion of antibiotic prescriptions for UTI, as the available data on antimicrobial use from the National Antimicrobial Utilisation Surveillance Program (NAUSP) do not include the

indication for which antimicrobials are utilised (Government of South Australia, 2013). These data are collected by NAUSP for compilation and processing from contributing health facilities who submit their total monthly antimicrobial usage and bed occupancy data (Government of South Australia, 2013). The treatment protocol for UTI in Australia is based on the Therapeutic Guidelines: Antibiotic (Antibiotic Expert Groups, 2014). In many cases, treatment is wholly empirical, meaning that the prescribed antibiotic is not reviewed against microbiology results but the antibiotic choice is based on prior knowledge of the most likely causative agent along with its local resistance patterns (Leekha et al., 2011). Therefore it is important to monitor the resistance patterns of these pathogens to make sure that patients are provided with the most appropriate antimicrobial treatment (Jancel & Dudas, 2002; Linhares et al., 2013). 'Appropriate' treatment is described as treatment to which the pathogen is susceptible (Paul et al., 2010). The reverse, that is, inappropriate treatment, leads to the development of resistant pathogens and recurrence of infection (Trautner, 2010).

In Australia, first choice treatment for acute uncomplicated UTI is trimethoprim, cephalexin, amoxycillin-clavulanate or nitrofurantoin (Antibiotic Expert Groups, 2014). Fluoroquinolones such as ciprofloxacin are not recommended as first-line UTI drugs (Antibiotic Expert Groups, 2014). Their use has been restricted in Australia since the early 1990s as they are classified as a reserve antimicrobial drug (Cheng et al., 2012). Likewise in the US, the Infectious Diseases Society of America (IDSA) guidelines recommend their use for treatment of more severe infections other than acute cystitis as they are associated with the development of resistance (Gupta et al., 2011). Fluoroquinolones have been listed by the WHO as one of the highest priority critically important antimicrobials alongside third and fourth generation cephalosporins, macrolides and glycopeptides (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, 2012). An important use of this list is to address prevalence data gaps on these critically important antimicrobials (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, 2012). Fluoroquinolones have an important role in the treatment of more severe infections, such as septicaemia; therefore resistance to fluoroquinolones can have serious clinical consequences. Fluoroquinolones are also one of few existing treatment options available for serious *Salmonella* species and *E. coli* infections. Resistance to fluoroquinolones emerges fast, and it should therefore be used with caution and reserved for severe infections, and be preceded by antimicrobial

susceptibility testing of the bacteria involved (Mcquiston et al., 2013). Ciprofloxacin is the most commonly prescribed fluoroquinolone for UTI (Schaeffer, 2002). This antimicrobial agent has broad Gram-negative organism coverage, and is well absorbed from the gastrointestinal tract after oral administration with high urinary excretion rate (Schaeffer, 2002).

To date, fluoroquinolones, particularly ciprofloxacin, are used as the drugs of choice for UTI in countries where the level of resistance to other antimicrobials such as ampicillin or trimethoprim-sulfamethoxazole is high (Liu et al., 2011; Maraki et al., 2013). A growing resistance to trimethoprim-sulfamethoxazole may have favoured the use of ciprofloxacin, leading to a rise in ciprofloxacin-resistant *E. coli* UTI. The increasing resistance to ciprofloxacin has important implications for the treatment of bacterial infections like UTI. The lack of quantitative syntheses of overall ciprofloxacin-resistant *E. coli* UTI prevalence is a major gap identified in the literature and the first study of this research program will address this gap.

Non-antimicrobial treatment of uncomplicated UTI has been investigated as a potential means to reduce unnecessary antimicrobial prescribing and subsequent resistance. Published evidence from randomised controlled trials comparing antimicrobial treatment with placebo or alternative treatment options such as delayed (48 hours) antimicrobials or ibuprofen showed that patients in the placebo and delayed antimicrobial groups had significant delays in improvement of symptoms and bacteriological cure (Little et al., 2010; Gágyor et al., 2012). Although patients in the ibuprofen group had significantly fewer antimicrobial courses, they had a significantly higher total symptom burden with more patients having pyelonephritis (Gágyor et al., 2015). Further research with larger sample sizes are recommended to further investigate the effectiveness of non-antimicrobial approaches in treatment of UTI.

As antimicrobials are considered the mainstay of treatment for UTI, the next section discusses antimicrobial use in humans and provides evidence to show increase in antimicrobial use, including those used for the treatment of UTI.

2.4 Quantifying antimicrobial use in humans

This section provides an overview of the current state of global and national antimicrobial consumption in humans. First, global antimicrobial use is discussed and suggested methods for quantifying antimicrobial use to allow for comparison of rates between countries are described. Evidence to support the increase in global antimicrobial use for human health is provided, including antimicrobials used in the treatment of UTI. The second part of this section discusses antimicrobial use in Australia. The organisations responsible for monitoring antimicrobial use in Australia are stated. Current estimates of antimicrobial use in Australian hospitals as well as the community setting are also provided. The effect of antimicrobial use in livestock production is not central to the thesis and will not be covered.

2.4.1 Global antimicrobial use

Appropriate measurement of antimicrobial use is essential to determine consumption trends over time as well as compare consumption levels between countries (Filius et al., 2005). A number of methods have been suggested for quantifying this antimicrobial use. Initial estimates of antimicrobial use were reported as the proportion of patients who were prescribed antimicrobials while hospitalised (Polk, Fox, Mahoney, Letcavage, & MacDougall, 2007). The current and most widely used measurement is the WHO recommended number of defined daily doses per 100 patient days (Filius et al., 2005). The Defined Daily Dose (DDD) is defined as "the assumed average maintenance dose per day for a drug used for its main indication in adults" (WHO Collaborating Centre for Drug Statistics Methodology, 2015, p. 22). Although this unit allows for drug use comparisons between countries, it is simply a measurement unit and is not a reflection of the recommended or prescribed dose in patients (Monnet, 2007). Some authors have proposed using the number of days of therapy (DOT) as an alternative measure given the limitations of the DDD, which include a lack of DDDs for some antimicrobials, inability to apply DDDs to paediatric patients as well as the potential to underestimate antimicrobial exposure when there are reductions in the administered daily dose for patients with impaired kidney function (Monnet, 2007; Polk et al., 2007). Further still, antimicrobial drug use has been measured by other authors using standard units which refer to a single dose of the drug, which may be in form of a pill, capsule or ampoule (Van Boeckel et al., 2014). Despite the different measurements described above, the DDD is still recognised as the standard measurement worldwide because it is endorsed by the WHO and can be reliably used globally for measuring

antimicrobial use in both inpatients and outpatients (Monnet, 2007). Furthermore, its widespread use allows for worldwide comparison to facilitate benchmarking (Monnet, 2007).

Antimicrobial use for human health is said to be increasing considerably (World Health Organization, 2015a). Between 2000 and 2010, antimicrobial use was estimated to have increased by 36% in 71 countries, from approximately 50 billion to 70 billion standard units (number of doses) (Van Boeckel et al., 2014). Reporting of antimicrobial use with DDDs was not possible given the lack of global data using DDDs, hence the use of standard units which was the measurement unit used in the most comprehensive global dataset for this study (Van Boeckel et al., 2014). The largest absolute increases in antimicrobial use were for cephalosporins, broad-spectrum penicillins and fluoroquinolones, antimicrobial classes used in the treatment of UTI. Countries rated as the top global consumers in 2010 were India, China and the US (Van Boeckel et al., 2014). Among the high-income countries, antimicrobial use increased substantially between 2000 and 2010 in Australia and New Zealand, from 25 to 87 units per person and 26 to 70 units per person respectively (Van Boeckel et al., 2014).

It is estimated that about 20% of antimicrobials are used in hospitals with about 80% used in community settings, which include general practitioner clinics and non-prescription use (Kotwani & Holloway, 2011). Over-the-counter or non-prescription antimicrobial use varies by country depending on whether prescription-only laws are enforced. A systematic review investigating global non-prescription antimicrobial use reported that for countries outside Europe and the US, estimates of over-the-counter antimicrobial use ranged from 19% to over 90% (Morgan, Okeke, Laxminarayan, Perencevich, & Weisenberg, 2011). Additional evidence from the systematic review showed that levels of antimicrobial-resistant bacteria were higher in populations with frequent use of non-prescription antimicrobials (Morgan et al., 2011). A number of limitations were noted, including combining studies with diverse population groups as well as the use of a search period spanning over 40 years, which may have resulted in the inclusion of studies which no longer mirror current prescription practices in some countries.

2.4.2 Antimicrobial use in Australia

In Australia, systematic surveillance of antimicrobial use in humans is provided by voluntary participation NAUSP (Gottlieb & Nimmo, 2011). The National Antimicrobial Prescribing Survey (NAPS) is a voluntary annual point prevalence survey undertaken by health facilities (Australian Commission on Safety and Quality in Health Care, 2016). The NAPS also reports data on the appropriateness of antimicrobial prescribing. The recent development of the Aged Care National Antimicrobial Prescribing Survey (acNAPS) also allows for collection of antimicrobial use data in Australian residential aged care facilities (Australian Commission on Safety and Quality in Health Care, 2016). Surveillance of antimicrobial use enables hospital administrators and clinicians to examine their antimicrobial use over time, and compare with similar hospitals.

There are approximately 22 million antibiotic prescriptions written annually in Australian primary care settings, ranking Australia as one of the major users of antibiotics in the industrialised world (McKenzie et al., 2013). Over 1100 prescriptions per 1000 population are dispensed in Australia (Australian Commission on Safety and Quality in Health Care, 2016). This is higher than the US, England, Scotland, Canada and Sweden which have prescriptions of less than 850/1000 population (Australian Commission on Safety and Quality in Health Care, 2016). In regards to community antimicrobial use, compared to Denmark, the Netherlands and Sweden, with a DDD of less than 15/1000 population/day, Australia's DDD is almost double this value at nearly 23/1000 population/day (McKenzie et al., 2013). Australia's hospital antimicrobial use is higher than Netherlands, Norway, Sweden, Canada and Denmark, at an estimated 3 DDD/1000 population per day in Australia but lower than England and Scotland, which both have DDDs over 4/1000 population per day (Australian Commission on Safety and Quality in Health Care, 2016). It should be noted that given the variability in the data collection processes in different countries, these comparisons are not absolute.

It is estimated that about 38% of all Australian hospital patients are prescribed an antimicrobial agent on any given day (Australian Commission on Safety and Quality in Health Care, 2015). In 2014, based on data from the 129 participating hospitals that submitted data to NAUSP, total antimicrobial use in hospitals was 936 DDD/1000 occupied bed days (Australian Commission on Safety and Quality in Health Care, 2015). Hospital antimicrobial

use was found to vary by states and territories with the highest rate reported in Tasmania and the lowest in Queensland. Urinary tract infections were the fourth most common indication (6.7%) requiring an antimicrobial prescription. In the community, 46% of Australian residents were prescribed at least one antimicrobial agent in 2014 (Australian Commission on Safety and Quality in Health Care, 2015). This equates to almost half of the total population surveyed. Antimicrobial use was estimated to be 24 DDD/1000 population per day and was noted to be highest in children below 9 years of age and adults above 65 years. The highest source of antimicrobial prescriptions (88%) was from general practitioners. Data from the first pilot of the acNAPS undertaken in 186 residential aged care facilities in 2015 showed that about 11% of residents received an antimicrobial, with UTI identified as the second most common reason (17%) requiring an antimicrobial (Australian Commission on Safety and Quality in Health Care, 2016). The acNAPS data are not representative of all Australian aged care facilities, given that this was a pilot study in preparation for national surveillance (Australian Commission on Safety and Quality in Health Care, 2016).

Overuse of antimicrobials in humans has significant health implications, particularly AMR which is discussed in the next section.

2.5 Antimicrobial resistance

The WHO has described AMR as an international threat to public health, threatening the successful prevention and treatment of bacterial, viral, parasitic and fungal infections (World Health Organization, 2012, 2014). As such, research into its prevention and reduction is very important. Antimicrobial resistance is on the rise globally and available data suggest that this problem is neither country nor region specific, affecting both industrialised and non-industrialised countries. In all six WHO regions (Africa, Americas, Eastern Mediterranean, European, South-East Asia and Western Pacific), high resistance have been noted in bacteria that cause common hospital- and community-acquired infections such as UTI and pneumonia (World Health Organization, 2014). This section discusses the health and economic burden of AMR in general and specifically in resistant *E. coli* UTI. International and national evidence in support of the rising AMR in *E. coli* UTI are also discussed.

2.5.1 Health and economic implications of AMR in general

In general, AMR has significant health implications to society. It results in increased patient morbidity and mortality. Antimicrobial resistance may lead to treatment failure, resulting in death, especially in already critically unwell patients who are more at risk because of their relative immune deficiency and high exposure to antimicrobial agents (Tenover, 2006). In the European Union, Iceland and Norway, approximately 25,000 patients died in 2007 from antimicrobial resistant infections. About two-thirds of these deaths were due to Gram-negative bacterial infections from third-generation cephalosporin-resistant *E. coli, Klebsiella pneumoniae* and carbapenem-resistant *Pseudomonas aeruginosa* (European Centre for Disease Prevention and Control, 2009). However, AMR is also a problem for Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* (European Centre for Disease Prevention and Control, 2009). Infection control problems may arise from spread of resistant bacteria in both healthcare facilities and in the community. Spread within the community creates significant concerns for infection control in long-term care facilities and day care centres, due to increased population mobility (Tenover, 2006). Antimicrobial resistance prolongs the duration of illness, increases the risks of complications and leads to longer hospital stay, thereby leading to greater healthcare costs for patients.

The authors of the report of the Review on Antimicrobial Resistance in the UK estimate that by 2050 the global financial cost of AMR will be approximately US$100 trillion, with ten million lives at risk of developing a resistant infection each year if the issue of AMR is not addressed (O'Neill, 2016). The economic burden of AMR also includes loss of productivity and increased cost of diagnostics and treatment (World Health Organization, 2012). Direct healthcare costs in the US have been estimated to be as high as $20 billion with loss of productivity costing $35 billion per year (Centers for Disease Control and Prevention, 2013). In the European Union the estimated total cost to society of AMR is €1.5 billion each year (European Centre for Disease Prevention and Control, 2009). The estimated cost of AMR to the health budget in Australia is over $250 million annually and double this amount to the community (Shaban et al., 2013). Most determinations of costs attributable to AMR are approximate values because they have been derived from small, often non-representative databases. Furthermore, these estimates were derived from reports or websites of notable

organisations, demonstrating the lack of published research on the overall health and economic burden associated with AMR at the population level.

2.5.2 Health and economic implications of AMR in UTI caused by *E. coli*

According to the most recent WHO report on AMR surveillance, *E. coli* is reported as one of the nine bacterial pathogens of global concern that are responsible for some of the common infections that occur in community and hospital settings (World Health Organization, 2014). Antimicrobial resistance in *E. coli* poses significant health and economic implications for society. Results from a recent systematic review showed significant increases in mortality for patients with third-generation cephalosporin and fluoroquinolone-resistant *E. coli* infections, compared to those with non-resistant infections (World Health Organization, 2014). Furthermore, the review results also showed that patients with a resistant bacterial infection had higher care needs compared to those without a resistant infection. The proportion of patients needing admission to the intensive care unit was significantly higher (P=0.03) for patients with a fluoroquinolone resistant *E. coli* compared to those with non-resistant infections (World Health Organization, 2014). The presence of confounding in the majority of the included studies was identified as a concern by the reviewers as well as the inclusion of studies with small sample sizes (World Health Organization, 2014). For example, a case control study aimed at determining the outcomes of patients with ciprofloxacin-gentamicin-resistant *E. coli* UTI found that *E. coli* bacteremia, septic shock and death within 30 days were more common among cases than controls (P=0.01, P=0.02 and P=0.04 respectively). Cases were also more likely than controls to relapse within two months (P=0.002). These associations were statistically significant (Pépin, Plamondon, Lacroix, & Alarie, 2009). Although controls were selected from the same population as cases, matching was not done, introducing the potential for confounding. While this study was also limited to a single hospital and the findings may not be generalisable to all populations, especially given that resistance has been shown to vary geographically, it illustrates some of the common study design problems.

Regarding the economic burden of antimicrobial resistant UTI, a study from the UK reported that between 2002 and 2004, patients with an antibiotic (specifically ampicillin)-resistant *E. coli* UTI received general practice costs that were £3.64 higher on average (P=0.008) than those of patients with an antibiotic sensitive UTI (Alam et al., 2009). Another study from

Thailand reported that between 2003 and 2004, hospitalisation costs were higher in patients with community-onset ESBL-producing *E. coli* infections (median, US $528 vs $108, respectively; *P*<0.001) compared to patients without infections (Apisarnthanarak et al., 2007). The authors acknowledge that their sample size of 46 patients with community-onset ESBL-producing *E. coli* was small and therefore limited the ability to detect other possible outcomes in these patients. Although the study evaluated community-onset infections, data were obtained from inpatients and may not be applicable to outpatients and those with hospital-onset infections. Again, design flaws are evident in regards to the conduct of AMR studies.

Knowledge of the health and economic implications of AMR, including the impact of AMR on patient outcomes, is important for both hospitals and clinicians as it provides motivation to hospitals to implement programs tailored towards control of AMR and antimicrobial use (Eliopoulos, Cosgrove, & Carmeli, 2003). The review of literature identified a dearth of research quantifying the health and economic implications of antimicrobial resistant *E. coli* UTI in Australia, highlighting the need for more research on AMR in *E. coli* UTI in Australia.

2.5.3 Antimicrobial resistance patterns and trends in *E. coli* infections

There is evidence to show that resistance to antimicrobials used to treat *E. coli* infections is increasing globally (World Health Organization, 2015a). Available data on global resistance published in 2014 by WHO showed at least 50% resistance to fluoroquinolones and third generation cephalosporins in *E. coli* in five WHO regions (World Health Organization, 2014). Data from ResistanceMap, developed by Centre for Disease Dynamics, Economics and Policy (CDDEP), also provides information on global AMR in *E. coli* isolates (Figure 1) (Center for Disease Dynamics, Economics and Policy, 2015b). Resistance for the four antimicrobial classes assessed were noted to be highest in India, which is a developing country, compared to South Africa, UK, US and Australia, which are developed countries. In comparison to international data from South Africa, UK and US, Australian *E. coli* resistance were lower. The aggregated data from ResistanceMap represent invasive *E. coli* isolates sourced from blood and cerebrospinal fluid, highlighting the lack of global data on AMR specific to *E. coli* isolates sourced from urinary samples. There was a lack of published studies comparing antimicrobial resistant *E. coli* UTI in developing versus developed countries. This evidence gap will be addressed in the first study of the research program.

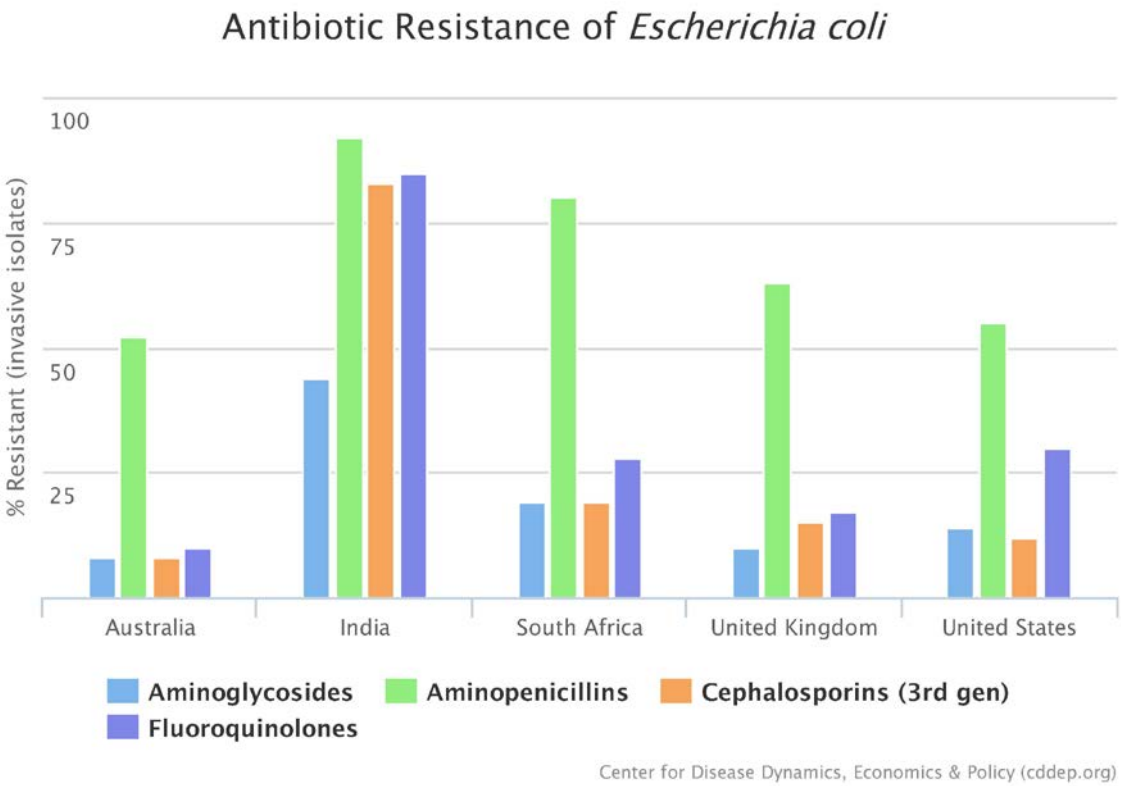

Figure 1 Antibiotic resistance for *Escherichia coli* isolates

Source: CDDEP 2015 sources include: AGAR (Australia); SRL Diagnostics (India); SASCM (South Africa); EARS-Net (Europe) and TSN (USA).

The chart displays the data for each country from ResistanceMap (CDDEP 2015). Data collection year varies by country (Australia-2013; India-2014; South Africa-2014; United Kingdom-2013; United States-2012).

Various international studies demonstrate an increase in the incidence and prevalence of resistance of *E. coli* UTI to commonly prescribed antimicrobials (Blaettler et al., 2009; Kronvall, 2010; Linhares et al., 2013; Maraki et al., 2013; Swami, Liesinger, Shah, Baddour, & Banerjee, 2012; Tadesse et al., 2012). Some of these studies also evaluated resistance patterns, taking into consideration individual patient risk factors such as sex and age (Blaettler et al., 2009; Linhares et al., 2013; Swami et al., 2012). The recently released first Australian report on antimicrobial use and AMR also reported increases in AMR in *E. coli*. Urinary *E. coli* resistance in 2014 were highest for ampicillin (amoxicillin) (42.3%) (Australian Commission on Safety and Quality in Health Care, 2016). Resistance also varied by states and territories although this finding was based on blood culture *E. coli* isolates (Australian Commission on Safety and Quality in Health Care, 2016). Results from a population-based retrospective study conducted in Tasmania, Australia, showed that *E. coli* resistance was

highest for amoxicillin (35%) and trimethoprim (14%) (Meumann, Mitchell, McGregor, McBryde, & Cooley, 2015). In addition, a significant increase in resistance was noted for amoxycillin clavulanate, from 3.4% in 2010 to 4.1% in 2012 (*P*=0.03) (Meumann et al., 2015). These Australian data do not include information about the age, sex, comorbidities, prior antibiotic exposure of patients and other clinical data. In addition, these data report on AMR prevalence with no available Australian data to provide information on the risk of disease occurrence, that is, incidence of antimicrobial resistant UTI over time. Hence collection of AMR data, which includes additional information on potential risk factors for AMR as well as more detailed statistical analyses, are required to better describe the issue of AMR in *E. coli* UTI in Australia. The second and third studies of the research program will address these gaps.

Previously published international studies have shown that AMR prevalence differs for hospital- and community-acquired UTI (Cullen et al., 2012; Ma & Wang, 2013; Perrin, Donnio, Heurtin-Lecorre, Travert, & Avril, 1999). In Australia, the Australian Group on Antimicrobial Resistance (AGAR) collects data on AMR prevalence in Gram-negative bacteria, including *E. coli* and *Klebsiella* species (Australian Group on Antimicrobial Resistance, 2011). The data collected by AGAR are not specifically UTI data, but include data on bacteria that may cause UTI. The surveys conducted by AGAR occur annually but alternate between community-onset and hospital-onset infections (Australian Group on Antimicrobial Resistance, 2013). Since the first community-onset Australian AMR prevalence survey conducted by AGAR in 2008, a gradual rise has been observed in the overall percentage of *E. coli* strains resistant to beta-lactam antibiotics (Australian Group on Antimicrobial Resistance, 2013). From 2008 to 2012, gentamicin resistance increased from 3.5% to 4.3%. Ciprofloxacin resistance appears to be increasing (from 4.2% in 2008, 5.4% in 2010, to 7.0% in 2012) despite government restrictions on its use in both the community and in hospitals (Australian Group on Antimicrobial Resistance, 2011, 2013; Cheng et al., 2012). Also, multi-resistance (acquired resistance to more than three agents) in *E. coli* increased from 4.5% in 2008 to 7.6% in 2012 (Australian Group on Antimicrobial Resistance, 2013). Similarly, since the first hospital-onset AMR prevalence surveys conducted by AGAR in 2009, resistance to beta-lactam agents has also been on the increase (Australian Group on Antimicrobial Resistance, 2011). *E. coli* resistance to ampicillin rose from 47.8% in 2009 to 50.5% in 2011 (Australian Group on Antimicrobial Resistance, 2011). Likewise, urinary *E.*

coli resistance to ciprofloxacin increased from 8% in 2009 to 11% in 2011 (Australian Group on Antimicrobial Resistance, 2011).

Although the AGAR undertakes hospital- and community- onset AMR prevalence surveys within Australia, the data are limited. Isolates categorised as community-onset are those obtained from non-hospitalised UTI patients, outpatient and patients from emergency departments or general practitioners, while hospital-onset isolates were obtained from various clinical specimens in patients hospitalised for over 48 hours. Although similar bacterial isolates (that is, *E. coli*) are included in both surveys, the use of varied clinical samples prevents direct comparison of these data between both groups and also among other studies. The second study of the research program will address this limitation by providing results of directly comparable data on hospital- and community-acquired *E. coli* UTI.

In recent years, a growing concern has been the emergence of extended spectrum beta-lactamase (ESBL) producing *E. coli* (Nicolle, 2008). Extended spectrum beta-lactamase producing organisms are resistant to beta-lactams, cephalosporins and other classes of antimicrobials, with carbapenems recognised as the most suitable treatment option for infections caused by ESBL-producing *E. coli* (Mazzulli, 2012). Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) found the prevalence of ESBL was 17.9% in urinary *E. coli* isolates obtained from hospital inpatients in countries worldwide (Hoban, Nicolle, Hawser, Bouchillon, & Badal, 2011). In Australia, about 7% to 12% of *E. coli* was found to be ESBL-producing *E. coli* (Australian Commission on Safety and Quality in Health Care, 2016). The presence of ESBL-producing *E. coli* isolates in both hospital and community settings has the potential to impact considerably on UTI treatment, as these pathogens are susceptible to a limited number of antimicrobials.

The Australian data reported in this section were obtained from reports and only one peer reviewed published paper on AMR in *E. coli* UTI in Australia was identified during the literature search (Meumann et al., 2015). In addition, data from reports were mainly based on *E. coli* isolates from a number of sources and not specifically urine isolates. This highlights the lack of rigorously conducted studies evaluating AMR in urinary *E. coli* isolates in Australia.

The next section brings together sections 2.4 and 2.5 by providing evidence to demonstrate the potential link between antimicrobial use and AMR.

2.6 The potential link between antimicrobial use and resistance in humans

Antimicrobial overuse or misuse is a known risk factor for the development of AMR in humans (Costelloe, Metcalfe, Lovering, Mant, & Hay, 2010). Demonstrating an association between antimicrobial use and urinary *E. coli* resistance is therefore important in developing strategies to combat AMR and improve understanding of the epidemiology of AMR (Hillier, Magee, Howard, & Palmer, 2002; Schechner, Temkin, Harbarth, Carmeli, & Schwaber, 2013). Several international studies have demonstrated this association. For example, in a study involving 26 European countries, the results showed statistically significant correlations for ciprofloxacin (Spearman correlation=0.74; 95% confidence interval=0.35-0.91; *P*=0.002) and cotrimoxazole (Spearman correlation=0.71; 95% confidence interval=0.29-0.90; *P*=0.005) use and their resistance in *E. coli* in 14 of the 26 countries (Goossens et al., 2005). Bergman et al. (2009) also found a number of associations between *E. coli* resistance and antimicrobial use. Significant associations were seen for: nitrofurantoin use and nitrofurantoin resistance (*P*<0.0001); cephalosporin use and nitrofurantoin resistance (*P*=0.029); fluoroquinolone use and ampicillin resistance (*P*=0.005); as well as amoxicillin use and fluoroquinolone resistance (*P*=0.003). In these studies, antimicrobial consumption was not stratified by length of therapy or number of antimicrobial drugs. It has been suggested that the varying associations for antimicrobial use and resistance may be attributable to a number of factors, including geographical differences in the resistance selection pressure, different statistical models used for assessing associations, differences in age and gender distribution and the use of patient or population level resistance and antimicrobial use data (Bergman et al., 2009; Goossens et al., 2005).

The association between ciprofloxacin use and the development of its resistance in UTI pathogens is also clearly documented in international studies. A recent Irish study involving 72 general practices found higher ciprofloxacin resistance levels (5.5%) in practices with 10 prescriptions per month, compared with resistance levels of 3% in practices with one prescription per month (Vellinga, Murphy, Hanahoe, Bennett, & Cormican, 2010). Although widespread use of this agent may have thus resulted in a rise in ciprofloxacin resistance, the

authors clearly state that their results should be interpreted with caution as data on antimicrobial prescribing was not representative of the entire population. In the Netherlands and US, an association has also been shown between high fluoroquinolone prescriptions and a rise in bacterial resistance (Goettsch et al., 2000; Zervos et al., 2003). These associations were statistically significant.

A detailed search of the literature revealed a lack of published Australian data aimed at determining the association between antimicrobial use and resistance in urinary *E. coli* isolates, with only one study identified. Findings from this study showed a statistically significant association between amoxicillin and amoxicillin-clavulanic acid use and resistance to these antimicrobials (Meumann et al., 2015).

2.7 Importance of the microbiology laboratory in AMR surveillance

The role of the microbiology laboratory is essential both in the detection and surveillance of AMR in the hospital and community (World Health Organization, 2001). Processing and examination of urine samples makes up a large part of the workload undertaken in microbiology laboratories. These laboratories are tasked with the job of performing urine cultures and antimicrobial susceptibility testing to obtain information on the causative pathogen and preferred antimicrobial(s) for treatment (Burd & Kehl, 2011; Wilson & Gaido, 2004). Microbiology laboratories are also responsible for collating susceptibility data to facilitate analysis for monitoring resistance prevalence and incidence and the possible detection of resistance trends. They are the initial sources for identification of emerging AMR patterns (Cantón, 2005; Reller et al., 2001). Therefore, accurate implementation of procedures by the microbiology laboratory as well as interpretation of susceptibility testing results is crucial to the quality of data produced for AMR reporting.

Collation and analysis of data from both public and private microbiology laboratories is highly valuable in providing an understanding of microbes and their susceptibility patterns (Coxeter et al., 2013). Useful data exist within various laboratory services across Australia (Shaban et al., 2013) but are not widely used and certainly not coordinated nationally. By retrospectively reviewing large amounts of these data for specific bacterial species over a period of time, the information obtained can help evaluate resistance patterns over time and assist in developing treatment guidelines (Shaban et al., 2013).

2.8 Gaps in global AMR surveillance

The global issue of AMR has been highlighted with evidence from the literature review showing the presence of antimicrobial resistant *E. coli* isolates in various countries and world regions (World Health Organization, 2014). In response to this crisis, the global action plan on AMR was approved at the World Health Assembly in May 2015 (World Health Organization, 2015a). A number of priority areas have been identified, representing knowledge gaps that need to be addressed through surveillance of AMR and research. These include: collection and analysis of data to identify the incidence and prevalence of resistant pathogens and geographical AMR patterns; understanding the development and spread of resistance; characterisation of emerging resistance in pathogens with an understanding of the mechanisms; understanding the social and behavioural factors that propagate resistance; clinical studies on the prevention and treatment of frequently occurring bacterial infections; and research on the economics and costs of AMR (World Health Organization, 2015a).

Furthermore, the WHO's global report on AMR surveillance also highlighted information gaps on AMR patterns in bacteria of significant public health importance, one of which is *E. coli* (World Health Organization, 2014). The report also documented the lack of an international standard for the collection and reporting of AMR data in human health as well as the absence of a global forum for rapid dissemination of AMR information (World Health Organization, 2014).

The knowledge gaps in AMR surveillance described above have been noted on a global level but are also applicable to the current AMR surveillance situation in Australia. This is discussed in the next section.

2.9 Gaps in Australian AMR surveillance

There are major gaps in our knowledge of the magnitude of AMR in Australia; and also hospital and community resistance levels, specifically for UTI pathogens. The lack of information about the magnitude of AMR in Australia has mainly been due to the absence of well-developed systems to evaluate antibiotic use and levels of AMR in various settings (Shaban et al., 2013). With the recent establishment of the Antimicrobial Use and Resistance in Australia (AURA) Surveillance System, better coordination of AMR and antimicrobial use

data is expected. Improvements in coordination have already been demonstrated with the release of Australia's first report on antimicrobial use and resistance in human health, in June 2016 (Australian Commission on Safety and Quality in Health Care, 2016), integrating data from a range of sources. Despite the establishment of the AURA Surveillance System, there is still work to be done in regards to ensuring adequate surveillance of AMR. Some of these gaps are: improvement in the analysis and interpretation of AMR data; wide coverage of AMR data from various geographical locations and patient settings; improvement in data collection methods to facilitate proper benchmarking, and continued monitoring of resistance patterns (Australian Commission on Safety and Quality in Health Care, 2016). Strengthening of the surveillance process is needed to ensure collection of relevant data which can inform action aimed at control of AMR.

Another important gap in surveillance of AMR in Australia is linkage of AMR data to other important data such as antimicrobial usage (Australian Government, 2015; Nimmo, Bell, & Collignon, 2003). There is potential to use the longitudinal antimicrobial use data from NAUSP and AMR data from microbiological laboratories to explore associations between antimicrobial consumption and resistance at hospital level (McNeil, Cruickshank, & Duguid, 2010), but to date in Australia these data have not been fully utilised specifically for UTI pathogens such as *E. coli*.

The AMR summit held in Australia in 2011 listed surveillance of AMR as one of the main action plans. Knowledge of hospital and community resistance levels, bacteria type and location were key recommendations for surveillance. The summit also recommended that the UTI causing bacteria, specifically *E. coli* and other Gram-negative bacteria, be given priority as the emerging resistance in these organisms poses a major new threat (Gottlieb & Nimmo, 2011). According to Gottlieb and Nimmo (2011), the incidence of AMR in Australia is still poorly described and measuring its extent in community-acquired and healthcare-associated infections is essential to describing the issue.

As highlighted in previous sections of this chapter (sections 2.3.4 and 2.5.3), some of these gaps will be addressed in the research program. Specifically, the first study will address the knowledge gaps on the global incidence and prevalence of resistant *E. coli* UTI and geographical AMR patterns, by systematically reviewing the literature to comprehensively

evaluate AMR resistance in *E. coli* UTI, specifically in ciprofloxacin, a high priority critically important antimicrobial agent. The systematic review also provided an opportunity for me to gain an in-depth understanding of the methodological issues in regards to undertaking AMR studies using laboratory-based data. Hence the research program also retrospectively reviews AMR data on *E. coli* UTI from a microbiology laboratory to address some of the gaps described above. The second study of the research program evaluates AMR temporal trends and compares the prevalence of AMR in hospital- and community-acquired *E. coli* UTI at the Canberra Hospital over a five-year period. The third study evaluates the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of ACT residents over a five-year period. Obtaining both incidence and prevalence data are necessary to provide information about the health status of people and contribute to disease management decisions (Buttner & Muller, 2011). In light of the impact of antimicrobial usage patterns on AMR highlighted in the literature review, as well as potential to use antimicrobial use data from NAUSP, determining antimicrobial use trends at the hospital level is undertaken as part of the second study of the research program.

The research program aims to address some of the gaps identified from the literature review and use the research findings to contribute towards strengthening AMR surveillance in Australia and also globally. Rich data sources exist in Australia and the potential for use of these data in the control of AMR and overuse or misuse of antimicrobials are currently not maximised. This research program exploits these data and makes available information on AMR patterns in *E. coli* UTI at the hospital and community levels, including trends in antimicrobial use at the hospital level.

2.10 Summary

Urinary tract infections are common infections that occur worldwide and have a significant health, economic and financial burden on society. Bacteria which cause UTI, most commonly *E. coli*, are becoming more resistant to currently prescribed antimicrobials. Therefore, monitoring AMR patterns is an essential part of guiding therapy in patients with UTI and can also provide data which can be used for the development of policies aimed at controlling further development and spread of AMR in *E. coli*.

This comprehensive review of the literature has identified that there are knowledge gaps on global prevalence and incidence of resistant *E. coli* UTI and geographical AMR patterns. The review of the literature also identified: the lack of published studies on AMR in *E. coli* UTI in Australia as well as considerable gaps in knowledge regarding hospital and community resistance levels; incidence of antimicrobial resistant *E. coli* UTI over time; and risk factors for the presence of resistant *E. coli* UTI in Australia. It is apparent that rich data, which can be analysed thoroughly using detailed statistical techniques to produce more comprehensive findings, exist in Australia. These rich data that exist within microbiology laboratories in Australia, which have been identified as key to controlling the spread of resistance, are currently being underutilised and they have the potential to inform strategies to prevent further development and spread of resistance.

This literature review has provided insight into the evidence gaps for AMR surveillance in *E. coli* UTI on a global level as well as in Australia. This research program is timely, especially given the recent release of the national AMR strategy in Australia. The outcomes of the three studies that make up this research program have the potential to: inform AMR control policies; influence therapy for UTI as it relates to the locality; contribute methodologically to analysis of microbiological laboratory data; and also contribute to enhancing AMR surveillance at the territory and national level. The following chapter discusses the design and methodology for undertaking each of the studies in the research program.

Chapter 3: Research methodology

3.1 Overview

This chapter outlines the individual methods used to undertake each study and address each study's aims. The first section of this chapter describes the methodological issues taken into consideration during the conduct of these studies. To avoid repetition of the methods used for undertaking the individual studies, this chapter provides an overview of the study methods with justification for the choice of methodological approaches undertaken. Specific information on the data collection processes used in each study, as well as details of the statistical analysis procedures, is presented in the study chapters. This chapter concludes with a discussion of the ethical concerns of informed consent, confidentiality and data security.

The methodological approach to conducting the three studies, which this research program comprises, is underpinned by the positivist paradigm. The French philosopher, August Comte, who first referred to the term 'positivism' in the 19th century, argued that the truth about reality can only be revealed through scientific knowledge (Kaboub, 2008; Mack, 2010). He believed that knowledge generation could only be achieved through the use of observation or human reasoning using a scientific methodology (Kaboub, 2008). This is likened to constructing an experiment, in a controlled environment or laboratory, in an objective manner without any interactions with the external world (Aliyu, Bello, Kasim, & Martin, 2014). The positivist research paradigm follows a scientific and systematic approach to the conduct of research and has been shown to support the use of quantitative methodology in data collection and analysis (Mackenzie & Knipe, 2006; Mukherji & Albon, 2009). This scientific paradigm aims to provide predictions and generalisation of research findings to the wider population. The methods employed often generate quantitative data with the use of descriptive and inferential statistical analysis tests (Scotland, 2012). The positivist paradigm has been criticised for its lack of subjectiveness, as reality is constructed on multiple factors and it is impossible to exclude subjective human involvement in constructing reality (Tuli, 2010). In response to this, those in favour of the positivist paradigm have argued that rather than claim absolute objectivity, the positivist approach seeks a certain degree of objectivity (Mack, 2010). Nevertheless, the choice of research paradigm depends on the research question that is being answered. This research program

therefore follows the positivist philosophical approach, as the aims of the three studies were most effectively addressed using research designs, data collection methods and data analyses techniques consistent with the quantitative methodological approach, to determine the prevalence and incidence of antimicrobial resistant urinary *E. coli*.

3.2 Methodological considerations

The use of microbiological laboratory data poses methodological challenges. These challenges relate to the appropriate and universally acceptable threshold for microbiological laboratory confirmation of a UTI, definitions for categorisation of infections based on setting of acquisition (e.g., hospital versus community) and the use of antimicrobial susceptibility testing (AST) categories in determining resistance. This section discusses these topics and also outlines the decisions made regarding the analytic methods used in this research program.

3.2.1 Threshold for laboratory confirmation of UTI

Processing urine cultures forms a large part of the workload undertaken in microbiology laboratories and clinicians mainly rely on the generation of accurate results from the laboratory to make therapeutic recommendations for management of patients with UTI (Burd & Kehl, 2011). Traditionally, laboratory UTI diagnosis has been based on a quantitative culture of a urine sample containing a pathogen of greater than or equal to 10^5 colony forming unit (cfu) per millilitre of urine (Kass, 1957; Kass & Finland, 2002; Stamm et al., 1982). However, this criterion was established in studies using women with acute pyelonephritis and asymptomatic bacteriuria (Kass, 1957; Kass & Finland, 2002). While the majority of patients with UTI are females, most of them present with acute uncomplicated cystitis and studies have shown that more than one-third of these patients have bacterial colony counts less than 10^5 cfu/ml (Stamm et al., 1982). The 10^5 cfu/ml cut-off, although chosen for its high specificity, has also been shown to have a sensitivity of about 50% (Burd & Kehl, 2011; Orenstein & Wong, 1999). Hence many microbiology laboratories use lower counts as a cut-off for interpreting results of urine cultures (Burd & Kehl, 2011; Wilson & Gaido, 2004). To increase the sensitivity of the urine culture test without making it impractical for use by clinicians and microbiology laboratories, a count of 10^4 cfu per millilitre (10^7cfu/L) of urine is commonly used (Wilson & Gaido, 2004).

Australian Capital Territory Pathology reports colony counts per litre of urine as opposed to per millilitre of urine. Hence using a conversion factor of 1000 (as 1L = 1000mL and 1 µL = 1000mL), a colony count of 10^5cfu/mL equates to 10^8cfu/L. Urine culture is done with a 1µL loop; for example, 100 colonies on the plate (100cfu in 1 µL of urine) will be equivalent to a colony count of either $100x10^3$ cfu/mL or $100x10^6$ cfu/L. When there are between 10–100 colonies on the plate, more often nearer 100, the result is recorded as 10^7–10^8cfu/L. These urine samples may come from patients who have commenced empirical antibiotic treatment or those with a significant cell count with a leucocyte response, hence making the cut-off of 10^7–10^8cfu/L clinically significant. Therefore, for the two studies of this research program that utilised data from ACT Pathology microbiology laboratory, a colony count of 10^7 cfu/L (10^4 cfu/ml) was used to confirm the diagnosis of UTI. This 10^7 cfu/L cut-off is commonly used as it increases the sensitivity of the urine culture test, making it a practical threshold (Wilson & Gaido, 2004) – a criterion used by various studies reporting on AMR of urinary *E. coli* (Laupland et al., 2007; Linhares et al., 2013; McGregor, Elman, Bearden, & Smith, 2013). Furthermore, this urine culture cut-off point has been used in reports by the National Association of Testing Authorities accredited clinical microbiology laboratory.

3.2.2 Categorisation of healthcare-associated, hospital- and community-acquired infections

To ensure appropriate interpretation of the study findings, especially in relation to empirical antimicrobial therapy, the allocation of the place of acquisition of infection is important because this relates to the range of potential organisms causing infection, providing an opportunity to identify areas to target interventions that aim to reduce AMR development.

Infections have traditionally been categorised as hospital- or community-acquired based on the setting of acquisition (Cardoso et al., 2014; Kollef et al., 2008), which also applies to UTI. However, the last ten years witnessed changes in healthcare delivery in many countries with procedures that were primarily performed during hospital admission now conducted routinely as outpatient procedures (Horcajada et al., 2013). These changes have led to the emergence of a group of infections, which could not be solely categorised as hospital- or community-acquired infections, belonging to a wider category known as healthcare-associated infections (HAI) (Cardoso et al., 2014). Evidence shows that patients with HAI have similar pathogens and outcomes when compared to patients with hospital-acquired infections (Bishara et al., 2012).

Furthermore, the changes in healthcare delivery make it difficult to clearly define what constitutes a healthcare setting (Sydnor & Perl, 2011). Patients freely transition within sometimes loosely defined levels of the healthcare system, for example between long-term care or rehabilitation services to acute-care centres (Sydnor & Perl, 2011), and this further complicates the definition of a healthcare setting, making it harder to define HAI. The Centers for Disease Control and Prevention (CDC) defines HAI as "a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s). There must be no evidence that the infection was present or incubating at the time of admission to the acute care setting" (Horan, Andrus, & Dudeck, 2008, p. 309). Infections present on admission are considered to be 'community-acquired' and these are not reported as healthcare associated (Horan et al., 2008). Although the CDC definition is commonly used, a recent systematic review assessing HAI definitions used in clinical studies found that there was still no consensus on the most appropriate definition (Cardoso et al., 2014). For example, Friedman et al. (2002) have broadly described HAI as infections present at the time of, or within 48 hours of, hospital admission with the fulfilment of specific criteria, some of which include receiving intravenous therapy within 30 days before infection, being hospitalised for two days or more within the previous 90 days or residing in a long-term care facility. Table 1 displays other widely used definitions for healthcare associated, hospital-acquired and community-acquired infections. While the exact reason for having so many definitions is unknown, possible explanations could be the creation of definitions to suit specific patient populations or being based on hospital protocols. Of note, some of these definitions overlap, for example those used by Cullen et al. (2012) and AGAR (Australian Group on Antimicrobial Resistance, 2011, 2013) are similar. Furthermore, some are very general (National Audit Office, 2009) and others are more specific (Friedman et al., 2002).

Table 1 Definitions for healthcare associated, hospital-acquired and community-acquired infections

Reference	Healthcare-associated	Hospital-acquired	Community-acquired
Centers for Disease Control and Prevention, 2016, p. 2–4	"The date of event of the National Healthcare Safety Network site-specific infection criterion occurs on or after the 3rd calendar day of admission to an inpatient location where day of admission is calendar day 1"		"The date of event of the National Healthcare Safety Network site-specific infection criterion occurs during the present on admission time period, which is defined as the day of admission to an inpatient location (calendar day 1), the 2 days before admission, and the calendar day after admission"
National Audit Office, 2009, p. 62	"An infection acquired via the provision of healthcare in either a hospital or community setting"	"An infection that was neither present nor incubating at the time of a patient's admission which normally manifests itself more than three nights after the patient's admission to hospital"	
Friedman et al., 2002, p. 792	"Infection present at the time of hospital admission or within 48 hours of admission if the patient fulfilled any of the following criteria: (a) Received intravenous therapy at home; received wound care or specialized nursing care through a health care agency, family, or friends; or had self-administered intravenous medical therapy in the 30 days before the bloodstream infection. Patients whose only home therapy was oxygen use were excluded. (b) Attended a hospital or hemodialysis clinic or received intravenous chemotherapy in the 30 days before the bloodstream infection. (c) Was hospitalized in an acute care hospital for 2 or more days in the 90 days	"Infection present in patients who had been hospitalized for 48 hours or longer"	"Infection present at the time of hospital admission or within the 48 hours after hospital admission"

	before the bloodstream infection. (d) Resided in a nursing home or long-term care facility”		
Perrin et al., 1999, p. 274		“Infections occurring more than 72 hours after admission”	“Infections were considered community-acquired when they occurred within the first 72 hours of entering the care centre”
Meier, Weber, Zbinden, Ruef, & Hasse, 2011, p. 334			“Infections were rated as community-acquired if they did not fulfill any of the following criteria: (1) patient received intravenous therapy or specialized wound care at home; (2) patient received hemodialysis treatment or antineoplastic chemotherapy in the 30 days before the infection; (3) patient was hospitalized in an acute care center 2 days in the 90 days before infection; (4) patient resided in a nursing home or long-term care facility”
Cullen et al., 2012, p. 1200		“Hospital in-patients with urine samples limited to those sent more than 48 h after admission”	“Samples originated from the offices of referring GPs were grouped with samples arriving at the laboratory from the emergency room. These samples were grouped as ‘community samples’ and comprise the pathogens seen outside of the hospital setting”
Australian Group on Antimicrobial Resistance, 2012, p. 6		“Isolates were from different patients hospitalised for more than 48 hours”	
Australian Group on Antimicrobial Resistance, 2013, p. 9			“All isolates were collected from non-hospitalised patients with urinary tract infections, including those presenting to emergency departments, outpatient departments or to community practitioners”

A limitation of most studies reporting on UTI as acquired in the hospital or community is their inconsistent description of these settings (Kouchak & Askarian, 2012). For example, some papers have included all hospitalised patients when referring to hospital-acquired UTI (Piéboji et al., 2004) as opposed to applying a timeframe to define infection cut-off. Other studies have included patients after 48 hours of admission (Ma & Wang, 2013) and, further still, after 72 hours of admission (Perrin et al., 1999), demonstrating the variability in defining infection cut-off. These inconsistencies in definitions also relate to community-acquired UTI (Gupta, Sahm, et al., 2001; Horcajada et al., 2013; Linhares et al., 2013; Perrin et al., 1999). Nonetheless, the most widely used cut-off period to define a community-acquired UTI is 48 hours, based on criteria from the CDC (Horan et al., 2008). Infections present on or after the third calendar day of hospital admission (that is, day three and onwards) are considered HAI (Centers for Disease Control and Prevention, 2016). Infections present within two calendar days of admission, the day of admission and the day after admission, are considered to be community-acquired (Centers for Disease Control and Prevention, 2016).

The use of the terminologies 'acquired' versus 'onset' is also debatable. It has been suggested by experts in the field of microbiology (P. Collignon, personal communication, May 5, 2016) that 'onset' might be a more appropriate term as it is often not certain where exactly these infections are specifically 'acquired' but it is assumed the symptoms and/or signs begin to manifest in either the hospital or community setting. In a paper reporting on blood stream infections, 'community-acquired' infections were categorised as a subset of 'community-onset' infections (Hoenigl et al., 2014), which further demonstrates the variability in the use of these terms. There is still much ongoing debate about these terms with no specific 'correct term' agreed upon, and with these terms often used interchangeably.

For the purpose of this research program, I used the terms 'community-acquired UTI' versus 'hospital-acquired UTI' as opposed to a wider definition of healthcare-associated UTI. Given the lack of consensus on an appropriate definition for HAI as well as the wide scope of this definition, which may not be accurately reflected when using retrospective laboratory data, the main data source for this research program, it was reasonable to use the terms hospital- and community-acquired. Published research has demonstrated the challenges of using the

wider healthcare-associated definition, which may require additional work by staff collecting the data to identify HAI cases, validation of these data and potential bias in the absence of an internationally accepted definition (Mitchell, Collignon, McCann, Wilkinson, & Wells, 2014). The use of the 48 hour rule to define hospital-acquired UTI based on the CDC definition makes the studies undertaken in this research program comparable to internationally published research. Antimicrobial resistance patterns will also be investigated in community-acquired UTI using this rule, with community-acquired infections defined as infections occurring in the community or within 48 hours of admission.

3.2.3 The use of antimicrobial susceptibility testing categories in determining resistance

Antimicrobial susceptibility testing (AST) has been described as the most important task carried out by the microbiology laboratory with relevance to the management of patients with infections (Doern, 2011). The aim of undertaking AST is to identify the presence of AMR in individual bacterial isolates and to confirm susceptibility to antimicrobial agents used for treating a specific infection (Reller, Weinstein, Jorgensen, & Ferraro, 2009). Furthermore, the capacity of the microbiology laboratory to adequately perform AST has the potential to influence surveillance of AMR (World Health Organization, 2014). When antimicrobial susceptibility tests are performed for isolates, the results are categorised by the microbiology laboratory as susceptible (also known as sensitive), intermediate or resistant to an antimicrobial, using interpretative criteria known as cut-offs or breakpoints (Hindler & Stelling, 2007; Turnidge & Paterson, 2007).

The susceptible category means that isolates are inhibited by the normally attainable levels of the antimicrobial agent when the recommended dosage for that site of infection is used (Reller et al., 2009; Turnidge & Paterson, 2007). The intermediate category refers to isolates for which the clinical response is likely to be less than those of susceptible isolates. This category also serves as a buffer zone to prevent an isolate from being categorised as susceptible at a certain time, and then resistant at another time, because of uncontrollable factors during testing (Reller et al., 2009; Rodloff, Bauer, Ewig, Kujath, & Müller, 2008; Turnidge & Paterson, 2007). The resistant category means that isolates are not inhibited by the usually attainable concentrations of the antimicrobial agent when the normal recommended dosage is used, and are likely to be associated with therapeutic failure (Reller et al., 2009; Rodloff et al., 2008; Turnidge & Paterson, 2007). These terms assist clinicians in

making therapeutic decisions to inform effective management of patients. Specifically, they provide information on whether an infecting pathogen is likely to respond to a certain antimicrobial when prescribed at the recommended dosage for the infection site (Jenkins & Jerris, 2011). It is important that the laboratory accurately categorises isolates using the most current recommended criteria, to prevent inaccurate reporting of patients' test results, as clinicians depend on this information to decide on the choice of antimicrobial in the management of patients (Reller et al., 2009).

While determination of the cut-offs (also referred to as breakpoints) has been the responsibility of the American Clinical and Laboratory Standards Institute (CLSI) – also formerly known as the National Committee for Clinical Laboratory Standards (NCCLS) – and the European Union Committee for Antimicrobial Susceptibility Testing (EUCAST), these two organisations are separate and often provide differing breakpoints for the same antimicrobial (Doern, 2011; Schreckenberger & Binnicker, 2011). This lack of one uniform international standard for AST prevents meaningful comparison of published resistance prevalence and incidence between countries and regions. Issues may also arise in within-country comparison, as even in Australia different susceptibility testing standards are currently being used across the country (Australian Government, 2015). The inconsistencies in interpretation can be such that pathogens categorised as resistant in one laboratory could be categorised as susceptible when tested in another laboratory (World Health Organization, 2014). For this research program, the CLSI criteria were applied by ACT Pathology to the data used in studies two and three. The reasons for this choice, as opposed to EUCAST, were that the CLSI criteria were the most commonly used criteria and the CLSI guidelines were freely available and acceptable internationally, with the EUCAST criteria only becoming freely available in the last couple of years. Also, the CLSI criteria are still being predominantly used in most Australian laboratories, allowing for comparisons (A. Das, personal communication, 19 June, 2015).

In analysing AST data, a decision has to be made on which of the three categories (susceptible, intermediate or resistant isolates) will be the focus of the analysis. For microbiologists and epidemiologists, the emphasis is on monitoring changes in antimicrobial resistance trends, therefore the proportion of resistant isolates is of greater interest in comparison to the proportion of susceptible isolates (Hindler & Stelling, 2007). Also, analysis

of resistance data has the potential to identify possible emerging resistances (Hindler & Stelling, 2007), which in turn could inform strategies to promptly curtail further development and spread of resistance. In regards to the use of intermediate isolates when analysing AST data, the CLSI recommends excluding intermediate isolates altogether from analysis of susceptibility data because clinicians generally do not prescribe antimicrobials to patients based on this category (Hindler & Stelling, 2007). Despite this, some studies reporting on urinary *E. coli* resistance have included intermediate isolates with resistant isolates in the calculation of resistance (Maraki et al., 2013; Sorlozano et al., 2014; Swami et al., 2012). Others have included intermediate isolates with susceptible isolates (Kahlmeter & Poulsen, 2012), while some studies have designated a 'non-susceptible category' which includes both resistant and intermediate isolates (Ironmonger et al., 2015). Given the definition of intermediate isolates, there may be some basis to their inclusion but there is still much debate on whether they should be included or not. For this research program, only isolates considered to be resistant based on AST data were included in the final analysis dataset.

The five-year cumulative antimicrobial susceptibility data obtained from ACT Pathology for the second and third studies of this research program included all three categories, that is, susceptible, intermediate and resistant isolates. As the focus of this research program is monitoring resistance trends with the aim of using the results to guide local empirical therapy, make policy recommendations for prevention of further development of resistance and methodological contributions to the analysis of microbiological laboratory data, the main emphasis for studies two and three was the reporting and interpretation of resistance data, based on the CLSI recommendations.

3.2.4 Approach to managing duplicate isolates

For many patients, repeated infections requiring multiple specimen or sample collection is common. Repeated infections with the same or different pathogens may occur, giving rise to duplicate isolates from the same individual. These duplicate isolates are usually included in a single antibiogram (Lee et al., 2004). An antibiogram is a report of cumulative AST results for a specified period of time (Hindler & Stelling, 2007; Lee et al., 2004). The presence of duplicate isolates per patient has the potential to overestimate resistance (Huovinen, 1985; Lee et al., 2004; Shannon & French, 2002a).

To prevent overestimation or inflation of the reported resistance, efforts should be made to exclude duplicate isolates per patient from the antibiogram prior to data analysis, and a number of approaches or methods have been proposed. These include identification using patient-based approaches (first isolate per patient) and identification based on antibiogram or phenotypic pattern of the isolates (Cornaglia et al., 2004). The patient-based approach, also known as the time criterion, eliminates duplicate isolates by using only the first isolate per patient during a specific timeframe (Cornaglia et al., 2004; Hindler & Stelling, 2007). Each patient has an equal contribution to the total number of isolates (Hindler & Stelling, 2007). Different time periods have been suggested to be used as a cut-off for considering an isolate as a duplicate. This cut-off has ranged from five days (Sahm, Marsilio, & Piazza, 1999) to 365 days (Shannon & French, 2002a), with longer time limits giving rise to lower resistance. A study investigating three time limits (5, 30 and 365 days) for exclusion of duplicate isolates for antimicrobial resistance surveillance found that 365 days was the most appropriate and excluded them after this period. This time limit was considered to be the best option given that patients may be admitted in hospital for long periods or may need therapy that requires frequent re-admission to the hospital (Shannon & French, 2002a).

The method of identifying the first isolate per patient has been by far the most commonly used in published studies (Shannon & French, 2002a, 2002b). The CLSI guidelines also recommend using this approach when calculating resistance and reporting antimicrobial susceptibility data (Hindler & Stelling, 2007). A study comparing resistance trends over three years, when using the first isolate per patient and all isolates, found that the rates of methicillin-resistant *Staphylococcus aureus* did not significantly differ over time when all isolates were used (P=0.86), but the rates significantly decreased over the study period when the first isolate per patient was used (P=0.006) (Lee et al., 2004). This difference was attributed to the varying proportions of duplicate isolates each year (Lee et al., 2004). It is advised that analysis of AMR data should include each individual isolate, in order to ensure sensitivity, but this isolate should only be included once to guarantee specificity (Cornaglia et al., 2004). This is especially true for UTI where multiple samples are often sent from the same patient. This approach is also consistent with published studies on resistance in UTI pathogens including *E. coli* (McGregor et al., 2013; Swami et al., 2012). In addition, according to Bax et al. (2001) who evaluated the strengths and weaknesses of AMR surveillance systems, using only the first isolate per patient is reported as usual practice

when calculating resistance. Despite the widespread use and acceptability of this approach, there are a number of limitations. Using the first isolate per patient approach may lead to an underestimation of the resistance, by selecting only the first isolate from a single patient within the observation period. Also, detection of resistance selection is not possible using this approach (Cornaglia et al., 2004). However, this method is straightforward and can be easily undertaken as long as there is a unique patient identifier provided in the antibiogram (Cornaglia et al., 2004).

A second approach involves identifying duplicate isolates from the same patient based on the antibiogram pattern or specific phenotypic characteristics. This involves including only the first isolate with a similar antimicrobial susceptibility pattern to the other identified isolates during the surveillance period (Cornaglia et al., 2004). For this approach, time limits are not required for eliminating duplicate isolates. The presence of at least one major difference in the antimicrobial susceptibility pattern of two isolates from a single patient signifies that these are different isolates and not duplicates (Cornaglia et al., 2004). To ideally identify duplicate isolates using the antibiogram or phenotypic pattern approach, antimicrobial susceptibility tests should include marker antibiotics and when these are not available, the differences used to identify the duplicate isolates must be clearly stated (Cornaglia et al., 2004). The advantage of this approach in identifying duplicate or multiple isolates, compared to the first isolate per patient approach, is its ability to detect selection of resistance (Rodríguez, Sirvent, López-Lozano, & Royo, 2003). However, using the antibiogram pattern criterion requires expertise from microbiological staff, making it subjective and difficult to verify (Rodríguez et al., 2003). Also, deciding on what characteristics to use to distinguish isolates may be challenging (Hindler & Stelling, 2007).

Other approaches that have been suggested for identifying duplicate isolates include selecting the last isolate, selecting only the most resistant isolate or the most susceptible isolate and also calculating a weighted average of an individual patient's susceptibility. These approaches are not considered more accurate than the first isolate per patient approach and are not generally recommended (Cornaglia et al., 2004; Hindler & Stelling, 2007).

Despite the arguments for eliminating duplicate isolates from the antibiogram to prevent overestimation of prevalence, there are certain situations where the inclusion of all isolates is required. For example, in determining incidence of AMR, it is necessary to include all isolates in the antibiogram to accurately identify the first resistant isolate, as incidence by definition refers to following up an individual until development of the outcome (Buttner & Muller, 2011); in this case, an antimicrobial resistant *E. coli* UTI. Subsequent duplicate resistant isolates are of no importance for the incidence and need not be included after identifying the first resistant isolate. Without the presence of all isolates from an individual in the antibiogram, calculating the incidence will not be feasible because all isolates are needed to identify the incident case for that individual. In addition, in cases where it is necessary to estimate the length of time a patient has had a resistant infection, the presence of duplicate isolates are required (Cornaglia et al., 2004). Also, inclusion of all isolates in the antibiogram is necessary when calculating laboratory workload and costs (Bax et al., 2001). It is recommended that when duplicate isolates are identified for a single patient, these isolates are labelled and excluded, depending on the indication of the analysis.

For the two studies in this research program, which utilised microbiology data from the ACT Pathology laboratory, both methods were used. In calculating the prevalence of resistance, the first isolate per patient per year was used for analysis in accordance with the CLSI criteria. This approach was essential to prevent overestimation of the AMR prevalence. For determining the incidence of resistant *E. coli* UTI, all isolates per patient were included in the dataset. Each patient was followed up until the occurrence of the outcome to identify incident cases, after which successive occurrences of the outcome, if any, were censored. Further details of the analyses are provided in the study chapters.

3.3 Systematic review and meta-analysis of observational studies

The first study in this research program aimed to systematically review the literature and conduct a meta-analysis of observational studies published in the last ten years, investigating ciprofloxacin resistance in community-acquired and hospital-acquired *E. coli* UTI. As described by the Cochrane Collaboration, “a systematic review attempts to collate all empirical evidence that fits pre-specified eligibility criteria in order to answer a specific research question” (Higgins and Green 2011, p. 1.2.2). The systematic review approach

makes use of an unbiased, objective and transparent process to identify, appraise and synthesize all literature specific to the research question (Egger & Smith, 2008). Given the methodological rigour of systematic reviews, they have been identified as a reference standard for synthesizing evidence in healthcare (Moher et al., 2015). Systematic reviews may include the use of statistical methods to combine and summarise the results of two or more primary studies in a technique referred to as meta-analysis (Greenhalgh, 1997b; Moher et al., 2015). Meta-analyses have the potential to provide more accurate estimates of the effect of the outcome on the study population compared to the estimates from single studies (Moher et al., 2015). They also enable investigations of the uniformity of evidence across studies, and provide an opportunity for investigation of differences between studies when they exist (Higgins & Green, 2011).

Traditionally, systematic reviews and meta-analyses have been applied to randomised controlled trials (Stroup et al., 2000). However, certain research questions are not suited to the randomised controlled trial design and can only be answered using an observational study design, which may include cross-sectional, cohort or case-control designs (Stroup et al., 2000). For example, in studies of risk factors where it is deemed unethical to expose patients to harmful risk factors or in the case of this research program where I aimed to answer a research question which can only be undertaken using data from observational studies. In such situations, systematic reviews and meta-analyses of observational data are the only feasible choice. Systematic reviews and meta-analyses of observational studies have gained popularity in research and are now as common as those of randomised trials (Egger, Smith, & Schneider, 2008), despite a number of arguments about the inclusion of observational studies in systematic reviews. These mainly relate to the absence of randomisation in observational studies and differences in study designs which may lead to confounding and various biases (Egger et al., 2008; Shrier et al., 2007; Stroup et al., 2000). Notwithstanding, systematic reviews and meta-analyses of observational studies, when conducted with standard systematic review principles, have the potential to provide important evidence to inform clinical and policy decision making, including the ability to make recommendations for future research (Egger et al., 2008; Shrier et al., 2007; Stroup et al., 2000).

3.3.1 Conduct of the systematic review and meta-analysis

The systematic review undertaken as part of this research program used a fixed step-by-step process as recommended by the Cochrane Collaboration (Higgins & Green, 2011).

Preparation and registration of the systematic review protocol

First, in order to reduce the potential for bias in the systematic review process, the proposed methods to be used for undertaking the systematic review were carefully planned and documented prior to commencement of the review (Higgins & Green, 2011). These included the review question, search strategies, inclusion and exclusion criteria, data extraction process, assessment of quality of the included studies and data synthesis (Garg, Hackam, & Tonelli, 2008). A research protocol was prepared and registered, which is an essential part of the review process as it reduces the effect of reviewer bias, promotes consistency and transparency of the review process and reduces the likelihood for duplication of reviews (Higgins & Green, 2011; Moher et al., 2015). Furthermore, a protocol is especially important for a review of observational studies given the higher propensity for potential confounding factors in the individual studies (Higgins & Green, 2011). The protocol for undertaking this systematic review and meta-analysis providing details of the a priori methods was registered on the International Prospective Register of Ongoing Systematic Reviews (PROSPERO). This international register was established by the Centre for Reviews and Dissemination, University of York (Moher et al., 2015). Details of the protocol are provided in Appendix D.

Formulation of the systematic review question

The research or review question was defined precisely, based on the study Population, Intervention or exposure of interest, Comparator (if relevant) and study Outcomes, which are generally referred to using the acronym PICO (Higgins & Green, 2011). For this review, the population included studies of patients with *E. coli* UTI. The exposure of interest was studies of patients in a hospital or community setting. A comparator or control was not applicable and the outcome was the prevalence or incidence of ciprofloxacin resistant *E. coli* UTI in both settings. The focused approach to defining the review question was important because this determined whether each potentially relevant paper identified was included or excluded (Greenhalgh, 1997b).

Inclusion and exclusion criteria

The inclusion and exclusion criteria for this systematic review were based on the components of the review question as well as the types of observational studies that were specific to answering the question. All observational study types were considered. Other factors considered included the distinction of the study settings into hospital or community and the diagnosis of UTI. As studies included in this systematic review were from different parts of the world, with varying laboratory protocols and patient populations in regards to defining UTI and setting of infection acquisition respectively, it was essential to choose a standard reference to make comparison in regards to the differences in study locations and populations. For these reasons, a colony count of 10^7 cfu/L (10^4 cfu/ml) which was used to confirm the diagnosis of UTI for the two studies of this research program that utilised data from ACT Pathology microbiology laboratory (discussed in section 3.2.1) could not be used for the systematic review. The CDC definition of microbiologically confirmed UTI (≥10^5 cfu/ml) was selected and studies that did not use the CDC definitions were excluded. The widely accepted CDC definition was also used, as discussed in section 3.2.2, to classify UTI into hospital- or community-acquired using the 48 hour rule. For the purpose of this review, all studies published in non-English were retrieved but, given the time and cost constraints to obtain different language interpreters, these studies were later excluded. Further details of the study inclusion and exclusion criteria are provided in Chapter Four.

Search strategy

For this review, I undertook searches using MEDLINE, EMBASE and Cochrane electronic bibliographic databases. These databases are regarded as important bibliographic databases to use for undertaking searches of relevant literature (Higgins & Green, 2011). They index a large number of journal articles with some degree of reference overlapping depending on the topic (Higgins & Green, 2011). Although MEDLINE indexes more journals in more languages than EMBASE (Garg et al., 2008), EMBASE is considered to have more recent articles than MEDLINE, with better coverage of European literature (Greenhalgh, 1997a). In addition to these three electronic databases, other electronic databases searched were CINAHL and Scopus. These are subject specific databases which are also useful to obtain relevant information (Higgins & Green, 2011). Hand-searching the reference lists of articles identified from the databases for additional papers was also done. Authors of included studies were contacted when deemed necessary to obtain additional information and only

published studies were included. Although biomedical journals and bibliographic databases are commonly used sources of information for a systematic review, hand-searching the reference lists of retrieved articles is also an important source of identifying articles relevant to the systematic review and meta-analysis. Grey literature, which includes unpublished studies, was not included. It is suggested that they should also be included when relevant to the review question to prevent distortion of the results (Egger & Smith, 2008). However, the inclusion of unpublished studies has the potential to include data of lesser quality, given the high possibility that unpublished data are not peer reviewed and also may have been obtained through less rigorous methodological techniques, therefore making these data prone to bias (Crowther & Cook, 2007).

A comprehensive search strategy was developed to undertake this systematic review and meta-analysis. For example, although the review question focused on ciprofloxacin resistance, the search parameters also included broad terms such as 'antimicrobial resistance' and 'antibiotic resistance'. The use of broad search terms tailored to the PICO parameters ensured the systematic review process was focused enough to prevent inclusion of unnecessary studies but wide enough to ensure relevant studies were included.

Study selection and data extraction

After the identified studies were screened against the study inclusion and exclusion criteria, I proceeded with extracting the relevant information from the studies that met the criteria. A copy of the data extraction form is included as an appendix (Appendix E). The literature strongly recommends that the process of screening papers against the study inclusion and exclusion criteria, including extraction of data from eligible papers, should be undertaken independently by at least two reviewers to reduce subjectivity (Egger & Smith, 2008; Higgins & Green, 2011; Lyman & Kuderer, 2005). As this systematic review and meta-analysis was undertaken by me as partial fulfilment towards a doctoral degree, I was primarily responsible for undertaking the systematic review. To ensure objectivity and rigour during the review process, 10% of retrieved papers were screened by my supervisors and data from 10% of eligible papers were extracted by my supervisors. Kappa coefficient was used to assess agreement between me and each supervisor.

Quality and risk of bias assessment

Assessment of methodological quality and risk of bias of eligible papers was also undertaken. As the risk of bias tool developed by the Cochrane Collaboration is better suited to randomised controlled trials and not observational studies, it was not applicable for use in this systematic review. Therefore the Newcastle-Ottawa Scale (NOS), recommended by the Cochrane Collaboration and shown to be valid and reliable, was used (Wells et al., 2014). The NOS is a bias tool which judges a study against eight items categorised into three domains, namely, selection of the study groups, group comparability and ascertainment of exposure or outcome (Wells et al., 2014). It uses a star system to assess the quality of studies and assigns a maximum of nine stars for studies with the lowest risk of bias when rated against all three domains (Wells et al., 2014). I first pilot tested the Scale and the original items of the Scale were subsequently modified to be in line with the systematic review question. A copy of the modified version of the NOS used for this review is included as an appendix (Appendix E).

The Cochrane Collaboration recommends against using scales that produce a summary quality score (Higgins & Green, 2011). This is because scales have been found to be unreliable tools for assessment of validity and are also less transparent (Higgins & Green, 2011). Furthermore, the summary quality score involves assigning 'weights' to different items in the scale, and it is difficult to justify the weights assigned. Therefore a criterion-based evaluation, in which critical assessments are made separately for different criteria with a focus on the major components of the design of the studies, is preferred (Higgins & Green, 2011; Stroup et al., 2000).

Data synthesis

Based on the data obtained from eligible studies, an initial descriptive analysis of the studies was performed. For example, the demographic characteristics of the included studies were described, such as the sex of the study populations, geographic location of the studies and study designs. Synthesis of the data also involved undertaking a meta-analysis. Meta-analysis is a statistical approach that involves combining data from two or more individual studies (Higgins & Green, 2011). This quantitative analytical technique has the potential to increase the power and provide a more precise effect estimate than from a single study. It also provides an opportunity to resolve conflicting findings that may be seen in individual

studies (Haidich, 2011; Higgins & Green, 2011). Meta-analysis can be undertaken under the assumption of a fixed-effects model or a random-effects model (Egger & Smith, 2008). In a fixed-effects model, the assumption is that the true effect in each study is the same or is 'fixed', while the random-effects model incorporates variation, allowing for heterogeneity between the studies (Blettner, Sauerbrei, Schlehofer, Scheuchenpflug, & Friedenreich, 1999; Higgins & Green, 2011). While there is no perfect method, if the choice is to use the random-effects model then the approach should be to identify and explain sources of heterogeneity rather than overlook it (Egger & Smith, 2008). A random-effects model is considered to be more conservative than the fixed effects model (Crowther & Cook, 2007) and was used in this systematic review and meta-analysis, specifically the DerSimonian and Laird method (DerSimonian & Laird, 1986; Higgins & Green, 2011). This method incorporates an estimate of the between-study variation into both the study weights and the standard error of the estimate of the common effect. The precision of an estimate from each included study was represented by the inverse of the variance of the outcome pooled across all studies. If the value of the pooled prevalence was within the 95% confidence interval, then the effect size was statistically significant at the 5% level (P<0.05). A single meta-analysis of all included studies was not undertaken for this review due to the presence of clinical heterogeneity in regards to the study setting, that is, hospital and community settings. Also, as the aim of the study was to compare ciprofloxacin resistance in both settings, comparison of the pooled rates from each setting was required. Therefore a separate meta-analysis was performed for studies undertaken in each setting. The outcome was the prevalence or incidence of ciprofloxacin resistant *E. coli* UTI. Meta-analysis involved a combination of both prevalence and incidence values with no differentiation between these two different statistics. Although it was very difficult to differentiate between these two statistics, due to underreporting of published studies, the majority of the studies were prevalence and only a few were considered to be incidence studies. The poor methodological reporting of most studies, which posed a difficulty in differentiating between the two statistics, was stated as a limitation in this study, presented in Chapter Four.

Investigation of heterogeneity

Every review process is likely to identify studies with diverse study designs, patient populations, interventions and methodological quality (Garg et al., 2008). Therefore, the

decision on whether or not it is appropriate to interpret the results of a meta-analysis should be made after investigating heterogeneity. Heterogeneity can either be clinical (for example, diverse patient populations and interventions) or statistical (inconsistent results or effect estimates across studies) (Crowther & Cook, 2007). An important statistic for quantifying statistical heterogeneity across studies is the I^2 statistic (Higgins & Green, 2011). The I^2 statistic describes the percentage variability across studies due to heterogeneity and not by chance (Higgins, Thompson, Deeks, & Altman, 2003). Values below 25% indicate low heterogeneity, 25–75% moderate heterogeneity and over 75% high heterogeneity (Higgins et al., 2003). For this review, heterogeneity was quantified using the I^2 statistic. In addition to quantifying heterogeneity, an important part of undertaking a meta-analysis is investigating potential sources of heterogeneity (Garg et al., 2008). Heterogeneity was investgated in this review by undertaking subgroup analyses and by running meta-regressions.

Analysis of subgroups

Subgroup analysis was planned for as documented in the systematic review protocol. Subgroup analysis may help to provide a clearer picture on the sources of heterogeneity (Deeks, Altman, & Bradburn, 2008; Higgins & Green, 2011). It involves sorting out studies into categories based on certain study characteristics and undertaking a separate meta-analysis for each category (Gagnier, Moher, Boon, Beyene, & Bombardier, 2012). Subgroup analyses are usually used when the study characteristic can be grouped as categorical variables (Deeks et al., 2008). The choice of study characteristics or covariates to include in the subgroup analysis was based on knowledge of the published literature in regards to potential risk factors for AMR. For instance, published evidence shows that AMR varies by age (Adam et al., 2013) and geographical location (Gupta, Sahm, et al., 2001; Schito et al., 2009), hence separate meta-analyses for subsets of participants based on age group and geographical region were undertaken for this systematic review and meta-analysis. In addition, the choice of covariates to include in the analysis was guided by my clinical expertise and supervisors.

Meta-regression

Meta-regression explores the sources of heterogeneity by comparing the effect size to the characteristics of the included studies (Thompson & Higgins, 2002). This approach is used

for study characteristics that are continuous variables and also those that are categorical variables (Deeks et al., 2008; Thompson & Higgins, 2002). Meta-regression uses an approach similar to linear or logistic regression where the outcome variable is predicted by the values of one or more covariates (Higgins & Green, 2011; Thompson, 2008). The outcome variable refers to the effect estimate (Higgins & Green, 2011), which in this research program is the ciprofloxacin resistance, while the covariates are the characteristics of the included studies that have the potential to modify the size of the effect estimate (Higgins & Green, 2011). An advantage of meta-regression is the ability to allow multiple covariates to be evaluated simultaneously (Baker, White, Cappelleri, Kluger, & Coleman, 2009; Higgins & Green, 2011). Despite this advantage, the lack of complete data from individual studies, as well as the inclusion of a limited number of studies in most systematic reviews, has been identified as limitations to undertaking a meta-regression as there may be a potential bias to interpreting the results (Thompson & Higgins, 2002). It is therefore recommended that this statistical procedure is not used when there are less than ten studies included in the meta-analysis (Higgins & Green, 2011). As there were more than ten studies included in the meta-analysis for this systematic review, a meta-regression was feasible.

Similar to meta-analysis, the fixed-effects and random-effects models also apply to meta-regression (Baker et al., 2009). In a fixed-effects model, the effect estimate among studies is assumed to be the same or fixed and any difference noted between studies is as a result of chance, while the random-effects model assumes that the effect estimate is different across the studies and allows for incorporation of the heterogeneity that cannot be explained by the covariates (Baker et al., 2009; Thompson & Higgins, 2002). For this systematic review and meta-analysis, I chose to use a random-effects meta-regression to take into account any residual variability between the studies not due to the covariates. The meta-regression analysis produces a regression coefficient which describes changes to the outcome variable based on a unit increase in a covariate (Baker et al., 2009; Higgins & Green, 2011). A positive coefficient indicates an increase in the outcome variable while a negative coefficient is consistent with a decrease (Baker et al., 2009). The test of statistical significance indicates whether or not a linear relationship exists between the outcome variable and covariate (Higgins & Green, 2011).

Assessment of publication bias

Publication bias is described as the favoured publication of studies with statistically significant results (also encompasses studies published in English (language bias), studies that are freely available or cheap to purchase (cost bias) (Rothstein, Sutton, & Borenstein, 2006)). Including such studies alone in a systematic review and meta-analysis has the potential to distort the review findings (Egger, Dickersin, & Smith, 2008). Assessment of publication bias was undertaken by visual inspection of the funnel plot analysis. The funnel plot is a graph of the effect estimates from the individual studies included in the systematic review against the sample size (Egger, Smith, Schneider, & Minder, 1997). It is similar to a simple scatter plot but differs in that the effect estimate is plotted on the horizontal axis and the sample size on the vertical axis (Sterne, Egger, & Smith, 2008). It appears as an inverted funnel when there is no bias with the effect estimates from small studies scattering more widely at the lower end of the graph and narrowing upwards with large studies. An asymmetrical funnel plot may signify presence of publication bias (Sterne et al., 2008).

Reporting of the systematic review and meta-analysis

Reporting of the systematic review and meta-analysis, which was undertaken as part of this research program, was guided by and complied with the guidelines on preferred reporting items for systematic reviews and meta-analyses (PRISMA) (Liberati et al., 2009). Guidelines, for example PRISMA and Meta-analysis Of Observational Studies in Epidemiology, have been developed to assist authors with the process of reporting systematic reviews and meta-analyses, to decrease the likelihood of misleading conclusions and reduce the inadequacy of reporting (Moher, Liberati, Tetzlaff, & Altman, 2009; Stroup et al., 2000). Chapter Four provides the results of the systematic review and meta-analysis.

3.4 Statistical analysis of microbiological laboratory surveillance data

The second and third studies of this research program utilised retrospective data from a regional microbiological laboratory, ACT Pathology. Study two aimed to evaluate AMR temporal trends and compare the prevalence of AMR in hospital-acquired and community-acquired *E. coli* UTI at the Canberra Hospital over a five-year period. Study three aimed to evaluate the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of ACT residents over a five-year period. The microbiological laboratory, as previously stated in the literature review chapter (Chapter Two), has been identified as a rich source of untapped

data in regards to surveillance of AMR (Cornaglia et al., 2004). Retrospective studies evaluate events that have already occurred before the study onset, for example, evaluating previously collected patient records in a database (Buttner & Muller, 2011; Sedgwick, 2013b). In comparison, prospective studies evaluate events that occur after the study begins. Retrospective designs allow timely access to data and overcome the long follow-up period to development of the outcome associated with prospective designs (Euser, Zoccali, Jager, & Dekker, 2009). The main disadvantage of retrospective studies is the likelihood for incomplete data with respect to potential risk factors associated with the outcome under study, as the researcher has no control over data that have been collected in the past (Euser et al., 2009; Sedgwick, 2014; Song & Chung, 2010). However, retrospective studies analysing large amounts of AST data over a period of time are able to provide important data on AMR trends as well as changes in resistance and also generate information to guide clinical decision making (Shaban et al., 2013). The results from analyses of these data will assist with the development of policies towards control and further development of AMR in hospital and community settings and also at a population level. These results can be achieved by applying different statistical analyses, which may have some methodological challenges. The following sections discuss the statistical techniques used in undertaking studies two and three of this research program. When a statistical technique was applied to only one of these two studies, this will be stated.

3.4.1 Approach to calculating the prevalence of resistance

Study two evaluated AMR temporal trends and compared the prevalence of AMR in hospital-acquired and community-acquired *E. coli* UTI at the Canberra Hospital from January 2009 to December 2013. This study followed a cross sectional design, making it a 'period prevalence study' because of the five-year period. Prevalence quantifies the proportion of people with the disease either at a specified point in time (point prevalence) or during a particular period of time (period prevalence) (Buttner & Muller, 2011). In period prevalence, both prevalent (pre-existing) and incident (new) cases of the disease are included in the numerator and the denominator is usually the total population during the observation period (Buttner & Muller, 2011). Prevalence is generally used to evaluate the occurrence or burden of a specific disease in the hospital, community and population as a whole.

In calculating the prevalence of antimicrobial resistant *E. coli* UTI, the data were analysed in a stepwise fashion. The first step was a descriptive analysis of the whole dataset containing information on the distribution of all urine samples processed by the microbiology laboratory for Canberra Hospital during the five-year period. The descriptive analysis entailed calculating the yearly and overall five-year proportions of positive bacterial urine samples and specifically positive *E. coli* urine samples, including the number of samples requested per patient each year. This analysis was important to determine whether there had been any change in the urine sample requesting pattern over the study period. The next step involved calculating the overall prevalence of resistance as well as the prevalence of resistance based on setting of acquisition, that is, hospital or community. The dataset was limited to the first isolate per patient based on the principles discussed previously (section 3.2.4), in regards to handling duplicate isolates. Hence, only the first positive *E. coli* UTI per patient per year was included in the analysis. It is important to note that this first positive culture was not necessarily resistant. The numerator was the number of antimicrobial resistant *E. coli* UTI. Urinary *E. coli* resistance was checked for against 12 antimicrobials, namely: ampicillin/amoxicillin; amoxycillin-clavulanate; cefazolin/cephalexin; trimethoprim; nalidixic acid; ciprofloxacin; nitrofurantoin; gentamicin; ceftriaxone; trimethoprim-sulphamethoxazole; meropenem, and piperacillin-tazobactam. The choice of an appropriate denominator for calculating the prevalence required careful consideration because of the complexities associated with analysing microbiological laboratory data. This is discussed in the next paragraph.

A major challenge of using microbiological laboratory data is the occurrence of selection bias due to selection of patients who have urine samples sent for culture. Cultures are often biased towards patients with recurrent and complicated UTI as well as those with resistant infections (Gupta et al 2011). For example, the Australian Therapeutic Guidelines described in section 1.3.1 of the Introduction chapter (Chapter One) recommends that urine samples for cultures and susceptibility testing should be obtained from pregnant women, men, aged care residents, patients who have recently taken antimicrobials or failed treatment, patients with recurrent UTI and those who have travelled internationally within the past six months. Therefore, evaluating resistance based on selection of samples from these patients has the potential to skew the data to more resistant cases and falsely elevate the resistance levels.

Another challenge worth highlighting is the potential for testing bias resulting from the analytic methods used in laboratories (Cornaglia et al., 2004; Hindler & Stelling, 2007). Some laboratories, including ACT Pathology, undertake routine first-line AST followed by more extensive testing with second-line antimicrobials only for isolates resistant to at least three of the routine antimicrobials. The routine first-line antimicrobials tested for the research study were ampicillin/amoxicillin, amoxycillin-clavulanate, cephalexin/cefazolin, trimethoprim, nalidixic acid, ciprofloxacin, nitrofurantoin and gentamicin. The isolates which were found to be resistant to at least three of the routinely tested antimicrobials were tested against second-line antimicrobials, namely ceftriaxone, trimethoprim-sulphamethoxazole, meropenem and piperacillin-tazobactam. Calculation of resistance prevalence for urinary *E. coli* isolates tested against second-line antimicrobials, using the total number of isolates tested against each agent as the denominator, may bias the results towards a higher prevalence of resistance (Cornaglia et al., 2004; Hindler & Stelling, 2007). Preliminary analysis using the total number of isolates tested against each antimicrobial as the denominator demonstrated that this potentially could overestimate the resistance for the second-line antibiotics. To overcome this potential limitation, isolates which had only first-line antimicrobials tested were separated from the ones which had additional testing with second-line antimicrobials. The total number of isolates tested was used as the denominator for those isolates that were tested against second-line antimicrobials. This was the most appropriate denominator choice to use based on the assumption that isolates were initially not tested for second-line antimicrobials, because they were considered highly unlikely to be resistant to these antimicrobials. The denominator therefore also included those isolates tested for first-line antimicrobials. Using this approach reduced the risk of overestimating resistance prevalence but, on the other hand, it could have underestimated the true resistance prevalence.

3.4.2 Comparison of resistance prevalence in hospital- and community-acquired UTI

Comparison of data based on different patient characteristics, including setting of infection acquisition, is essential for evaluating resistance trends (Cornaglia et al., 2004). Often, data needed for these comparisons are lacking in terms of quality. This may be attributed to poor recording of patient clinical information on laboratory forms, including an absence of computing systems that are able to link patient medical records to laboratory records (Cornaglia et al., 2004). The wide reach of ACT Pathology, the microbiology laboratory from

which data were obtained for studies two and three, further contributed to the uniqueness of the dataset in regards to undertaking comparisons based on the setting of infection acquisition, that is, hospital or community. The ACT Pathology laboratory provides specialist pathology services to: patients of public and private hospitals; specialist and general practitioner clinics; nursing homes, and the general community. Given the wide reach of ACT Pathology with the potential for incompleteness of data, comparison based on the site of infection acquisition was only undertaken in study two, which evaluated the prevalence of AMR at a single hospital.

As described earlier in this chapter (section 3.2.2), positive urine cultures were classified into hospital- and community-acquired UTI using criteria from the CDC definition (Horan et al., 2008). Samples collected 48 hours or more after admission and within 48 hours after discharge were termed hospital-acquired, and samples collected within 48 hours of admission or from outpatients were classified as community-acquired. To ensure valid comparisons based on setting of acquisition, the data had to be thoroughly managed and evaluated to detect any errors. The admission dataset was obtained separately from the medical record service of the hospital and contained information on admission and discharge dates. This required merging with the laboratory dataset. Factors which posed methodological challenges included incorrectly entered and missing unique patient identification numbers as well as patient administrative discharges that did not necessarily indicate separate hospital admissions. An administrative discharge refers to patients who have been technically discharged and no longer in full care of the hospital, for example, geriatric patients waiting to be transferred to aged care facilities (Department of Health Western Australia, 2012). For such patients, the first admission date and last discharge date were considered. All observations without a unique patient identification number were excluded from the final analysis as it was impossible to classify samples from these patients as hospital- or community-acquired. Also, these could have included duplicates of existing resistance isolates. Although these observations were not included in the main analysis, they were included as part of the descriptive analysis for all urine samples described above, in section 3.4.1. After the cleaning and merging of the two datasets (laboratory and admission datasets), date of admission to the hospital was used to calculate the interval between admission and urine sample collection. The discharge date was used to correlate for hospital-acquired infections to ensure the sample was taken when the patient was an

inpatient or within 48 hours after discharge. Observations with a unique patient identification number but no admission date were grouped as community samples, as these were outpatients who did not require hospital admission although they were managed in the hospital (A. Das, personal communication, February 11, 2015).

The calculated resistance prevalence was compared between hospital- and community-acquired *E. coli* UTI. Sample size was estimated to ensure the study was adequately powered to detect a clinically significant difference in resistance between the two groups. Some of the considerations when calculating a sample size include the type I error (also referred to as alpha), power, the clinically relevant difference and variability (Noordzij et al., 2010). Type I error occurs when we incorrectly reject the null hypothesis when it is actually true that there is no difference between the groups (Jones, Carley, & Harrison, 2003). Conventionally, the alpha or type I error is set at 0.05 meaning that the probability of reaching a false positive conclusion and rejecting the null hypothesis is less than 5% (Noordzij et al., 2010). The power of a study refers to the probability of correctly detecting a difference between two groups if it truly exists (Whitley & Ball, 2002). Power prevents the occurrence of a type II error (also referred to as beta), which means falsely accepting the null hypothesis (false negative conclusion) when there is in fact a difference between the two groups (Jones et al., 2003; Noordzij et al., 2010). The beta is conventionally set at 0.20, meaning that the probability of reaching a false negative conclusion and accepting the null hypothesis is less than 20% (Noordzij et al., 2010). The clinically relevant difference is the smallest effect of interest between the two groups that is hoped to be detected by undertaking the study and the variability refers to the standard deviation of the variable of interest in the study population (Jones et al., 2003; Noordzij et al., 2010). The clinically relevant difference and variability are usually estimated using data from similar published studies or pilot studies and may be combined to give an effect size (Noordzij et al., 2010). In calculating the sample size for hospital- and community-acquired UTI in this research program, an alpha of 0.05%, power of 80% and effect size estimated from published research (Cullen et al., 2012) were used. Further details of the methods for study two are provided in Chapter Five.

3.4.3 Approach to calculating the incidence of resistance

Study three evaluated the incidence and AMR trend in a cohort of ACT residents over a five-year period using a laboratory-based retrospective cohort study design (Buttner & Muller, 2011). Incidence quantifies the number of new (incident) cases of a condition (e.g., a disease) in people at risk of developing the condition during a specified time period (Buttner & Muller, 2011; Porta, 2014). Incidence may be expressed as a proportion (sometimes called cumulative incidence) or as a rate (referred to as incidence rate or person-time rate). The cumulative incidence refers to the proportion of people who are initially disease-free and develop the disease during a specified time period (Buttner & Muller, 2011; Centers for Disease Control and Prevention, 2012). The denominator for the cumulative incidence is the initial people who are at risk. It can also be described as the probability of developing a disease over a specific period of time and as such is a measure of risk. In contrast to cumulative incidence, the incidence rate incorporates 'time' into the denominator and is calculated as the number of new cases of disease during a specified period of time divided by the total disease-free time each person was observed (Buttner & Muller, 2011; Centers for Disease Control and Prevention, 2012).

For estimating incidence, it is necessary to define the initial disease-free people who are at risk of developing the disease, as incidence involves following up people who are healthy to detect those who develop the disease or otherwise stay disease-free. Such cohort studies may involve observing a large group of people for a sufficient number of years to generate reliable incidence data, using persons or person-time of observation as the denominator. The denominator for calculating incidence should exclude all pre-existing disease cases (prevalent cases) to clearly identify the initial disease-free population at risk for developing the outcome (Buttner & Muller, 2011). In reality it may be difficult to obtain an accurate estimate of the disease-free people at risk and this limitation should be acknowledged when reporting results of cumulative incidence (Buttner & Muller, 2011). Furthermore, as people are enrolled or enter studies at different times, and also leave at different times for various reasons (development of the outcome or change location), the dynamic nature of populations constantly changes the people at risk (Buttner & Muller, 2011; Vandenbroucke & Pearce, 2012).

For this research project, the incidence of antimicrobial resistant *E. coli* UTI was measured using the cumulative incidence or incidence proportion. The five-year AST microbiological laboratory data was such that people submitted urine samples to ACT Pathology at different calendar time periods, highlighting the dynamic nature of populations with people entering and leaving the study at various time points. Multiple samples were also submitted to the laboratory from the same individuals. All urine samples submitted by people in the cohort over the five-year period were included in the analysis dataset. Hence the inclusion of all urine samples in the dataset, with the presence of multiple rows per person, allowed the creation of a 'follow-up' of each person as opposed to restricting the dataset to the first isolate per person. This demonstrates how AST data can be used not only for identifying prevalence of resistance but also incidence. The date of submission of first urine sample to the laboratory during the study period was taken as the follow-up start date for each person in the cohort. All urine samples submitted were followed up until the development of the outcome, which is antimicrobial resistant *E. coli* UTI. The first development of the outcome in each person was identified as an incident case comprising the numerator. Determination of the denominator involved defining all the people at risk of developing the outcome. These were people who submitted a urine sample to ACT Pathology, were ACT residents and were free of an antimicrobial resistant *E. coli* UTI at the start of follow-up. To identify the people at risk, it was important to define a period free of infection. As this was a retrospective study with data obtained from the 1st of January 2009, ideally all those with the outcome on this date needed to be excluded. Given that data from December 2008 were not available to distinguish people who had an infection carried from the previous year from those who truly had an incident infection on the 1st of January 2009, excluding people with the outcome on the 1st of January had the potential to underestimate the incidence of resistance. As stated in the previous paragraph, determination of the people at risk is generally one of the difficulties when estimating cumulative incidence, more so with laboratory data which are not collected for research purposes. Hence for this research program, the denominator used for calculating the five-year cumulative incidence was the cohort of ACT residents who submitted urine samples to ACT Pathology for processing over the five-year period. The denominator for calculating the annual cumulative incidence was the cohort of ACT residents who submitted urine samples each year.

Calculating an incidence rate as opposed to a cumulative incidence was not practical although it overcomes the problems associated with dynamic populations (Buttner & Muller, 2011). The incidence rate relies on the total time each person was observed until the development of the outcome, loss to follow-up, death or end of study (Centers for Disease Control and Prevention, 2012). As retrospective microbiological laboratory data were used for determining the incidence and individuals were not actually 'followed up', precise calculation of the person-time of observation was not feasible. Individuals who did not develop the outcome were censored at the end of follow-up. It is possible that some may have redeveloped symptoms of UTI but not have had a urine sample taken or, further still, the urine sample may have been processed elsewhere.

Therefore to maximize use of the available laboratory data, while acknowledging the strengths and limitations of both cumulative incidence and incidence rate, the cumulative incidence was used to determine incidence of AMR for this research program. Further methodological details and results of the incidence of AMR are reported in Chapter Six.

An important issue I considered when evaluating the incidence of resistance in the cohort of ACT residents was determining how representative of the whole ACT population was the cohort of residents who submitted urine samples for processing to ACT Pathology. As mentioned in Chapter One, the ACT Pathology laboratory provides specialist pathology services to patients in the region and this includes patients in public and private hospitals, specialist clinics, general practice clinics, nursing homes and the community (ACT Government, 2015a). Almost 100% of urine samples from all inpatients of public hospitals in the ACT, as well as people attending emergency departments and specialist outpatient clinics of the public hospitals, are processed by ACT Pathology. The non-public hospital samples are processed by either ACT Pathology or private laboratories in the ACT (P. Collignon, personal communication, 8 November, 2016). This mix of laboratories makes it difficult to estimate incidence. In addition, the AST method used by private laboratories differs slightly from the CLSI method used by ACT Pathology, which was described above in section 3.2.3 (A. Das, personal communication, 8 November, 2016), further limiting the estimation of resistance incidence. To obtain as close an estimate as possible for the number of non-public hospital urine samples (private hospital and community samples) processed by ACT Pathology, data were obtained from the Medicare statistics website

(Medicare Australia, 2016). As previously mentioned in the introduction chapter (Chapter One), the funding contribution by the Australian Government towards healthcare includes the Medicare scheme (Australian Institute of Health and Welfare, 2014). Medicare provides free or subsidised healthcare to all Australians and this includes the cost of most laboratory investigations (Australian Institute of Health and Welfare, 2014). Urine sample examination, which includes examination for cell count, culture, colony count and AST, is covered under Medicare item number 69333 (Medicare Australia, 2016). There were a total of 283,543 requests for this item billed to Medicare for the five-year study period from January 2009 to December 2013 in the ACT (Medicare Australia, 2016). This number excludes urine sample requests ordered for public patients in public hospitals, which are funded under a different arrangement (Medicare Australia, 2016; A. Das, personal communication, 11 November, 2016). A total of 146,915 urine samples belonging to patients whose residential status was ACT were processed over the five years by ACT Pathology. Of these samples, 110,791 belonged to inpatients of public hospitals in the ACT as well as people attending emergency departments and specialist outpatient clinics of the public hospitals. It is important to note that for billing purposes, emergency department patients are categorised as public hospital patients (A. Das, personal communication, 11 November, 2016). The remaining 36,124 samples of the 146,915 samples processed by ACT Pathology belonged to non-public hospital patients (that is, private hospital and community samples), which are billed to Medicare by ACT Pathology (A. Das, personal communication, 11 November, 2016) and therefore inclusive of the 283,543 samples billed to Medicare over the five years. Private laboratories therefore process the majority of private hospital and community samples in the ACT which are billed to Medicare (283,543-36,124=247,419).

Based on the available information described above, I therefore estimated that ACT Pathology processes approximately 100% of urine samples from inpatients, emergency department and specialist outpatient clinic patients of public hospitals, and at least 13% of urine samples from patients in the community and private hospitals whose residential status is ACT. The cohort comprised 57,873 ACT residents whose urine samples were processed at ACT Pathology during the period of 1st January 2009 to 31st December 2013, of whom an estimated 71% were inpatients, emergency department and specialist outpatient clinic patients of public hospitals and the remaining 29% were patients in the

community and from private hospitals. Although the incidence of resistance evaluated in this research program is not representative of the ACT population, the results reported in the thesis are the best available incidence to date on *E. coli* UTI resistance in the ACT.

3.4.4 Estimating the effect of factors that may predict AMR

During statistical analysis, it is important to adjust for the presence of covariates or patient-related factors because this may provide information on the risk of development of AMR in individuals. Patient-related factors include continuous variables such as age or categorical variables like sex (Bradburn, Clark, Love, & Altman, 2003; Clark, Bradburn, Love, & Altman, 2003). For studies two and three of the research program, certain variables potentially available in the microbiological laboratory dataset were considered to account for potential risk factors for resistance. These variables were: age; sex; hospital length of stay; hospital ward; presence of catheter; diagnosis related group (DRG) codes; origin of urine sample, and socioeconomic status. The DRG is a classification system that categorises the episodes of care of patients admitted to hospital into groups with similar medical conditions and usage of hospital resources (Australian Institue of Health and Welfare, 2016). The sample origin refers to the health service requesting or ordering the urine sample test. Socioeconomic status was determined using the Australian Socio-Economic Indexes for Areas, based on the residential postcode. This index score ranks areas in Australia based on their relative socioeconomic indicators of advantage and disadvantage, with lower scored areas being more disadvantaged than higher scored areas.

To allow for proper comparison, variables that were only available for one comparison group were not used. These included the hospital length of stay, hospital ward and DRG, which were only available for patients with hospital-acquired UTI. In addition, there were limitations to stratifying the data based on the hospital ward variable because patients may not necessarily be admitted on a ward based on their diagnosis. Data on the presence or absence of catheter could not be included in the analysis due to the poor quality of reporting on this variable, with a lot of missing data. Administrative coding data procedural codes (International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10)) were considered for use as an alternative to assess for presence of catheter specimens, but as evidence shows that coding data underestimates catheter use in hospitals (Gardner, Mitchell, Beckingham, & Fasugba, 2014), evaluating this potential risk

factor was not feasible. Although the variables described above were recognised as potential risk factors for resistance development, examination of the data fields revealed the methodological problems associated with analysing retrospective microbiological laboratory data. These data are not collected primarily for the purpose of the research being undertaken, with the likelihood for missing data on potential risk factors. Based on the available data, the potential confounders to account for resistance in the analysis were age, sex, origin of urine sample and socioeconomic status.

Multiple covariates can be adjusted for using multivariate modelling techniques. The first technique I considered for studies two and three was the Cox Proportional Hazards Regression, which is a semi-parametric survival analysis or "time-to-event" analysis technique that describes the relationship between the incidence of an event and a set of covariates (Bradburn et al., 2003; Cox, 1972). Microbiological laboratory data is such that patients submit samples at different times, meaning that participants enter and leave the study as occurs in a dynamic population. Therefore, not all patients are observed for the same length of time and some have not developed the event of interest at the end of the follow-up period and are therefore right censored (Bradburn et al., 2003; Buttner & Muller, 2011). Right censoring occurs when a patient does not experience the outcome by the end of the study, or is lost to follow-up during the study, or experiences an event outside the outcome that prevents further follow-up (Clark et al., 2003). These features make microbiological laboratory data suitable for the application of survival analysis approaches, including Cox analysis and Kaplan-Meier plots. Kaplan-Meier plots describe and compare graphically the time to event by variables of interest (Balakrishnan, 2014). As study two evaluated prevalence and Cox analysis is better suited to incidence studies, Cox analysis was no longer considered a suitable technique for study two. Time series models were used for study two, which is discussed below in the subsequent section (section 3.4.5).

For this research program, the time to event is the time to occurrence of an antimicrobial resistant *E. coli* UTI. The period of observation starts from the day a urine sample is submitted to ACT Pathology for processing and finishes either when the event occurs, last healthcare encounter or the study end date. In preparing the dataset for study three for Cox analysis, preliminary analysis showed that restricting the data to the first positive *E. coli* UTI per person, an approach used by other studies which assessed incidence of resistant urinary

E. coli isolates (Swami et al., 2012), did not allow Cox regression analysis to be undertaken. This was because it excluded the time to event component of the data and did not allow the follow-up period to be clearly defined. Kaplan-Meier curves generated were also not indicative of incidence. Hence to appropriately define the follow-up period for each individual to undertake Cox analysis, inclusion of all urine samples belonging to the cohort of ACT residents over the five-year period was necessary. This has been previously described in section 3.4.3 above, discussing the approach to calculating the incidence of resistance. After undertaking the Cox regression analysis, it is important to verify that the proportional hazard assumption holds to ensure meaningful interpretation of the results. This assumption means that the hazard of the event in a group is a constant multiple of the hazard in the other group. That is, both hazard curves are proportional over time and do not overlap (Bradburn et al., 2003; Clark et al., 2003). Violation of the proportional hazard assumption was verified in the data, using the scaled Schoenfeld residuals statistical test (Grambsch & Therneau, 1994), preventing the use of Cox regression analysis for study three.

The logistic regression model was considered as a statistical method to adjust for the effect of multiple covariates on resistance risk because it is appropriate for binary outcomes (in this instance, resistance – 'yes' or 'no'). Logistic regression is a statistical analysis technique that is able to investigate the relationship between an outcome variable (for example, antimicrobial resistance) and one or more covariates or predictor variables (age, sex etc.) simultaneously (Balakrishnan, 2014; Sedgwick, 2013a). It is well suited to binary outcome variables because it is a flexible and easily used mathematical function and also provides a meaningful interpretation of the analysis (Balakrishnan, 2014). It estimates the probability of occurrence of the outcome, based on the values of the covariates or predictor variables (Sedgwick, 2013a). In the presence of two or more covariates, the logistic regression model is extended to the multiple or multivariate logistic regression model (Tai & Machin, 2013). The logistic regression results are typically presented as odds ratios, which refer to the ratio of the probability of occurrence of the outcome in the presence of a covariate to the probability of the occurrence of the outcome in the absence of the covariate (Bland & Altman, 2000). This method was therefore used for study three.

The Hosmer-Lemeshow test was used to assess the goodness of fit of the model (Archer & Lemeshow, 2006). In this research program, multivariate logistic regression models were

constructed for each antimicrobial agent and assessment of the models showed good fit to the data. Additional detail is provided in Chapter Six, which discusses study three.

3.4.5 Approach to calculating change in resistance patterns over time

To examine the changes in AMR patterns over time in study two, time series analysis was considered an appropriate statistical analysis technique because the dataset contained observations with a time-ordered sequence. That is, observations in the dataset are arranged according to the time of AMR development. This time-ordered sequence is referred to as time series and the statistical method applied to time series data is called time series analysis (Wei, 2013). The three main concepts in time series analysis include trend, serial dependence and stationarity (Crawley, 2012). Trend refers to a consistent upward or downward direction of the data. Serial dependence means that observations in a time series may be correlated with one another, and stationarity means that the time series data does not change over time, that is, no trend (Crawley, 2012). In certain situations, such as for antimicrobial susceptibility test data, the data demonstrate non-stationary phenomenon with time-varying resistance patterns. In addition, time series data may demonstrate seasonal cycles (Wei, 2013). Seasonality is a pattern that shows periodic repetitive fluctuations over time (Wei, 2013).

Time series data can be fitted to one of three models. These include the autoregressive model, moving average model and autoregressive moving average model (Box & Jenkins, 1976). As the name implies, the autoregressive model regresses the variable under observation against past values of itself (Crawley, 2012). The moving average model averages the random variations in observations over a certain time period. It is the easiest way of identifying patterns in time series data (Crawley, 2012). The autoregressive moving average model includes the properties of both the autoregressive and moving average models. These models are incorporated in commonly used statistical analysis software. For the purpose of this research program, time series analysis was used to identify trends and seasonal variation in resistance patterns. A non-stationary autoregressive model was constructed and multiple time series models were fitted to also account for patient risk factors. The Dickey-Fuller and augmented Dickey-Fuller tests were used to assess a unit root in the time series data. These unit root tests investigate whether a time series variable (in this instance, resistance) is non-stationary using the autoregressive model (Dickey & Fuller,

1979). Both the Dickey-Fuller and augmented Dickey-Fuller statistics are negative numbers; the more negative, the stronger the rejection of the null hypothesis (that there is unit root at some level of confidence).

The choice of time unit used in the time series analysis was based on exploratory analysis I undertook, using various time intervals. Yearly rates were not a suitable choice due to loss of power with too few data points. Also, the time series plots did not show the true picture of the fluctuating rates. Of all available analytic options, quarterly data provided a better picture of the trend in antimicrobial resistance patterns and this was further investigated on a seasonal basis. Further information is provided in Chapter Five, which reports on study two.

3.5 Ethical considerations

The *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council, Australian Research Council, & Australian Vice-Chancellors' Committee, 2014) informed the design, ethics and conduct of this research program. For study one of the research program ethics approval was not obtained because the systematic review and meta-analysis undertaken in this study utilised aggregate data from published studies, for which ethics approval had already been granted. Furthermore, there are currently no processes in place for ethical approval of systematic reviews (Vergnes, Marchal-Sixou, Nabet, Maret, & Hamel, 2010). Despite this, the research program complied with the guidelines for merit and integrity. For studies two and three, ethics approval was obtained from ACT Health Human Research Ethics Committee (ETHLR.14.223) and Australian Catholic University Human Research Ethics Committee (2014 276N) (See Appendices F.1 and F.2 respectively for approval letters). Additional approval for the supplementary data on antimicrobial use included in study two was obtained from ACT Health Human Research Ethics Committee and the Executive Director Performance Information Branch, ACT Health (See Appendices F.3 and F.4). The specific principles relevant to these studies are informed consent, and privacy and confidentiality (National Health and Medical Research Council et al., 2014), which are discussed below.

3.5.1 Informed consent

Consent from individual patients to participate in studies two and three was not obtained and approval was granted to waive consent. Section 2.3.9 of the *National Statement on Ethical Conduct in Human Research* outlines the principles for waiving consent. It was impracticable to obtain consent for a number of reasons, consistent with the rationale in the National Statement. These reasons are discussed.

The research is low risk with no interventions and no harm or discomfort as a result. Also, the results of the research are not individualised or, indeed, patient identifiable. Conduct of the research did not require direct involvement of patients, but only collected existing information obtained as a result of their contact with the laboratory. The second and third studies undertaken in this research program aggregated existing data in a manner which enabled the proposed research question to be answered. Furthermore, no other alternatives for fuller disclosure were possible. A patient will have a urine sample taken based on the clinical assessment and decisions of the treating medical practitioner. A urine sample can be taken for any number of reasons and any number of organisms may be found. If consent from the patient was required in this instance, it would mean that every person who had a urine sample collected would need to consent to the study. This is not only impractical but impossible to ensure it was implemented. Verbal consent for taking a urine sample would be expected for clinical purposes, consistent with current practices. Obtaining consent from each person would also necessitate me having access to a greater level of personal information (such as address and telephone numbers). Once data were collected and matched across databases, no re-identifiable patient information was kept. If consent was required and results of the study communicated to patients, re-identifiable information would need to be kept by me, potentially for an extended period of time.

In addition, the potential benefits of the research to the public and wider community outweigh the risks or harm associated with not obtaining consent. When UTI is treated inappropriately, pathogens causing UTI such as *E. coli* may develop resistance to antimicrobials (Trautner, 2010). Resistance to commonly prescribed antimicrobials used in the treatment of UTI has adverse health consequences, with an increased risk of morbidity and mortality in patients (World Health Organization, 2014). This research program seeks to provide a better understanding of antimicrobial resistant *E. coli* UTI in Australia. In doing so,

it is envisaged this will guide therapeutic recommendations for UTI and provide information on patient risk factors associated with resistant UTI pathogens, thereby assisting in the designing and evaluation of interventions to reduce antimicrobial resistant *E. coli* UTI, including public health measures.

3.5.2 Confidentiality and data security

Data for studies two and three were collected from the microbiology laboratory records but, in doing this, privacy of data was maintained. The privacy and confidentiality of patients and patient data was maintained by ensuring that no re-identifiable information was stored after the conclusion of data analysis. I required re-identifiable data, specifically the patients' unit record number, to ensure accurate linkage of data obtained from the pathology department and the Clinical Record Service. Following the data analysis process, information that allowed the data to be re-identifiable was permanently deleted. The research results were produced and published in such a way that a patient's information cannot be identified or cause harm of any description to patients. No individual data were reported.

There were additional measures to ensure patient privacy. The review of patients' microbiology laboratory records was undertaken by ACT Pathology staff and I was only provided with information specific to the research program, under supervision of the ACT Health staff member who is also a co-investigator of studies two and three. In doing so, an additional safeguard was in place to ensure that only information relevant to studies two and three were reviewed. I did not directly review the medical records of patients or have access to the systems that retrieve this information. The data collected were stored in a password protected Microsoft Excel document and only stored through my own password protected computer access on a secure computer network at the Australian Catholic University.

The non-identifiable data for studies two and three will be kept for a period of five years from the point of any publication relating to the research. This timeframe is chosen to comply with point 2.1.1 of the *Australian Code for the Responsible Conduct of Research* (National Health and Medical Research Council, Australian Research Council, & Australian Vice-Chancellors' Committee, 2007). After this period, the computer file (Microsoft Excel spreadsheet) will be permanently deleted and as such includes deleting the file from the

recycle bin on the relevant computers. The study undertaken is deemed low risk as there are no interventions and no harm or discomfort likely to be caused by the study. It can also be argued that it is in the community's interest to ensure access to information about UTI and AMR as these conditions pose a significant problem for patient safety (Tenover, 2006; World Health Organization, 2014).

3.6 Summary

This chapter has provided a detailed discussion of the methodological approaches to undertaking the three studies in this research program. For study one, important methodological considerations for the systematic review and meta-analysis include: the development of a protocol; explicitly stated inclusion and exclusion criteria; development of the search strategy; an appropriate quality and risk of bias assessment tool; adequate data synthesis, and reporting the review based on recommended reporting guidelines. For studies two and three, analysis of microbiological laboratory data had to take into account certain methodological issues prior to undertaking the analysis. These issues include: classification of samples based on the setting of infection acquisition; the use of AST categories in calculating resistance; identification and exclusion of duplicate isolates when required, and laboratory threshold for defining UTI. An understanding of these factors is essential in the analysis of published AMR data and data obtained from a microbiological laboratory database, as they ultimately affect the interpretation and reporting of AMR prevalence and incidence. Using evidence from the literature, a rationale for how these issues will be handled in undertaking the studies in this research program has been provided.

An explanation of the methods used for undertaking each study, along with the rationale for the approaches undertaken, has also been provided. Application of standard systematic review principles to published data for study one provided the opportunity to identify potential areas where further research is required; in particular the methodological quality of published studies, some of which were addressed in studies two and three. Despite the complexities associated with analysing microbiological laboratory data for studies two and three, this research program has been able to demonstrate the wide applicability of AST data using a number of statistical analysis techniques, and thereby provide methodological contributions to the research community in regards to analysing this type of data. In many

AMR studies, the terms ‘prevalence’ and ‘incidence’ are commonly used interchangeably, with prevalence more often evaluated (Bax et al., 2001). The methods outlined in this chapter show that both prevalence and incidence, two statistical calculations serving different purposes, are possible using data from a regional microbiological laboratory. In addition, the methodological issues with analysing these data have been highlighted.

This chapter has also outlined the ethical principles that informed the design, ethics and conduct of this research program, focusing on informed consent as well as confidentiality and data security in relation to studies two and three. The next chapter reports the findings of study one, the systematic review and meta-analysis of observational studies investigating ciprofloxacin resistance in hospital-acquired and community-acquired *E. coli* UTI.

Chapter 4: Study one – Systematic review of ciprofloxacin resistance in *E. coli* UTI

4.1 Overview

Fluoroquinolones are ranked as one of the highest priority critically important antimicrobials because they are a key treatment measure for severe infections, such as septicaemia. Fluoroquinolones, predominantly ciprofloxacin, are now frequently prescribed for the treatment of UTI because of the increasing resistance of *E. coli* to other commonly used antimicrobials, such as trimethoprim-sulphamethoxazole (Mehnert-Kay, 2005). It is believed that excessive use of this group of antimicrobials may have led to the increase in resistance of urinary *E. coli* isolates to these agents observed in many countries (Nickel, 2007). Increasing resistance to fluoroquinolones may therefore have serious clinical consequences for patients with severe infections.

There is evidence to support the association between AMR and ciprofloxacin use (Goettsch et al., 2000; Vellinga et al., 2010). Although restrictions have been placed on the use of ciprofloxacin in countries such as Australia (Cheng et al., 2012), there is evidence both nationally and internationally to show that resistance to this agent is still increasing. Reports from AGAR show that *E. coli* resistance to ciprofloxacin increased in the community from 4.2% in 2008 to 7.0% in 2012 and in hospitals from 8% in 2009 to 11% in 2011 (Australian Group on Antimicrobial Resistance, 2011, 2013). In addition, a retrospective ecological study in Denmark, which assessed the effects of patent loss and generic introduction of ciprofloxacin on ciprofloxacin resistance rates, found that within a year following patent loss the number of ciprofloxacin brands increased from three to ten (Jensen et al., 2010). Ciprofloxacin use increased from 0.13 to 0.33 DDD/1000 inhabitant-days in the four years following patent loss and resistance to urinary *E. coli* isolates increased by 200% during the same period (Jensen et al., 2010).

To preserve the use of this critically essential antimicrobial in the treatment of patients, it is necessary to provide comprehensive evidence in support of the increasing resistance, which will be used to inform policy decisions both nationally and internationally in relation to antimicrobial stewardship and, in this instance, regulatory control for ciprofloxacin use. This chapter presents the published manuscript of the first study undertaken as part of this

research program (Fasugba et al., 2015). In study one, I systematically reviewed the literature and undertook a meta-analysis of eligible studies examining ciprofloxacin resistance in community- and hospital-acquired urinary *E. coli* infections. The findings of study one have been published and are presented in section 4.2 along with recommendations for limiting ciprofloxacin use to prevent further increases in resistance to this agent. Supplementary tables (or Additional files) in the published paper are provided in Appendix G of the thesis.

4.2 Publication one: Ciprofloxacin resistance in community- and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies

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Ciprofloxacin resistance in community- and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies

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4.2.1 Abstract

Background: During the last decade the resistance rate of urinary *Escherichia coli* (*E. coli*) to fluoroquinolones such as ciprofloxacin has increased. Systematic reviews of studies investigating ciprofloxacin resistance in community- and hospital-acquired *E. coli* urinary tract infections (UTI) are absent. This study systematically reviewed the literature and where appropriate, meta-analysed studies investigating ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTI.

Methods: Observational studies published between 2004 and 2014 were identified through Medline, PubMed, Embase, Cochrane, Scopus and Cinahl searches. Overall and sub-group pooled estimates of ciprofloxacin resistance were evaluated using DerSimonian-Laird random-effects models. The I^2 statistic was calculated to demonstrate the degree of heterogeneity. Risk of bias among included studies was also investigated.

Results: Of the identified 1134 papers, 53 were eligible for inclusion, providing 54 studies for analysis with one paper presenting both community and hospital studies. Compared to the community setting, resistance to ciprofloxacin was significantly higher in the hospital setting (pooled resistance 0.38, 95% CI 0.36-0.41 versus 0.27, 95% CI 0.24-0.31 in community-acquired UTI, $P<0.001$). Resistance significantly varied by region and country with the highest resistance observed in developing countries. Similarly, a significant rise in resistance over time was seen in studies reporting on community-acquired *E. coli* UTI.

Conclusions: Ciprofloxacin resistance in *E. coli* UTI is increasing and the use of this antimicrobial agent as empirical therapy for UTI should be reconsidered. Policy restrictions on ciprofloxacin use should be enhanced especially in developing countries without current regulations.

4.2.2 Background

Urinary tract infections (UTI) are one of the most frequent bacterial infections affecting people both in the community and in hospitals (Laupland et al., 2007). It is estimated that about 150 million people per annum are diagnosed with UTI worldwide (Gupta, Hooton, & Stamm, 2001). A recent World Health Organization (WHO) report on antimicrobial resistance (AMR) surveillance specified nine bacteria of international concern which are responsible for some of the most common infections in community and hospital settings (World Health Organization, 2014). *Escherichia coli* (*E. coli)*, the pathogen most often implicated in UTI, is listed as one of the nine. In all six WHO regions (Africa, Americas, Eastern Mediterranean, European, South-East Asia and Western Pacific) high rates of antimicrobial resistance have been observed in this pathogen (World Health Organization, 2014).

Ciprofloxacin is the most commonly prescribed fluoroquinolone for UTI because it is available in oral and intravenous preparations (Schaeffer, 2002). It is well absorbed from the gastrointestinal tract after oral administration. It also has a documented safety profile,

broad Gram-negative organism coverage and high urinary excretion rate (Schaeffer, 2002). During the last decade the resistance rate of *E. coli* to fluoroquinolones such as ciprofloxacin has increased (Mcquiston et al., 2013). A ten year analysis of urinary *E. coli* specimens in Switzerland, found an increasing trend in resistance to ciprofloxacin, from 1.8% to 15.9% (Blaettler et al., 2009). Fluoroquinolones are ranked as one of four of the highest priority critically important antimicrobials (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, 2012) as they have an important role in the treatment of more severe infections, such as septicaemia. Therefore resistance to fluoroquinolones can have serious clinical consequences. They are one of few available therapies for serious *Salmonella* spp. and *E. coli* infections (Mcquiston et al., 2013). Resistance to fluoroquinolones emerges quickly, and this is likely to be related to the biology of resistance as well as a direct response to drug pressure (Redgrave, Sutton, Webber, & Piddock, 2014). They should therefore be used with caution and reserved for severe infections, and preceded by antimicrobial susceptibility testing of the bacteria involved (Mcquiston et al., 2013). The most recent Infectious Diseases Society of America (IDSA) guidelines recommend that fluoroquinolones should be reserved for important uses due to their propensity for ecological unfavorable effects of antimicrobial therapy such as the selection of drug-resistant pathogens and colonisation or infection with multidrug-resistant organisms (Gupta et al., 2011).

Recent prescribing guidelines recommend reserving ciprofloxacin use for more severe infections and resistance to this agent is increasing prompting further research in this area (Blaettler et al., 2009; Linhares et al., 2013; Ma & Wang, 2013). Published quantitative syntheses of overall ciprofloxacin-resistant *E. coli* UTI prevalence and incidence in hospital and community settings are absent. This systematic review of observational studies therefore aims to compare ciprofloxacin resistance in both settings. Knowledge about ciprofloxacin resistance in hospital- and community-acquired *E. coli* UTI will provide information for control of resistant pathogens. This review also has the potential to provide a basis for which future interventions can be evaluated. The findings will, in addition, make available information on ciprofloxacin resistance in various regions of the world providing some evidence for further regulatory control of ciprofloxacin use globally.

4.2.3 Methods

Protocol and registration: The protocol for conducting this review has been registered and can be accessed on the International prospective register of systematic reviews (PROSPERO) (available at www.york.ac.uk/inst/crd with registration number: CRD42014014473). Prior to registration, the protocol was reviewed by a reviewer external to the study team. Ethics approval was not sought as this review synthesized data from published studies for which approval had already been obtained.

Search strategy: We conducted a systematic review of observational (cross sectional, cohort and case control) studies published in the last eleven years (2004-2014) reporting on ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTI. This time limit is based on changes in the microbiology and epidemiology of antimicrobial resistant pathogens which occurred in the past decade with subsequent changes in treatment regimens and patient outcomes (United States Interagency Task Force on Antimicrobial Resistance, 2011). Reporting of this review complied with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) (Liberati et al., 2009).

The electronic bibliographic databases MEDLINE/PubMed, EMBASE, Cochrane, CINAHL and Scopus were searched. Searches were conducted for words in the title or abstract or within the full text of the papers. These included both keywords only and keywords with medical subject headings (MeSH) using the search terms 'resistance', 'urinary tract infection' and '*Escherichia coli*' from 1st January 2004 to 31st December 2014 (see Additional file 1). The reference lists of papers identified from the electronic databases were hand-searched for additional papers.

Inclusion and exclusion criteria: Papers were included if they reported prevalence or incidence rates of ciprofloxacin resistance in community- or hospital-acquired *E. coli* UTI. Papers reporting on urinary *E. coli* ciprofloxacin susceptibility in which resistance rate could be calculated were also included. We included papers involving adults and/or children. Only peer reviewed manuscripts were considered. Grey material which includes unpublished literature, conference abstracts, letters to editors, newsletters and reports were excluded. Non-peer reviewed literature were also excluded. Papers written in languages other than English were also excluded. In addition, papers not clearly specifying the setting (hospital-acquired or community-acquired); drug (ciprofloxacin) or sample (urine) were excluded. Papers that focused on specific sub-populations (e.g. diabetics and patients with recurrent UTI) were also excluded as these did not represent the general population. This review

included only papers that used the Centers for Disease Control and Prevention (CDC) definition of microbiologically confirmed UTI (≥10^5 colony forming unit/ml) (Centers for Disease Control and Prevention, 2015).

Definitions: For the purpose of this review, a study was defined as all data from a published paper with the only distinction being 'hospital' or 'community' setting. Therefore, if a single paper meeting the eligibility criteria reported data on both settings, they were included as two separate studies.

Community-acquired UTI was defined as positive samples obtained from (i) outpatient clinics; (ii) general practice (GP) clinics; (iii) emergency departments; (iv) within 48 hours of hospital admission or (v) from nursing homes or residential aged care facilities (Bouchillon, Badal, Hoban, & Hawser, 2013; Cullen et al., 2012; Sanchez, Master, Karlowsky, & Bordon, 2012).

Hospital-acquired UTI was defined as positive samples obtained (i) after 48 hours of hospital admission or (ii) within 48 hours of hospital discharge (Bouchillon et al., 2013).

Important changes in healthcare delivery over the last few years have seen some usually inpatient procedures now more often than not performed on an outpatient basis (Horcajada et al., 2013). Patients transition freely within sometimes loosely defined levels of the health care system, for example between long-term care or rehabilitation services, to acute-care centres (Henderson et al., 2013; Sydnor & Perl, 2011). This study only considered hospital-acquired UTI as opposed to a wider definition of healthcare-associated UTI, to avoid this confusion.

Study selection: The titles and abstracts of all papers identified in the electronic databases were examined and assessed for relevance and appropriateness to the principal objective of the systematic review. Irrelevant studies were excluded. Full texts of the potentially relevant papers were printed and carefully assessed against the systematic review inclusion and exclusion criteria. Those not meeting the criteria were excluded. The remaining papers deemed to have data relevant to the systematic review and meta-analysis were assessed for quality and risk of bias.

The study selection process and other stages of the review were performed by the lead author (OF). At each stage, 10% of papers identified were also screened against the study criteria independently by other authors (AG, GM and BM). Discrepancies in either the application of inclusion or exclusion of papers, quality assessment or on data extraction were discussed among all authors to make the final decision.

Data extraction process: Data were extracted by one author (OF) and 10% of papers eligible for data extraction were independently extracted by another author (AG). Data extraction was compared between AG and OF demonstrating 100% agreement for all items except the study design. This variable was therefore assessed by all authors. Where there was missing information on the study design of papers to be included in the meta-analysis, attempts were made to contact the authors. When there was no response, consensus on the study design was reached by all authors. Agreement between authors was assessed using Kappa coefficient. The agreement between all authors in deciding on the study design was 71% (Kappa (95% CI) = 0.429 (0.154-0.703), *P* Value=0.003). Papers for which no agreement could be reached on the design, based on insufficient information, were assigned as non-classifiable. Any other missing information in the included papers was recorded as 'not stated'.

The first author, year of study, country of study, study setting, age and sex distribution, co-morbidities, sample size, study design, study aim, antimicrobial susceptibility testing method, ciprofloxacin resistance rate, risk factors for ciprofloxacin resistance (i.e. previous antibiotic use) and mortality data (if reported) were extracted. Where the ciprofloxacin resistance rate was not available, the susceptibility rate was used to determine resistance.

Risk of bias in individual studies: Quality and risk of bias of the final papers included in the review was conducted using a modified version of the Newcastle-Ottawa Scale (NOS) which is a risk of bias assessment tool for observational studies recommended by the Cochrane Collaboration (Higgins & Green, 2011; Wells et al., 2014). Content validity and inter-rater reliability of this tool have been established (Wells et al., 2014). Studies were rated by assigning a judgment of 'Low risk' of bias, 'High risk' of bias, or 'Unclear risk' of bias according to published criteria (Higgins & Green, 2011).

4.2.4 Data analysis

Pooled ciprofloxacin resistance proportions (with 95% confidence intervals) in patients with *E. coli* UTI were separately calculated and compared between hospital and community settings using a random-effects meta-analysis model based on DerSimonian and Laird method (Cooper, Hedges, & Valentine, 2009; DerSimonian & Laird, 1986). This method incorporates an estimate of the between-study variation into both the study weights and the standard error of the estimate of the common effect. The precision of an estimate from each included study was represented by the inverse of the variance of the outcome pooled

across all studies. If the value of the pooled prevalence was within the 95% CI, then the effect size was statistically significant at the 5% level ($P<0.05$). The heterogeneity among studies was assessed by using the I^2 statistic with a P value of <0.05 considered statistically significant, and I^2 values below 25% indicating low heterogeneity, 25-75% moderate heterogeneity and over 75% high heterogeneity (Higgins et al., 2003). Subgroup analyses were done by risk of bias, study duration, age group, UTI symptoms, world region and economy of country (categorised as developed and developing using the World Bank classification (World Bank Group, 2015)). A meta-regression analysis was used to determine the effect of measured covariates on the observed heterogeneity in resistance estimates across studies (Cooper et al., 2009). Assessment of publication bias was estimated using funnel plots. Further analysis was undertaken to examine pooled ciprofloxacin resistance over time using the median study year. For studies occurring over 2 years, the first year was used; for studies occurring over 4 years, the 2nd year was used; for those over 6 years, the 3rd year was used. The non-parametric Spearman's rho correlation coefficient was calculated to determine significance in resistance trend over time. Statistical analyses were undertaken using -Stata statistical software (version 13) (StataCorp, 2013).

4.2.5 Results

Study selection: Electronic database searches identified 15,062 potential studies and 31 additional studies were identified through hand searching. After 11,397 duplicates were removed, 3696 articles remained for title and abstract screening. We assessed 1134 as potentially eligible and retrieved the full text of these articles. After applying inclusion and exclusion criteria, 53 papers (5%) were deemed to have data relevant to the systematic review and meta-analysis. These 53 papers consisted of 54 studies comprising three hospital-acquired *E. coli* UTI studies and 51 community-acquired *E. coli* UTI studies. There was one paper that compared resistance in both hospital and community settings hence reported as two studies (Bouchillon et al., 2013). The PRISMA flow chart describing the papers identified from the search strategy and reasons for exclusion is shown in Figure 1.

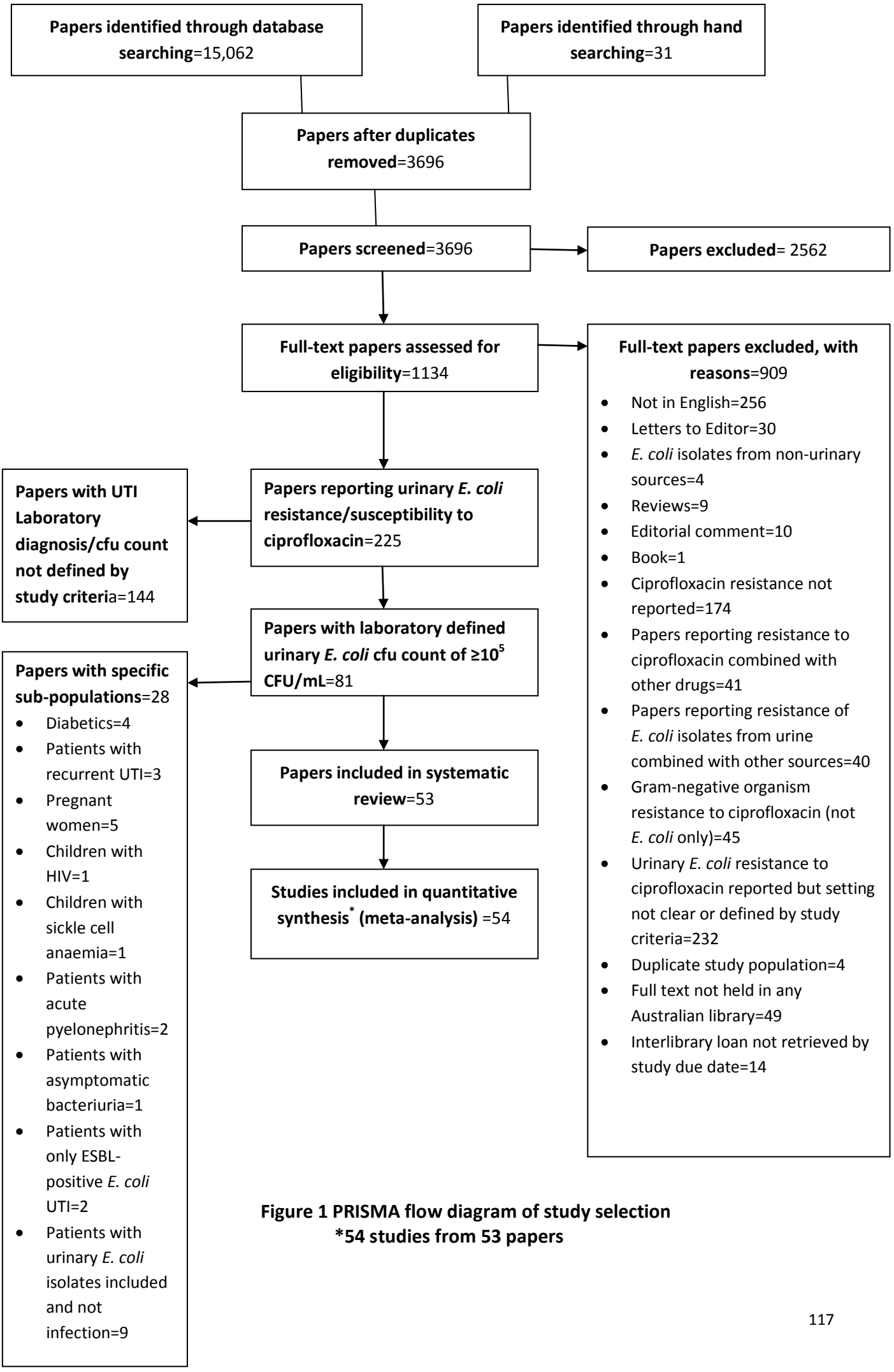

Figure 1 PRISMA flow diagram of study selection
***54 studies from 53 papers**

Study characteristics: Geographically, 53 of the 54 studies were carried out in Asia (28%; n=15), Europe (24%; n=13), Middle East (15%; n=8), Africa (13%; n=7), North America (11%; n=6) and South America (7%; n=4). The remaining study was conducted in multiple countries [28]. There were 17 (31%) studies conducted in developed countries and 36 (67%) in developing countries. The majority of the studies (80%) followed a cross sectional design. The duration of studies ranged from 2 months to 84 months (median=15.5; IQR=12.0-30.0). The mean age and sex proportion of patients with an *E. coli* UTI were stated in 13% (n=7) and 44% (n=24) of studies respectively. Most study populations included patients of both sexes although 19% (n=10) included only women. Antimicrobial susceptibility testing and interpretation was performed using the disk diffusion method (74%) and Clinical and Laboratory Standards Institute (CLSI) criteria (83%) respectively in most studies. Table 1 provides further details on the description of the included studies.

Table 1 Description of studies included in meta-analysis

Study author	Country	Design*	Setting	Risk of bias	Study duration¥ (months)	Number of positive *E. coli* UTI samples+	Number of ciprofloxacin resistant *E. coli*	Proportion resistant (95% CI)	Standard error	Weight# (%)
Ahmad, 2012	India	Cross sectional	Community	Unclear	24	318	48	0.15 (0.11, 0.19)	0.02	2.09
Akoachere et al, 2012	Cameroon	Cross sectional	Community	Low	12	43	11	0.26 (0.13, 0.39)	0.07	1.61
Akram et al, 2007	India	Cross sectional	Community	High	12	61	42	0.69 (0.57, 0.80)	0.06	1.70
AlSweih et al, 2005	Kuwait	Cross sectional	Community	High	12	1535	81	0.05 (0.04, 0.06)	0.01	2.15
Al-Tawfiq et al, 2009	Saudi Arabia	Cohort	Community	High	12	2281	592	0.26 (0.24, 0.28)	0.01	2.14
Ansbach et al, 2013	USA	Cross sectional	Community	High	7	98	2	0.02 (-0.01, 0.05)	0.01	2.12
Arabi et al, 2013	Iran	Cross sectional	Community	Low	33	103	23	0.22 (0.14, 0.30)	0.04	1.91
Araujo et al, 2011	Brazil	Cross sectional	Community	Unclear	24	391	36	0.09 (0.06, 0.12)	0.01	2.12
Arslan et al, 2005	Turkey	Cross sectional	Community	Low	5	514	135	0.26 (0.22, 0.30)	0.02	2.09
Astal, 2005	Palestine	Cross sectional	Community	High	6	252	30	0.12 (0.08, 0.16)	0.02	2.09
Azap et al, 2010	Turkey	Cohort	Community	Unclear	12	464	139	0.30 (0.26, 0.34)	0.02	2.08
Bahadin et al, 2011	Singapore	Cross sectional	Community	Unclear	12	90	22	0.24 (0.16, 0.33)	0.05	1.86

Study author	Country	Design*	Setting	Risk of bias	Study duration¥ (months)	Number of positive *E. coli* UTI samples+	Number of ciprofloxacin resistant *E. coli*	Proportion resistant (95% CI)	Standard error	Weight# (%)
Biswas et al, 2006	India	Cross sectional	Community	High	36	354	124	0.35 (0.30, 0.40)	0.03	2.05
Bouchillon et al, 2013	USA	Cross sectional	Community	High	24	723	234	0.32 (0.29, 0.36)	0.02	2.10
Bouchillon et al, 2013	USA	Cross sectional	Hospital	High	24	253	103	0.41 (0.35, 0.47)	0.03	11.83
Dash et al, 2013	India	Cross sectional	Community	Low	30	397	212	0.53 (0.48, 0.58)	0.03	2.05
Dimitrov et al, 2004	Kuwait	Cross sectional	Community	High	84	780	92	0.12 (0.10, 0.14)	0.01	2.13
Farshad et al, 2011	Iran	Cross sectional	Community	Low	12	90	8	0.09 (0.03, 0.15)	0.03	2.01
Ghadiri et al, 2012	Iran	Cross sectional	Hospital	High	24	200	80	0.40 (0.33, 0.47)	0.03	9.41
Gobernado et al, 2007	Spain	Cross sectional	Community	Low	12	2292	418	0.18 (0.17, 0.20)	0.01	2.14
Ho et al, 2010	Hong Kong	Cross sectional	Community	Low	24	271	35	0.13 (0.09, 0.17)	0.02	2.09
Hoban et al, 2011	Multiple countries	Cross sectional	Hospital	High	24	1643	624	0.38 (0.36, 0.40)	0.01	78.76
Ismaili et al, 2011	Belgium	Cohort	Community	High	24	189	5	0.03 (0.00, 0.05)	0.01	2.13
Kashef et al, 2010	Iran	Cross sectional	Community	High	30	578	180	0.31 (0.27, 0.35)	0.02	2.09

Study author	Country	Design*	Setting	Risk of bias	Study duration¥ (months)	Number of positive *E. coli* UTI samples+	Number of ciprofloxacin resistant *E. coli*	Proportion resistant (95% CI)	Standard error	Weight# (%)
Kiffer et al, 2007	Brazil	Cross sectional	Community	Unclear	48	22679	2699	0.12 (0.11, 0.12)	0.002	2.15
Killgore et al, 2004	USA	Case-control	Community	Low	12	120	40	0.33 (0.25, 0.42)	0.04	1.89
Kimando et al, 2010	Kenya	Cross sectional	Community	Unclear	6	92	6	0.07 (0.01, 0.12)	0.03	2.05
Kothari et al, 2008	India	Cross sectional	Community	High	6	361	260	0.72 (0.67, 0.77)	0.02	2.06
Kurutepe et al, 2005	Turkey	NC	Community	High	72	880	174	0.20 (0.17, 0.22)	0.01	2.12
Lau et al, 2004	Taiwan	Cross sectional	Community	Unclear	13	80	14	0.17 (0.09, 0.26)	0.04	1.89
Ljuca et al, 2010	Bosnia & Herzegovina	Cross sectional	Community	High	36	43	4	0.09 (0.01, 0.18)	0.04	1.87
Longhi et al, 2012	Italy	NC	Community	Low	6	154	36	0.23 (0.17, 0.30)	0.03	1.98
Martinez et al, 2012	Colombia	Cross sectional	Community	High	2	102	39	0.38 (0.29, 0.48)	0.05	1.83
Miragliotta et al, 2008	Italy	Cohort	Community	Low	60	2589	422	0.16 (0.15, 0.18)	0.01	2.14
Molina-Lopez et al, 2011	México	Cross sectional	Community	High	48	119	65	0.55 (0.46, 0.64)	0.05	1.86
Moreira et al, 2006	Brazil	Cross sectional	Community	Unclear	15	544	65	0.12 (0.09, 0.15)	0.01	2.12
Murugan et al, 2012	India	Cohort	Community	High	12	204	144	0.71 (0.64, 0.77)	0.03	2.00

Study author	Country	Design*	Setting	Risk of bias	Study duration¥ (months)	Number of positive *E. coli* UTI samples+	Number of ciprofloxacin resistant *E. coli*	Proportion resistant (95% CI)	Standard error	Weight# (%)
Muvunyi et al, 2011	Rwanda	Cross sectional	Community	Low	6	72	23	0.32 (0.21, 0.43)	0.05	1.75
Mwaka et al, 2011	Uganda	Cross sectional	Community	High	NS	27	9	0.33 (0.16, 0.51)	0.09	1.32
Ni Chulain et al, 2005	Ireland	Cross sectional	Community	High	5	723	18	0.02 (0.01, 0.04)	0.01	2.15
Olson et al, 2012	USA	Cross sectional	Community	Unclear	16	95	4	0.04 (0.00, 0.08)	0.02	2.08
Otajevwo, 2013	Nigeria	Cross sectional	Community	High	6	5	4	0.80 (0.45, 1.15)	0.18	0.63
Prakash et al, 2013	India	Cross sectional	Community	Low	NS	23	16	0.70 (0.51, 0.88)	0.10	1.26
Randrianirina et al, 2007	Madagascar	Cross sectional	Community	Low	28	607	100	0.16 (0.14, 0.19)	0.02	2.12
Rani et al, 2011	India	Cross sectional	Community	Unclear	6	208	151	0.73 (0.67, 0.79)	0.03	2.01
Shaifali et al, 2012	India	Cross sectional	Community	Unclear	12	46	28	0.61 (0.47, 0.75)	0.07	1.54
Shariff et al, 2013	India	Cross sectional	Community	High	18	491	160	0.33 (0.28, 0.37)	0.02	2.08
Sire et al, 2007	Senegal	Cross sectional	Community	Low	33	1010	157	0.16 (0.13, 0.18)	0.01	2.13
Sood et al, 2012	India	NC	Community	High	30	214	160	0.75 (0.69, 0.81)	0.03	2.02
Stratchounski et al, 2006	Russia	NC	Community	Low	48	423	18	0.04 (0.02, 0.06)	0.01	2.14

Study author	Country	Design[*]	Setting	Risk of bias	Study duration[¥] (months)	Number of positive *E. coli* UTI samples[+]	Number of ciprofloxacin resistant *E. coli*	Proportion resistant (95% CI)	Standard error	Weight[#] (%)
Vellinga et al, 2012	Ireland	Case-control	Community	Low	9	633	78	0.12 (0.10, 0.15)	0.01	2.12
Wang et al, 2014	China	Cross sectional	Community	High	8	129	91	0.71 (0.63, 0.78)	0.04	1.92
Yildirim et al, 2010	Turkey	Cross sectional	Community	Unclear	24	450	85	0.19 (0.15, 0.23)	0.02	2.10
Yolbas et al, 2013	Turkey	Cross sectional	Community	High	12	113	24	0.21 (0.14, 0.29)	0.04	1.93

[*]Non-classifiable design

[¥]Not stated

[+]Study denominator

[#]Weights are from random-effects analysis using DerSimonian-Laird model

Pooled ciprofloxacin resistance: Figures 2 and 3 show the forest plots of studies reporting on ciprofloxacin resistance in community-acquired *E. coli* UTI by economy and region, respectively. Figure 4 shows the forest plot of studies reporting on ciprofloxacin resistance in hospital-acquired *E. coli* UTI. Compared with the community setting, resistance to ciprofloxacin in *E. coli* UTI was significantly higher in the hospital setting (P<0.001). Overall, the pooled rate for ciprofloxacin resistance in patients with community-acquired *E. coli* UTI was 0.27 (95% CI: 0.240-0.310), compared with 0.38 (95% CI: 0.360-0.410) in the hospital setting. There was substantial heterogeneity among the community setting studies (I^2=98.8%, P<0.0001), but very little in the hospital ones (I^2=<0.010%, P=0.641). Further analysis of studies reporting on community-acquired *E. coli* UTI by region (figure 3) showed that Asia had the highest pooled resistance. Analysis by economy based on the World Bank classification (figure 2) showed a higher pooled resistance in developing countries.

Resistance over time in community-acquired UTI studies: Figure 5 shows the scatter plot of ciprofloxacin resistance in 47 studies reporting on community-acquired UTI using the median study year for each study. Four studies did not provide data on the year(s) the study was conducted and were excluded from this analysis (Kimando, Okemo, & Njagi, 2010; Mwaka, Mayanja-Kizza, Kigonya, & Kaddu-Mulindwa, 2011; Prakash & Saxena, 2013; Shaifali, Gupta, Mahmood, & Ahmed, 2012). The results of the Spearman's rho correlation test showed a statistically significant rise in resistance over time (n=47, r_s=0.431, P=0.003). Similar findings were observed for developing countries. There was no significant rise in resistance over time in developed countries.

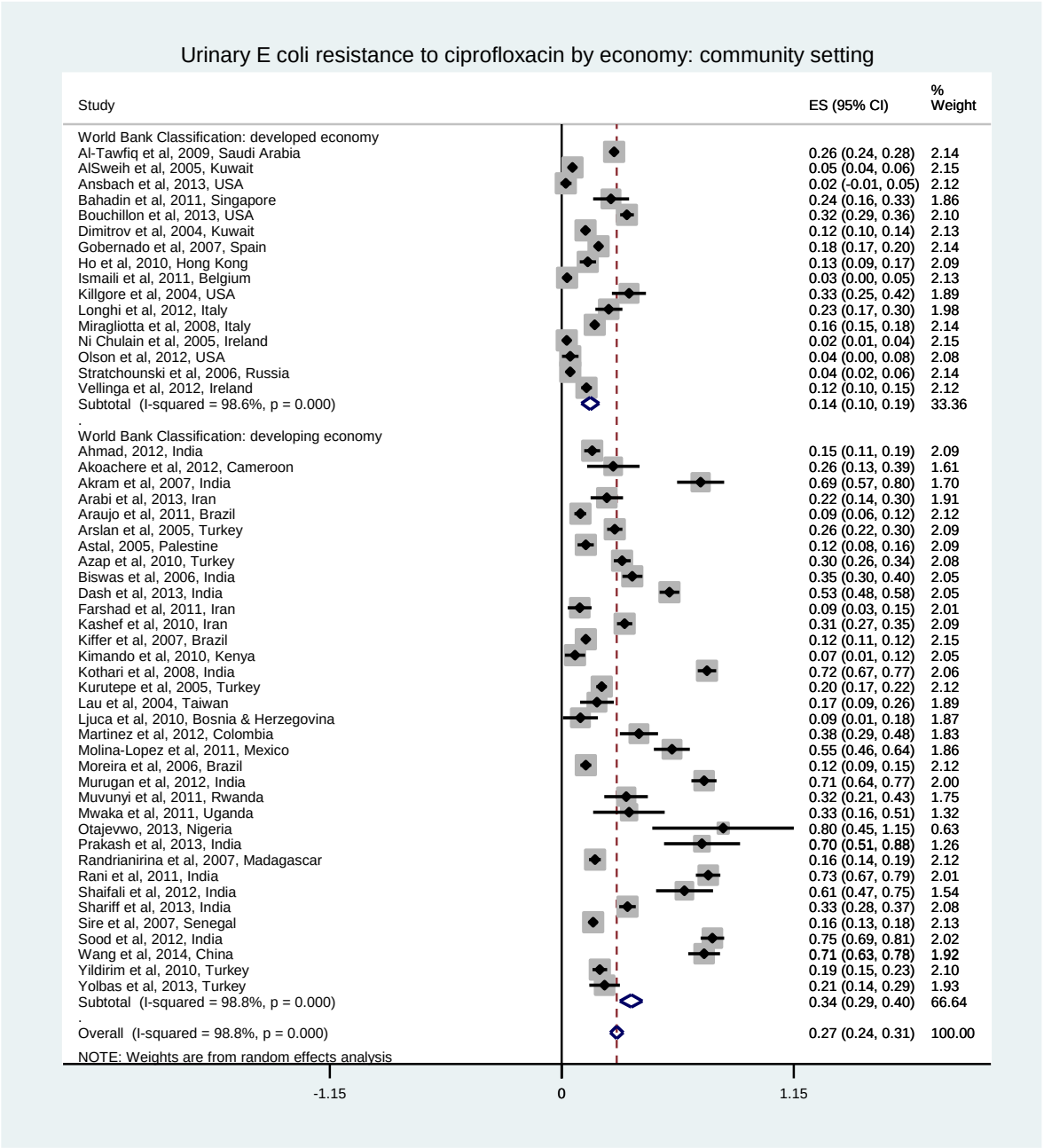

Figure 2 Forest plot of ciprofloxacin resistance in community-acquired *E. coli* UTI by economy

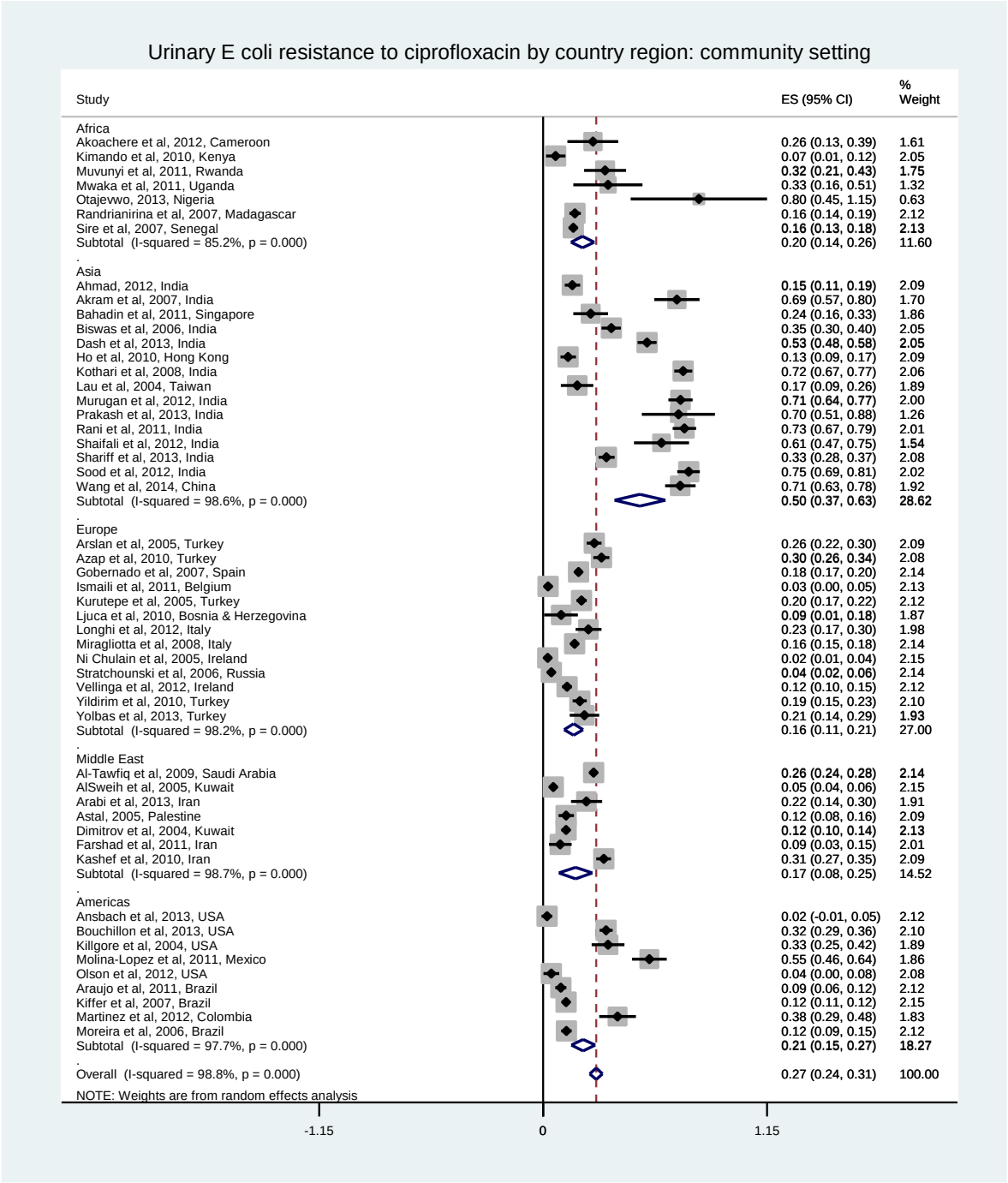

Figure 3 Forest plot of ciprofloxacin resistance in community-acquired *E. coli* UTI by region

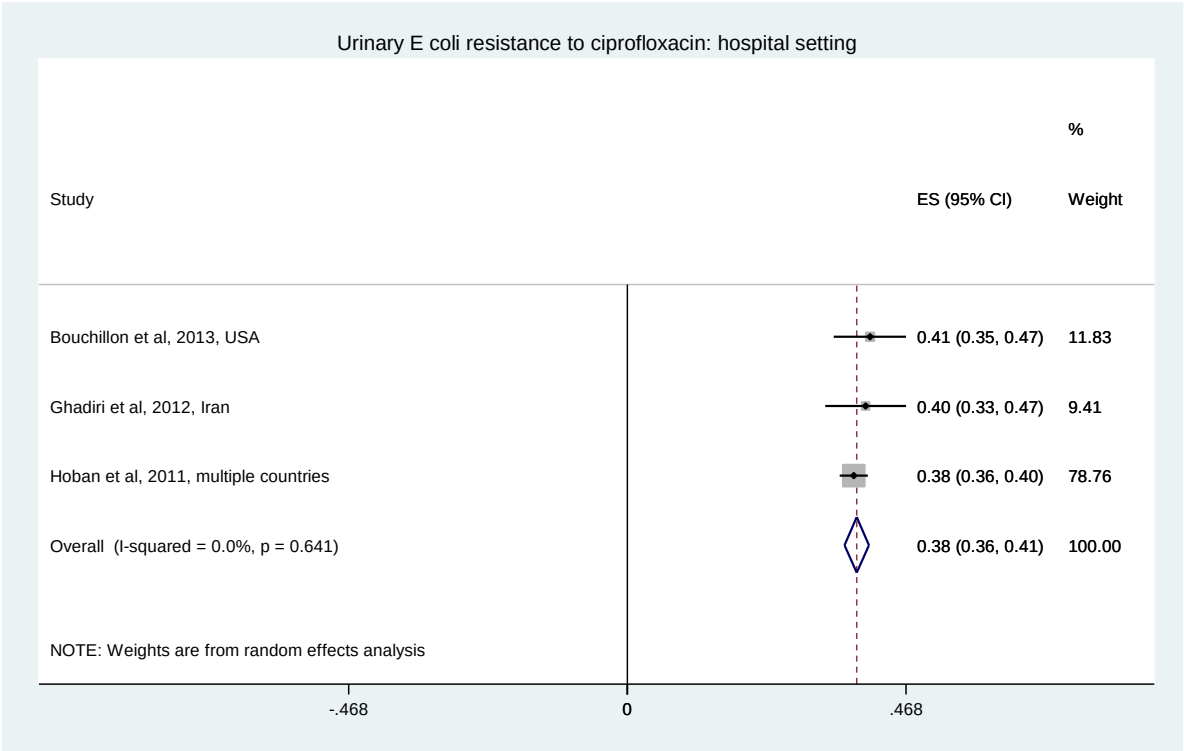

Figure 4 Forest plot of ciprofloxacin resistance in hospital-acquired *E. coli* UTI

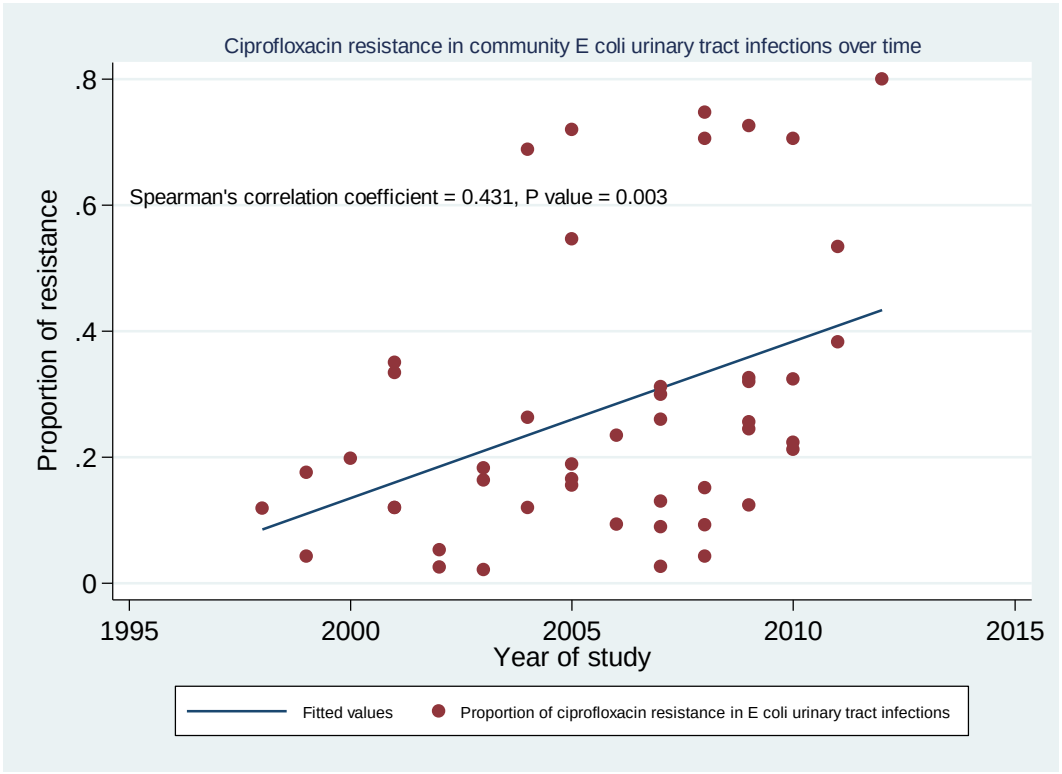

Figure 5 Scatter plot of ciprofloxacin resistance in community-acquired UTI by year of study (1998-2012)

N=47 (4 studies excluded due to missing information on year study was conducted)

Subgroup analyses: Sub-group analysis was conducted within each major setting. For community-acquired UTI studies (Table 2), there was a significant difference in the pooled resistance within each subgroup examined (risk of bias, study duration, economy, region, age group and UTI symptoms). The subgroup analyses results for studies reporting on hospital-acquired *E. coli* UTI (see Additional file 2) showed no difference in the pooled resistance within the subgroups examined (region, economy and UTI symptoms). When both settings were compared (see Additional file 3), there were significant differences noted for risk of bias (high), study duration (>12 months), economy (developed), region (Americas), age group (adults and children) and UTI symptoms (P<0.001). There were no data available on mortality for comparison between settings.

Table 2 Subgroup analyses of pooled ciprofloxacin resistance in community setting

	Subgroup	Community Setting N=51	*P* value*
		Pooled resistance	
Risk of bias	Low and unclear n=28 studies	0.221	<0.0001
	High n=23 studies	0.337	
Study duration*	≤12 months n=25 studies	0.323	<0.0001
	>12months n=24 studies	0.219	
Economy	Developed n=16 studies	0.141	<0.0001
	Developing n=35 studies	0.345	
Region	Africa, Asia and Middle East n=29 studies	0.361	<0.0001
	Europe, North and South America n=22 studies	0.174	
Age group*	Adults and children ‡ n=24 studies	0.265	<0.0001
	Adults only n=19 studies	0.302	
UTI symptoms	Symptomatic and asymptomatic patients n=11 studies	0.185	<0.0001
	Symptomatic patients only n=40 studies	0.295	

n=number of studies reporting on community-acquired UTI
*****Comparing pooled resistance for difference in subgroup in community setting
*Studies with missing information on this sub-analysis were not included
‡ Studies reporting resistance in adults and children or children only

Meta-regression analyses: Random-effects meta-regression analyses of studies reporting on community-acquired *E. coli* UTI showed that country's economy (*P*=0.008), Asia as a region (*P*=0.002), high risk of bias (*P*=0.003), year of study (*P*=0.020) and studies using only children as the study population (*P*=0.030) were the study factors significantly accounting for the observed heterogeneity, responsible for 61% of the between study variance (Adjusted R^2) in ciprofloxacin resistance.

Risk of bias: When studies were assessed for risk of bias using the Newcastle-Ottawa scale, 30% (n=16) were assessed as having a low risk of bias; 22% (n=12) unclear risk of bias and 48% (n=26) were deemed to have a high risk of bias. Further analysis of the 16 low risk studies only was consistent with findings reported from the analysis of all studies. An increasing resistance trend over time was also observed, however this increase did not reach statistical significance because of reduced statistical power.

4.2.6 Discussion

The findings of this systematic review and meta-analysis highlight the higher ciprofloxacin resistance in hospital-acquired *E. coli* UTI when compared to community-acquired UTI. There is also substantial evidence that ciprofloxacin resistance in community-acquired *E. coli* UTI has been increasing in recent years. Resistance was also found to be significantly higher in developing countries reporting on *E. coli* UTI in community settings.

Antimicrobial resistance has been described as an international hazard to public health threatening the successful prevention and treatment of bacterial, viral, parasitic and fungal infections (World Health Organization, 2012, 2014). As such, research into its prevention and reduction is very important. Our estimated pooled ciprofloxacin resistance of 27% and 38% in community and hospital-acquired *E. coli* UTI respectively could not be compared to any other systematic review findings because, to our knowledge, this is the first systematic review and meta-analysis comparing ciprofloxacin resistance in community and hospital-acquired *E. coli* UTI. However, national data from five WHO regions show at least 50% resistance to fluoroquinolones (ciprofloxacin, norfloxacin or ofloxacin) in *E. coli* (World Health Organization, 2014). Data on *E. coli* in the WHO report are from various settings and sources (including blood and urine) hence cannot be directly compared with the results from our systematic review. Another recent review on global fluoroquinolone resistance

epidemiology reported a range of 2% to 69% for fluoroquinolone resistance in uncomplicated community-acquired UTI and up to 98% in complicated cases, with fluoroquinolone resistance in healthcare-associated UTI ranging from 6% to 62% (Dalhoff, 2012a). The findings from our systematic review are within the above reported ranges. However, the latter ranges were wide and the data were from a number of different Gram-negative uropathogens and not specifically *E. coli* accounting for the higher rates. Available published data show relatively high rates of urinary *E. coli* resistance to ciprofloxacin (Azap et al., 2010; Hassanzadeh & Motamedifar, 2007; Killgore, March, & Guglielmo, 2004; Molina-López et al., 2011; Otajevwo, 2013; Sood & Gupta, 2012; Wang et al., 2014) prompting the need for a renewed effort in the further prevention of spread of resistance to this antimicrobial agent.

We found that urinary *E. coli* resistance to ciprofloxacin was higher in the hospital compared to the community setting. Our finding is comparable to individual studies which have assessed urinary *E. coli* resistance to ciprofloxacin in both, hospital and community settings (Chulain, Murray, Corbett-Feeney, & Cormican, 2005; Ljuca, Zvizdic, Hamzic, Kalajdzija, & Ljuca, 2010; Longhi et al., 2012; Prakash & Saxena, 2013; Shariff, Shenoy, Yadav, & Radhakrishna, 2013; Wang et al., 2014). However, often studies do not apply the criterion of 48 hours post admission used in our systematic review for identifying hospital-acquired UTI (Al Sweih, Jamal, & Rotimi, 2005; Shariff et al., 2013). The Canadian national surveillance study (CANWARD), a large population-based study undertaken from 2007 to 2009, further confirms our finding of higher resistance in the hospital setting (Karlowsky et al., 2011). Inpatients had a significantly higher urinary *E. coli* resistance to ciprofloxacin. Similar findings were reported by Cullen et al. in Dublin (Cullen et al., 2012). This is not an unusual finding and may be attributed to the selective pressure resulting from antimicrobial use in hospital settings (Karlowsky et al., 2011). Patients in hospital, already acutely ill, become more at risk of developing a resistant infection because of potential immune deficiency and relative high exposure to antimicrobial agents (Tenover, 2006). Furthermore, hospitalized patients are more likely to be exposed to practices that result in cross infection or transmission of organisms. These and other risk factors enable the spread of resistance. This has significant implications for patient care as antimicrobial resistance may lead to treatment failure resulting in death.

The results of our systematic review showed a significant rise in resistance over time in the community setting. This finding is supported by a number of US-based studies investigating antimicrobial resistance trend in outpatients. A fivefold increase (from 3% to 17.1%) in ciprofloxacin resistance was observed from 2000 to 2010 by Sanchez et al. (2012) in comparison with other antibiotics investigated (Maraki et al., 2013). Our findings are also consistent with Blaettler et al. (2009) who found that over a ten year period (1997-2007), similar to the timeframe for our review, resistance increased significantly for ciprofloxacin from 1.8% to 15.9% in Switzerland. This increase coincided with a rise in ciprofloxacin use in Switzerland (Blaettler et al., 2009). These findings suggest that with increase in the use of fluoroquinolones generally over time, resistance ciprofloxacin is likely to further increase. It is now known that antimicrobial overuse or misuse is a risk factor for the development of AMR (Costelloe et al., 2010). The specific effect of ciprofloxacin use on the development of its resistance in UTI pathogens is also clearly documented. A recent Irish study involving 72 general practices found higher ciprofloxacin resistance levels (5.5%) in practices with 10 prescriptions per month compared with resistance levels of 3% in practices with one prescription per month (Vellinga et al., 2010). Wide spread use of this agent may have thus resulted in a rise in ciprofloxacin resistance. In the Netherlands and United States, an association has also been shown between high fluoroquinolone prescriptions and a rise in bacterial resistance (Goettsch et al., 2000; Zervos et al., 2003). Furthermore, changes in antimicrobial prescribing practices have been shown to precede changes in resistance rates. A study by Gottesman et al. (2009) in Israel found a significant decrease in *E. coli* resistance to ciprofloxacin following a nationwide restriction on ciprofloxacin use. Resistance decreased from 12% in the pre-intervention period to 9% in the intervention period. Our results pose a strong argument for the development of more stringent criteria limiting ciprofloxacin use. In addition, other strategies such as adequate surveillance and monitoring, reinforcement of existing infection prevention and control measures as well as new technological advancement will help reduce the widespread problem of antimicrobial resistance (Australian Commission on Safety and Quality in Health Care, 2013b; Australian Government, 2015; Dancer, 2013) but these aspects are not within the scope of this paper.

Our finding of a significant rise in resistance over time also has implications for the development of treatment guidelines. The national recommendations for first-choice empiric antibiotic treatment of UTI vary considerably (Mcquiston et al., 2013). In countries

like Spain, Taiwan and Turkey, the treatment choice for uncomplicated UTI are fluoroquinolones (Infectious Diseases Society of the Republic of China, 2000; Karaca et al., 2005; Mcquiston et al., 2013). In 2000, fluoroquinolones were prescribed for treatment of uncomplicated UTI in Switzerland in 64% of cases (Naber, 2000). There is concern that resistance to ciprofloxacin resulting from its first-line use may be associated with an increase in multidrug resistance (Olson & Haith, 2012). The most recent IDSA guidelines (Gupta et al., 2011) advise using nitrofurantoin, trimethoprim-sulphamethoxazole, fosfomycin or pivmecillinam for first-line treatment of acute uncomplicated cystitis. Fluoroquinolones should be reserved for important uses other than acute cystitis or used as an alternative only when these recommended agents cannot be used (Gupta et al., 2011). We recommend that ciprofloxacin should not be used as a first-line treatment option for UTI as continuous increases in resistance to ciprofloxacin further weaken the effectiveness of this drug.

Additional findings from the meta-analysis showed that resistance was significantly higher in developing countries compared to developed countries. A major factor accounting for this difference is the use of over-the-counter or non-prescription antibiotics which occur commonly in developing countries (Okeke et al., 2005; Okeke, Lamikanra, & Edelman, 1999). Although this review did not directly consider antimicrobial resistance in relation to prescribing for the included studies, evidence shows that over-the-counter or non-prescription use results in unnecessary and excessive use of antibiotics. Some of the included studies in our review clearly state that there are no restrictions for over-the-counter prescribing of antimicrobials within their countries (Ahmad, 2013; Akoachere, Yvonne, Akum, & Seraphine, 2012; Akram, Shahid, & Khan, 2007; Arabi & Banazadehi, 2013; Astal, 2005; Dash, Padhi, Mohanty, Panda, & Parida, 2013; Kashef, Djavid, & Shahbazi, 2010; Kimando et al., 2010; Kothari & Sagar, 2008; Murugan, Savitha, & Vasanthi, 2012; Sire et al., 2007). A recent systematic review investigating global non-prescription antimicrobial use found that resistance was common in communities with frequent non-prescription antimicrobial use (Morgan et al., 2011). Non-prescription use was highest in Africa, Asia and Middle East at 100%, 58% and 39% respectively (Morgan et al., 2011). In our review, further analyses by region showed that Asia had the highest pooled resistance to ciprofloxacin with a significantly higher resistance in Africa, Asia and Middle East combined compared with Europe and the Americas. Our finding is supported by a recent paper by Dalhoff (2012b)

reporting that fluoroquinolone resistance was highest in the Asia-Pacific region and moderate to low in Europe and North America. Furthermore, there is evidence to show that countries that have developed control policies to regulate non-prescription use have seen a decrease in antimicrobial use and resistance rates (Morgan et al., 2011). Based on our findings, we therefore emphasise the need for the development of policies restricting over-the-counter antimicrobial use in countries that do not have such policies thereby contributing to the prevention of patient morbidity and mortality associated with resistant infections. It is noteworthy to mention that another important factor contributing to antimicrobial resistance is the use of antibiotics in livestock for growth promotion (Maron, Smith, & Nachman, 2013). Extensive antimicrobial use in food animal production has been associated with antimicrobial resistance globally (Maron et al., 2013). This has considerable implications for human health with the need to protect the efficacy of these antimicrobials to ensure their effectiveness for the treatment of humans.

A large variation in ciprofloxacin resistance was found in studies reporting on community-acquired UTI. This variation highlights the significance of local resistance monitoring to guide the development of local antibiotic guidelines. The random-effects meta-regression model confirmed that a number of factors significantly accounted for the variations in ciprofloxacin resistance. These include economy (developed and developing), Asia as a region, year of study, studies including only children and studies with a high risk of bias. The first three factors have been discussed in detail in the preceding paragraphs. We found that resistance was lower in studies involving only children. This finding is in line with a number of studies which have compared resistance in adults and children showing significantly higher ciprofloxacin resistance in adults compared to children (Owumi, Banaei, & Shortliffe, 2014; Storby, Österlund, & Kahlmeter, 2004). Increased age has also been shown to be significantly associated with ciprofloxacin resistance (Blaettler et al., 2009; Karlowsky et al., 2011). Given that children are less exposed to antimicrobials with limited ciprofloxacin use in the paediatric age group, this finding is expected (Adefurin, Sammons, Jacqz-Aigrain, & Choonara, 2011; Owumi et al., 2014; Storby et al., 2004). Although the importance of intrafamilial cross-infection of resistant pathogens is yet to be confirmed, it has been suggested that fluoroquinolone resistance may to some extent be dependent on cross-infection with transfer from adults to children (Storby et al., 2004). Given this assumption, it is necessary to also monitor resistance levels in children to prevent further resistance

development in this vulnerable age group. Other likely causes of higher resistance in adults may be the greater likelihood of comorbidities with more frequent contact with healthcare settings (Karlowsky et al., 2011). The last factor found to account for heterogeneity between studies was high risk of bias. Most of the studies included in the review were found to have a high risk of bias as assessed using the NOS scale. These studies lacked methodological rigour including absence of the inclusion of possible confounding factors (such as age, sex and previous use of an antimicrobial) in the design and analysis of the studies. The poor reporting of observational studies poses limitations for conducting meta-analysis of these studies. Better presentation of definitions would enable inclusion in systematic reviews of some categories that had to be excluded in this review. Observational studies are more prone to confounding bias (von Elrn, 2004) further emphasising the need for adherence to reporting guidelines such as such as that based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (Vandenbroucke et al., 2007) to ensure clear and comprehensive reporting prior to publication acceptance. The poor quality of many studies initially retrieved for this review resulted in a large number being excluded. Therefore the information provided in this systematic review and meta-analysis of 54 observational studies may not sufficiently address ciprofloxacin resistance globally but may provide satisfactory evidence to inform future interventions.

In addition, this systematic review highlights the weaknesses in the quality of antimicrobial resistance data that are being collected in various regions. These weaknesses have implications for development of effective surveillance systems to monitor resistance globally and strategies to prevent further resistance development. The need for the implementation of national and global surveillance systems to detect and continuously monitor AMR cannot be overemphasised. These systems would enable prospective studies to be conducted and would play a major role in curtailing the widespread effect of antimicrobial resistance and help healthcare providers in deciding on the most appropriate empirical therapy for UTI to ensure proper management of patients. Governments need to put in place policies to restrict over-the-counter use and inappropriate prescribing of ciprofloxacin and other antimicrobials to prevent further development of resistance.

Strengths and limitations

There are a number of notable strengths to our review. To our knowledge, this is the first systematic review to compare the overall prevalence of ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTI. We undertook a comprehensive literature search process to identify and screen articles against eligibility criteria. Given that generic versions of ciprofloxacin were first marketed at different times in various countries, our choice of 2004 as the start date was therefore made on the basis of changes in the epidemiology of antimicrobial resistant pathogens which had resulted in changes to treatment regimens. A further strength of this systematic review is the development of a peer reviewed, registered protocol prior to undertaking the review. For studies to be included in the review, they were restricted to those that used a standard laboratory UTI criterion of $\geq 10^5$ cfu/mL as recommended by the CDC. Although applying the internationally recognised CDC criteria may definitely be considered a strength as it ensures the quality and uniformity of included studies, this criterion limited the number of hospital-acquired UTI studies included in our systematic review. Despite this, resistance was still found to be higher in the hospital setting compared to the community setting similar to published studies. While lower counts of uropathogens are relevant for acute episodes of uncomplicated cystitis, the use of different colony counts makes comparison of data between studies difficult. Including all urinary *E. coli* isolates was considered but not done because this existing surveillance criterion ($\geq 10^5$ cfu/mL and 48 hours cut off) is usually applied to defining infections not isolates. Also, including all isolates carries the risk of including duplicates. This approach poses some degree of ascertainment bias as our systematic review focuses on laboratory identified UTI which may not only underestimate the total number of UTI but also lead to selection of samples from complicated cases thereby overestimating resistance. Another limitation is the wide variation of resistance estimates between studies and the inclusion of studies having substantial clinical and methodological heterogeneity. Visual inspection of the funnel plot (figure 6) showed asymmetry suggesting evidence of publication bias, with studies reporting high resistance rates being more likely to be published posing a limitation to this review. Also, the quality and risk of bias of some of the studies included in the review were assessed as high. These limitations were addressed by undertaking a random-effects meta-analysis with subsequent subgroup analyses and random-effects meta-regression to explain the sources of heterogeneity. For studies in which the design was not stated, the review authors faced

difficulties in categorising such studies hence some of these studies were grouped as non-classifiable. These studies did not provide clear and explicit information on the methods used for conducting the studies. This emphasises the need for implementation and adherence to clear reporting standards prior to publication of papers. Furthermore, in some included studies, adjustments were not made for important confounding factors relevant to antimicrobial resistance such as antibiotic use and patient demographics including age and sex. For this systematic review, studies on samples obtained from emergency department (ED) patients were classified as community-acquired samples. Included papers did not provide any information on whether some of these patients may have returned from a recent hospitalisation and represented to the ED. Ideally, these should be considered as hospital-acquired infections as some of these patients may have been discharged in the previous 48 hours. For the purpose of this review and to overcome inherent variations in how individual studies have defined these patients, we classified all papers reporting on ED patients as community-acquired UTI studies. It was not possible to determine the potential effect of samples obtained from nursing home or residential aged care studies on the pooled resistance because this participant group did not meet the inclusion criteria for analysis. Furthermore, classification of this setting as hospital or community remains controversial. Finally, validity issues may have arisen from the use of different antimicrobial susceptibility test and interpretation methods with differing breakpoints which tend to change over the years. To date, there is still no worldwide consensus on the most suitable antimicrobial susceptibility testing method with the fact that various countries and even laboratories within the same country use different tests and interpretative criteria. Subgroup analysis for AST method was considered but not done because almost all studies used the disk diffusion method and CLSI criteria.

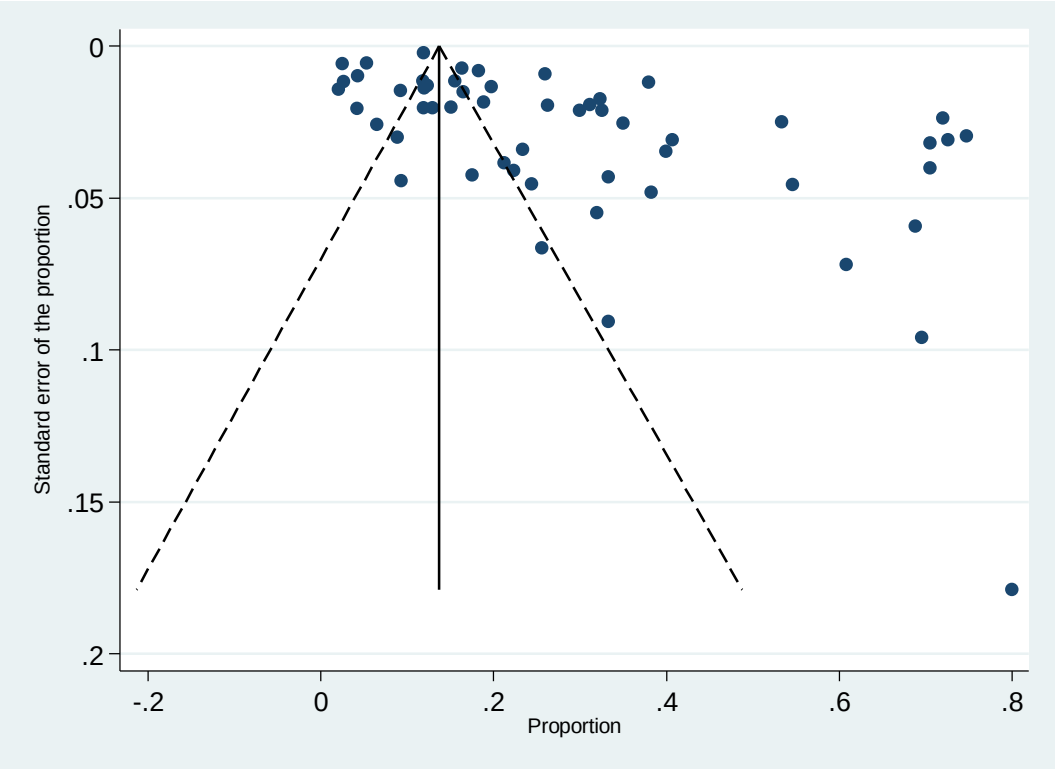

Figure 6 Funnel plot of studies included in meta-analysis

4.2.7 Conclusion

Ciprofloxacin resistance in *E. coli* UTI is increasing. The use of this antimicrobial agent as empirical therapy for UTI should be reconsidered and efforts should be made to limit its use to clinical conditions for which there are clear therapeutic indications. Policy restrictions on ciprofloxacin use need to be developed and enforced especially in developing countries that are yet to have such policies put in place. Further research is needed to describe ciprofloxacin resistance in hospital-acquired *E. coli* UTI using widely accepted definitions.

4.3 Summary

This is the most comprehensive published systematic review to date that has compared ciprofloxacin resistance rates in hospital- and community-acquired UTI. This systematic review and meta-analysis of observational studies published over a period of eleven years has identified that ciprofloxacin resistance is present in both the hospital and community. Worldwide evidence obtained from this study shows that resistance to this agent is on the increase regardless of country. Given the rising resistance rates to ciprofloxacin, despite recommendations against its use for first-line empiric treatment of UTI, undertaking this systematic review and meta-analysis was timely. The results of this systematic review and

meta-analysis provide justification for limiting the use of this agent, not only in hospital settings where higher resistance was noted, but also in the community where an increasing trend was noted for both developed and developing countries. This published manuscript further emphasises that the issue of AMR is neither country nor region specific. The study findings provide an opportunity to inform policy and practice, especially in relation to antimicrobial prescribing. Continuous surveillance and monitoring of ciprofloxacin resistance rates in all regions of the world is recommended to ensure that this antimicrobial agent, as well as other agents within the fluoroquinolone group, is preserved for important use. This systematic review also highlights the poor quality of AMR data and lack of methodological rigour when conducting AMR studies in various countries. This has implications for development of interventions to control further development and spread of resistance.

The next chapter presents the findings of the second study. The focus of subsequent studies will now be on antimicrobial susceptibility test data obtained from ACT Pathology microbiology laboratory. Study two reports on a time series analyses of five-year antimicrobial resistant urinary *E. coli* prevalence data at the Canberra Hospital.

Chapter 5: Study two – Prevalence of antimicrobial resistance in *E. coli* UTI

5.1 Overview

Escherichia coli has been identified by the WHO as one of the key bacteria demonstrating significant increases in resistance levels to antimicrobials, which is a major concern (World Health Organization, 2015a). Increasing resistance of urinary *E. coli* to antimicrobials has also been demonstrated in a number of studies published worldwide (Bouchillon et al., 2013; Linhares et al., 2013; Maraki et al., 2013), highlighting the importance of continued monitoring of resistance patterns in this bacterium to ensure implementation of strategies to prevent further resistance development and spread. Previously, antimicrobial resistant infections were primarily associated with healthcare settings, but during the last ten years increasing levels of antimicrobial resistant infections have been noted in the community as well (O'Neill, 2016). This emphasises the need to ensure resistance monitoring occurs both in hospital and community settings.

There is published evidence to demonstrate the association between AMR and antimicrobial use (Goossens et al., 2005; Gottesman et al., 2009; López-Lozano et al., 2000). Resistance rates have been correlated with antimicrobial prescribing at the hospital level (Gallini et al., 2010; Vernaz et al., 2011) as well as in community settings (Goossens et al., 2005; Gottesman et al., 2009). To fully comprehend the epidemiology and impact of AMR, evaluating both AMR and antimicrobial use data is important (O'Neill, 2016). The information obtained can inform clinical practice and contribute to development of policies to control further development and spread of AMR, thereby improving patient outcomes (O'Neill, 2016). Evaluating AMR and antimicrobial use can also provide baseline data to monitor the effectiveness of future interventions.

Chapter Five (section 5.2) presents the published manuscript of the second study (Fasugba et al., 2016) undertaken as part of the research program. The supplementary (S1) table cited in the published manuscript is provided in Appendix G of the thesis. In study two, I used data from a microbiological laboratory to describe temporal trends of resistance prevalence and seasonality of antimicrobial resistant urinary *E. coli* isolates over five years, from January 2009 to December 2013, at the Canberra Hospital. Evaluation of the data also involved comparison of the prevalence of resistance in hospital- and community-acquired

urinary *E. coli* infections. Study two was also informed by findings from study one (reported in Chapter Four), which identified methodological issues with the conduct and reporting of AMR studies, one of which was calculation of the prevalence of resistance.

Given the strong evidence in support of the association between antimicrobial use and development of resistance, additional data on antimicrobial use for Canberra Hospital were obtained. However, the analysis of antimicrobial use data was not included in the manuscript along with the resistance data for a number of reasons. First, the antimicrobial use data are ecological and are for all infections, not only UTI. Second, the antimicrobial use data are for all Canberra Hospital inpatients, which are a small subset of the resistance data (80% of which are community-acquired), making the antimicrobial use and AMR datasets non-comparable. Hence, the analysis of supplementary antimicrobial use data is included separately (section 5.3). Section 5.3 extends the study further by undertaking a time series analysis to evaluate trends and seasonal variation in antimicrobial use at the Canberra Hospital over a five-year period.

5.2 Publication two: Five-year antimicrobial resistance patterns of urinary *Escherichia coli* at an Australian tertiary hospital: time series analyses of prevalence data

PLOS ONE

RESEARCH ARTICLE

Five-Year Antimicrobial Resistance Patterns of Urinary *Escherichia coli* at an Australian Tertiary Hospital: Time Series Analyses of Prevalence Data

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5.2.1 Abstract

This study describes the antimicrobial resistance temporal trends and seasonal variation of *Escherichia coli* (*E. coli)* urinary tract infections (UTI) over five years, from 2009 to 2013, and

compares prevalence of resistance in hospital- and community-acquired *E. coli* UTI. A cross sectional study of *E. coli* UTI from patients attending a tertiary referral hospital in Canberra, Australia was undertaken. Time series analysis was performed to illustrate resistance trends. Only the first positive *E. coli* UTI per patient per year was included in the analysis. A total of 15,022 positive cultures from 8724 patients were identified. Results are based on 5333 first *E. coli* UTI, from 4732 patients, of which 84.2% were community-acquired. Five-year hospital and community resistance rates were highest for ampicillin (41.9%) and trimethoprim (20.7%). Resistance was lowest for meropenem (0.0%), nitrofurantoin (2.7%), piperacillin-tazobactam (2.9%) and ciprofloxacin (6.5%). Resistance to amoxycillin-clavulanate, cefazolin, gentamicin and piperacillin-tazobactam were significantly higher in hospital- compared to community-acquired UTI (9.3% versus 6.2%; 15.4% versus 9.7%; 5.2% versus 3.7% and 5.2% versus 2.5%, respectively). Trend analysis showed significant increases in resistance over five years for amoxycillin-clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin, trimethoprim-sulphamethoxazole, cefazolin, ceftriaxone and gentamicin ($P<0.05$, for all) with seasonal pattern observed for trimethoprim resistance (augmented Dickey-Fuller statistic=4.136; $P=0.006$). An association between ciprofloxacin resistance, cefazolin resistance and ceftriaxone resistance with older age was noted. Given the relatively high resistance rates for ampicillin and trimethoprim, these antimicrobials should be reconsidered for empirical treatment of UTI in this patient population. Our findings have important implications for UTI treatment based on setting of acquisition.

5.2.2 Introduction

Urinary tract infections (UTI) are predominantly bacterial infections affecting people both in the community and in hospitals (Laupland et al., 2007). Over 80% are caused by *Escherichia coli (E. coli)*, a Gram-negative bacillus (Nicolle, 2008). Data from the combined National Ambulatory Health Care Surveys in the United States (US) for 2009-2010 showed that UTI accounted for approximately 9.8 million visits to ambulatory care settings such as primary care, outpatient and emergency departments (Centers for Disease Control and Prevention & National Center for Health Statistics, 2015). Visits due to UTI were estimated to be 0.8% of all ambulatory care visits (Centers for Disease Control and Prevention & National Center for Health Statistics, 2015). In Australia, national data on UTI are unavailable but recent estimates from 82 hospitals and 17 aged care facilities reported a point prevalence of 1.4% and 1.5% respectively for healthcare-associated UTI (Mitchell, Fasugba, et al., 2016).

While UTI are a major infection burden globally, the growing problem of antimicrobial resistance (AMR) can result in treatment failures and increased cost of healthcare (Howard, Scott, Packard, & Jones, 2003). There is evidence to show that the AMR pattern of urinary *E. coli* is increasing (Blaettler et al., 2009). In Switzerland, an analysis of urinary *E. coli* specimens obtained from a university hospital from 1997 to 2007 found an increasing trend in resistance to trimethoprim/sulfamethoxazole, ciprofloxacin and amoxycillin/clavulanic acid (from 17.4% to 21.3%, 1.8% to 15.9%, and 9.5% to 14.5%, respectively) (Blaettler et al., 2009). The Australian Group on Antimicrobial Resistance (AGAR) which undertakes AMR prevalence surveys within Australia also noted a gradual rise in overall percentage of *E. coli* strains resistant to beta-lactam antibiotics and ciprofloxacin (Australian Group on Antimicrobial Resistance, 2012). From 2009 to 2011, resistance of hospital-onset *E. coli* isolates to ampicillin and ciprofloxacin increased from 48% to 51% and 8% to 11% respectively (Australian Group on Antimicrobial Resistance, 2012). Furthermore, the resistance rates of urinary *E. coli* to various antimicrobials show large inter-country variability (Karlowsky, Kelly, Thornsberry, Jones, & Sahm, 2002). Only a few studies have shown that *E. coli* resistance rates differ for hospital-acquired and community-acquired UTI (Cullen et al., 2012; Ma & Wang, 2013; Perrin et al., 1999). Measuring and comparing the levels of AMR in both hospital- and community-acquired UTI is essential because although effects of AMR are mainly felt in healthcare facilities, the greatest use of antimicrobials occurs in the community (Coxeter et al., 2013). Comparing resistance rates in hospital- and community-acquired UTI may influence therapeutic recommendations for UTI based on setting of acquisition.

The prevalence of AMR including hospital and community urinary *E. coli* resistance levels is not completely known in Australia. Obtaining this information is important because it not only provides knowledge about the health status of a population, but also contributes to disease management decisions (Buttner & Muller, 2011). This study describes the AMR temporal trends and seasonal variation of *E. coli* UTI over five years at an Australian tertiary hospital. The study also compares the prevalence of resistance between hospital- and community-acquired *E. coli* UTI.

5.2.3 Materials and Methods

Study design and setting

A retrospective cross sectional design was used. The study was conducted with data from ACT Pathology which is based at a tertiary referral hospital, the Canberra Hospital and Health Services. This is Australian Capital Territory's (ACT) main hospital which provides acute and specialist care services to over 600,000 people in the surrounding region. The 600 bed publicly-funded hospital which includes an emergency department and intensive care unit, offers a comprehensive range of health services such as acute inpatient and day services, outpatient services, women's and children's services and pathology services. Solid organ transplant services are not offered in Canberra.

Human research ethics approval was granted by ACT Health Human Research Ethics Committee's Low Risk Sub-Committee and Australian Catholic University Human Research Ethics Committee. Consent from patients was not obtained as a waiver of consent was granted by the ethics committees.

Urine sample and data collection

The microbiology records of inpatients and those attending Canberra Hospital who had urine samples processed at ACT Pathology from January 2009 to December 2013 were retrospectively reviewed. Demographic data and clinical information such as date of birth, gender, admission date, specimen collection date and antimicrobial susceptibility test result were obtained from the microbiology laboratory database and administrative record system.

Bacterial isolation and identification

Urine samples were analysed and processed based on the microbiology laboratory standards (Australian Capital Territory Pathology, 2013). For this study, a culture with presence of $\geq 10^7$ colony forming unit (cfu) per litre of urine was considered positive for UTI based on the laboratory recommendations. This 10^7 cfu/L cut-off is commonly used as it increases the sensitivity of the urine culture test making it a practical threshold (Wilson & Gaido, 2004). The criterion has also been used by several studies reporting on antimicrobial resistance of urinary *E. coli* (Laupland et al., 2007; Linhares et al., 2013; McGregor et al., 2013). Cultures with three or more bacterial species isolated were considered contaminated

and excluded. Only the first positive *E. coli* UTI per patient per year was included in the final analysis.

Definitions

Urine cultures were classified based on the setting of acquisition of infection (hospital-acquired and community-acquired, also known as hospital-onset and community-onset) using criteria from the Centers for Disease Control and Prevention definitions (Horan et al., 2008). Positive *E. coli* urine cultures obtained within the first 48 hours of admission (including cultures from non-admissions such as outpatient clinics) were defined as community-acquired UTI. Positive cultures obtained more than 48 hours after admission and within 48 hours of discharge were defined as hospital-acquired UTI.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed by a disc diffusion method and the automated minimum inhibitory concentration (MIC) method using Vitek2 (Biomerieux Diagnostics). Interpretation was based on Clinical Laboratory Standard Institute (CLSI, formerly NCCLS) criteria (Clinical and Laboratory Standards Institute, 2014). Based on a stepwise laboratory testing protocol used during the study period, all significant *E. coli* (>10^7cfu/L) isolated after overnight incubation on culture had disc susceptibility testing done**.** The antibiotic discs used for these tests were ampicillin (10µg), amoxycillin-clavulanate (augmentin) (30µg), cephalexin/cefazolin (30 µg), trimethoprim (5µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg) and gentamicin (10µg). The isolates which were found to be resistant to at least three of the routinely tested antibiotics were then sent for Vitek2 testing to determine the MICs for ceftriaxone, trimethoprim-sulphamethoxazole, meropenem and piperacillin-tazobactam in addition to the routinely tested antibiotics. Direct susceptibility testing method on urine specimens for *E. coli* has been validated at ACT Pathology and is comparable to the CLSI recommended methods.

The quality control strains used for disc diffusion tests were *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and for Vitek *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213.

Extended spectrum beta lactamase (ESBL) confirmation

Detection of ESBL-producing isolates was performed with combination discs of cefotaxime (30µg), cefotaxime/clavulanic acid (30/10µg), ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10µg) whenever required according to CLSI guidelines (Wilson & Gaido, 2004). Extended spectrum beta lactamase production was inferred when the zone diameter of the disc with clavulanate was ≥5mm larger than the disc without clavulanate for the same antibiotic. *K. pneumoniae* ATCC 700603 was used as the quality control strain.

5.2.4 Data analysis

The overall 5-year and yearly resistance rates of *E. coli* to the routinely tested first-line antimicrobials on over 4,000 isolates (ampicillin, amoxycillin-clavulanate, cephalexin/cefazolin, ciprofloxacin, gentamicin, nalidixic acid, and trimethoprim) were calculated by dividing the number of urinary *E. coli* isolates resistant to each antimicrobial by the number of isolates tested against an individual antimicrobial agent. For the isolates which were sent for further susceptibility testing on Vitek2 against second-line antimicrobials (ceftriaxone, trimethoprim-sulphamethoxazole, meropenem, piperacillin-tazobactam and nitrofurantoin), the denominator used in calculating the resistance rates was the total number of isolates included in the study. This denominator was used based on the assumption that isolates were initially not tested for the Vitek2 antibiotics because they were considered highly unlikely to be resistant to these antibiotics. Hence in order not to overestimate the resistance rates of these isolates the denominator included all isolates tested on both antibiotic discs and Vitek2. The binomial exact 95% confidence intervals (CI) of the resistance percentages were calculated. The 5-year resistance rates were compared for community- and hospital-acquired isolates. The chi-square test was used to check for statistically significant differences in AMR between both groups. Mean differences in age between the two groups were tested using Student's t-tests. A time series analysis was performed separately for all antimicrobials tested to identify patterns in resistance (trends and seasonal variation) over the five-year period. Seasonality is a pattern that shows periodic repetitive fluctuations over time. An autoregressive (AR) model was constructed to assess time-varying resistance patterns (i.e., resistance is non-stationary, or changing, over time) and multiple time series models were fitted to also account for age and sex. The analysis on age and sex followed an ecological study design because these variables were aggregated for each season. The Dickey-Fuller (DF) and the augmented Dickey-Fuller (ADF)

tests were used to assess a unit root in the time series data. Both DF and ADF statistics are negative numbers; the more negative, the stronger the rejection of the null hypothesis (that there is unit root at some level of confidence). These unit root tests investigate whether a time series variable (e.g., resistance) is non-stationary using the AR model (Dickey & Fuller, 1979). Urinary *E. coli* isolates for which the antimicrobial showed an intermediate susceptibility category (amoxycillin-clavulanate, trimethoprim, and ciprofloxacin) were excluded from the final analysis. A significance level of $P < 0.05$ was used. Data were analysed using STATA statistical software (version 13, StataCorp).

5.2.5 Results

A total of 106,512 urine samples from 47,727 patients attending Canberra Hospital from 2009 to 2013 were processed by ACT Pathology. Of these, 14.1% (n=15,022) had positive cultures with *E. coli* being the most common organism isolated in 7670 (51.1%) samples. The distribution of samples by study year is shown in S1 Table.

Of the 7670 *E. coli* cultures, most (7103 isolates) could be further classified as community- or hospital-acquired UTI based on available data. The data were then restricted to the first positive *E. coli* UTI per patient per year of which there were 5346 positive *E. coli* UTI but only 5333 had susceptibility test results. Hence 5333 *E. coli* UTI belonging to 4732 patients in the 5-year period were included in the final analysis. The majority (84.2%, n=4492) of UTI were classified as community-acquired and 15.8% (n=841) as hospital-acquired. The mean age of all patients was 57.0 years (SD=27.5) and patients were mostly female (80.2%, n=3795). There was a significant difference in age between patients with hospital- and community-acquired *E. coli* UTI (mean age 67.2 years versus 55.1 years, $P<0.001$) but no significant differences in gender.

Antimicrobial resistance

All 5333 isolates had routine susceptibility testing performed against first-line antimicrobials and the overall 5-year and stratified (hospital- and community-acquired) AMR rates are summarised in Table 1. Of the 5333 isolates, 1599 (29.9%) were sent for further antimicrobial susceptibility testing for second-line antimicrobials on Vitek2. The overall 5-year resistance rates to these second-line antimicrobials are reported in Table 2.

Table 1 Resistance profile of urinary *E. coli* isolates sent for routine susceptibility testing from 2009 to 2013 by setting

		COMMUNITY			HOSPITAL			TOTAL		
Antibiotic	Year	Number of community isolates tested	R n (%)	95% CI of resistance percentage	Number of hospital isolates tested	R n (%)	95% CI of resistance percentage	Total number of isolates tested*	R n (%)	95% CI of resistance percentage
Ampicillin	2009	835	331 (39.6)	36.3-43.1	143	71 (49.7)	41.2-58.1	978	402 (41.1)	38.0-44.3
	2010	897	358 (39.9)	36.7-43.2	182	70 (38.5)	31.4-45.9	1079	428 (39.7)	36.7-42.7
	2011	1037	443 (42.7)	39.7-45.8	189	91 (48.2)	40.8-55.5	1226	534 (43.6)	40.8-46.4
	2012	939	412 (43.9)	40.7-47.1	173	74 (42.8)	35.3-50.5	1112	486 (43.7)	40.8-46.7
	2013	784	315 (40.2)	36.7-43.7	154	71 (46.1)	38.1-54.3	938	386 (41.2)	38.0-44.4
	Total	4492	1859(41.4)	39.9-42.8	841	377 (44.8)	41.4-48.3	5333	2236(41.9)	40.6-43.3
AMC	2009	785	24 (3.1)	2.0-4.5	133	6 (4.5)	1.7-9.6	918	30 (3.3)	2.2-4.6
	2010	832	49 (5.9)	4.4-7.7	172	11 (6.4)	3.2-11.2	1004	60 (6.0)	4.6-7.6
	2011	981	61 (6.2)	4.8-7.9	170	17 (10.0)	5.9-15.5	1151	78 (6.8)	5.4-8.4
	2012	895	71 (7.9)	6.2-9.8	161	19 (11.8)	7.3-17.8	1055	89 (8.4)	6.8-10.3
	2013	754	58 (7.7)	5.9-9.8	145	23 (15.9)	10.3-22.8	899	81 (9.0)	7.2-11.1
	Total	4247	263 (6.2)	5.5-6.9	781	76 (9.3)	7.7-12.0	5027	338 (6.7)	6.0-7.5
Cefazolin	2009	821	60 (7.3)	5.6-9.3	129	14 (10.9)	6.1-17.5	950	74 (7.8)	6.2-9.7
	2010	885	96 (10.9)	8.9-13.1	179	24 (13.4)	8.8-19.3	1064	120 (11.3)	9.4-13.3
	2011	1019	103 (10.1)	8.3-12.1	178	30 (16.9)	11.7-23.2	1197	133 (11.1)	9.4-13.0
	2012	917	82 (8.9)	7.2-11.0	168	26 (15.5)	10.4-21.8	1085	108 (10.0)	8.2-11.9
	2013	776	89 (11.5)	9.3-13.9	151	30 (19.9)	13.8-27.1	927	119 (12.8)	10.8-15.2
	Total	4418	430 (9.7)	8.9-10.6	805	124 (15.4)	13.0-18.1	5223	554 (10.6)	9.8-11.5

		COMMUNITY			HOSPITAL			TOTAL		
Antibiotic	Year	Number of community isolates tested	R n (%)	95% CI of resistance percentage	Number of hospital isolates tested	R n (%)	95% CI of resistance percentage	Total number of isolates tested*	R n (%)	95% CI of resistance percentage
Trimethoprim	2009	830	153 (18.4)	15.9-21.2	143	28 (19.6)	13.4-27.0	973	181 (18.6)	16.2-21.2
	2010	897	172 (19.2)	16.6-21.9	181	33 (18.2)	12.9-24.6	1078	205 (19.0)	16.7-21.5
	2011	1036	217 (20.9)	18.5-23.6	189	42 (22.2)	16.5-28.8	1225	259 (21.1)	18.9-23.5
	2012	939	200 (21.3)	18.7-24.1	173	40 (23.1)	17.1-30.1	1112	240 (21.6)	19.2-24.1
	2013	784	181 (23.1)	20.2-26.2	154	36 (23.4)	16.9-30.9	938	217 (23.1)	20.5-26.0
	Total	4486	923 (20.6)	19.4-21.8	840	179 (21.3)	18.6-24.2	5326	1102(20.7)	19.6-21.8
Nalidixic acid	2009	826	63 (7.6)	5.9-9.7	143	12 (8.4)	4.4-14.2	969	75 (7.7)	6.1-9.6
	2010	892	73 (8.2)	6.5-10.2	182	12 (6.6)	3.5-11.2	1074	85 (7.9)	6.4-9.7
	2011	1034	109 (10.5)	8.7-12.6	188	22 (11.7)	7.5-17.2	1222	131 (10.7)	9.0-12.6
	2012	755	56 (7.4)	5.7-9.5	140	17 (12.1)	7.2-18.7	895	73 (8.2)	6.4-10.1
	2013	585	33 (5.6)	3.9-7.8	103	11 (10.7)	5.5-18.3	688	44 (6.4)	4.7-8.5
	Total	4092	334 (8.2)	7.3-9.0	756	74 (9.8)	7.8-12.1	4848	408 (8.4)	7.6-9.2
Ciprofloxacin	2009	808	33 (4.1)	2.8-5.7	139	7 (5.0)	2.0-10.1	947	40 (4.2)	3.0-5.7
	2010	701	35 (5.0)	3.5-6.9	150	4 (2.7)	0.7-6.7	851	39 (4.6)	3.3-6.2
	2011	795	52 (6.5)	4.9-8.5	156	10 (6.4)	3.1-11.5	951	62 (6.5)	5.0-8.3
	2012	749	56 (7.5)	5.7-9.6	143	11 (7.7)	3.9-13.3	892	67 (7.5)	5.9-9.4
	2013	631	60 (9.5)	7.3-12.1	135	17 (12.6)	7.5-19.4	766	77 (10.1)	8.0-12.4
	Total	3684	236 (6.4)	5.6-7.2	723	49 (6.8)	5.1-8.9	4407	285 (6.5)	5.8-7.2

		COMMUNITY			HOSPITAL			TOTAL		
Antibiotic	Year	Number of community isolates tested	R n (%)	95% CI of resistance percentage	Number of hospital isolates tested	R n (%)	95% CI of resistance percentage	Total number of isolates tested*	R n (%)	95% CI of resistance percentage
Gentamicin	2009	514	17 (3.3)	1.9-5.2	85	5 (5.9)	1.9-13.2	599	22 (3.7)	2.3-5.5
	2010	893	23 (2.6)	1.6-3.8	182	2 (1.1)	0.1-3.9	1075	25 (2.3)	1.5-3.4
	2011	1036	38 (3.7)	2.6-5.0	189	12 (6.4)	3.3-10.8	1225	50 (4.1)	3.0-5.3
	2012	931	40 (4.3)	3.1-5.8	172	12 (7.0)	3.7-11.9	1102	52 (4.7)	3.5-6.1
	2013	783	36 (4.6)	3.2-6.3	154	10 (6.5)	3.2-11.6	937	46 (4.9)	3.6-6.5
	Total	4157	154 (3.7)	3.2-4.3	782	41 (5.2)	3.8-7.0	4938	195 (3.9)	3.4-4.5

*Note that not all 5333 isolates were tested against each antimicrobial. Isolates not tested: AMC=3; Cefazolin=110; Trimethoprim=3; Nalidixic acid=485; Ciprofloxacin=893; Gentamicin=395

Number of isolates with intermediate susceptibility to an antimicrobial: AMC=303; Trimethoprim=4; Ciprofloxacin=33

R=Resistant

n= Number of isolates

AMC=Amoxycillin-clavulanate; TMP-SMX=Trimethoprim-sulphamethoxazole

Table 2 Resistance profile of urinary *E. coli* isolates sent for further testing on Vitek2 from 2009 to 2013 by setting

Year	Setting	N	Antibiotic									
			Ceftriaxone		TMP-SMX		MER		PIT		NIT	
			R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage
2009	CA	835	12 (1.4)	0.7-2.5	58 (6.9)	5.3-8.9	0 (0.0)	-	2 (0.2)	0.0-0.9	14 (1.7)	0.9-2.8
	HA	143	2 (1.4)	0.2-5.0	12 (8.4)	4.4-14.2	0 (0.0)	-	0 (0.0)	-	4 (2.8)	0.8-7.0
	Total	978	14 (1.4)	0.8-2.4	70 (7.2)	5.6-9.0	0 (0.0)	-	2 (0.2)	0.0-0.7	18 (1.8)	1.1-2.9
2010	CA	897	22 (2.5)	1.5-3.7	76 (8.5)	6.7-10.5	0 (0.0)	-	27 (3.0)	2.0-4.3	15 (1.7)	0.9-2.7
	HA	182	4 (2.2)	0.6-5.5	11 (6.0)	3.1-10.6	0 (0.0)	-	4 (2.2)	0.6-5.5	5 (2.7)	0.9-6.3
	Total	1079	26 (2.4)	1.6-3.5	87 (8.1)	6.5-9.9	0 (0.0)	-	31 (2.9)	2.0-4.1	20 (1.9)	1.1-2.8
2011	CA	1037	46 (4.4)	3.3-5.9	99 (9.5)	7.8-11.5	0 (0.0)	-	19 (1.8)	1.1-2.8	17 (1.6)	1.0-2.6
	HA	189	13 (6.9)	3.7-11.5	30 (15.9)	11.0-21.9	0 (0.0)	-	9 (4.8)	2.2-8.8	3 (1.6)	0.3-4.6
	Total	1226	59 (4.8)	3.7-6.2	129 (10.5)	8.9-12.4	0 (0.0)	-	28 (2.3)	1.5-3.3	20 (1.6)	1.0-2.5
2012	CA	939	43 (4.6)	3.3-6.1	102 (10.9)	8.9-13.0	1 (0.1)	0.0-0.6	33 (3.5)	2.4-4.9	35 (3.7)	2.6-5.1
	HA	173	13 (7.5)	4.1-12.5	22 (12.7)	8.1-18.6	0 (0.0)	-	15 (8.7)	4.9-13.9	5 (2.9)	0.9-6.6
	Total	1112	56 (5.0)	3.8-6.5	124 (11.1)	9.4-13.1	1 (0.1)	0.0-0.5	48 (4.3)	3.2-5.7	40 (3.6)	2.6-4.9
2013	CA	784	45 (5.7)	4.2-7.6	87 (11.1)	9.0-13.5	0 (0.0)	-	30 (3.8)	2.6-5.4	42 (5.4)	3.9-7.2
	HA	154	15 (9.7)	5.6-15.6	25 (16.2)	10.8-23.0	0 (0.0)	-	16 (10.4)	6.1-16.3	4 (2.6)	0.7-6.5
	Total	938	60 (6.4)	4.9-8.2	112 (11.9)	9.9-14.2	0 (0.0)	-	46 (4.9)	3.6-6.5	46 (4.9)	3.6-6.5
Total	CA	4492	168 (3.7)	3.2-4.3	422 (9.4)	8.6-10.3	1 (0.0)	0.0-0.1	111 (2.5)	2.0-3.0	123 (2.7)	2.3-3.3
	HA	841	47 (5.6)	4.1-7.4	100 (11.9)	9.8-14.3	0 (0.0)	-	44 (5.2)	3.8-7.0	21 (2.5)	1.6-3.8
	Total	5333	215 (4.0)	3.5-4.6	522 (9.8)	9.0-10.6	1 (0.0)	0.0-0.1	155 (2.9)	2.5-3.4	144 (2.7)	2.3-3.2

R=Resistant

N= Number of isolates tested

CA=Community isolates; HA=Hospital isolates

TMP-SMX=Trimethoprim-sulphamethoxazole; MER=Meropenem; PIT=Piperacillin-tazobactam; NIT=Nitrofurantoin

The highest overall 5-year resistance rates to urinary *E. coli* for both hospital and community isolates combined were seen for ampicillin (41.9%; 95% CI=40.6-43.3) and trimethoprim (20.7%; 95% CI=19.6-21.8). The lowest resistance rates were for meropenem (0.0%), nitrofurantoin (2.7%; 95% CI=2.3-3.2) and piperacillin-tazobactam (2.9%; 95% CI=2.5-3.4). Resistance to amoxycillin-clavulanate, cephalexin/cefazolin, gentamicin and piperacillin-tazobactam was significantly higher in hospital- compared to community-acquired UTI (*P*<0.001, *P*<0.001, *P*=0.043 and *P*=0.002, respectively). For ampicillin, trimethoprim, nalidixic acid, ciprofloxacin, ceftriaxone and trimethoprim-sulphamethoxazole, resistance rates were also higher for hospital- compared with community-acquired UTI but this did not reach statistical significance (Fig 1).

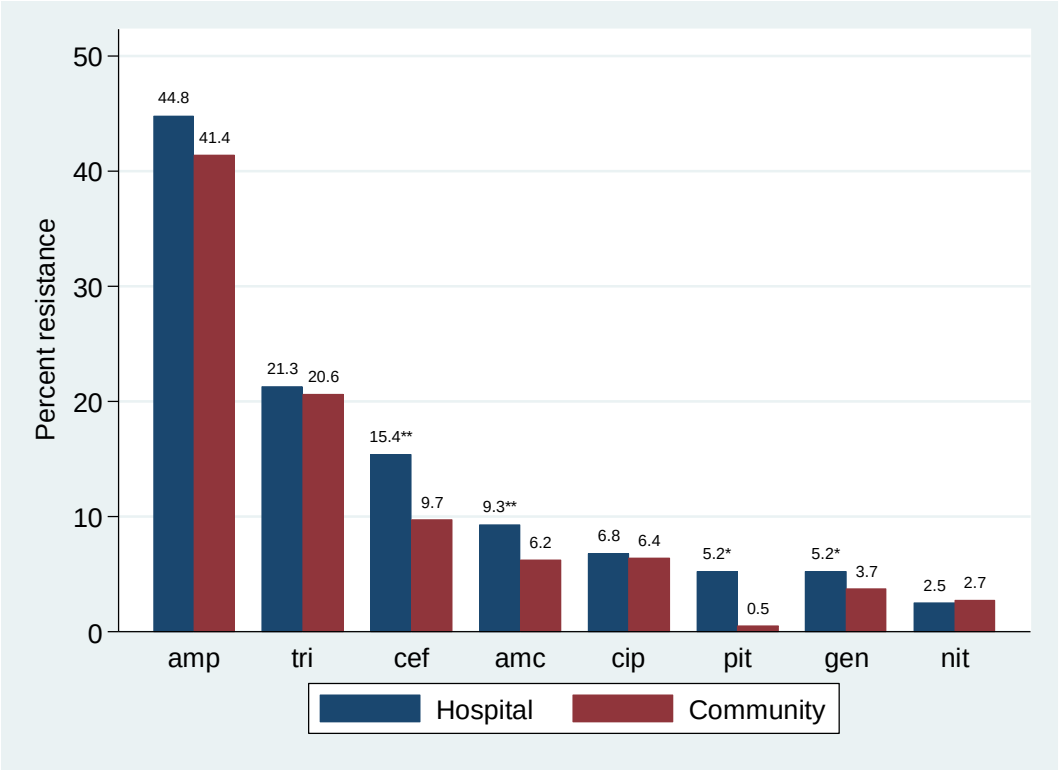

Figure 1 Five-year resistance rates of hospital- and community-acquired *E. coli* UTIs by selected antibiotics

amp=ampicillin; tri=trimethoprim; cef=cefazolin; amc=amoxycillin-clavulanate; cip=ciprofloxacin; pit=piperacillin-tazobactam; gen=gentamicin; nit=nitrofurantoin
** 0.001 < *P* value < 0.05
** *P* < 0.001

Trend analysis showed a significant increase in resistance to amoxycillin-clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin, trimethoprim-sulphamethoxazole, cefazolin,

ceftriaxone and gentamicin over the five-year period (Fig 2). There was no significant increase in resistance for ampicillin, nalidixic acid, meropenem and piperacillin-tazobactam. A seasonal pattern was only observed for trimethoprim (ADF statistic=-4.136; *P*=0.006) with higher resistance rates for this antimicrobial seen in the summer months. Regression analysis indicated an association between increasing age and resistance to ciprofloxacin (regression coefficient=0.01; *P*=0.004), cefazolin (regression coefficient=0.004; *P*=0.038) and ceftriaxone (regression coefficient=0.01; *P*=0.002).

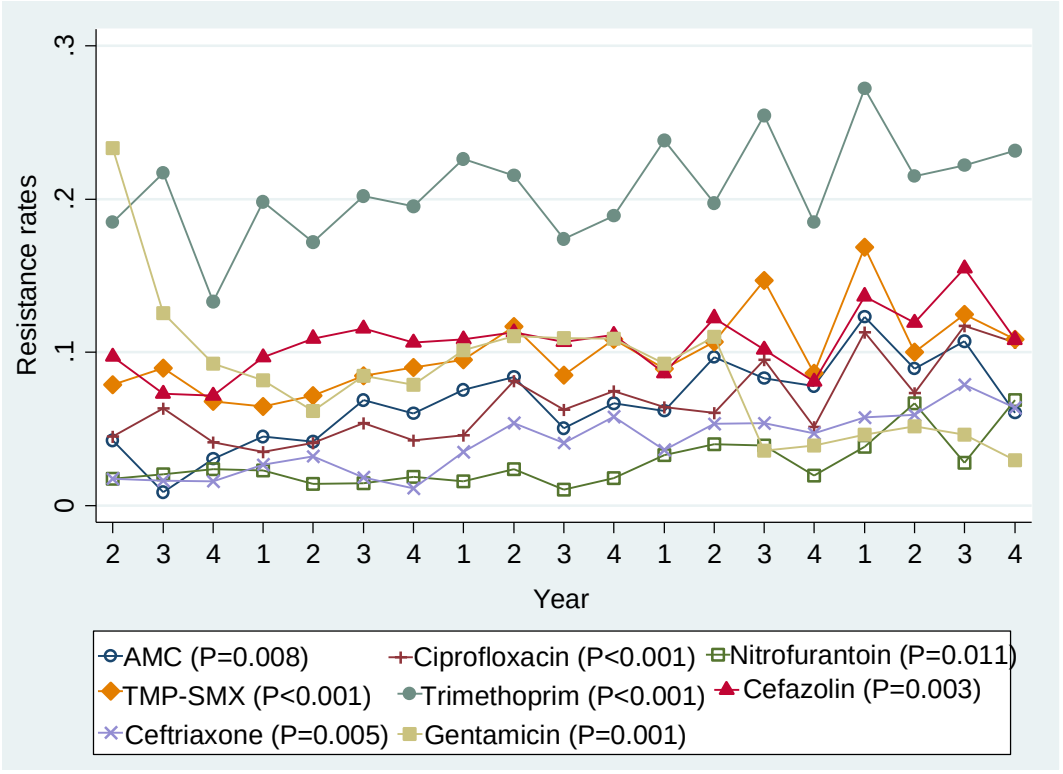

Figure 2 Seasonal antimicrobial resistance rates for *E. coli* UTIs

1=Summer; 2=Autumn; 3=Winter; 4=Spring
P=significance level for an increasing trend
AMC=Amoxycillin-clavulanate; TMP-SMX=Trimethoprim-sulphamethoxazole

ESBL production

Overall 5-year prevalence of ESBL-producing *E. coli* isolates was 1.9% (95% CI=1.5-2.3; n=100). Extended spectrum beta-lactamase production was low by international standards but was significantly higher in hospital-acquired (3.0%; 95% CI=1.9-4.4; n=25) compared with community-acquired UTI (1.7%; 95% CI=1.3-2.1; n=75, P=0.01). The levels of ESBL-producing *E. coli* increased from 0.7% (95% CI=0.0-3.8) in hospital-acquired UTI in 2009 to 6.5% (95% CI=3.2-11.6) in 2013. An increase was also noted for community-acquired UTI

(0.6%; 95% CI=0.2-1.4 in 2009 to 3.7%; 95% CI=2.5-5.3 in 2013). The increasing trend in ESBL production over the five years was statistically significant for both hospital (*P*=0.035) and community-acquired UTI (*P*<0.001).

5.2.6 Discussion

This study provides information about the AMR pattern of *E. coli* UTI in an Australian tertiary hospital. To our knowledge this is the first Australian study to compare AMR in hospital- and community-acquired *E. coli* UTI and assess AMR temporal trends and seasonal variation of *E. coli* UTI over time. Our results showed that overall resistance was highest for ampicillin and trimethoprim. We also found significantly higher resistance rates in hospital- compared to community-acquired UTI for amoxycillin-clavulanate, cephalexin/cefazolin, gentamicin and piperacillin-tazobactam with an increasing resistance trend for eight of the twelve antimicrobials tested which include the four commonly used antimicrobials for first-line treatment of UTI in Australia.

In Australia, trimethoprim, cephalexin, amoxycillin-clavulanate or nitrofurantoin are recommended for first-line treatment of UTI (Antibiotic Expert Groups, 2014). The Infectious Diseases Society of America (IDSA) and European Society for Microbiology and Infectious Diseases recommend trimethoprim-sulphamethoxazole as an appropriate treatment choice if local resistance rates do not exceed 20%. The IDSA guidelines also recommend that amoxycillin or ampicillin should not be used alone for empirical treatment because of the relatively poor efficacy and the relatively high prevalence of AMR to these agents worldwide (Gupta et al., 2011). Given the high levels of resistance to ampicillin and trimethoprim identified in this study, the appropriateness of these antimicrobials in the management of UTI in this patient population should be assessed. The IDSA suggests that beta-lactam agents, including amoxycillin-clavulanate are appropriate choices for therapy when other recommended agents cannot be used (Gupta et al., 2011). Based on our findings, the majority of UTIs have very low resistance to amoxycillin-clavulanate and nitrofurantoin which are commonly used for UTI treatment in Canberra. Ciprofloxacin, which is recommended in Australia for complicated UTI, was also found to have a low resistance rate. Through the national pharmaceutical subsidy scheme, the use of quinolones in humans has been restricted in Australia. Quinolone use in food-producing animals is also not permitted. Therefore, fluoroquinolone resistance in the community has been slow to

emerge and has remained at low levels in important pathogens such as *E. coli* compared to most countries (Cheng et al., 2012). Our overall resistance rates are also generally lower than reported for other single site studies (Cullen et al., 2012; Wang et al., 2014), demonstrating that resistance may vary geographically, as shown in a recent meta-analysis (Fasugba et al., 2015). The explanation for the varying resistance rates is not clearly understood but possible reasons have been postulated. A study conducted in the US demonstrated a geographic gradient in resistance with the highest resistance rates noted in the Pacific region and lowest rates in the South Atlantic region (Sannes, Kuskowski, & Johnson, 2004). It was suggested that geographic clustering of resistance phenotypes may have accounted for the geographic differences in resistance. It is therefore possible that the lower rates we found in comparison to those reported for other single site studies may be due to lower levels of bacteria with resistance phenotypes in our locality. Another possible suggestion for geographic variation in resistance is the differences in antimicrobial use (Gupta et al., 2001; Sannes et al., 2004). Several studies have demonstrated an association between antimicrobial use and resistance (Bergman et al., 2009; Goossens et al., 2005; Vellinga et al., 2010). Hence it is probable that the lower resistance noted may be as a result of lower antimicrobial use resulting in lower antimicrobial selection pressure. This emphasises the need for continued local monitoring of resistance patterns to ensure appropriate treatment for people in the locality.

The sample size of the data was able to detect some significant differences between community- and hospital-acquired UTI resistance rates but for some antimicrobials the differences observed could not be confirmed statistically, possibly due to an insufficient sample size. Overall, we found lower rates of antibiotic resistance for community- compared with hospital-acquired *E. coli* UTI, consistent with other studies (Bouchillon et al., 2013; Cullen et al., 2012). The difference in resistance rates is however only small and supports the view that *E. coli,* a bacterium carried in the bowel and acquired in the community, is brought into hospital usually by patients themselves rather than being hospital-acquired. This finding may also have been partially dictated by our methodology from using the first positive UTI per person per year. The different resistance rates for hospital- and community-acquired urinary *E. coli* isolates seen in this study are comparable with findings reported previously (Ma & Wang, 2013; Perrin et al., 1999). Similar results have been seen in blood culture isolates of *E. coli* in Canberra (Kennedy et al., 2008). While the difference in resistance rates

was not large and most antimicrobial use occurs in the community, the proportion of patients receiving antimicrobials is much higher in the hospital and hence explains the difference seen (Zarb et al., 2012). We agree with recommendations that to accurately represent *E. coli* resistance rates, antibiograms should be stratified by setting of infection onset (Swami et al., 2012).

The increasing resistance trend noted in our study for the eight antimicrobials is consistent with previously reported Australian data and published studies from other developed countries (Australian Group on Antimicrobial Resistance, 2011; Blaettler et al., 2009; Cullen et al., 2012; Hsu et al., 2010; Wong et al., 2014). The increasing trend may be attributable to antimicrobial overuse or misuse which is a known risk factor for the development of AMR (Costelloe et al., 2010). However, clinical data on hospital antimicrobial use at the study location showed stable rates for most antimicrobials tested (data not shown). We also found seasonal increases in trimethoprim resistance especially in summer months. The literature suggests a possible seasonality with UTI incidence (Freeman, Anderson, & Sexton, 2009; Perencevich et al., 2008) but this was not demonstrated in our study. It is possible that seasonality in UTI may lead to seasonal variation in antimicrobial use with subsequent seasonal resistance patterns although to our knowledge, this is yet to be demonstrated in published studies. Evidence currently exists to show higher use of antimicrobials in winter months which is likely related to the increased incidence of respiratory tract infections during that period with consequent increases in resistance during winter (Dagan et al., 2008; Sun, Klein, & Laxminarayan, 2012). Therefore the seasonal trimethoprim resistance is a potentially important finding which should be explored in future studies especially in relation to antimicrobial use. The ecological analysis conducted in this study showed an association between older age and resistance to ciprofloxacin, cefazolin and ceftriaxone consistent with published studies (Adam et al., 2013; Blaettler et al., 2009; Swami et al., 2012). The association between increasing age and increased resistance is not surprising given that the physiological changes caused by aging and increased comorbidities predispose to a higher risk of infection leading to more contact with healthcare settings and hence more frequent exposure to antibiotics (Adam et al., 2013).

It is worth emphasising that our overall ESBL rate of 1.9% was low compared to most other published studies (Bouchillon et al., 2013). Results from the 2009-2011 SMART study in the

US reported an ESBL rate of 6.8% for *E. coli* UTI (Bouchillon et al., 2013). Although our reported ESBL rate is relatively low, the presence and increasing trend of ESBL-producing *E. coli* in both hospital- and community-acquired UTI pose considerable public health concern. This is because this organism renders many of the conventional empirical treatment options for UTI ineffective especially in community-acquired UTI where options for oral antibiotic therapy appear to be limited (Falagas & Karageorgopoulos, 2009). For hospital-acquired UTI caused by ESBL-producing *E. coli*, carbapenems are considered the treatment of choice (Falagas & Karageorgopoulos, 2009). In our study, the lowest resistance rate reported was for meropenem, a carbapenem.

This study has some limitations. As most UTIs are treated empirically, it is possible that samples submitted to the laboratory included patients with recurrent UTIs and asymptomatic bacteriuria thereby overestimating the resistance rates. In addition, inclusion of the first positive *E. coli* UTI per person per year may have underestimated the resistance rates reported in our study. Evidence suggests that analysis of antimicrobial resistance data should include each individual positive isolate in order to ensure sensitivity, but this positive isolate should only be included once to guarantee specificity (Cornaglia et al., 2004). This approach of using only the first positive isolate per patient per year is also consistent with published studies on resistance in UTI pathogens including *E. coli* (McGregor et al., 2013; Swami et al., 2012). It is unlikely that repeated isolates are correlated but there is a small possibility that this could occur although it was not accounted for in the analysis. The 5-year period prevalence study could therefore have overestimated the resistance. The use of routinely collected microbiology data also posed some limitations as clinical information on patients including comorbidities and presence of indwelling urethral catheters was often missing. The incompleteness of this information prevented its inclusion in the analysis. This study was based on retrospective antimicrobial susceptibility data from a National Association of Testing Authorities, Australia (NATA) accredited clinical microbiology laboratory. The stepwise laboratory testing protocol involved routine first-line antibiotic sensitivity testing followed by more extensive testing with second-line antibiotics only for isolates resistant to at least three of the routine antibiotics. Although this laboratory approach is widely used (Cornaglia et al., 2004) there is the potential for testing bias and/or selection bias with consequent overestimation of resistance rates. Given the lack of consensus on an appropriate denominator using this testing approach and to prevent

possible overestimation of the resistance rates against second-line antibiotics, the denominator therefore included all isolates tested, which, in turn, may have under-estimated resistance rates of broad spectrum antimicrobials. Determining the resistance rate can be influenced by the extent of laboratory testing which in turn influences the selection of the denominator. Using the total number of isolates tested or the number of isolates tested against second-line antibiotics alone as the denominator will either underestimate or overestimate the resistance rates respectively. Although using all isolates for calculating resistance rates for second-line antibiotics has its limitations, this was an appropriate denominator choice to make the findings relevant for use in the clinical setting. For ideal comparison of susceptibility patterns, all isolates would need to be tested against the extended panel of antibiotics in a properly designed prospective study. Regardless of these limitations, our reported resistance rates are low compared to other studies. The use of ecological data to account for the effects of age and sex on resistance also poses limitations to interpretation of these results at the individual patient level. Although our data are from a single tertiary hospital and may not be generalisable to other populations, the data were reported by a NATA accredited laboratory and are therefore satisfactory to provide recommendations to guide local empirical therapy.

5.2.7 Conclusion

Antimicrobial resistance poses grave concerns for antimicrobial effectiveness in treating infections such as UTI. This study demonstrates the increasing resistance of urinary *E. coli* to commonly prescribed antimicrobials. Amoxycillin-clavulanate and nitrofurantoin are still effective for empirical treatment of UTI in this population. Overuse of ampicillin and trimethoprim should be avoided given the high resistance rates reported. In developing local antimicrobial prescribing guidelines, the choice of antimicrobial in the treatment of UTI should be based on setting (community or hospital) of acquisition.

5.3 Analysis of supplementary antimicrobial use data at the Canberra Hospital

Increasing use of antimicrobials in hospitals has been noted in a number of countries (de With et al., 2004; Janknegt, Lashof, Gould, & Van der Meer, 2000; Müller-Pebody et al., 2004). Compared with Denmark, Canada, Sweden, Norway and the Netherlands, antimicrobial use in Australian hospitals is higher (Australian Commission on Safety and Quality in Health Care, 2016). In 2014, 24.3% of antimicrobial prescriptions in Australian

hospitals were said to be non-compliant with the therapeutic guidelines (Australian Commission on Safety and Quality in Health Care, 2016). Despite the high usage of antimicrobials in Australia, to my knowledge there are no published studies investigating antimicrobial use patterns and trends in Australian hospitals, although a limited number of studies have investigated changes in antimicrobial use in the community (Meumann et al., 2015).

Regular monitoring of antimicrobial use data can inform the development of antimicrobial stewardship programs (McNeil et al., 2010). Antimicrobial stewardship is described as a combined set of strategies aimed at improving the appropriateness and unintended effects of antimicrobial use, such as AMR, toxic effects and costs (McKenzie et al., 2013; Schuts et al., 2016). A cross sectional survey of acute care hospitals in the US in 2011 found that 64% of hospitals surveyed had an antimicrobial stewardship policy (Pogorzelska-Maziarz et al., 2015). Hospital antimicrobial stewardship programs have been shown to decrease inappropriate antimicrobial use. Implementation of an antimicrobial stewardship program at a tertiary hospital showed a decrease in broad spectrum antimicrobial use of 17% and 10% in intensive care and non-intensive care units, respectively, following implementation of the program (Cairns et al., 2013). In addition, findings from a recent systematic review showed that antimicrobial stewardship had beneficial effects on patients' clinical outcomes, such as decreased mortality and hospital AMR (Schuts et al., 2016). Although policies restricting antimicrobial use have been in existence in some hospitals for years, it was not until the last five to ten years that formal antimicrobial stewardship programs were developed (Cairns, Roberts, Cotta, & Cheng, 2015). Guidelines for implementing antimicrobial stewardship in hospitals in Australia were published in 2011 (Duguid & Cruickshank, 2011) and antimicrobial stewardship is now one of the mandatory requirements of the National Safety and Quality Health Service Standards (Standard 3.14), that all hospitals must meet for accreditation (Australian Commission on Safety and Quality in Health Care, 2012).

Data on antimicrobial usage for inpatients in acute care settings in Australia is managed by NAUSP (SA Health, 2016). The data are expressed in the internationally recognised defined daily dose (DDD) per 1000 occupied bed days (OBD), as recommended by the WHO (SA Health, 2016). As described previously in the literature review chapter (Chapter Two), the DDD is defined as "the assumed average maintenance dose per day for a drug used for its

main indication in adults" (WHO Collaborating Centre for Drug Statistics Methodology, 2015, p. 22). The OBD is the total number of bed days of all patients admitted during a specific month with the exclusion of those admitted and discharged on the same day (National Antimicrobial Utilisation Surveillance Program, 2015). Using the DDD/1000 OBD as the measure of antimicrobial use facilitates benchmarking between Australian hospitals and jurisdictions as well as other countries (Australian Commission on Safety and Quality in Health Care, 2016). The data submitted to NAUSP by participating hospitals are aggregated as most hospitals in Australia do not have the systems in place to provide patient-level data (McNeil et al., 2010).

The NAUSP provides bimonthly and annual reports on antimicrobial usage to contributing hospitals (SA Health, 2016). Hospitals use the data to compare their antimicrobial use to other hospitals and to their previous reports, but there is no evidence of whether any suspected increase or decrease in usage noted is statistically significant. Statistical significance does not necessarily imply clinical significance (LeFort, 1993), especially in relation to antimicrobial use, which is recognised as the main factor contributing to antimicrobial resistance (López-Lozano et al., 2000), but it is still important to demonstrate whether or not any increase or decrease in antimicrobial usage is a true difference or likely to be due to chance alone. Hence the analysis of these data as part of the research program aims to evaluate trends and seasonal variation in antimicrobial use at the Canberra Hospital over a five-year period. The results of this analysis have the potential to identify specific antimicrobials that require targeted interventions to minimise overuse and also improve antimicrobial prescribing at the hospital.

5.3.1 Methodological approach to calculating antimicrobial use trend

Monthly data on antimicrobial use for the Canberra Hospital for the five-year study period (January 2009 to December 2013) were retrospectively obtained from NAUSP. An amendment was submitted to the ACT Health Human Research Ethics Committee to obtain these data from NAUSP, with additional approval received from the Executive Director Performance Information Branch, ACT Health (See Appendices F.3 and F.4 respectively). The antimicrobial agents assessed for this analysis were: ampicillin; amoxycillin-clavulanic acid; cefazolin; ceftriaxone; ciprofloxacin; gentamicin; meropenem; nitrofurantoin; piperacillin-tazobactam; trimethoprim, and trimethoprim-sulphamethoxazole. These were 11 of the 12

antimicrobials included in the analysis for resistance prevalence reported in section 5.2. Antimicrobial use data on the 12th antimicrobial, nalidixic acid, was unavailable. The antimicrobial usage rates were calculated in DDD per 1000 OBD, using the total number of DDD for each drug consumed as the numerator and the number of OBD as the denominator.

Changes in antimicrobial use over time or trend were undertaken for the 11 antimicrobials. A time series analysis was performed separately for each antimicrobial to identify trends and seasonal variation in antimicrobial use over the five-year period. Time series analysis is a statistical analysis technique applied to observations with a time-ordered sequence (Wei, 2013). The methodological concepts of the time series analysis have been previously described in the methods chapter in section 3.4.5. The trend analysis followed an ecological study design because antimicrobial use data were aggregated at the hospital level and not individual patient data. A significance level of $P < 0.05$ was used. Data were analysed using STATA statistical software (version 13, StataCorp).

5.3.2 Results of analysis of antimicrobial use data

For the overall five-year period, antimicrobial usage at the Canberra Hospital was highest for amoxicillin-clavulanate, cefazolin and ampicillin, and lowest for trimethoprim and nitrofurantoin. Overall antimicrobial use decreased by 10.7%, from 409.3 DDD/1000 OBD in 2009 to 365.4 DDD/1000 OBD in 2013. Total antimicrobial use peaked in 2010 to 418.0 DDD/1000 OBD. Ampicillin, gentamicin and ciprofloxacin demonstrated a gradual decrease in the antimicrobial usage rates over the five-year period, while broad spectrum antibiotics, piperacillin-tazobactam and meropenem, demonstrated a gradual increase in usage rates over the same period. Table 3 provides details of the overall five-year and yearly antimicrobial usage at the Canberra Hospital for each of the antimicrobials included in the analysis. These rates were standardised for bed days for all years.

Table 3 Overall 5-year and yearly antimicrobial use at the Canberra Hospital*

Antimicrobial agent	2009	2010	2011	2012	2013	Overall 5-year
AMC	75.8	88.3	86.7	82.8	76.6	82.0
Cefazolin	81.3	80.1	81.2	77.3	81.9	80.3
Ampicillin	68.3	65.4	59.0	43.4	38.8	54.3
Gentamicin	52.5	48.6	43.7	32.7	20.7	39.0
Ciprofloxacin	44.0	38.6	34.0	33.0	24.1	34.4
Ceftriaxone	35.1	32.8	36.7	34.6	29.4	33.6
PIT	8.6	14.8	26.1	31.5	39.5	24.7
TMP-SMX	16.7	22.0	14.7	24.2	23.9	20.5
Meropenem	16.0	17.0	19.2	19.5	19.3	18.3
Trimethoprim	9.0	8.4	9.9	8.9	9.6	9.2
Nitrofurantoin	2.0	2.0	1.4	1.6	1.6	1.7
Total	409.3	418.0	412.6	389.5	365.4	398.0

*Expressed in defined daily dose/1000 occupied bed days
AMC=Amoxicillin-clavulanate; TMP-SMX=Trimethoprim-sulphamethoxazole;
PIT=Piperacillin-tazobactam

Trend analysis of antimicrobial use

Trend analysis showed that antimicrobial use was relatively stable for amoxicillin-clavulanate, cefazolin, trimethoprim-sulphamethoxazole, trimethoprim and nitrofurantoin, with no significant change over the five-year period. Ampicillin, gentamicin and ciprofloxacin showed decreasing trends, although these did not reach statistical significance (Figure 3). Trend analysis for piperacillin-tazobactam and meropenem demonstrated gradual increase in their use, but significance was only found for meropenem. Seasonal patterns (periodic repetitive fluctuations over time) of antimicrobial usage were observed for ceftriaxone, predominantly during the winter months.

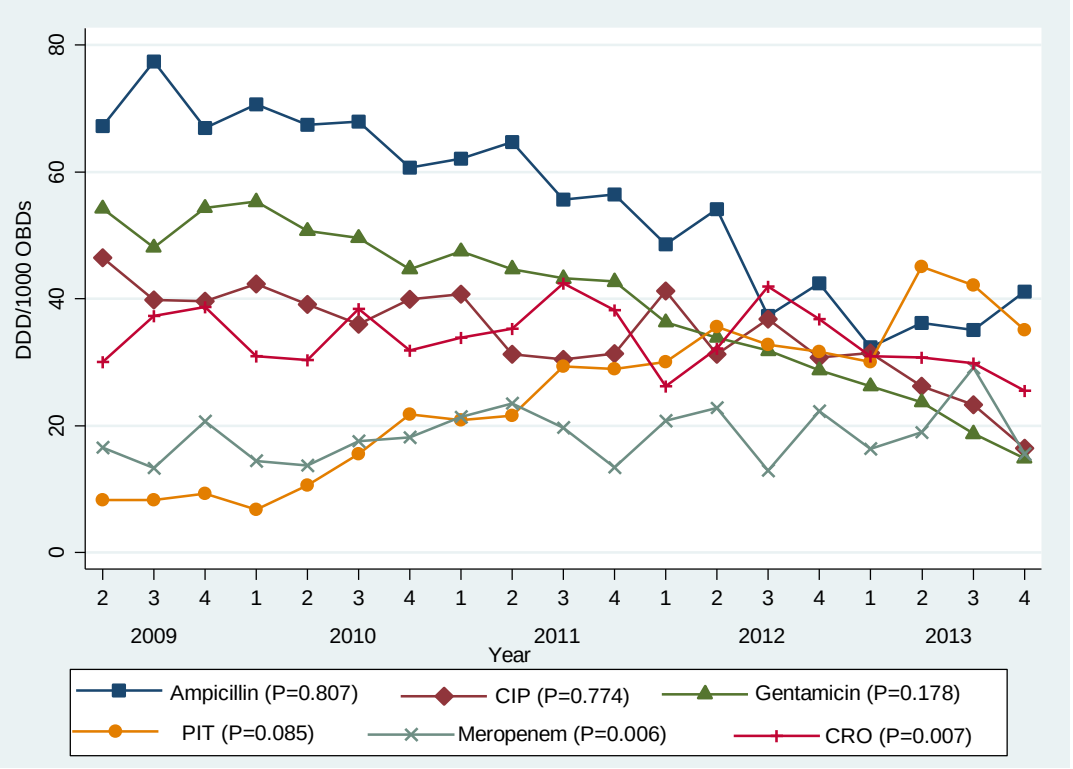

Figure 3 Trend of antimicrobial use for Canberra Hospital from 2009 to 2013

1=Summer; 2=Autumn; 3=Winter; 4=Spring
AMC=Amoxycillin-clavulanate; TMP-SMX=Trimethoprim-sulphamethoxazole

5.3.3 Discussion

The finding of a decrease in overall antimicrobial use from 2009 to 2013 is consistent with the latest national report on antimicrobial use and resistance in acute care hospitals (Australian Commission on Safety and Quality in Health Care, 2016). When antimicrobial use data from 2005 to 2014 were evaluated, total antimicrobial use in Australian hospitals peaked in 2010 and declined gradually thereafter (Australian Commission on Safety and Quality in Health Care, 2016). One possible explanation for the decrease in overall antimicrobial use is the implementation of antimicrobial stewardship initiatives, as demonstrated in other studies (Kaki et al., 2011; Schuts et al., 2016), but it is not possible to say whether this accounted for the findings observed at the Canberra Hospital. The infectious diseases and microbiology departments provided clinical services to the hospital in regards to control of antimicrobial use prior to the implementation of formal antimicrobial stewardship initiatives at the hospital in 2013 (A. Das, personal communication, 26 August, 2016). Despite demonstrating a decrease in overall quantity of antimicrobial use at the hospital over the study period, effective monitoring of antimicrobial

use also requires data on the quality or appropriateness of antimicrobial prescribing (Duguid & Cruickshank, 2011), which was not possible in this study. Data on appropriateness of antimicrobial use would provide evidence to show that any reported decrease in antimicrobial use is associated with effective prescribing in accordance with prescribing guidelines (Duguid & Cruickshank, 2011). These guidelines are developed based on local resistance patterns (Antibiotic Expert Groups, 2014). Hence, to enable adequate interpretation of the decrease in overall antimicrobial use at the hospital, data on the appropriateness of antimicrobial use must also be evaluated.

The decreasing trend for antimicrobial use was evident for ampicillin, gentamicin and ciprofloxacin, although statistical significance was not demonstrated. Gentamicin and ciprofloxacin, the most frequently used aminoglycoside and fluoroquinolone respectively, are recognised as antimicrobials most likely to promote spread of resistance in hospitalised patients (Australian Commission on Safety and Quality in Health Care, 2016). Hence the decreasing antimicrobial usage rates observed for these antimicrobials are notable. Reduction in gentamicin use may be attributed to the recommendation provided in the recent Australian therapeutic guidelines on its empirical use, which states that empirical therapy with the aminoglycoside class of antibiotics should not extend beyond 48 hours, given its 'post-antibiotic' or long lasting effect in the body (Antibiotic Expert Groups, 2014). Through the national pharmaceutical subsidy scheme, ciprofloxacin use has been restricted in Australia since the early 1990s as it is classified as a reserve antimicrobial drug (Cheng et al., 2012).

Although a decrease in overall antimicrobial use was noted, findings showed that use of the broad spectrum antimicrobials, piperacillin-tazobactam and meropenem, increased. The newer broad spectrum antimicrobials are costly and have been associated with the emergence of resistance (Kritsotakis et al., 2006). They are also regarded as last resort antimicrobials (ESPAUR Writing Committee, 2015). The exact reason for the observed increases in use is not clear. Results from the English Surveillance Programme on Antimicrobial Use and Resistance showed that from 2010 to 2014 prescribing of broad spectrum antimicrobials, specifically piperacillin-tazobactam and carbapenems, increased by 55% and 36% respectively in UK hospital patients (ESPAUR Writing Committee, 2015). An increased use of broad spectrum and newer antimicrobials was also noted in hospitals in

Greece (Kritsotakis et al., 2006) and Denmark (Müller-Pebody et al., 2004). A possible reason proposed for this shift in antimicrobial use was that in a bid for doctors to cover for all possible causes of bacterial infections in patients, and because of pressure to ensure quick recovery of patients, doctors in hospitals were more likely to use broad spectrum antimicrobials for empirical therapy (Müller-Pebody et al., 2004). They were also less likely to change this to narrow spectrum antimicrobials when the microbiological results were available, as there was already clinical improvement in the patient's condition (Müller-Pebody et al., 2004).

The results of analysis of the antimicrobial use data from Canberra Hospital also showed that five-year antimicrobial usage rates were highest for the broad spectrum antimicrobials, amoxicillin-clavulanate and cefazolin, consistent with national data, with amoxicillin-clavulanate recognised as the most commonly used antimicrobial in Australian hospitals, with cefazolin ranking third (Australian Commission on Safety and Quality in Health Care, 2016). These findings are also comparable to published findings from a general hospital in Spain where, over a five-year period from 1996 to 2000, amoxicillin-clavulanate was the most frequently used antimicrobial (Hermosilla, Canut, Ulibarrena, Abasolo, & Carlos, 2003). Other recent data from outpatients in 33 European countries, including Spain, also showed that amoxicillin-clavulanate was the most frequently used penicillin (Versporten et al., 2011).

A seasonal pattern of antimicrobial use, specifically in the winter months, was shown for ceftriaxone. This finding is consistent with national data and is said to reflect the use of ceftriaxone in the treatment of lower respiratory tract infections (Australian Commission on Safety and Quality in Health Care & SA Health, 2015). Seasonal variation in antimicrobial use has also been demonstrated in published national and international studies (Achermann et al., 2011; Meumann et al., 2015; Sun et al., 2012). The seasonality of antimicrobials has been attributed to the possible inappropriate prescribing of antimicrobials for respiratory tract infections during the winter months (Suda, Hicks, Roberts, Hunkler, & Taylor, 2014). This further emphasises the need to assess the appropriateness of antimicrobial use.

Limitations to the analysis of the antimicrobial use data included the inability to compare the data directly with AMR data on *E. coli* UTI, because the indications (including organisms)

for which these antimicrobials were used were not available. Stratification of the data based on hospital wards or intensive care unit was not done, which may have allowed important antimicrobial use patterns within specific hospital areas to be identified. Despite these limitations, these results provide important information on the antimicrobial use trends at the Canberra Hospital and complement the main study two findings.

5.4 Summary

This chapter reports on the second study of the research program. The first and main part of the chapter discusses the analysis of AMR *E. coli* UTI data from the Canberra Hospital, which is incorporated in the thesis as a publication. This study is the first of its kind in Australia. The findings have highlighted the increasing resistance of urinary *E. coli* to commonly prescribed antimicrobials both in the hospital and community setting. Study two also identified low levels of ESBL-producing *E. coli* UTI. Although the levels of ESBL-producing *E. coli* are low, their presence further emphasises the need for the development of effective AMR control policies, as infection with this bacterium is usually non-responsive to the conventional empirical antimicrobial agents used in the treatment of UTI.

The second part of this chapter provides time series analysis of supplementary antimicrobial use data at the Canberra Hospital. These data comprise all antimicrobials used during the five-year period (not limited to those used to treat UTI) but the findings complement the main study findings. A decrease in overall antimicrobial use was noted over the study period, but with an increasing use of newer broad spectrum antimicrobials signifying the need for evaluation of appropriateness of antimicrobial use or quality of antimicrobial prescribing at the hospital. The seasonal use of ceftriaxone at the hospital, particularly in the winter months, further emphasises the importance of undertaking further research on the appropriateness of antimicrobial prescribing.

The findings on AMR and antimicrobial use presented in this chapter provide baseline data for the hospital for which future interventions can be measured against. Continued monitoring of AMR and antimicrobial use in both hospital and community settings is important for several reasons. Analysis of the data can identify areas with high antimicrobial usage and resistance, which can inform the development of interventions aimed at limiting overuse and misuse of antimicrobials and implementation of antimicrobial resistance

control policies. Antimicrobial use data can also be linked to AMR surveillance data to provide information on the antimicrobial use and resistance association for specific bacterium-drug combinations.

The next chapter presents the findings of the third study. Study three reports on the five-year risk and incidences of single drug-, multidrug-, extensively drug- and pandrug-resistant *E. coli* UTI in a cohort of ACT residents.

Chapter 6: Study three – Incidence of antimicrobial resistance in *E. coli* UTI

6.1 Overview

Evaluating not only the prevalence, but also the incidence, of AMR in urinary *E. coli* is important to gain a better understanding of the magnitude and impact of the issue of AMR. Incidence quantifies the number of new cases of a condition, in this instance newly diagnosed cases of resistant *E. coli* UTI in people at risk during a specified time period (Buttner & Muller, 2011; Porta, 2014). Evidence from the review of the literature (Chapter Two) highlighted the lack of Australian data on the incidence of antimicrobial resistant *E. coli* UTI over time, including estimates on the risk of disease occurrence. These evidence gaps will be addressed in the third study of the research program. Furthermore, study one identified the lack of inclusion of potential risk factors for AMR in the design and analysis of published AMR studies, demonstrating the need to collect these data and include them in the analysis of microbiological laboratory data.

The emergence of multidrug-resistant urinary *E. coli* has been reported frequently overseas (Eshetie et al., 2015; Khawcharoenporn, Vasoo, & Singh, 2013; Yadav, Adhikari, Khadka, Pant, & Shah, 2015). Multidrug-resistant infections are a major concern and pose a serious threat to public health as there are only a limited number of antimicrobials effective against multidrug-resistant bacteria, leading to difficulty in treatment and potentially high mortality rates (Eshetie et al., 2015; Nikaido, 2009). In Australia, prevalence of multidrug-resistant *E. coli* has been investigated by AGAR, although not specifically in *E. coli* UTI. There are no available incidence data on multidrug-, extensively drug- and pandrug-resistant *E. coli* UTI in Australia. Given the high mortality associated with infections caused by these bacteria, knowledge of the extent of multidrug-resistance in urinary *E. coli*, including potential risk factors, will contribute to the development of interventions aimed at controlling AMR.

This chapter presents the results of the third study undertaken as part of this research program. The study aim was to evaluate the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of ACT residents over a five-year period. In study three, I investigated the incidence and risk of single drug-resistant, multidrug-resistant, extensively drug-resistant and pandrug-resistant *E. coli* UTI. The manuscript reporting the findings of study three is presented in section 6.2. While the thesis was under examination, the manuscript was

finalised and submitted to the Medical Journal of Australia with feedback received from the journal. Given the narrowed focus of request from the journal for the manuscript to be presented as a short report focusing on the incidence findings, the previously submitted version of the manuscript has been included in the thesis and a revised short report focusing on the incidence findings is being prepared for resubmission to the Medical Journal of Australia.

6.2 Publication three: Incidence of single-drug-, multidrug-, and extensively drug-antimicrobial resistance of *Escherichia coli* urinary tract infections: an Australian laboratory-based retrospective cohort study

6.2.1 Abstract

Objectives: To evaluate incidence and risk of antimicrobial resistant *Escherichia coli* (*E. coli*) urinary tract infections (UTI) in a cohort of Australian Capital Territory (ACT) residents.

Design, setting and participants: Laboratory-based retrospective cohort study of ACT residents who submitted urine samples to ACT Pathology between January 2009 and December 2013.

Main outcome measures: Incidence and risk for single-drug, multidrug-, extensively drug- and pandrug-resistant *E. coli* UTI.

Results: A total of 146,915 urine samples from 57,837 ACT residents were identified over five years. Mean age of people in the cohort was 48 years (standard deviation=26 years) and 64.4% were females. Five-year incidence of single-drug resistant *E. coli* UTI was high for ampicillin, trimethoprim and cefazolin (6.8%, 3.5% and 1.9% respectively). No pandrug-resistant *E. coli* UTI was detected. Five-year incidences of multidrug- and extensively drug-resistant *E. coli* UTI were 1.9% and 0.2% respectively. In multivariate logistic regressions, female sex and age over 38 years were significantly associated with single- and multidrug-resistance. Compared to hospitals, office-hours general practices, community and specialist health services, risk of single-drug resistance was significantly higher in samples from after-hours general practices (adjusted-odds ratio (OR) and 95% confidence intervals (CI) 2.6 (2.2–3.1)). There was higher risk of resistance to ciprofloxacin (OR 1.3) with high socioeconomic status.

Conclusions: In this cohort, incidence of multidrug- and extensively drug-resistant *E. coli* UTI are low in comparison to international data. Our findings have significant implications for

antimicrobial prescribing given identified risk factors for resistance development, especially in patients attending after-hours general practices.

6.2.2 Introduction

Escherichia coli (*E. coli)* is recognised as the most common cause of urinary tract infections (UTI) (Nicolle, 2008). The prevalence of resistance in urinary *E. coli* is increasing in Australia (Australian Group on Antimicrobial Resistance, 2013; Fasugba et al., 2016) but the resistance incidence is not well described. Australian studies that investigated urinary *E. coli* resistance provided no separate data on incidence of single- and multidrug-resistant (MDR) isolates nor did they identify patient risk factors for resistance development (Kennedy et al., 2008; Meumann et al., 2015). Recent data from the United States (US) showed that incidence of fluoroquinolone-resistant *E. coli* bacteriuria had significantly increased from 464 to 1116 per 100,000 person years between 2005 and 2009 in patients older than 80 years (Swami et al., 2012).

The emergence of *E. coli* resistance to multiple antimicrobials poses a significant threat to public health with limited antimicrobials available for treating MDR infections. These infections may complicate UTI treatment leading to poorer patient outcomes (Magiorakos et al., 2012). In Australia, MDR *E. coli* prevalence increased from 4.5% in 2008 to 7.6% in 2012 (Australian Group on Antimicrobial Resistance, 2013). In the US, incidence of MDR *E. coli* was 37 per 100,000 person-years in 2009 (Swami et al., 2012).

The recently developed Australian antimicrobial resistance strategy lists *E. coli* as a priority organism for resistance surveillance due to its impact in hospital and community settings (Australian Commission on Safety and Quality in Health Care, 2016). Given the newly established Antimicrobial Use and Resistance in Australia (AURA) surveillance system (Australian Commission on Safety and Quality in Health Care, 2016), evaluating incidence and resistance trends at a jurisdictional level will contribute important information to understanding the distribution and impact of resistant *E. coli* UTI. This study, the first of its kind in Australia, evaluated incidence and five-year risk of resistant *E. coli* UTI in a large cohort of Australian Capital Territory (ACT) residents. The study also provides novel information on incidence of MDR, extensively drug-resistant (XDR) and pandrug-resistant

(PDR) *E. coli* UTI, with evidence to inform clinicians, policy makers and other stakeholders about emerging trends that may impact on public health.

6.2.3 Methods

Study design, setting and population: We utilised a laboratory-based retrospective cohort design (Buttner & Muller, 2011) using data from a cohort of ACT residents who had urine samples processed at ACT Pathology. Based on available data from Medicare statistics (Medicare Australia, 2016) and ACT Pathology (A. Das, personal communication, 11 November, 2016), it is estimated that ACT Pathology processes approximately 100% of urine samples from inpatients, emergency department and specialist outpatient clinic patients of public hospitals, and at least 13% of urine samples from patients in the community and private hospitals whose residential status is ACT. The population of the ACT in 2011 was 367,985. The study cohort comprised 57,873 ACT residents whose urine samples were processed at ACT Pathology during the period of 1st January 2009 to 31st December 2013, of whom an estimated 71% were inpatients, emergency department and specialist outpatient clinic patients of public hospitals and the remaining 29% were patients in the community and from private hospitals. Residence in the ACT was based on postcode, and those with postcodes from NSW and elsewhere were excluded. Samples collected outside ACT and from patients without unique identifiers were also excluded.
Ethics approval was granted by ACT Health and Australian Catholic University (ETHLR.14.223 and 2014276N).

Bacterial isolation and identification and antimicrobial susceptibility testing: Urine samples were processed based on microbiology laboratory standards described elsewhere (Fasugba et al., 2016).

Definitions: The incidence of single-drug resistance was separately assessed for ampicillin, amoxycillin-clavulanate, trimethoprim, norfloxacin, gentamicin, cefazolin, ceftriaxone, trimethoprim-sulphamethoxazole, nalidixic acid, ciprofloxacin, meropenem, piperacillin-tazobactam and nitrofurantoin. Non-susceptibility to at least one agent in three or more antimicrobial categories was defined as MDR; non-susceptibility to at least one agent in all but two or fewer antimicrobial categories was defined as XDR; and non-susceptibility to all agents in all antimicrobial categories was defined as PDR based on published international

standardised definitions (Magiorakos et al., 2012). For these definitions to be comparable, isolates must be tested against all antimicrobial agents within the antimicrobial categories. In cases of incomplete testing, it is difficult to distinguish reliably between XDR and PDR isolates. In ACT Pathology nine of the 13 potential antimicrobial categories were tested for hence these isolates were classified as 'possible PDR' or 'possible XDR' (Magiorakos et al., 2012).

6.2.4 Data analysis

All ACT residents who submitted urine samples to ACT Pathology during the five years were followed until they developed their first antimicrobial resistant *E. coli* UTI or died or were right censored at the end of follow-up (December 2013). Follow-up was based on presence or absence of a subsequent urine sample submitted to ACT Pathology during the study five-year period. Five-year cumulative incidence of resistance were calculated by dividing number of incident cases of antimicrobial resistant *E. coli* UTI by total number of individuals with urine samples included in the study. The incidence was expressed per 100,000 people whose urine samples were tested by ACT Pathology, that is, the number of people with a resistant *E. coli* UTI among every 100,000 people submitting urine samples to ACT Pathology for testing.

Data were also analysed yearly to establish the changing cumulative incidence. Kaplan-Meier (KM) curves were plotted to show the effect of sex and age on resistance incidence to any antimicrobials tested. Differences in resistance between sexes and between age groups were evaluated using the Log-rank test (Cox, 1972). Multivariate logistic regression models were constructed for each antimicrobial to determine the effect of age, sex, socioeconomic status (SES), and urine sample origin on risk of resistance. The Hosmer–Lemeshow test was performed to assess goodness of fit of logistic regressions with $P > 0.05$ indicating good fit.

Age was assessed within five categories based on age distribution in the data (≤23, 24-37, 38-56, 57-73 and ≥74 years). The Australian Socio-Economic Indexes for Areas (SEIFA) based on residential postcodes derived from 2006 Australian census data was used to determine SES (Australian Bureau of Statistics, 2008). This validated ecological index score ranks areas in Australia based on relative socioeconomic indicators of advantage and disadvantage, with lower scored areas being more disadvantaged than higher scored areas (Walker & Hiller,

2005). For this analysis, SES was assessed within three categories (low, middle and high) based on the dataset SEIFA score distribution.

The sample origin refers to the health service requesting the urine sample test and was grouped into seven categories, namely: public acute hospitals; private acute hospitals; GP clinics; after-hours GP clinics; community health services including Aboriginal and Torres Strait Islander and youth health services; specialist health services including minor surgical and procedural outpatient units; and 'others'. This last category included samples sent from non-acute hospitals, correctional services, dialysis clinics, dental clinics, hospice, ambulance services and a life insurance organisation. Further analyses were performed to determine levels of MDR, XDR and PDR for isolates. A significance level of 0.05 was used. Data were analysed using STATA statistical software (version 14, StataCorp).

6.2.5 Results

A total of 196,385 urine samples were processed over the five-year period. When restricted to samples belonging to ACT residents and collected within the ACT, there were 163,792 samples. Of these, unique patient identifiers were missing for 16,877 samples (10.3%) resulting in 146,915 samples belonging to 57,837 ACT residents over five years being included in the final analysis. The mean age of people in the cohort at first sample submitted was 48 years (standard deviation=26 years) and most were female (64.4%, n=37,234). Of all included samples, positive cultures accounted for 15.9% (n=23,486) with 56.9% (n=13,371) of positive cultures being *E. coli* UTIs.

Overall 5-year and yearly incidence of single-drug resistant *E. coli* UTI per 100,000 people whose urine samples were tested by ACT Pathology are shown in Table 1. Five-year resistance incidence (expressed as percentages) was highest for ampicillin, trimethoprim and cefazolin (6.8%, 3.5% and 1.9% respectively) and lowest for meropenem, nitrofurantoin and piperacillin-tazobactam (0.002%, 0.5% and 0.5% respectively).

Table 1 Overall five-year and yearly cumulative incidence of antimicrobial resistant *E. coli* UTI per 100,000 people whose urine samples were tested by ACT Pathology

	2009*	2010*	2011*	2012*	2013*	5-year total*
Antibiotic	Rate (n)	Rate (n)	Rate (n)	Rate (n)	Rate (n)	Rate (n)
Ampicillin	4697.8 (722)	5181.6 (863)	5814.6 (1015)	5247.3 (956)	5469.0 (835)	6760.4 (3910)
Trimethoprim	2322.9 (357)	2635.8 (439)	3001.8 (524)	2629.1 (479)	2908.0 (444)	3483.9 (2015)
Cefazolin	1067.1 (164)	1495.0 (249)	1638.4 (286)	1432.6 (261)	1644.0 (251)	1974.5 (1142)
TMP-SMX	943.5 (145)	1296.9 (216)	1598.3 (279)	1427.1 (260)	1441.0 (220)	1751.5 (1031)
Nalidixic acid	982.5 (151)	1128.8 (188)	1409.3 (246)	905.6 (165)	615.7 (94)	1345.2 (778)
AMC	481.5 (74)	732.5 (122)	928.0 (162)	1092.3 (199)	1152.7 (176)	1201.7 (695)
Ciprofloxacin	442.4 (68)	450.3 (75)	658.8 (115)	697.1 (127)	903.9 (138)	824.7 (477)
Norfloxacin	396.9 (61)	438.3 (73)	658.8 (115)	680.6 (124)	884.2 (135)	802.3 (464)
Ceftriaxone	214.7 (33)	366.3 (61)	618.7 (108)	735.5 (134)	792.5 (121)	741.7 (429)
Gentamicin	292.8 (45)	420.3 (70)	532.8 (93)	526.9 (96)	635.3 (97)	629.4 (364)
PIT	39.0 (6)	402.3 (67)	383.8 (67)	499.5 (91)	576.4 (88)	539.4 (312)
Nitrofurantoin	240.7 (37)	276.2 (46)	297.9 (52)	384.2 (70)	648.4 (99)	506.6 (293)
Meropenem	0.0 (0)	6.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	1.7 (1)
Any antibiotic[¥]	5446.0 (837)	5619.9 (936)	6261.5 (1093)	5554.6 (1012)	5901.2 (901)	7311.9 (4229)

*Number of residents (2009=15,369; 2010=16,655; 2011=17,456; 2012=18,219; 2013=15,268; five year period=57,837)

[¥]Resistance to at least one of the thirteen antibiotics

AMC=Amoxycillin-clavulanate; TMP-SMX=Trimethoprim-sulphamethoxazole; PIT=Piperacillin-tazobactam

Risk of developing a resistant UTI was significantly higher in females compared to males (Kaplan-Meier analysis, Log-rank test P<0.001). Ages below 23 years and over 38 years were significantly associated with resistant infection; risk of resistance was highest for people over 74 years.

A separate multivariate logistic regression model was constructed for each antimicrobial (excluding meropenem which had only one resistant isolate identified) adjusting for age, sex, SES and sample origin. Although results varied for each antimicrobial, consistent findings included significantly higher odds of resistance in females and older people (Appendix S1). The risk of ampicillin, cefazolin, trimethoprim and trimethoprim-sulphamethoxazole resistance was significantly higher in people aged below 23 years. Significantly higher odds of ciprofloxacin, norfloxacin and nalidixic acid resistance (odds ratios (OR) 1.3, 1.3 and 1.2 respectively) were seen in people with high SES. The multivariate logistic regression model for resistance to any of the 13 antimicrobials tested found higher odds of resistance in females and older aged people (Table 2). Samples from after-hours GP

clinics had the highest odds of being resistant (ORs and 95% confidence intervals (CIs) 2.6 (2.2-3.1); P<0.05). The Hosmer–Lemeshow tests showed good fit for all regression models (P>0.05).

Table 2 Multivariate logistic regression model for the effects of age, gender, socioeconomic status and urine source on the risk of developing urinary *E. coli* resistance to any of the 13 antimicrobial agents tested or a multidrug-resistant infection

Variable	Categories	N	Any resistance*		Multidrug-resistance	
			Odds ratio	95% CI	Odds ratio	95% CI
Age (years)	≤23	11,570	1.3	1.2–1.5	1.0	0.8–1.3
	38–56	11,566	1.3	1.1–1.4	1.6	1.3–2.0
	57–73	11,567	1.9	1.7–2.1	2.5	2.1–3.1
	≥74	11,567	2.9	2.6–3.2	3.3	2.7–4.0
	24–37 (reference)	11,567	1.0		1.0	
Gender	Female	37,234	3.3	3.0–3.5	2.6	2.2–3.0
	Male (reference)	20,593	1.0		1.0	
SES	Middle	20,450	1.0	1.0–1.1	1.0	0.9–1.1
	High	16,168	1.0	0.9–1.1	1.0	0.9–1.2
	Low (reference)	21,051	1.0		1.0	
Sample origin	Private acute hospitals	1,313	0.7	0.6–0.9	1.0	0.7–1.5
	GP clinics	12,446	1.1	1.0–1.2	1.0	0.9–1.2
	After-hours GP clinics	1,024	2.6	2.2–3.1	1.6	1.1–2.3
	Community health services	1,284	1.4	1.1–1.7	1.5	1.0–2.1
	Specialist health services	533	1.1	0.8–1.4	1.8	1.1–2.9
	Others[#]	363	1.3	0.9–1.9	2.3	1.3–4.0
	Public acute hospitals (reference)	40,874	1.0		1.0	

*Antimicrobials include ampicillin, amoxycillin-clavulanate, cefazolin, ceftriaxone, trimethoprim, trimethoprim-sulphamethoxazole, nalidixic acid, ciprofloxacin, norfloxacin, gentamicin, nitrofurantoin and piperacillin-tazobactam

[#]non-acute hospitals, correctional services, dialysis clinics, dental clinics, a hospice, ambulance services and a life insurance organisation

N=Number of residents (missing: Gender=10; SES=168)

SES=Socioeconomic status; GP=General practice

Hosmer Lemeshow test for Any resistance model: Chi square statistic=8.13; *P*=0.42

Hosmer Lemeshow test for multidrug- resistance model: Chi square statistic=7.65; *P*=0.47

Further analyses showed no possible PDR *E. coli* but 5-year incidence of MDR and possible XDR *E. coli* was found to be 1.9% and 0.2% respectively. Female sex and age over 38 years were significantly associated with MDR (Table 2). After adjusting for age, sex and SES, urine samples from after-hours GP clinics, specialist health services and the "other" category were associated with significantly higher odds of MDR (OR and 95% CIs 1.6 (1.1-2.3), 1.8 (1.1-2.9)

and 2.3 (1.3-4.0) respectively). Given the low incidence of possible XDR *E.coli* UTI, and because this group was a subset of MDR, logistic regression analysis was not performed.

6.2.6 Discussion

To our knowledge, this is the first study examining incidence and risk of developing a resistant *E. coli* UTI in Australia. Notably, this study is the first in Australia to assess the risk of *E. coli* UTI resistance related to the health service requesting the urine sample test. This study has identified (1) high incidence of resistance to ampicillin, trimethoprim and cefazolin; (2) presence of MDR and possible XDR *E. coli* UTI in this cohort; (3) significantly higher risk of resistance in females and people over 38 years; (4) significantly higher risk of resistance to some antimicrobials in the high socioeconomic group; and (5) risk of resistance varied by health service requesting urine sample.

The UTI resistance incidence was highest for ampicillin, trimethoprim and cefazolin consistent with national and territory blood culture prevalence (Australian Commission on Safety and Quality in Health Care, 2016). The high resistance for these three antimicrobials, recommended for first-line UTI therapy raises concern about selection of an appropriate empirical treatment agent for UTI. The low resistance for last-line antimicrobials such as meropenem, ceftriaxone, ciprofloxacin and gentamicin in comparison to published European data is notable (Allocati, Masulli, Alexeyev, & Di Ilio, 2013; van der Donk et al., 2012). Our study also reports MDR and possible XDR *E. coli* UTI in this cohort, although in relatively low numbers compared to countries like the Netherlands (van der Donk et al., 2012). Globally, Australia is considered one of the countries with high antimicrobial use (Australian Commission on Safety and Quality in Health Care, 2016). Despite this, the incidence of single-drug resistant, MDR and XDR *E. coli* is relatively low. Likely reasons may be that many people acquire resistant bacteria through food, water, travel and the environment rather than through antimicrobial use (Collignon, 2015). Although no PDR *E. coli* UTI were identified in our study, the presence of MDR and possible XDR isolates raises concern because these pathogens are associated with poorer patient outcomes due to limited availability of drugs for treatment.

Resistance risk was higher in females and people over 38 years in this study. There are inconsistent findings for the effect of sex on resistance (Lagacé-Wiens et al., 2011; McGregor

et al., 2013; Sahm et al., 2001). Possible explanations include higher predisposition to UTIs in females resulting in more antimicrobial prescriptions (Al-Badr & Al-Shaikh, 2013). Our finding of higher resistance risk in older age groups has been previously described and may be due to increasing cumulative exposure to antimicrobials and healthcare settings with age (Lagacé-Wiens et al., 2011). We also found a significantly higher risk of ampicillin, cephazolin and trimethoprim-sulphamethoxazole resistance in people below 23 years, supported by a recent systematic review (Bryce et al., 2016). Interventions aimed at reducing further development and spread of resistance should take account of patient age and gender. Ciprofloxacin and nalidixic acid resistance were found to be significantly associated with high SES, consistent with published studies (Kristiansson et al., 2009). This may be due to higher antimicrobial use by more affluent families (Kristiansson et al., 2009), including overseas colonisation with resistant *E. coli* as those with high SES are more likely to travel often (Kennedy & Collignon, 2010). Socioeconomic status in the ACT is relatively uniform across postcodes hence further research is needed to confirm the possible association between SES and resistance.

The highest resistance risk was noted in samples from after-hours GP clinics. Patients receiving treatment from these clinics typically require medical services outside regular office-hours usually between 6pm to 8.30am on weekdays and during weekends. Evidence shows higher resistance in hospital compared to community settings (Fasugba et al., 2015), hence higher resistance risk in samples from after-hours GP clinics is surprising. The reason for this finding is unknown but it could be postulated that patients attending after-hours GP clinics are similar to patients presenting at emergency departments. These patients are likely to be too unwell to wait for appointments during regular office-hours, and more likely to require antimicrobials. In the UK, when compared to regular office-hours GPs and hospitals, after-hours GPs and other community health services had higher antimicrobial prescribing rates (Public Health England, 2015). As antimicrobial use is a major factor contributing to resistance (Australian Commission on Safety and Quality in Health Care, 2016), this finding highlights the importance of judicious antimicrobial prescribing by clinicians. Education campaigns should be tailored towards clinicians consulting in after-hours clinics and health services should develop systems that continuously monitor antimicrobial prescriptions to ensure their rational use.

Our study has limitations. This study comprised patients whose samples were processed by ACT Pathology which captures all inpatient public hospital samples, samples from outpatients attending public hospital emergency departments and specialist clinics and approximately 13% of private hospital and community samples. Hence the calculated resistance incidence is based on sample data and may not be directly generalised to the total ACT population. These findings are however the most reliable estimates to date of ACT *E. coli* UTI resistance incidence. Population-level analysis based on setting of infection onset, that is community versus inpatients, was considered. This analysis was not possible because data from Medicare statistics website used to estimate the proportion of community samples processed by ACT Pathology does not separate community from private hospital inpatient samples. In defining incident UTI, there was a possibility that pre-existing infections (not verified) were carried over from the year before study commencement. Due to insufficient data, UTI was not classified based on setting of acquisition. Exclusion of samples from patients treated empirically without laboratory culture and likely inclusion of multiple samples from people with recurrent UTI potentially overestimated resistance incidence. Patient risk factors including comorbidities and urinary tract instrumentation which could influence resistance incidence could not be accounted for in analysis because retrospective data were used. Given the composition of de-identified data, 10% of samples were excluded due to lack of unique patient identifiers thereby underestimating resistance incidence.

6.2.7 Conclusion

The detection of single drug-resistant, MDR and possible XDR *E.coli* UTI emphasises the need for continued monitoring of resistance to ensure suitable empirical therapeutic agents remain available. This study highlights the importance for development of interventions aimed at reducing resistance based on patient risk factors. Higher risk of resistance in patients attending after-hours GP clinics necessitates further research to investigate antimicrobial prescribing practices within these health services.

6.3 Summary

This is the first Australian study to examine incidence and risk of antimicrobial resistant *E. coli* UTI. Incidence studies as opposed to prevalence studies are able to provide information on the rate at which new cases of a disease occur as well as identify at risk groups. This five-

year study has identified that incidence of single drug-resistance to urinary *E. coli* was highest for ampicillin, trimethoprim and cefazolin. Kaplan-Meier curves and Log-rank tests, showing the effect of sex and age on the incidence of resistance to any of the antimicrobials tested, were also produced and are included as appendices (Appendix G). Evidence obtained from study three also demonstrates potential patient risk factors that may influence resistance development. The detection of MDR and XDR *E. coli* UTI, although relatively low, emphasises the need for continued surveillance of AMR in urinary *E. coli* infections. Close monitoring of resistance in this pathogen with concurrent implementation of strategies is necessary to prevent further development of MDR. If not, there is the possibility that PDR *E. coli* UTI may subsequently emerge, leading to dire public health ramifications for patients with PDR infections. The findings of the third study of the research program will also contribute to policy and practice, especially measures targeted towards health service providers who prescribe antimicrobials for patients with UTI.

The sample used for study three was previously described in the introduction chapter (Chapter One) and the limitation of the representativeness of the sample was discussed in detail in the methods chapter (Chapter Three). Given that the whole ACT population was not sampled but only a subset, albeit a considerable one, the findings of the incidence of resistance should be interpreted with caution. However, these are the most comprehensive results available to date in Australia reporting on the incidence of resistance in *E. coli* UTI and are therefore noteworthy. Furthermore, study three provides evidence of a new methodological approach to identifying incident cases of resistant *E. coli* UTI using microbiological laboratory data. The inclusion of private laboratories in the surveillance of AMR has been identified as being important to appropriately estimating AMR prevalence and incidence (Gandra, Merchant, & Laxminarayan, 2016). Whilst there may be barriers to obtaining data from private laboratories, effort should be made to include private laboratories in all surveillance activities to ensure a complete picture of the risk and burden of AMR. The new methodological approach demonstrated in study three can then be replicated using a dataset more representative of a geographical population.

The next chapter, Chapter Seven, is the final chapter of the thesis. The discussion and conclusion of the research program is presented by synthesising the results of the three studies undertaken in the research program. The contribution of the research program to

knowledge is detailed as well as recommendations for policy, clinical practice and future research.

Chapter 7: Discussion and conclusion

7.1 Overview

The thesis subject of AMR in *E. coli* UTI has been investigated in this research program. Urinary tract infections caused by the *E. coli* bacterium are common and resistance to antimicrobials used to treat urinary *E. coli* infections is increasing, prompting the need for continued research in this area. This research program is the first of its kind in Australia to have comprehensively and rigorously examined AMR in urinary *E. coli* infections in a large sample of ACT residents. This research is also the first to compare urinary *E. coli* resistance to ciprofloxacin in hospital and community settings at a global level.

The findings of the three studies undertaken over the last three years are synthesised in this chapter to address the overall aim of the research program. The first part of the chapter reviews the main findings of the research program against each study aim. The strengths and limitations of the research are also outlined. The novel contributions of the research findings to knowledge are discussed. Finally, the chapter ends with a summary of recommendations for policy, clinical practice and research.

7.2 The study aims achieved

The overall aim of the research program was to contribute to the body of knowledge about AMR in *E. coli* UTI. Based on the gaps identified from the review of literature in Chapter Two, three specific aims were identified and each aim was addressed in a separate study. The three separate but interrelated studies focused on providing knowledge about AMR in urinary *E. coli* infections. A summary of the main findings from each study undertaken is presented in Table 1, alongside the individual study aims.

Table 1 Summary of main findings from research study

Study	Aim	Main findings
Study One	Systematically review the literature and conduct a meta-analysis of observational studies published in the last ten years investigating ciprofloxacin resistance in community-acquired and hospital-acquired *E. coli* UTI	• Resistance to ciprofloxacin was statistically significantly higher in the hospital compared to the community setting across the world • Resistance statistically significantly varied by region and country • Resistance was highest in developing countries compared to developed countries • A statistically significant rise in resistance over time was seen in studies reporting on community-acquired *E. coli* UTI • Poor methodological quality of published studies investigating AMR in *E. coli* UTI was identified
Study Two	(a) Evaluate AMR temporal trends and compare the prevalence of AMR in hospital-acquired and community-acquired *E. coli* UTI at the Canberra Hospital over a five-year period (b) Evaluate trends and seasonal variation in antimicrobial use at the Canberra Hospital over a five-year period	• Prevalence of resistance for hospital- and community-acquired *E. coli* UTI combined was high for ampicillin and trimethoprim • Resistance for hospital- and community-acquired *E. coli* UTI combined was low for meropenem, nitrofurantoin, piperacillin-tazobactam and ciprofloxacin • Resistance to amoxycillin-clavulanate, cefazolin, gentamicin and piperacillin-tazobactam was statistically significantly higher in hospital- compared to community-acquired *E. coli* UTI • Statistically significant increases in resistance over the five-year study period were noted for amoxycillin-clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin and trimethoprim-sulphamethoxazole for hospital- and community-acquired *E. coli* UTI combined • Ciprofloxacin resistance was found to be associated with older age • Using supplementary data, overall antimicrobial usage (assessed only for inpatients at the hospital) decreased over the study period • Using supplementary data, an increase in the use of newer broad spectrum antimicrobials was noted among hospital inpatients only

Study	Aim	Main findings
Study Three	Evaluate the incidence and risk of antimicrobial resistant *E. coli* UTI in a cohort of ACT residents	• Incidence of resistance was highest for ampicillin, trimethoprim and cefazolin • Incidence of multidrug- and extensively drug-resistant *E. coli* UTI was low • Female sex and age over 38 years were statistically significantly associated with single- and multidrug-resistance • People with high socioeconomic status had higher odds of resistance to ciprofloxacin, norfloxacin and nalidixic acid • Higher odds of resistance were noted in samples sent from after-hours GP clinics

A detailed discussion of how each aim was achieved is provided in the sections below.

7.2.1 Study one: Systematic review of ciprofloxacin resistance in *E. coli* UTI

Study one addressed a research gap in global knowledge of ciprofloxacin resistance in *E. coli* UTI. Ciprofloxacin, a high priority critically important antimicrobial, is recommended for second-line treatment of UTI. Despite the recommendations in internationally recognised treatment guidelines that ciprofloxacin should not be used as an empirical therapeutic agent for UTI, evidence from individual studies shows that resistance to this agent is increasing. Hence, ciprofloxacin was selected as an appropriate antimicrobial to examine for global evidence on AMR in *E. coli* UTI. Study one systematically reviewed the literature and meta-analysed studies investigating ciprofloxacin resistance in hospital- and community-acquired *E. coli* UTI. This systematic review of the literature also informed the research aims for studies two and three.

The findings from study one (presented in Chapter Four) showed that: across the world ciprofloxacin resistance is present in both hospital- and community-acquired UTI, although higher in hospital-acquired UTI; ciprofloxacin resistance is increasing internationally in the community; developing countries have higher resistance to ciprofloxacin compared to developed countries; and there is a lack of methodological rigour in the design and analysis of published studies investigating AMR in *E. coli* UTI. Higher resistance in hospital as opposed to community settings may not be surprising due to the effect of antimicrobial use

in hospitals, but the novel finding identified from this systematic review is the increasing resistance to ciprofloxacin in the community setting. The exact cause for this increasing international trend in the community is not certain but a strong possibility may be an increase in prescribing of ciprofloxacin in the community. The use of non-prescription antimicrobials and over-the-counter prescribing is relatively common in developing countries especially those countries without policy regulation on antimicrobial use hence this may account for the higher resistance in developing countries.

In summary, the study synthesised findings from published studies worldwide to estimate global resistance of urinary *E. coli* to ciprofloxacin, and also highlighted the notable increase in resistance in the community setting. Of particular importance is the poor methodological quality of the design and analysis of AMR studies identified during the systematic review. Studies two and three provided an opportunity for me to address some of these methodological issues using my own microbiological laboratory dataset.

7.2.2 Study two: Prevalence of antimicrobial resistance in *E. coli* UTI

The lack of published Australian studies that directly compare resistance patterns over time for hospital- and community-acquired UTI identified from the review of literature presented in Chapter Two, as well as the lack of methodological rigour in the design and analysis of AMR studies identified in study one, prompted the need to undertake the second study of the research program. Study two utilised retrospective microbiological laboratory data to evaluate AMR temporal trends and seasonal variation of *E. coli* UTIs from 2009 to 2013 in patients attending a tertiary referral hospital in Canberra, Australia. The prevalence of resistance in hospital- and community-acquired *E. coli* UTI was also compared. Previous international studies identified from the literature review utilised varying criteria to classify patients, based on the setting of infection acquisition into hospital- and community-acquired. Internationally accepted criteria from the CDC were applied in study two for the classification of UTI. Linkage of microbiological laboratory data with patient administrative data from a tertiary referral hospital, the Canberra Hospital, was undertaken to ensure UTI was appropriately classified. In addition, antimicrobial use data from the hospital inpatients was evaluated for changes over time.

Major findings from study two (detailed in Chapter Five) were that: resistance was highest for ampicillin and trimethoprim; resistance to amoxycillin-clavulanate, cefazolin, gentamicin and piperacillin-tazobactam were statistically significantly higher in hospital- compared to community-acquired UTI; and an increase in resistance over the five years was noted for amoxycillin-clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin and trimethoprim-sulphamethoxazole, with seasonal pattern observed for trimethoprim resistance for hospital- and community-acquired *E. coli* UTI combined. In addition, ciprofloxacin resistance was found to be associated with increasing age. Additional analysis of antimicrobial use data for inpatients only showed that overall antimicrobial use at the hospital decreased over the study period but with an increase in the use of newer broad spectrum antimicrobials.

In summary, study two confirmed the increasing resistance of urinary *E. coli* to commonly prescribed antimicrobials, identified differences in resistance patterns based on the setting of infection acquisition and provided information on inpatient antimicrobial use at the hospital. Findings from analysis of inpatient antimicrobial use data emphasise the need to consider not only the level of antimicrobial usage but also the appropriateness of this usage in addressing AMR.

7.2.3 Study three: Incidence of antimicrobial resistance in *E. coli* UTI

The third study in the research program focused on the incidence of antimicrobial resistant *E. coli* UTI using retrospective laboratory-based data from a cohort of ACT residents. As identified from the literature review, there are no incidence data on AMR in *E. coli* UTI in Australia. Determining the incidence of resistance among *E. coli* UTI provides a better understanding of the impact of AMR by making available information on the risk of development of resistance in individuals. Study three utilised microbiological laboratory data to evaluate the incidences and risk of multidrug-, extensively drug- and pandrug-resistant *E. coli* UTI in a cohort of ACT residents.

Study three (discussed in Chapter Six) found high incidence of single-drug resistance of urinary *E. coli* to ampicillin, trimethoprim and cefazolin. The incidence of multidrug- and extensively drug-resistant *E. coli* UTI was relatively low. Higher odds of resistance to any of the 13 antimicrobials tested were noted in females and people aged 38 years and above. Higher odds of resistance to some antimicrobials for people in the high socioeconomic

group were also seen. Urine samples belonging to people attending after-hours GP clinics had higher odds of being antimicrobial resistant.

In summary, study three provided new insights, not previously shown in Australia, into patient and health service characteristics that have the potential to influence the risk of development of AMR.

Overall, the findings show resistance of urinary *E. coli* to antimicrobials is increasing. The burden of resistance is higher in the hospital compared with the community setting but is increasing in the community. Also, patient risk factors play an important role in the development of AMR in *E. coli* UTI. The overall findings from the research program highlight the methodological issues with analysing AMR data. To address some of the methodological issues with analysing microbiological laboratory data, a new method for clearly identifying incident cases of resistant *E. coli* UTI was developed and good quality evidence of the impact of the choice of denominator used for calculating resistance prevalence was demonstrated.

7.3 Strengths of the research program

The strengths of the individual studies undertaken have been highlighted in Chapters Four, Five and Six, but it is important to also highlight the strengths of the research program as a whole. Comprehensive analysis of antimicrobial susceptibility test data from 54 published studies and a large dataset comprising 196,385 observations from one central testing microbiology laboratory, ACT Pathology, ensured large enough datasets (Hussein, 2011) to provide sound and reliable results for evaluating AMR in *E. coli* UTI.

The consistent application of definitions for infection cut-off (studies one and two) and antimicrobial susceptibility testing methodology (studies two and three) during the research program is another very important strength. Surveillance studies are strengthened by the use of standardised data collection and assessment methods because this allows for comparison of results between various sites both locally and internationally (Masterton, 2008). For studies one and two, the 48 hour timeframe to define infection cut-off as recommended by CDC (Centers for Disease Control and Prevention, 2016) was used for classifying hospital- and community-acquired UTI. For studies two and three, a single

uniform antimicrobial susceptibility testing methodology, namely the Clinical and Laboratory Standards Institute (CLSI) criteria, was employed over the study period from 2009 to 2013.

Existing data and/or datasets, either published or routinely collected microbiological laboratory data, were utilised for the analysis detailed in the research program. The use of readily accessible data and existing datasets for health research has been identified as a useful source of rich data to answer novel and significant research questions (Cheng & Phillips, 2014; Hussein, 2011; Schneeweiss & Avorn, 2005) and the findings from this research program confirm this. Using existing data for the research program allowed timely extraction and retrieval of data from published data and microbiological laboratory data respectively. The use of existing data also eliminated the constraints associated with designing questionnaires as well as data collection, providing time for preparation and comprehensive analysis of the data. The processes undertaken with these datasets can now easily be replicated using similar databases.

7.4 Limitations of the research program

The limitations of the research program undertaken as well as their potential impact on the findings are discussed in this section. The limitations discussed here relate primarily to the large laboratory database used for studies two and three, including the supplementary study on antimicrobial use provided in Chapter Five. Specific limitations for each study undertaken in the research program have already been identified in the relevant chapters (Chapters Four, Five and Six).

Secondary data sources were used for the research program. Secondary data are those which are not collected primarily for the purpose of the research to be undertaken (Cheng & Phillips, 2014). Study one synthesised existing published data while studies two and three evaluated existing routinely collected data from a laboratory database. Although the use of secondary data for health research has its advantages, a general limitation of using these datasets for research is incompleteness of data (Cheng & Phillips, 2014; Schneeweiss & Avorn, 2005). Given that secondary data were analysed in the research program, some variables of interest were not adequately documented and could not be included in the analysis. For example, in identifying potential individual risk factors for resistance development, the presence of urinary catheters has been shown to increase the odds of

development of AMR in patients (Ko et al., 2008). Presence of comorbidities in patients have also been shown to be risk factors for acquiring antimicrobial resistant infections (Nouvenne et al., 2014) as well as prior antimicrobial use (Hillier et al., 2007). These potential risk factors could not be taken into consideration in analysis given the lack of information in regards to these variables in the published data and laboratory dataset. The lack of information on important variables has been reported as a limitation of systematic reviews (Gopalakrishnan & Ganeshkumar, 2013), as systematic reviews rely on data from other studies which may not have been adequately documented. The issue of missing or incomplete data on potential risk factors has also been identified as a common problem in database studies (Sorenson, Sabroe, & Olsen, 1996). This research program provides empirical evidence of the impact of missing or incomplete data on research findings.

Another limitation noted during the research program for all three studies was the potential for selection bias. As stated in the literature review chapter (Chapter Two), the majority of patients with a UTI are treated empirically while awaiting results of the urine sample submitted to the microbiology laboratory for processing. This would likely have led to the increased selection of samples from patients who were unresponsive to initial antimicrobial therapy due to a resistant infecting bacterium as well as samples from those with complicated and recurrent UTI. Hence, evaluating resistance based on selection of samples from these patients has the potential to skew the data to more resistant cases and falsely elevate the resistance prevalence and incidence reported in the thesis. Therefore, the results from this research program need to be interpreted in this context.

For studies two and three, the protocol used by the microbiology laboratory involved testing first for resistance against routine first-line antimicrobials, followed by more extensive testing with second-line antimicrobials only for isolates resistant to at least three of the routine antimicrobials. The microbiology laboratory procedures arguably lead to the presence of testing bias with potential for overestimation of resistance for the second-line antimicrobials (Hindler & Stelling, 2007). Frequent claims from developed countries appear to be that this testing approach is commonly used in laboratories (Cornaglia et al., 2004; Standing Medical Advisory Committee & Sub-Group on Antimicrobial resistance, 1998) and is mainly attributed to the availability of funds provided to laboratories (Bax et al., 2001). Despite these limitations, with the potential for overestimation of resistance, the prevalence

and incidence reported in studies two and three of the research program was found to be lower when compared to data in published international literature.

The incidence of resistance evaluated in study three of this research program was based on microbiological laboratory data belonging to all public hospital inpatients, outpatients attending public hospital emergency departments and specialist clinics, and approximately 13% of private hospital and community patients. The remaining data belonging to patients in the community and those attending private health services were not included. Given that a subset of the ACT population, albeit considerable, was sampled, the calculated incidence of resistance cannot be generalised to the whole ACT population. However, the novel methodological approach was tested and the incidence is the most comprehensive available to date in Australia and therefore noteworthy.

In spite of the limitations described above, the findings of the research program contribute significantly to knowledge, as described in the next section.

7.5 Contribution to knowledge

The research program I have undertaken has not only confirmed existing evidence but also contributed new knowledge about AMR in *E. coli* UTI in hospital and community settings. The three main contributions to knowledge from the research program are:

1) Confirmation of existing evidence and improved understanding of the increase in resistance of urinary *E. coli* to antimicrobials, both globally and locally.
2) Identification of a new methodological approach, which can be applied by researchers to calculating incidence and prevalence of resistance using routinely collected antimicrobial susceptibility test data.
3) Identification of a hitherto unrecognised and potentially important patient group that may be at high risk of developing an antimicrobial resistant urinary *E. coli* infection, specifically those receiving care at after-hours GP clinics.

These knowledge contributions are woven through the research program and have been deemed significant by two peer reviewed journals. The three main contributions of this research program to knowledge will now be discussed in subsequent paragraphs of this section.

First, the findings from the research program confirmed the results from previously published studies, including prominent reports from the WHO that AMR in urinary *E. coli* is increasing. The increasing AMR to *E. coli* UTI identified both internationally in the systematic review and locally in the ACT using regional microbiological laboratory data, has implications for UTI treatment. The increase in resistance is especially important for antimicrobial agents like ciprofloxacin, which is considered a reserve antimicrobial for use in severe life threatening infections such as septicaemia. The research findings have also improved understanding of AMR in *E. coli* UTI by extending knowledge on the AMR trends as well as site of infection acquisition of urinary *E. coli*. This knowledge contribution is significant, both internationally in developed and developing countries and in Australia with the newly developed AURA Surveillance System, because the research has the potential to inform AMR surveillance by highlighting which antimicrobials to monitor closely. The findings emphasise the need for continued surveillance of AMR at the local, national and international levels. Monitoring of resistance through surveillance is considered an important means of controlling AMR, as the data obtained can provide information on new and emerging resistance patterns and also help identify antimicrobials that require interventions to be put in place to prevent further increases in resistance.

The second major contribution to knowledge of the research program is the new insight provided on the methodological approaches to calculating resistance incidence and prevalence, using routinely collected antimicrobial susceptibility test data from a regional microbiology laboratory. Incidence and prevalence are two separate statistical terms which have both been described extensively in the methods chapter of the thesis (Chapter Three). These terms are often used interchangeably in AMR surveillance studies with the prevalence most frequently reported as opposed to incidence (Bax et al., 2001). The method used by other researchers for calculating incidence involved the inclusion of the first positive urinary *E. coli* isolate per patient per year as incident cases (Swami et al., 2012). This approach for calculating incidence is not ideal because the first isolate may not be a true reflection of the first occurrence of the outcome, which is an antimicrobial resistant infection. By including all urine samples in the dataset used for the research program with the presence of multiple observations per person, I was able to 'follow-up' each person who submitted a urine sample until they developed a resistant *E. coli* UTI, as opposed to restricting the dataset to the first isolate per patient. This unique method to clearly identify incident cases of resistant

E. coli UTI in a laboratory dataset provides a new methodological approach not demonstrated in previously published studies and can now be used by other researchers analysing antimicrobial susceptibility test data for incidence of resistance. Furthermore, in estimating prevalence of resistance, different denominator choices have previously been applied. For example, some studies used the total number of isolates tested as the denominator (Alós, Serrano, Gómez-Garcés, & Perianes, 2005; Wu, Lee, Chen, Tuan, & Chiu, 2016). It is not clear from these studies if all isolates were tested against each antimicrobial, as if this were the case then it may be appropriate to use all isolates tested as the denominator. Meanwhile, other studies have used the number of isolates tested against each specific antimicrobial as the denominator (Kibret & Abera, 2014; Talan et al., 2016). The research I have undertaken provides empirical evidence to confirm that using these approaches will underestimate or overestimate the resistance prevalence respectively. Although there may be no ideal solution to this issue, the limitations of whichever denominator is used need to be clearly identified, something not done in other studies (Kibret & Abera, 2014; Wu et al., 2016). This will ensure proper interpretation of the resistance prevalence. Given the increasing resistance noted in the research program, perhaps it may be better to overestimate the resistance prevalence, as it could be argued that underestimation could cause complacency with the use of antimicrobials. To effectively address the increase in AMR, it is important to provide precise estimates of the true picture of the incidence and prevalence of AMR by applying appropriate methodological techniques to the available data, which has been achieved in the research program.

The third major original contribution to knowledge is the demonstrated potential for identification of previously unknown patient groups at high risk of developing an antimicrobial resistant urinary *E. coli* infection; in this case through the health service providing patient care. It has been demonstrated from my research that analysis of antimicrobial susceptibility data can identify potential high risk groups for antimicrobial resistant *E. coli* UTI, such as patients attending after-hours GP clinics. Information on available patient characteristics were included as part of the analysis of the laboratory data used in the research program as evidence shows that certain risk factors such as age and presence of comorbidities may be associated with a higher risk of resistance (Blaettler et al., 2009; Nouvenne et al., 2014). Given the limitation of using secondary data, as discussed previously in section 7.4, the previously identified risk factors identified from the literature

were likely to be incomplete. Hence, I sought to identify other potential risk factors. The identification of patients attending after-hours GP clinics as potentially at high risk of resistant *E. coli* UTI demonstrates the significance and importance of improving the quality of AMR data, as key findings such as this are able to be detected when valid and reliable AMR data are collected. To adequately manage AMR, insights on patient risk factors will assist with developing targeted interventions to control further development and spread of AMR.

After-hours GP clinics were developed as an alternative health service provider to address the increasing use of emergency department services attributed to low acuity patient presentations (Buckley, Curtis, & McGirr, 2010). Australian GPs are significantly more likely than GPs in Canada, Germany, New Zealand and UK to provide after-hours (before 8.30am, after 6pm and during weekends) patient care (Schoen et al., 2006). With the growing demand for after-hours care by patients (Salisbury, 2000) there is potential to use the research findings for the development of interventions targeted specifically towards patients attending after-hours GP clinics. Detection of a higher risk of resistance in patients attending after-hours GP clinics, a community health service, as opposed to a hospital setting might be unexpected. However evidence from study one, where an increasing resistance in the community was noted worldwide, supports this finding and emphasises the need to monitor resistance, particularly in community settings. Data from other countries suggest that prescribing in after-hours GP clinics is high in comparison to office hours GP clinics (ESPAUR Writing Committee, 2015; Huibers, Moth, Christensen, & Vedsted, 2014), although exact reasons for this are not known. High prescribing in this setting could account for the higher resistance risk found in this research program. Whilst there is no published evidence, it could be postulated that patients with UTI presenting to after-hours clinics are similar to patients presenting to the emergency department. These patients are likely to be too unwell to wait for an appointment with a GP during regular office hours and may be more likely to require antimicrobials. Another possible hypothesis could be that GPs working after-hours may be fatigued, which could affect their clinical decision, hence lead to inappropriate prescribing. General practitioners consulting during regular office hours have been found to prescribe antimicrobials more towards the end of the working day as opposed to earlier in the day (Linder et al., 2014). This hypothesis could be tested in after-hours clinics. A direct impact of this research program is to inform and direct decision-

making among the Royal Australian College of General Practitioners to develop specific protocols for after-hours GP clinics. This knowledge from my research may influence the way empirical treatment guidelines are developed, with the potential for guidelines to be tailored specifically to particular patient groups on the basis that those who attend after-hours GP clinics are more likely to have a resistant urinary *E. coli* infection. There is a need to replicate this study in other populations to determine whether similar findings will be seen.

Both national and international surveillance systems monitoring AMR and antimicrobial use in hospital and community settings have the potential to benefit from the knowledge provided in the research program. The knowledge generated from this research program will contribute specifically to ACT Health, as the data used for studies two and three were provided by this organisation and have not previously been analysed in such a way as undertaken in the research program. Feedback on the findings from the research program will be provided to staff of ACT Health with the aim of influencing clinical practice. The findings of the research program form the basis of recommendations for policy and clinical practice as well as potential areas for further research.

7.6 Recommendations

Antimicrobial resistance is a problem for not only humans but also animals and plants (Robinson et al., 2016). One Health is an initiative that recognises the interactions between the health of humans and animals as well as the environment (Gibbs, 2014). Given the complex web of interactions between humans, plants and animals in the environment, with the potential for transmission of resistance from one group to the other (Collignon, 2015; Hristovski, Cvetkovik, Cvetkovik, & Dukoska, 2010), other sectors such as the veterinary, agricultural and environmental health sectors are also directly involved in controlling the development and further spread of AMR. My research program on AMR in urinary *E. coli* infections in humans will contribute to the wider work of One Health.

This thesis focuses on microbiological laboratory data, which is only one aspect of what informs therapeutic management of *E. coli* UTI, which are often treated empirically. Therefore, the thesis could only inform some aspects of decision-making about prescribing given that the thesis aim is not about prescribing practices for treatment of UTI.

Based on the findings of this research program, which contributes to the body of knowledge about AMR in *E. coli* UTI, recommendations for policy, clinical practice and research are proposed. A summary of the recommendations is presented in Table 2. A brief discussion of each recommendation is provided thereafter.

Table 2 Summary of recommendations for addressing AMR in *E. coli* UTI

Topic	Recommendation
Policy	1. Linkage of microbiological laboratory and clinical databases 2. Compulsory completion of minimum patient data on the microbiological laboratory request form when requesting AST for urine samples 3. Development and implementation of policy limiting use of ciprofloxacin as an empirical agent for UTI management 4. Increased state and territory governments funding to microbiology laboratories to improve the range of testing urine samples and all other specimens sent for microbiological laboratory processing
Clinical practice	1. Ongoing education of GPs who consult at after-hours clinics on the judicious use of antimicrobials 2. Education of healthcare staff on the importance of adequately completing laboratory request forms
Research	**Design** 1. Well-designed prospective cohort studies undertaken using patient-based real-time AMR data with the aim of identifying other potential risk factors for AMR development 2. Examination of the association between policy regulation on antimicrobial use and AMR 3. Future studies aimed at linking AMR data to antimicrobial use data **Methodology** 4. Uptake of the new methodological approach for clearly identifying incident cases of resistant *E. coli* UTI 5. Further evaluation of the impact of the denominators selected for calculating resistance prevalence **Reporting** 6. Establishment of an expert panel to develop a standardised classification system for infections, based on the setting of acquisition to allow consistency in reporting as well as comparison of data 7. Improved compliance with reporting guidelines during journal submission processes

7.6.1 Policy

Linkage of microbiological laboratory and clinical databases is highly recommended. This research program has highlighted the weaknesses in the collection and quality of laboratory based data for research purposes and demonstrates the need for improvement in the data collection systems for AMR surveillance. Laboratory data do not contain detailed clinical information of patients and AMR surveillance should ideally involve collection and analysis of both clinical and microbiological data. Surveillance systems that combine clinical and laboratory data are recommended as the combination of both datasets strengthens the quality and richness of the data, thereby enhancing understanding of AMR (World Health Organization, 2002, 2015a). For example, to address the research gap of differences in AMR prevalence based on setting of infection acquisition, I had to manually link microbiological data to the hospital administrative system to obtain additional information needed to stratify samples into hospital- and community-acquired UTI. This recommendation reiterates national and international calls for integration of hospital and laboratory information systems, which will allow for easy extraction of clinical and laboratory data concurrently (Cornaglia et al., 2004; World Health Organization, 2002), although there are ethical issues that still need to be overcome. Linkage of data poses issues of patient privacy and confidentiality as data linkage requires the use of potentially identifying information to ensure accurate linkage, identified 20 years ago (Sibthorpe, Kliewer, & Smith, 1995) but still relevant (Xafis, 2015). Recommended safeguards to ensure protection of patient privacy includes: the submission to the overseeing ethics committee of a detailed research protocol, which conforms to the National Health and Medical Research Council guidelines; the adherence of researchers to ethical codes of conduct; and the organisation releasing the data undertaking the linkage before release to the researchers, without any identifying information (Sibthorpe et al., 1995). Evidence from the Western Australia Data Linkage System highlights the benefits of population level data linkage for health research (Trutwein, Holman, & Rosman, 2006). The recently developed AURA surveillance system should over time look at ways that the surveillance system can be linked with clinical data to improve the quality of the overall surveillance data. To appropriately control AMR, collection and analysis of accurate data must be done.

Compulsory completion of minimum patient data on the microbiological laboratory request form, when requesting AST for urine samples, is recommended. A limitation identified in all

three studies of the research program was the incompleteness of the AMR data, which prevented the detailed analysis of potential patient risk factors that may contribute to development of AMR. Study one of the research program identified issues with the reporting of AMR data in published studies, and studies two and three identified issues with the collection of AMR data in the microbiological laboratory dataset. To improve the quality of AMR data and allow for detailed analysis, compulsory completion of a minimum set of patient data fundamental to AMR control is recommended. The WHO, through the global AMR surveillance system, requires that data on the patient's age, gender, type of specimen, date of collection, site of specimen collection (i.e. hospital or community) and current antimicrobial use, are collected (World Health Organization, 2015b). Completion of these patient parameters is dependent on the requesting clinician and, in most cases, is poorly documented on the laboratory request form. Compulsory completion may be achieved through implementation of an electronic medical record system with electronic ordering of laboratory tests. Evidence suggests that the use of electronic medical records with electronic ordering of tests improves the quality of medical notes and test orders (Burke et al., 2015; Georgiou et al., 2012). By using this system, the requesting clinician will be unable to submit the electronic request form unless the mandatory patient parameters are completed. This approach will contribute to having more reliable AMR data with the potential for better AMR control. The ACT Pathology, through ACT Health, is in the process of trialling an electronic medical record system with electronic test orders (A. Das, personal communication, 11 August, 2016).

Development and implementation of policy limiting use of ciprofloxacin as an empirical agent for UTI management is recommended. The overall knowledge provided by the research program provides a platform on which to institute this recommendation at local, national, regional and international levels. An increasing resistance trend to ciprofloxacin was clearly identified in all three studies in the research program, prompting the need for a renewed effort in the further prevention of spread of resistance to this antimicrobial agent. Australia currently has a policy restricting ciprofloxacin use, which requires that clinicians adhere to specific indications authorised by the Pharmaceutical Benefits Scheme for using ciprofloxacin and other fluoroquinolones (Cheng et al., 2012). Countries without a similar policy need to ensure the development of policies limiting use of ciprofloxacin. Implementation of these policies is also recommended and this could be achieved, for

example, by ensuring that clinicians obtain permission to prescribe ciprofloxacin. Regular monitoring of ciprofloxacin resistance alongside usage rates should be advocated in antimicrobial use and resistance surveillance systems worldwide. This is extremely relevant to the recently developed AURA Surveillance System, which undertakes surveillance of antimicrobial use and resistance in Australia.

It is recommended that state and territory governments increase funding to microbiology laboratories to improve the range of testing urine samples and all other specimens sent for microbiological laboratory processing. This recommendation improves on current microbiological laboratory procedures, in which isolates are first tested against first-line antimicrobials with isolates that are identified as being resistant subjected to further testing against second-line agents. The use of this two phase approach has been attributed to availability of insufficient funds (Bax et al., 2001), hence the need to effectively utilise available resources. Given the potential for testing bias using this approach, with the resultant effect of overestimation of resistance (Hindler & Stelling, 2007), increased funding by state and territory governments to microbiology laboratories, to improve the quality of testing urine samples and all other specimens sent for laboratory processing, is recommended. Improvements in the quality of laboratory testing will ultimately improve the AMR data quality, enabling better interpretation of resistance.

7.6.2 Clinical practice

Whilst further exploration is needed to identify the exact cause, it is recommended that ongoing education on the judicious use of antimicrobial agents, and other risk factors contributing to AMR, is targeted specifically towards GPs who consult at after-hours clinics. This recommendation is guided by knowledge gained from the research program, which identified that patient groups receiving care at after-hours GP clinics were at high risk of developing an antimicrobial resistant urinary *E. coli* infection. As previously discussed the exact reason for this finding is unknown but may be as a result of inappropriate prescribing, therefore warranting the need for open discussion and considering the requirement for clinicians to receive approval from the infectious diseases or microbiology units prior to prescribing all antimicrobial agents. Currently in Australia, Pharmaceutical Benefits Scheme approval is required by clinicians for prescribing specific antimicrobials such as fluoroquinolones, in accordance with antimicrobial stewardship practices in hospitals

(Duguid & Cruickshank, 2011). Evidence from published studies shows that implementation of antimicrobial restriction policies in hospitals leads to decreased use of restricted antimicrobials, with subsequent decrease in their resistance as well as reduction in hospital costs for purchasing antimicrobials (Falagas et al., 2007; Martin et al., 2005). However, these studies have also shown that only restricting the use of some antimicrobials favours the use of unrestricted antimicrobials (Falagas et al., 2007; Martin et al., 2005). Therefore it may be worthwhile restricting the prescription of all antimicrobials to prevent the unwanted effect of subsequent increase in the use of unrestricted antimicrobials. This is an important consideration, especially given the increasing resistance noted for some antimicrobials in the research program. Limitations to the implementation of prescribing restrictions include additional time to contact the infectious diseases unit for approval as well as extra costs to hospitals for staff dedicated specifically for this purpose (Paterson, 2006b). Despite these limitations, implementation of restriction policies for all antimicrobials may eventually promote the judicious use of antimicrobials by clinicians.

Education of healthcare staff on the importance of adequately completing microbiological laboratory request forms is also recommended. As previously highlighted in section 7.4, incompleteness of data was a limitation noted in the research program, affecting the level of analysis undertaken, particularly in relation to individual patient risk factors for development of resistant *E. coli* UTI. Also noted in section 7.6.1, the quality of AMR data is dependent on the requesting clinician collecting the data and often tends to be poorly documented. Adequately completed laboratory request forms provide detailed information that, as well as being important for pathology staff, can be included in the analysis of AMR data and subsequently inform the development of AMR control policies and interventions.

7.6.3 Research

A number of areas that warrant further research were identified, as well as further testing of new methods and recommendations for the reporting of the findings of research. These recommendations are discussed below.

Design

It is recommended that well-designed prospective cohort studies using patient-based real-time AMR data are undertaken with the aim of identifying other potential risk factors for

AMR development. This research program focused on retrospective routine susceptibility data but there is a need to conduct prospective studies using patient-based real-time data, because this will help identify other predictors of AMR in patients with UTI not identified in the research program. Effective monitoring of resistance patterns with the use of real-time data will also provide detailed insights to guide the development of appropriate responses to controlling AMR.

Examination of the association between policy regulation on antimicrobial use and AMR is recommended. The first study in the research program found that developing countries have higher resistance to ciprofloxacin compared to developed countries. The reason for the higher resistance in developing countries was thought to be as a result of the use of non-prescription antimicrobials and over-the-counter prescribing, which is relatively common in developing countries (Sosa et al., 2010). If an association exists between policy regulations on antimicrobial use and AMR in countries that have such policies, in comparison to those that do not, this may provide further evidence to justify policy development regulating non-prescription antimicrobial use in countries lacking this policy.

It is also recommended that future studies should aim at linking AMR data to antimicrobial use data to enhance the interpretation of findings to inform development of interventions. Analysis of hospital antimicrobial use data (reported in Chapter Five) undertaken as part of the research program could not be linked to the only available AMR data because the latter comprised both hospital and community data. Furthermore, analysis of data on antimicrobial use should also include data on the appropriateness of use. Data on the appropriateness of antimicrobial use will provide information on effectiveness of prescribing practices in accordance with therapeutic guidelines.

Methods

Uptake of the new methodological approach for clearly identifying incident cases of resistant *E. coli* UTI is recommended for use internationally. The research program provides good quality evidence on a suitable and rigorous approach to calculating resistance incidence, given that most studies report on prevalence of resistance. The research program finding supports a minimum dataset that comprises: the unique patient identifier; all urine samples processed over a minimum of one year for each individual patient; date of urine

sample submission to the laboratory; all urine culture results with colony counts; antimicrobials tested; and antimicrobial susceptibility test results are recommended for calculation of the incidence of resistance. The approach discussed in the methods chapter (Chapter Three) of the thesis can be applied by other researchers to provide estimates of resistance incidence.

Further evaluation of the impact of denominators selected for calculating resistance prevalence is recommended. The research undertaken provides empirical evidence on how resistance prevalence may either be overestimated or underestimated, depending on the choice of denominator, which is using only the number of isolates tested against each antimicrobial agent or using the total number of isolates tested respectively. The impact of whichever denominator is selected on the resistance prevalence must be clearly evaluated in publications or reports on AMR, to enable clinicians to adequately interpret the resistance prevalence. Although using the number of isolates tested against each antimicrobial agent as the denominator may overestimate the resistance prevalence, clinicians may be more likely to refrain from overjudicious use of antimicrobials given higher resistance prevalence as opposed to using a denominator which appears to underestimate the resistance thereby giving a false impression that resistance is low, favouring greater use of antimicrobials.

Reporting

Establishment of an expert panel to develop an internationally accepted standardised classification system for infections, based on the setting of acquisition to allow consistency in reporting as well as comparison of data, is recommended. As highlighted in the methods chapter (Chapter Three), there are many definitions being applied to classify infections based on the setting of acquisition into the broad healthcare-associated infections, including hospital-acquired as well as community-acquired. An expert panel comprising representatives from notable organisations, such as the CDC and WHO, could develop a standardised classification system that can be used internationally thus facilitating consistency in reporting as well as comparison of data. This approach was used for defining multidrug-, extensively drug- and pandrug-resistant bacteria and the proposed definitions have been taken up internationally and frequently cited in published literature (Ansari et al., 2015; Basak, Singh, & Rajurkar, 2016; Bhatt, Tandel, Shete, & Rathi, 2015).

Improved compliance with reporting guidelines during journal submission processes is recommended. In regards to the methodological aspects of research, the poor reporting of research studies, which have been highlighted as a major issue (Altman & Simera, 2010), were also identified in the research program. This necessitates recommending better compliance with reporting guidelines during journal submission processes. Currently some peer reviewed journals add to the journal submission process the compulsory completion of reporting guidelines depending on the study design (such as the PRISMA guidelines for systematic reviews, STROBE guidelines for observational studies and CONSORT guidelines for randomised controlled trials), to optimise transparency and accuracy of reporting. All journals should include the completion of reporting guidelines as a compulsory component of the submission process, and not use tick boxes but mandate that prospective authors include the page numbers from their manuscript which provide the required information. Journals should aim to ask peer reviewers to assess the completed guidelines along with the manuscript as part of the peer review process.

7.7 Summary and conclusion

In the research program undertaken, I have obtained, collated, analysed and evaluated antimicrobial resistance data to evaluate antimicrobial resistance patterns in *E. coli* UTI. The overall research findings have contributed insight and new knowledge to better understand AMR patterns and trends in urinary *E. coli* infections. The research program provides an indication of the current state of ciprofloxacin resistance globally and also makes available information on AMR at a jurisdictional level. Hence the findings are applicable not only nationally but also on a wider scale. The research undertaken in the thesis has been especially timely because it coincided with the development of the AURA surveillance system, with the recent release of the first national report. Dissemination of the research findings at the local level to ACT Health staff, including my collaboration with ACT Pathology, will inform local and national level surveillance activities, improvements in the surveillance system and support the national agenda for monitoring of AMR in this critically important bacterium. It is hoped that the recommendations made in the thesis, based on the evidence from the research undertaken, can be instituted to help maintain the effectiveness of currently available antimicrobials.

The health and economic implications of antimicrobial resistant urinary *E. coli* infections justify the need to continue to undertake research in this area. Furthermore, the resistance patterns of microorganisms to antimicrobials are constantly changing, therefore regular surveillance must continue. The findings of this research program establish a baseline against which subsequent future progress in ACT jurisdictional level surveillance can be compared. In accordance with the One Health concept, AMR surveillance in humans alone cannot tackle the problem of resistance, hence the findings will add to the wider work on AMR being undertaken by other sectors directly involved with tackling the issue of AMR, such as the veterinary, agricultural and environmental health sectors.

In conclusion, the research program emphasises the urgency for all levels of government, including all public and private healthcare services, healthcare professionals, academic institutions and the general public, to be united and work together towards tackling the issue of AMR. Without a concerted effort on all sides, resistance may continue to rise and with the lack of development of new antimicrobial agents, global healthcare may return to the 'pre-antibiotic era', where antimicrobials were unavailable and death from infection was common.

References

Achermann, R., Suter, K., Kronenberg, A., Gyger, P., Mühlemann, K., Zimmerli, W., & Bucher, H. C. (2011). Antibiotic use in adult outpatients in Switzerland in relation to regions, seasonality and point of care tests. *Clinical Microbiology and Infection, 17*(6), 855-861.

ACT Government. (2015a). *About ACT pathology*. Retrieved from http://www.health.act.gov.au/our-services/act-pathology/about-act-pathology

ACT Government. (2015b). *About Canberra Hospital*. Retrieved from http://www.health.act.gov.au/our-services/canberra-hospital-campus/about-canberra-hospital

Adam, H. J., Baxter, M. R., Davidson, R. J., Rubinstein, E., Fanella, S., Karlowsky, J. A., . . . Zhanel, G. (2013). Comparison of pathogens and their antimicrobial resistance patterns in paediatric, adult and elderly patients in Canadian hospitals. *Journal of Antimicrobial Chemotherapy, 68*(suppl 1), i31-i37.

Adefurin, A., Sammons, H., Jacqz-Aigrain, E., & Choonara, I. (2011). Ciprofloxacin safety in paediatrics: A systematic review. *Archives of Disease in Childhood, 96*(9), 874-880.

Ahmad, S. (2012). Pattern of urinary tract infection in Kashmir and antimicrobial susceptibility. *Bangladesh Medical Research Council Bulletin, 38*(3), 79-83.

Akoachere, J. F., Yvonne, S., Akum, N. H., & Seraphine, E. N. (2012). Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. *BMC Research Notes, 5*(219). doi: 10.1186/1756-0500-5-219

Akram, M., Shahid, M., & Khan, A. U. (2007). Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobials, 6*(4). doi:10.1186/1476-0711-6-4

Alam, M. F., Cohen, D., Butler, C., Dunstan, F., Roberts, Z., Hillier, S., & Palmer, S. (2009). The additional costs of antibiotics and re-consultations for antibiotic-resistant *Escherichia coli* urinary tract infections managed in general practice. *International Journal of Antimicrobial Agents, 33*(3), 255-257.

Al-Badr, A., & Al-Shaikh, G. (2013). Recurrent urinary tract infections management in women: A review. *Sultan Qaboos University Medical Journal, 13*(3), 359-367.

Aldred, K. J., Kerns, R. J., & Osheroff, N. (2014). Mechanism of quinolone action and resistance. *Biochemistry, 53*(10), 1565-1574.

Al-Hasan, M. N., Eckel-Passow, J. E., & Baddour, L. M. (2010). Bacteremia complicating Gram-negative urinary tract infections: A population-based study. *Journal of Infection, 60*(4), 278-285.

Aliyu, A. A., Bello, M. U., Kasim, R., & Martin, D. (2014). Positivist and non-positivist paradigm in social science research: Conflicting paradigms or perfect partners? *Journal of Management and Sustainability, 4*(3), 79-95.

Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). *Escherichia coli* in Europe: An overview. *International Journal of Environmental Research and Public Health, 10*(12), 6235-6254.

Alós, J. I., Serrano, M. G., Gómez-Garcés, J. L., & Perianes, J. (2005). Antibiotic resistance of *Escherichia coli* from community-acquired urinary tract infections in relation to demographic and clinical data. *Clinical Microbiology and Infection, 11*(3), 199-203.

Al Sweih, N., Jamal, W., & Rotimi, V. (2005). Spectrum and antibiotic resistance of uropathogens isolated from hospital and community patients with urinary tract infections in two large hospitals in Kuwait. *Medical Principles and Practice, 14*(6), 401-407.

Al-Tawfiq, J. A., & Anani, A. A. (2009). Antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infections in a Saudi Arabian hospital. *Chemotherapy, 55*(2), 127-131.

Altman, D. G., & Simera, I. (2010). Responsible reporting of health research studies: Transparent, complete, accurate and timely. *Journal of Antimicrobial Chemotherapy, 65*(1). doi: 10.1093/jac/dkp410

Aminov, R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. *Frontiers in Microbiology, 1*(134). doi: 10.3389/fmicb.2010.00134

Ansari, S., Nepal, H. P., Gautam, R., Shrestha, S., Neopane, P., Gurung, G., & Chapagain, M. L. (2015). Community-acquired multi-drug resistant clinical isolates of *Escherichia coli* in a tertiary care center of Nepal. *Antimicrobial Resistance and Infection Control, 4*(15). doi:10.1186/s13756-015-0059-2

Ansbach, R. K., Dybus, K., & Bergeson, R. (2005). Uncomplicated *E. coli* urinary tract infection in college women: A follow-up study of *E. coli* sensitivities to commonly prescribed antibiotics. *Journal of American College Health, 54*(2), 81-84.

Antibiotic Expert Groups. (2014). *Therapeutic guidelines: Antibiotic. Version 15*. Melbourne: Therapeutic Guidelines Limited.

Apisarnthanarak, A., Kiratisin, P., Saifon, P., Kitphati, R., Dejsirilert, S., & Mundy, L. M. (2007). Clinical and molecular epidemiology of community-onset, extended-spectrum beta-lactamase-producing *Escherichia coli* infections in Thailand: A case-case-control study. *American Journal of Infection Control, 35*(9), 606-612.

Arabi, F., & Banazadehi, A. (2013). Prevalence and antimicrobial susceptibility patterns of uropathogens among patients referring to Valieasr laboratory in Najafabad, Isfahan. *Iran Middle Eastern Journal Science Research, 13*(1), 85-90.

Araújo, S. M. H. A., Mourão, T. C., Oliveira, J. L., Melo, I. F. S., Araújo, C. A. A., Araújo, N. A. A., . . . Daher, E. F. (2011). Antimicrobial resistance of uropathogens in women with acute uncomplicated cystitis from primary care settings. *International Urology and Nephrology, 43*(2), 461-466.

Archer, K. J., & Lemeshow, S. (2006). Goodness-of-fit test for a logistic regression model fitted using survey sample data. *Stata Journal, 6*(1), 97-105.

Arslan, H., Azap, O. K., Ergönül, O., Timurkaynak, F., Aydin, K., Bakir, M., . . . Willke-Topçu, A. (2005). Risk factors for ciprofloxacin resistance among *Escherichia coli* strains isolated from community-acquired urinary tract infections in Turkey. *Journal of Antimicrobial Chemotherapy, 56*(5), 914-918.

Astal, Z. (2005). Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in the Gaza Strip. *Singapore Medical Journal, 46*(9), 457-460.

Australian Bureau of Statistics. (2008). 2033.0.55.001 - *Census of Population and Housing: Socio-Economic Indexes for Areas (SEIFA), Australia, 2006*. Retrieved from http://www.abs.gov.au/ausstats/abs@.nsf/DetailsPage/2033.0.55.0012011?OpenDocument

Australian Bureau of Statistics. (2015). Catalogue Number 3235.0 - *Population by Age and Sex, Regions of Australia*. Retrieved from http://www.abs.gov.au/ausstats/abs@.nsf/Latestproducts/3235.0Main%20Features502015?opendocument&tabname=Summary&prodno=3235.0&issue=2015&num=&view=

Australian Bureau of Statistics. (2016). Catalogue Number 3101.0 - *Australian Demographic Statistics, Dec 2015*. Retrieved from http://www.abs.gov.au/ausstats/abs@.nsf/0/BCDDE4F49C8A3D1ECA257B8F00126F77?Opendocument

Australian Bureau of Statistics. (2016). 4839.0 - *Patient Experiences in Australia: Summary of Findings, 2015-16*. Retrieved from http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4839.0~2015-16~Main%20Features~General%20practitioners~2

Australian Capital Territory Pathology. (2013). *Microbiology department urine manual*. Australian Capital Territory: ACT Pathology.

Australian Commission on Safety and Quality in Health Care. (2012). *National Safety and Quality Health Service Standards*. Retrieved from https://www.safetyandquality.gov.au/wp-content/uploads/2011/09/NSQHS-Standards-Sept-2012.pdf

Australian Commission on Safety and Quality in Health Care. (2013a). *Australian One Health Antimicrobial Resistance Colloquium Background Paper*. Retrieved from https://safetyandquality.gov.au/wp-content/uploads/2013/07/Briefing-paper-for-One-Health-AMR-Colloquium-participants-Final-Jul-2013.pdf

Australian Commission on Safety and Quality in Health Care. (2013b). *A Report of the Australian One Health Antimicrobial Resistance Colloquium*. Retrieved from https://www.health.gov.au/internet/main/publishing.nsf/Content/1803C433C71415CACA257C8400121B1F/$File/Colloquium-Report-Final-Feb2014.pdf

Australian Commission on Safety and Quality in Health Care. (2015). *Antimicrobial prescribing practice in Australian hospitals: results of the 2014 National Antimicrobial Prescribing Survey*. Retrieved from https://www.safetyandquality.gov.au/wp-content/uploads/2015/07/Antimicrobial-prescribing-practice-in-Aust-hospitals-NAPS-2014-Results.pdf

Australian Commission on Safety and Quality in Health Care. (2016). *AURA 2016: first Australian report on antimicrobial use and resistance in human health*. Retrieved from https://www.safetyandquality.gov.au/wp-content/uploads/2016/06/AURA-2016-First-Australian-Report-on-Antimicroibal-use-and-resistance-in-human-health.pdf

Australian Commission on Safety and Quality in Health Care, & SA Health. (2015). *Antimicrobial use in hospitals: 2014 report of the National Antimicrobial Utlisation Surveillance Program.* Retrieved from https://www.safetyandquality.gov.au/wp-content/uploads/2015/09/2014-NAUSP-Report-AU-Australian-Hospitals.pdf

Australian Government. (2015). *Responding to the threat of antimicrobial resistance: Australia's First Antimicrobial Resistance Strategy 2015-2019*. Canberra ACT: Commonwealth of Australia. Retrieved from http://www.health.gov.au/internet/main/publishing.nsf/content/1803C433C71415CACA257C8400121B1F/$File/amr-strategy-2015-2019.pdf.

Australian Government. (2016a). *The Australian continent*. Retrieved from http://www.australia.gov.au/about-australia/our-country/the-australian-continent

Australian Government. (2016b). *State and Territory Government*. Retrieved from http://www.australia.gov.au/about-government/how-government-works/state-and-territory-government

Australian Government. (2017). *Medicare Benefits Schedule Book - The February 2017 Medicare Benefits Schedule*. Retrieved from http://www.mbsonline.gov.au/internet/mbsonline/publishing.nsf/Content/4A79A04857852120CA2580B1001E5978/$File/201702-MBS.pdf.

Australian Group on Antimicrobial Resistance. (2011). *2009 Antimicrobial Susceptibility Report: Gram-negative Survey*. Retrieved from http://www.agargroup.org/files/AGAR%20GNB09%20Final%20Report.pdf

Australian Group on Antimicrobial Resistance. (2012). *2011 Antimicrobial susceptibility report: Gram-negative Survey*. Retrieved from http://www.agargroup.org/files/AGAR%20GNB11%20Report%20FINAL.pdf

Australian Group on Antimicrobial Resistance. (2013). *2012 Antimicrobial Susceptibility Report: Gram-negative Survey*. Retrieved from http://www.agargroup.org/files/AGAR%20GNB12%20Report%20FINAL.pdf

Australian Institute of Health and Welfare. (2014). *Australia's health 2014*. Retrieved from http://www.aihw.gov.au/australias-health/2014/health-system/

Australian Institue of Health and Welfare. (2016). *Australian refined diagnosis-related groups (AR-DRG) data cubes*. Retrieved from http://www.aihw.gov.au/hospitals-data/ar-drg-data-cubes/

Azap, Ö., Arslan, H., Şerefhanoğlu, K., Çolakoğlu, Ş., Erdoğan, H., Timurkaynak, F., & Senger, S. (2010). Risk factors for extended-spectrum β-lactamase positivity in uropathogenic *Escherichia coli* isolated from community-acquired urinary tract infections. *Clinical Microbiology and Infection, 16*(2), 147-151.

Bahadin, J., Teo, S. S., & Mathew, S. (2011). Aetiology of community-acquired urinary tract infection and antimicrobial susceptibility patterns of uropathogens isolated. *Singapore Medical Journal, 52*(6), 415-420.

Bahagon, Y., Raveh, D., Schlesinger, Y., Rudensky, B., & Yinnon, A. (2007). Prevalence and predictive features of bacteremic urinary tract infection in emergency department patients. *European Journal of Clinical Microbiology & Infectious Diseases, 26*(5), 349-352.

Baker, W., White, C., Cappelleri, J., Kluger, J., & Coleman, C. (2009). Understanding heterogeneity in meta-analysis: the role of meta-regression. *International Journal of Clinical Practice, 63*(10), 1426-1434.

Balakrishnan, N. (2014). *Methods and applications of statistics in clinical trials, Volume 2 : Planning, analysis, and inferential methods*. Somerset, US: Wiley.

Basak, S., Singh, P., & Rajurkar, M. (2016). Multidrug resistant and extensively drug resistant bacteria: A study. *Journal of Pathogens.* doi:10.1155/2016/4065603

Bax, R., Bywater, R., Cornaglia, G., Goossens, H., Hunter, P., Isham, V., . . . Sahm, D. (2001). Surveillance of antimicrobial resistance—what, how and whither? *Clinical Microbiology and Infection, 7*(6), 316-325.

Bergman, M., Nyberg, S. T., Huovinen, P., Paakkari, P., Hakanen, A. J., & and the Finnish Study Group for Antimicrobial Resistance. (2009). Association between antimicrobial consumption and resistance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy, 53*(3), 912-917.

Bhatt, P., Tandel, K., Shete, V., & Rathi, K. (2015). Burden of extensively drug-resistant and pandrug-resistant Gram-negative bacteria at a tertiary-care centre. *New Microbes and New Infections, 8*, 166-170.

Bishara, J., Goldberg, E., Leibovici, L., Samra, Z., Shaked, H., Mansur, N., & Paul, M. (2012). Healthcare-associated vs. hospital-acquired *Staphylococcus aureus* bacteremia. *International Journal of Infectious Diseases, 16*(6), e457-e463.

Biswas, D., Gupta, P., Prasad, R., Singh, V., Arya, M., & Kumar, A. (2006). Choice of antibiotic for empirical therapy of acute cystitis in a setting of high antimicrobial resistance. *Indian Journal of Medical Sciences, 60*(2), 53-58.

Blaettler, L., Mertz, D., Frei, R., Elzi, L., Widmer, A., Battegay, M., & Flückiger, U. (2009). Secular trend and risk factors for antimicrobial resistance in *Escherichia coli* isolates in Switzerland 1997–2007. *Infection, 37*(6), 534-539.

Bland, J. M., & Altman, D. G. (2000). The odds ratio. *BMJ, 320*(7247), 1468.

Blettner, M., Sauerbrei, W., Schlehofer, B., Scheuchenpflug, T., & Friedenreich, C. (1999). Traditional reviews, meta-analyses and pooled analyses in epidemiology. *International Journal of Epidemiology, 28*(1), 1-9.

Bouchillon, S. K., Badal, R. E., Hoban, D. J., & Hawser, S. P. (2013). Antimicrobial susceptibility of inpatient urinary tract isolates of Gram-negative bacilli in the United States: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) Program: 2009− 2011. *Clinical Therapeutics, 35*(6), 872-877.

Box, G. E., & Jenkins, G. M. (1976). *Time series analysis: forecasting and control* (Revised ed.). San Francisco: Holden-Day.

Bradburn, M. J., Clark, T. G., Love, S., & Altman, D. (2003). Survival analysis part II: multivariate data analysis – An introduction to concepts and methods. *British Journal of Cancer, 89*(3), 431-436.

Bryce, A., Hay, A. D., Lane, I. F., Thornton, H. V., Wootton, M., & Costelloe, C. (2016). Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by *Escherichia coli* and association with routine use of antibiotics in primary care: Systematic review and meta-analysis. *BMJ, 352*. doi:10.1136/bmj.i939

Bryskier, A. (2005). *Antimicrobial agents: Antibacterials and antifungals (2)*. Washington, US: ASM Press.

Buckley, D. J., Curtis, P. W., & McGirr, J. G. (2010). The effect of a general practice after-hours clinic on emergency department presentations: A regression time series analysis. *Medical Journal of Australia, 192*(8), 448-451.

Burd, E. M., & Kehl, K. S. (2011). A critical appraisal of the role of the clinical microbiology laboratory in the diagnosis of urinary tract infections. *Journal of Clinical Microbiology, 49*(suppl 9), S34-S38.

Burke, H. B., Sessums, L. L., Hoang, A., Becher, D. A., Fontelo, P., Liu, F., . . . Baxi, N. S. (2015). Electronic health records improve clinical note quality. *Journal of the American Medical Informatics Association*, *22*(1), 199-205.

Buttner, P., & Muller, R. (2011). *Epidemiology*. Victoria, Australia: Oxford University Press.

Cairns, K. A., Jenney, A., Abbott, I. J., Skinner, M. J., Doyle, J. S., Dooley, M., & Cheng, A. C. (2013). Prescribing trends before and after implementation of an antimicrobial stewardship program. *Medical Journal of Australia, 198*(5), 262-266.

Cairns, K. A., Roberts, J. A., Cotta, M. O., & Cheng, A. C. (2015). Antimicrobial stewardship in Australian hospitals and other settings. *Infectious Diseases and Therapy, 4*(1), 27-38.

Callan, A., O'Shea, E., Galvin, S., Duane, S., Corry, O., Simple Team, T., & Vellinga, A. (2014). The economic cost of urinary tract infections in the community: Results from Ireland. *Value in Health, 17*(7), A468.

Cantón, R. (2005). Role of the microbiology laboratory in infectious disease surveillance, alert and response. *Clinical Microbiology and Infection, 11*(suppl 1), 3-8.

Cardoso, T., Almeida, M., Friedman, N. D., Aragão, I., Costa-Pereira, A., Sarmento, A. E., & Azevedo, L. (2014). Classification of healthcare-associated infection: a systematic review 10 years after the first proposal. *BMC Medicine, 12*(40). doi: 10.1186/1741-7015-12-40

Center for Disease Dynamics, Economics and Policy. (2015a). *State of the world's antibiotics, 2015*. Retrieved from https://cddep.org/sites/default/files/swa_2015_final.pdf

Center for Disease Dynamics, Economics and Policy. (2015b). *ResistanceMap*. Retrieved from http://resistancemap.cddep.org/resmap/resistance

Centers for Disease Control and Prevention. (2012). *Measures of risk principles of epidemiology in public health practice - An introduction to applied epidemiology and biostatistics (Third edition.).* Retrieved from http://www.cdc.gov/ophss/csels/dsepd/ss1978/ss1978.pdf

Centers for Disease Control and Prevention. (2013). *Antibiotic resistance threats in the United States*. Retrieved from http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf

Centers for Disease Control and Prevention. (2015). *Urinary tract infection (Catheter-Associated Urinary Tract Infection [CAUTI] and non-catheter-associated Urinary Tract Infection [UTI]) and other Urinary System Infection [USI]) events.* Retrieved from http://www.cdc.gov/nhsn/PDFs/pscManual/7pscCAUTIcurrent.pdf

Centers for Disease Control and Prevention. (2016). *Identifying Healthcare-associated Infections (HAI) for NHSN surveillance*. Retrieved from http://www.cdc.gov/nhsn/PDFs/pscManual/2PSC_IdentifyingHAIs_NHSNcurrent.pdf

Centers for Disease Control and Prevention, & National Center for Health Statistics. (2015). *2009-2010 Combined year tables - Combined NAMCS and NHAMCS.* Retrieved from http://www.cdc.gov/nchs/data/ahcd/combined_tables/2009-2010_combined_web_table01.pdf

Cheng, A. C., Turnidge, J., Collignon, P., Looke, D., Barton, M., & Gottlieb, T. (2012). Control of fluoroquinolone resistance through successful regulation, Australia. *Emerging Infectious Diseases, 18*(9), 1453-1460.

Cheng, H. G., & Phillips, M. R. (2014). Secondary analysis of existing data: opportunities and implementation. *Shanghai Archives of Psychiatry, 26*(6), 371-375.

Chulain, M. N., Murray, A.-M., Corbett-Feeney, G., & Cormican, M. (2005). Antimicrobial resistance in *E. coli* associated with urinary tract infection in the West of Ireland. *Irish Journal of Medical Science, 174*(4), 6-9.

Ciani, O., Grassi, D., & Tarricone, R. (2013). An economic perspective on urinary tract infection: the "costs of resignation". *Clinical Drug Investigation, 33*(4), 255-261.

Clark, T., Bradburn, M., Love, S., & Altman, D. (2003). Survival analysis part I: Basic concepts and first analyses. *British Journal of Cancer, 89*(2), 232-238.

Clinical and Laboratory Standards Institute. (2014). *Performance standards for antimicrobial susceptibility testing: Twenty-fourth informational supplement M100-S24*. Wayne, Pennsylvania USA: CLSI.

Cohen, M. L. (1992). Epidemiology of drug resistance: Implications for a post-antimicrobial era. *Science, 257*(5073), 1050-1055.

Collignon, P. (2015). Antibiotic resistance: are we all doomed? *Internal Medicine Journal, 45*(11), 1109-1115.

Cooper, H., Hedges, L. V., & Valentine, J. C. (2009). *The handbook of research synthesis and meta-analysis* (2nd Edition). New York: Russell Sage Foundation.

Cornaglia, G., Hryniewicz, W., Jarlier, V., Kahlmeter, G., Mittermayer, H., Stratchounski, L., & Baquero, F. (2004). European recommendations for antimicrobial resistance surveillance. *Clinical Microbiology and Infection, 10*(4), 349-383.

Costelloe, C., Metcalfe, C., Lovering, A., Mant, D., & Hay, A. D. (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: Systematic review and meta-analysis. *BMJ, 340*. doi:10.1136/bmj.c2096

Cox, D. R. (1972). Regression models and life-tables. *Journal of the Royal Statistical Society. Series B (Methodological), 34*(2), 187-220.

Coxeter, P., Looke, D., Hoffmann, T., Lowe, J., & Del Mar, C. (2013). The antibiotic crisis: Charting Australia's path towards least resistance. *Australian and New Zealand Journal of Public Health, 37*(5), 403-404.

Craig, J. C., Irwig, L. M., Knight, J. F., & Roy, L. P. (1997). Trends in the health burden due to urinary tract infection in children in Australia. *Journal of Paediatrics and Child Health, 33*(5), 434-438.

Crawley, M. J. (2012). *The R Book* (2nd ed.). United Kingdom: Wiley.

Crowther, M. A., & Cook, D. J. (2007). Trials and tribulations of systematic reviews and meta-analyses. *ASH Education Program Book, 2007*(1), 493-497.

Cullen, I. M., Manecksha, R. P., McCullagh, E., Ahmad, S., O'Kelly, F., Flynn, R. J., . . . Fennell, J. P. (2012). The changing pattern of antimicrobial resistance within 42,033 *Escherichia coli* isolates from nosocomial, community and urology patient-specific urinary tract infections, Dublin, 1999–2009. *BJU International, 109*(8), 1198-1206.

Dagan, R., Barkai, G., Givon-Lavi, N., Sharf, A. Z., Vardy, D., Cohen, T., . . . Greenberg, D. (2008). Seasonality of antibiotic-resistant *Streptococcus pneumoniae* that causes acute otitis media: A clue for an antibiotic-restriction policy? *The Journal of Infectious Diseases, 197*(8), 1094-1102.

Daghrir, R., & Drogui, P. (2013). Tetracycline antibiotics in the environment: a review. *Environmental Chemistry Letters, 11*(3), 209-227.

Dalhoff, A. (2012a). Global fluoroquinolone resistance epidemiology and implications for their clinical use. *Interdisciplinary Perspectives on Infectious Diseases*. doi:10.1155/2012/976273

Dalhoff, A. (2012b). Resistance surveillance studies: a multifaceted problem—the fluoroquinolone example. *Infection, 40*(3), 239-262.

Dancer, S. J. (2013). Infection control in the post-antibiotic era. *Healthcare Infection, 18*(2), 51-60.

Dash, M., Padhi, S., Mohanty, I., Panda, P., & Parida, B. (2013). Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India. *Journal of Family and Community Medicine, 20*(1), 20-26.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417-433.

de With, K., Bergner, J., Bühner, R., Dörje, F., Gonnermann, C., Haber, M., . . . Kern, W. V. (2004). Antibiotic use in German university hospitals 1998–2000 (Project INTERUNI-II). *International Journal of Antimicrobial Agents, 24*(3), 213-218.

Deeks, J. J., Altman, D. G., & Bradburn, M. J. (2008). Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In M.

Egger, G. D. Smith & D. G. Altman, *Systematic Reviews in Health Care* (pp. 285-312). London, UK: BMJ Publishing Group.

Department of Health Western Australia. (2012). *Admission, readmission, discharge and transfer policy for WA Health Services*. Retrieved from http://www.health.wa.gov.au/circularsnew/attachments/718.pdf

DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. *Controlled Clinical Trials, 7*(3), 177-188.

Dickey, D. A., & Fuller, W. A. (1979). Distribution of the estimators for autoregressive time series with a unit root. *Journal of the American Statistical Association, 74*(366a), 427-431.

Dimitrov, T. S., Udo, E. E., Awni, F., Emara, M., & Passadilla, R. (2004). Etiology and antibiotic susceptibility patterns of community-acquired urinary tract Infections in a Kuwait Hospital. *Medical Principles and Practice, 13*(6), 334-339.

Doern, G. V. (2011). Antimicrobial susceptibility testing. *Journal of Clinical Microbiology, 49*(suppl 9), S4.

Duguid, M., & Cruickshank, M. (2011). *Antimicrobial stewardship in Australian hospitals*. Retrieved from https://www.safetyandquality.gov.au/wp-content/uploads/2011/01/Antimicrobial-stewardship-in-Australian-Hospitals-2011.pdf

Egger, M., Dickersin, K., & Smith, G. (2008). Problems and limitations in conducting systematic reviews. In M. Egger, G. D. Smith & D. G. Altman, *Systematic Reviews in Health Care* (pp. 43-68). London, UK: BMJ Publishing Group.

Egger, M., & Smith, G. (2008). Principles of and procedures for systematic reviews. In M. Egger, G. D. Smith & D. G. Altman, *Systematic Reviews in Health Care* (pp. 23-42). London, UK: BMJ Publishing Group.

Egger, M., Smith, G., & Schneider, M. (2008). Systematic Reviews of Observational Studies. In M. Egger, G. D. Smith & D. G. Altman, *Systematic Reviews in Health Care* (pp. 211-227). London, UK: BMJ Publishing Group.

Egger, M., Smith, G., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ, 315*(7109), 629-634.

Eliopoulos, G. M., Cosgrove, S. E., & Carmeli, Y. (2003). The impact of antimicrobial resistance on health and economic outcomes. *Clinical Infectious Diseases, 36*(11), 1433-1437.

Eshetie, S., Unakal, C., Gelaw, A., Ayelign, B., Endris, M., & Moges, F. (2015). Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. *Antimicrobial Resistance and Infection Control, 4*(12). doi: 10.1186/s13756-015-0054-7

Public Health England. (2015). *English surveillance programme for antimicrobial utilisation and resistance (ESPAUR) 2010 to 2014.* Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/477962/ESPAUR_Report_2015.pdf

European Centre for Disease Prevention and Control. (2009). *The bacterial challenge: Time to react*. Retrieved from http://ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf

European Centre for Disease Prevention and Control. (2013). *Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2011-2012*. Retrieved from http://ecdc.europa.eu/en/publications/publications/healthcare-associated-infections-antimicrobial-use-pps.pdf

Euser, A. M., Zoccali, C., Jager, K. J., & Dekker, F. W. (2009). Cohort studies: Prospective versus retrospective. *Nephron Clinical Practice, 113*(3), c214-c217.

Falagas, M., Bliziotis, I., Michalopoulos, A., Sermaides, G., Papaioannou, V., Nikita, D., & Choulis, N. (2007). Effect of a policy for restriction of selected classes of antibiotics on antimicrobial drug cost and resistance. *Journal of Chemotherapy, 19*(2), 178-184.

Falagas, M., & Karageorgopoulos, D. (2009). Extended-spectrum β-lactamase-producing organisms. *Journal of Hospital Infection, 73*(4), 345-354.

Farshad, S., Anvarinejad, M., Tavana, A. M., Ranjbar, R., Japoni, A., Zadegan, R. M., & Alborzi, A. (2011). Molecular epidemiology of *Escherichia coli* strains isolated from children with community acquired urinary tract infections. *African Journal of Microbiology Research, 5*(26), 4476-4483.

Fasugba, O., Gardner, A., Mitchell, B. G., & Mnatzaganian, G. (2015). Ciprofloxacin resistance in community-and hospital-acquired *Escherichia coli* urinary tract infections: A systematic review and meta-analysis of observational studies. *BMC Infectious Diseases, 15*(545). doi:10.1186/s12879-015-1282-4

Fasugba, O., Mitchell, B. G., Mnatzaganian, G., Das, A., Collignon, P., & Gardner, A. (2016). Five-year antimicrobial resistance patterns of urinary *Escherichia coli* at an Australian tertiary hospital: Time series analyses of prevalence data. *PLoS ONE, 11*(10). doi:10.1371/journal.pone.0164306

Filius, P., Liem, T. Y., van der Linden, P. D., Janknegt, R., Natsch, S., Vulto, A. G., & Verbrugh, H. A. (2005). An additional measure for quantifying antibiotic use in hospitals. *Journal of Antimicrobial Chemotherapy, 55*(5), 805-808.

Fishman, N. (2006). Antimicrobial stewardship. *The American Journal of Medicine, 119*(6A), S53-S61.

Fleming, A. (1929). On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *British Journal of Experimental Pathology, 10*(3), 226.

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology, 13*(5), 269-284.

Foxman, B. (1990). Recurring urinary tract infection: incidence and risk factors. *American Journal of Public Health, 80*(3), 331-333.

Foxman, B. (2002). Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. *The American Journal of Medicine, 113*(1), 5-13.

Foxman, B. (2003). Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. *Disease-a-Month, 49*(2), 53-70.

Foxman, B., & Brown, P. (2003). Epidemiology of urinary tract infections: Transmission and risk factors, incidence, and costs. *Infectious Disease Clinics of North America, 17*(2), 227-241.

Foxman, B., & Frerichs, R. R. (1985). Epidemiology of urinary tract infection: I. Diaphragm use and sexual intercourse. *American Journal of Public Health, 75*(11), 1308-1313.

Freeman, J., Anderson, D., & Sexton, D. (2009). Seasonal peaks in Escherichia coli infections: Possible explanations and implications. *Clinical Microbiology and Infection, 15*(10), 951-953.

Friedman, N. D., Kaye, K. S., Stout, J. E., McGarry, S. A., Trivette, S. L., Briggs, J. P., . . . Walton, A. L. (2002). Health care–associated bloodstream infections in adults: A reason to change the accepted definition of community-acquired infections. *Annals of Internal Medicine, 137*(10), 791-797.

Gagnier, J. J., Moher, D., Boon, H., Beyene, J., & Bombardier, C. (2012). Investigating clinical heterogeneity in systematic reviews: A methodologic review of guidance in the literature. *BMC Medical Research Methodology, 12*(111). doi: 10.1186/1471-2288-12-111

Gallini, A., Degris, E., Desplas, M., Bourrel, R., Archambaud, M., Montastruc, J.-L., . . . Sommet, A. (2010). Influence of fluoroquinolone consumption in inpatients and outpatients on ciprofloxacin-resistant *Escherichia coli* in a university hospital. *Journal of Antimicrobial Chemotherapy, 65*(12), 2650-2657.

Gandra, S., Merchant, A. T., & Laxminarayan, R. (2016). A role for private sector laboratories in public health surveillance of antimicrobial resistance. *Future Microbiology, 11*(6), 709-712.

Gardner, A., Mitchell, B., Beckingham, W., & Fasugba, O. (2014). A point prevalence cross-sectional study of healthcare-associated urinary tract infections in six Australian hospitals. *BMJ Open, 4*(7). doi:10.1136/bmjopen-2014-005099

Garg, A. X., Hackam, D., & Tonelli, M. (2008). Systematic review and meta-analysis: When one study is just not enough. *Clinical Journal of the American Society of Nephrology, 3*(1), 253-260.

Gágyor, I., Hummers-Pradier, E., Kochen, M. M., Schmiemann, G., Wegscheider, K., & Bleidorn, J. (2012). Immediate versus conditional treatment of uncomplicated urinary tract infection-a randomized-controlled comparative effectiveness study in general practices. *BMC Infectious Diseases, 12*(1), 146.

Gágyor, I., Bleidorn, J., Kochen, M. M., Schmiemann, G., Wegscheider, K., & Hummers-Pradier, E. (2015). Ibuprofen versus fosfomycin for uncomplicated urinary tract infection in women: randomised controlled trial. *Bmj, 351*, h6544.

Georgiou, A., Vecellio, E., Toouli, G., Eigenstetter, A., Li, L., Wilson, R., & Westbrook, J. (2012). *The impact of the implementation of electronic ordering on hospital pathology services.* Retrieved from http://www.aims.org.au/documents/item/295

Ghadiri, H., Vaez, H., Khosravi, S., & Soleymani, E. (2012). The antibiotic resistance profiles of bacterial strains isolated from patients with hospital-acquired bloodstream and urinary tract infections. *Critical Care Research and Practice, 2012*. doi:10.1155/2012/890797

Gibbs, E. (2014). The evolution of One Health: A decade of progress and challenges for the future. *Veterinary Record, 174*(4), 85-91.

Gobernado, M., Valdes, L., Alos, J. I., Garcia-Rey, C., Dal-Re, R., Garcia-de-Lomas, J., & Spanish Surveillance Group for Urinary Pathogens. (2007). Antimicrobial susceptibility of clinical *Escherichia coli* isolates from uncomplicated cystitis in women over a 1-year period in Spain. *Revista Espanola de Quimioterapia, 20*(1), 68-76.

Goettsch, W., Van Pelt, W., Nagelkerke, N., Hendrix, M., Buiting, A., Petit, P., . . . De Neeling, A. (2000). Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. *Journal of Antimicrobial Chemotherapy, 46*(2), 223-228.

Goossens, H., Ferech, M., Vander Stichele, R., & Elseviers, M. (2005). Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. *The Lancet, 365*(9459), 579-587.

Gopalakrishnan, S., & Ganeshkumar, P. (2013). Systematic reviews and meta-analysis: Understanding the best evidence in primary healthcare. *Journal of Family Medicine and Primary Care, 2*(1), 9-14.

Gottesman, B. S., Carmeli, Y., Shitrit, P., & Chowers, M. (2009). Impact of quinolone restriction on resistance patterns of *Escherichia coli* isolated from urine by culture in a community setting. *Clinical Infectious Diseases, 49*(6), 869-875.

Gottlieb, T., & Nimmo, G. R. (2011). Antibiotic resistance is an emerging threat to public health: an urgent call to action at the Antimicrobial Resistance Summit 2011. *Medical Journal of Australia, 194*(6), 281-283.

Government of South Australia. (2013). *National Antimicrobial Utilisation Surveillance Program*. Retrieved from http://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/clinical+resources/clinical+programs/antimicrobial+stewardship/national+antimicrobial+utilisation+surveillance+program+nausp

Grambsch, P. M., & Therneau, T. M. (1994). Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika, 81*(3), 515-526.

Greenhalgh, T. (1997a). How to read a paper. The Medline database. *British Medical Journal, 315*(7101), 180-183.

Greenhalgh, T. (1997b). Papers that summarise other papers (systematic reviews and meta-analyses). *British Medical Journal, 315*(7109), 672-675.

Gupta, K., Hooton, T. M., Naber, K. G., Wullt, B., Colgan, R., Miller, L. G., . . . Schaeffer, A. J. (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clinical Infectious Diseases, 52*(5), e103-e120.

Gupta, K., Hooton, T. M., & Stamm, W. E. (2001). Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. *Annals of Internal Medicine, 135*(1), 41-50.

Gupta, K., Sahm, D. F., Mayfield, D., & Stamm, W. E. (2001). Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: A nationwide analysis. *Clinical Infectious Diseases, 33*(1), 89-94.

Haidich, A. (2011). Meta-analysis in medical research. *Hippokratia, 14*(1), 29-37.

Hassanzadeh, P., & Motamedifar, M. (2007). The prevalence of asymptomatic bacteriuria in long term care facility residents in Shiraz, Southwest Iran: A cross-sectional study. *Pakistan Journal of Biological Sciences, 10*(21), 3890-3894.

Henderson, K. L., Muller-Pebody, B., Johnson, A. P., Wade, A., Sharland, M., & Gilbert, R. (2013). Community-acquired, healthcare-associated and hospital-acquired bloodstream infection definitions in children: A systematic review demonstrating inconsistent criteria. *Journal of Hospital Infection, 85*(2), 94-105.

Hermosilla, N. L., Canut, B. A., Ulibarrena, S. M., Abasolo, O. E., & Carlos, A. I. L. (2003). Trends in antimicrobial utilization at a Spanish general hospital during a 5-year period. *Pharmacoepidemiology and Drug Safety, 12*(3), 243-247.

Higgins, J., & Green, S. (2011). *Cochrane handbook for systematic reviews of interventions: Version 5.1.0 [updated March 2011].* Retrieved from http://handbook.cochrane.org/

Higgins, J., Thompson, S., Deeks, J., & Altman, D. (2003). Measuring inconsistency in meta-analyses. *BMJ, 327*(7414), 557-560.

Hillier, S., Magee, J., Howard, A., & Palmer, S. (2002). How strong is the evidence that antibiotic use is a risk factor for antibiotic-resistant, community-acquired urinary tract infection? *Journal of Antimicrobial Chemotherapy, 50*(2), 241-247.

Hillier, S., Roberts, Z., Dunstan, F., Butler, C., Howard, A., & Palmer, S. (2007). Prior antibiotics and risk of antibiotic-resistant community-acquired urinary tract infection: A case–control study. *Journal of Antimicrobial Chemotherapy, 60*(1), 92-99.

Hindler, J. F., & Stelling, J. (2007). Analysis and presentation of cumulative antibiograms: A new consensus guideline from the Clinical and Laboratory Standards Institute. *Clinical Infectious Diseases, 44*(6), 867-873.

Ho, P., Yip, K., Chow, K., Lo, J. Y. C., Que, T., & Yuen, K. (2010). Antimicrobial resistance among uropathogens that cause acute uncomplicated cystitis in women in Hong Kong: a prospective multicenter study in 2006 to 2008. *Diagnostic Microbiology and Infectious Disease, 66*(1), 87-93.

Hoban, D. J., Nicolle, L. E., Hawser, S., Bouchillon, S., & Badal, R. (2011). Antimicrobial susceptibility of global inpatient urinary tract isolates of *Escherichia coli*: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program 2009–2010. *Diagnostic Microbiology and Infectious Disease, 70*(4), 507-511.

Hoenigl, M., Wagner, J., Raggam, R. B., Prueller, F., Prattes, J., Eigl, S., . . . Zollner-Schwetz, I. (2014). Characteristics of hospital-acquired and community-onset blood stream infections, South-East Austria. *PLoS ONE, 9*(8). doi: 10.1371/journal.pone.0104702

Hooton, T. M. (2000). Pathogenesis of urinary tract infections: An update. *Journal of Antimicrobial Chemotherapy, 46*(suppl 1), 1-7.

Hooton, T. M. (2012). Uncomplicated urinary tract infection. *New England Journal of Medicine, 366*(11), 1028-1037.

Hooton, T. M., Scholes, D., Hughes, J. P., Winter, C., Roberts, P. L., Stapleton, A. E., . . . Stamm, W. E. (1996). A prospective study of risk factors for symptomatic urinary tract infection in young women. *New England Journal of Medicine, 335*(7), 468-474.

Horan, T. C., Andrus, M., & Dudeck, M. A. (2008). CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *American Journal of Infection Control, 36*(5), 309-332.

Horcajada, J. P., Shaw, E., Padilla, B., Pintado, V., Calbo, E., Benito, N., . . . Sociedad Española de Enfermedades Infecciosas y Microbiología, C. (2013). Healthcare-associated, community-acquired and hospital-acquired bacteraemic urinary tract infections in hospitalized patients: A prospective multicentre cohort study in the era of antimicrobial resistance. *Clinical Microbiology and Infection, 19*(10), 962-968.

Hounsom, L., Grayson, K., & Melzer, M. (2011). Mortality and associated risk factors in consecutive patients admitted to a UK NHS trust with community-acquired bacteraemia. *Postgraduate Medical Journal, 87*(1033), 757-762.

Howard, D. H., Scott, R. D., Packard, R., & Jones, D. (2003). The global impact of drug resistance. *Clinical Infectious Diseases, 36*(suppl 1), S4-S10.

Hristovski, M., Cvetkovik, A., Cvetkovik, I., & Dukoska, V. (2010). Concept of One Health - A new professional imperative. *Macedonian Journal of Medical Sciences, 3*(3), 229-232.

Hsu, L., Tan, T., Tam, V., Kwa, A., Fisher, D. A., Koh, T., & Network for Antimicrobial Resistance Surveillance (Singapore). (2010). Surveillance and correlation of antibiotic prescription and resistance of Gram-negative bacteria in Singaporean hospitals. *Antimicrobial Agents and Chemotherapy, 54*(3), 1173-1178.

Hsueh, P., Hoban, D. J., Carmeli, Y., Chen, S., Desikan, S., Alejandria, M., . . . Binh, T. Q. (2011). Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. *Journal of Infection, 63*(2), 114-123.

Huibers, L., Moth, G., Christensen, M. B., & Vedsted, P. (2014). Antibiotic prescribing patterns in out-of-hours primary care: A population-based descriptive study. *Scandinavian Journal of Primary Health Care, 32*(4), 200-207.

Huovinen, P. (1985). Recording of antimicrobial resistance of urinary tract isolates—effect of repeat samples on resistance levels. *Journal of Antimicrobial Chemotherapy, 16*(4), 443-447.

Hussein, S. (2011). *The use of 'large scale datasets' in UK social care research*. Retrieved from http://www.sscr.nihr.ac.uk/PDF/MR/MR5.pdf

Infectious Diseases Society of the Republic of China. (2000). Guidelines for antimicrobial therapy of urinary tract infections in Taiwan. *Journal of Microbiology, Immunology and Infection, 33*(4), 271-272.

Ironmonger, D., Edeghere, O., Bains, A., Loy, R., Woodford, N., & Hawkey, P. M. (2015). Surveillance of antibiotic susceptibility of urinary tract pathogens for a population of 5.6 million over 4 years. *Journal of Antimicrobial Chemotherapy*, *70*(6), 1744-1750.

Ismaili, K., Wissing, K. M., Lolin, K., Le, P. Q., Christophe, C., Lepage, P., & Hall, M. (2011). Characteristics of first urinary tract infection with fever in children: A prospective clinical and imaging study. *Pediatric Infectious Disease Journal, 30*(5), 371-374.

Jacobsen, S., Stickler, D., Mobley, H., & Shirtliff, M. (2008). Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clinical Microbiology Reviews, 21*(1), 26-59.

Jancel, T., & Dudas, V. (2002). Management of uncomplicated urinary tract infections. *Western Journal of Medicine, 176*(1), 51-55.

Janknegt, R., Lashof, A. O., Gould, I., & Van der Meer, J. (2000). Antibiotic use in Dutch hospitals 1991–1996. *Journal of Antimicrobial Chemotherapy, 45*(2), 251-256.

Jarvis, T. R., Chan, L., & Gottlieb, T. (2014). Assessment and management of lower urinary tract infection in adults. Aust Prescr, 37, 7-9.

Jenkins, S. G., & Jerris, R. C. (2011). Critical assessment of issues applicable to development of antimicrobial susceptibility testing breakpoints. *Journal of Clinical Microbiology, 49*(suppl 9), S5-S10.

Jensen, U. S., Muller, A., Brandt, C. T., Frimodt-Møller, N., Hammerum, A. M., Monnet, D. L., & DANRES Study Group. (2010). Effect of generics on price and consumption of ciprofloxacin in primary healthcare: the relationship to increasing resistance. *Journal of Antimicrobial Chemotherapy*, *65*(6), 1286-1291.

Johnson, J. R., Menard, M., Johnston, B., Kuskowski, M. A., Nichol, K., & Zhanel, G. G. (2009). Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. *Antimicrobial Agents and Chemotherapy, 53*(7), 2733-2739.

Jones, S., Carley, S., & Harrison, M. (2003). An introduction to power and sample size estimation. *Emergency Medicine Journal, 20*(5), 453-458.

Kaboub, F. (2008). Positivist paradigm. In F. Leong (Ed.), *Encyclopedia of counseling* (pp. 786-786). Thousand Oaks, CA: SAGE Publications Ltd.

Kahlmeter, G., & Poulsen, H. O. (2012). Antimicrobial susceptibility of *Escherichia coli* from community-acquired urinary tract infections in Europe: the ECO· SENS study revisited. *International Journal of Antimicrobial Agents, 39*(1), 45-51.

Kaki, R., Elligsen, M., Walker, S., Simor, A., Palmay, L., & Daneman, N. (2011). Impact of antimicrobial stewardship in critical care: A systematic review. *Journal of Antimicrobial Chemotherapy, 66*(6), 1223-1230.

Karaca, Y., Coplu, N., Gozalan, A., Oncul, O., Citil, B. E., & Esen, B. (2005). Co-trimoxazole and quinolone resistance in *Escherichia coli* isolated from urinary tract infections over the last 10 years. *International Journal of Antimicrobial Agents, 26*(1), 75-77.

Karlowsky, J. A., Kelly, L. J., Thornsberry, C., Jones, M. E., & Sahm, D. F. (2002). Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli*

from female outpatients in the United States. *Antimicrobial Agents and Chemotherapy, 46*(8), 2540-2545.

Karlowsky, J. A., Lagacé-Wiens, P. R., Simner, P. J., DeCorby, M. R., Adam, H. J., Walkty, A., . . . Zhanel, G. G. (2011). Antimicrobial resistance in urinary tract pathogens in Canada from 2007 to 2009: CANWARD surveillance study. *Antimicrobial Agents and Chemotherapy*, *55*(7), 3169-3175

Kashef, N., Djavid, G. E., & Shahbazi, S. (2010). Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran. *The Journal of Infection in Developing Countries, 4*(4), 202-206.

Kass, E. H. (1957). Bacteriuria and the diagnosis of infections of the urinary tract: with observations on the use of methionine as a urinary antiseptic. *AMA Archives of Internal Medicine, 100*(5), 709-714.

Kass, E. H., & Finland, M. (2002). Asymptomatic infections of the urinary tract. *The Journal of Urology, 168*(2), 420-424.

Kennedy, K. J., & Collignon, P. J. (2010). Colonisation with *Escherichia coli* resistant to "critically important" antibiotics: A high risk for international travellers. *European Journal of Clinical Microbiology & Infectious Diseases, 29*(12), 1501-1506.

Kennedy, K. J., Roberts, J. L., & Collignon, P. J. (2008). *Escherichia coli* bacteraemia in Canberra: incidence and clinical features. *Medical Journal of Australia, 188*(4), 209-213.

Khawcharoenporn, T., Vasoo, S., & Singh, K. (2013). Urinary tract infections due to multidrug-resistant Enterobacteriaceae: Prevalence and risk factors in a Chicago emergency department. *Emergency Medicine International.* doi:10.1155/2013/258517

Kibret, M., & Abera, B. (2014). Prevalence and antibiogram of bacterial isolates from urinary tract infections at Dessie Health Research Laboratory, Ethiopia. *Asian Pacific Journal of Tropical Biomedicine, 4*(2), 164-168.

Kiffer, C. R., Mendes, C., Oplustil, C. P., & Sampaio, J. L. (2007). Antibiotic resistance and trend of urinary pathogens in general outpatients from a major urban city. *International Brazilian Journal of Urology, 33*(1), 42-49.

Killgore, K. M., March, K. L., & Guglielmo, B. J. (2004). Risk factors for community-acquired ciprofloxacin-resistant *Escherichia coli* urinary tract infection. *Annals of Pharmacotherapy, 38*(7-8), 1148-1152.

Kimando, J., Okemo, P., & Njagi, E. (2010). Resistance to antibiotics in urinopathogenic bacteria isolated in patients attending Kenyatta University Health Clinic, Nairobi. *East African Medical Journal, 87*(3), 115-119.

Ko, M., Liu, C., Woung, L., Lee, W., Jeng, H., Lu, S., . . . Li, C. (2008). Species and antimicrobial resistance of uropathogens isolated from patients with urinary catheter. *The Tohoku Journal of Experimental Medicine, 214*(4), 311-319.

Kollef, M. H., Napolitano, L. M., Solomkin, J. S., Wunderink, R. G., Bae, I., Fowler, V. G., . . . Shorr, A. F. (2008). Health care-associated infection (HAI): A critical appraisal of the emerging threat—Proceedings of the HAI Summit. *Clinical Infectious Diseases, 47*(suppl 2), S55-S99.

Koningstein, M., van der Bij, A. K., de Kraker, M. E. A., Monen, J. C., Muilwijk, J., de Greeff, S. C., . . . van Hall, M. (2014). Recommendations for the empirical treatment of complicated urinary tract infections using surveillance data on antimicrobial resistance in the Netherlands. *PLoS ONE, 9*(1). doi: 10.1371/journal.pone.0086634

Kothari, A., & Sagar, V. (2008). Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: A multicenter study. *The Journal of Infection in Developing Countries, 2*(5), 354-358.

Kotwani, A., & Holloway, K. (2011). Trends in antibiotic use among outpatients in New Delhi, India. *BMC Infectious Diseases, 11*(99). doi: 10.1186/1471-2334-11-99

Kouchak, F., & Askarian, M. (2012). Nosocomial infections: The definition criteria. *Iranian Journal of Medical Sciences, 37*(2), 72-73.

Kristiansson, C., Grape, M., Gotuzzo, E., Samalvides, F., Chauca, J., Larsson, M., . . . Petzold, M. (2009). Socioeconomic factors and antibiotic use in relation to antimicrobial resistance in the Amazonian area of Peru. *Scandinavian Journal of Infectious Diseases*, *41*(4), 303-312.

Kritsotakis, E., Assithianakis, P., Kanellos, P., Tzagarakis, N., Ioannides, M., & Gikas, A. (2006). Surveillance of monthly antimicrobial consumption rates stratified by patient-care area: A tool for triggering and targeting antibiotic policy changes in the hospital. *Journal of Chemotherapy, 18*(4), 394-401.

Kronvall, G. (2010). Antimicrobial resistance 1979–2009 at Karolinska hospital, Sweden: Normalized resistance interpretation during a 30 year follow-up on *Staphylococcus aureus* and *Escherichia coli* resistance development. *APMIS, 118*(9), 621-639.

Kumar, S., Dave, A., Wolf, B., & Lerma, E. V. (2015). Urinary tract infections. *Disease-a-Month, 61*(2), 45-59.

Kurutepe, S., Surucuoglu, S., Sezgin, C., Gazi, H., Gulay, M., & Ozbakkaloglu, B. (2005). Increasing antimicrobial resistance in *Escherichia coli* isolates from community-acquired urinary tract infections during 1998-2003 in Manisa, Turkey. *Japanese Journal of Infectious Diseases, 58*(3), 159-161.

Lagacé-Wiens, P. R., Simner, P. J., Forward, K. R., Tailor, F., Adam, H. J., DeCorby, M., . . . Zhanel, G. G. (2011). Analysis of 3789 in- and outpatient *Escherichia coli* isolates from across Canada—results of the CANWARD 2007–2009 study. *Diagnostic Microbiology and Infectious Disease, 69*(3), 314-319.

Lau, S. M., Peng, M. Y., & Chang, F. Y. (2004). Resistance rates to commonly used antimicrobials among pathogens of both bacteremic and non-bacteremic community-acquired urinary tract infection. *Journal of Microbiology, Immunology and Infection, 37*(3), 185-191.

Laupland, K. B., Church, D. L., Vidakovich, J., Mucenski, M., & Pitout, J. D. (2008). Community-onset extended-spectrum β-lactamase (ESBL) producing *Escherichia coli*: Importance of international travel. *Journal of Infection, 57*(6), 441-448.

Laupland, K. B., Ross, T., Pitout, J., Church, D., & Gregson, D. (2007). Community-onset urinary tract infections: A population-based assessment. *Infection, 35*(3), 150-153.

Lee, S., Cho, Y. K., Kim, S., Lee, E. S., Park, S. Y., & Seo, Y. (2004). Comparison of trends of resistance rates over 3 years calculated from results for all isolates and for the first isolate of a given species from a patient. *Journal of Clinical Microbiology, 42*(10), 4776-4779.

Leekha, S., Terrell, C. L., & Edson, R. S. (2011). General principles of antimicrobial therapy. *Mayo Clinic Proceedings, 86*(2), 156-167.

LeFort, S. M. (1993). The statistical versus clinical significance debate. *Journal of Nursing Scholarship, 25*(1), 57-62.

Levy, S. B., & Marshall, B. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. *Nature Medicine, 10*(suppl 12), S122-S129.

Liao, Y., Yang, C., Kao, C., Dougherty, M. C., Lai, Y., Chang, Y., . . . Chang, L. (2009). Prevalence and impact on quality of life of lower urinary tract symptoms among a sample of employed women in Taipei: A questionnaire survey. *International Journal of Nursing Studies, 46*(5), 633-644.

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gotzsche, P. C., Ioannidis, J. P., . . . Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Medicine*. doi:10.1371/journal.pmed.1000100

Lichtenberger, P., & Hooton, T. M. (2008). Complicated urinary tract infections. *Current Infectious Disease Reports, 10*(6), 499-504.

Linder, J. A., Doctor, J. N., Friedberg, M. W., Nieva, H. R., Birks, C., Meeker, D., & Fox, C. R. (2014). Time of day and the decision to prescribe antibiotics. *Journal of the American Medical Association Internal Medicine, 174*(12), 2029-2031.

Linhares, I., Raposo, T., Rodrigues, A., & Almeida, A. (2013). Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000–2009). *BMC Infectious Diseases, 13*(9). doi: 10.1186/1471-2334-13-19

Little, P., Merriman, R., Turner, S., Rumsby, K., Warner, G., Lowes, J., . . . Mullee, M. (2010). Presentation, pattern, and natural course of severe symptoms, and role of antibiotics and antibiotic resistance among patients presenting with suspected uncomplicated urinary tract infection in primary care: Observational study. *BMJ, 340*. doi: 10.1136/bmj.b5633

Little, P., Moore, M., Turner, S., Rumsby, K., Warner, G., Lowes, J., . . . Arscott, A. (2010). Effectiveness of five different approaches in management of urinary tract infection: randomised controlled trial. *Bmj, 340*, c199.

Liu, H., Lin, H., Lin, Y., Yu, S., Wu, W., & Lee, Y. (2011). Antimicrobial susceptibilities of urinary extended-spectrum beta-lactamase-producing *Escherichia coli* and Klebsiella pneumoniae to fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. *Journal of Microbiology, Immunology and Infection, 44*(5), 364-368.

Liu, J. Y., Wu, Y. H., Cai, M., & Zhou, C. L. (2016). Point-prevalence survey of healthcare-associated infections in Beijing, China: A survey and analysis in 2014. *Journal of Hospital Infection, 93*(3), 271-279.

Ljuca, S., Zvizdic, S., Hamzic, S., Kalajdzija, M., & Ljuca, A. (2010). Antimicrobial susceptibility of bacteria isolated from inpatients and outpatients urine samples. *Medical Archives, 64*(3), 161-164.

Longhi, C., Conte, M., Marazzato, M., Lebba, V., Totino, V., Santangelo, F., . . . Schippa, S. (2012). Plasmid-mediated fluoroquinolone resistance determinants in *Escherichia*

coli from community uncomplicated urinary tract infection in an area of high prevalence of quinolone resistance. *European Journal of Clinical Microbiology & Infectious Diseases, 31*(8), 1917-1921.

López-Lozano, J., Monnet, D. L., Yagüe, A., Burgos, A., Gonzalo, N., Campillos, P., & Saez, M. (2000). Modelling and forecasting antimicrobial resistance and its dynamic relationship to antimicrobial use: A time series analysis. *International Journal of Antimicrobial Agents, 14*(1), 21-31.

Lyman, G. H., & Kuderer, N. M. (2005). The strengths and limitations of meta-analyses based on aggregate data. *BMC Medical Research Methodology, 5*(14). doi: 10.1186/1471-2288-5-14

Ma, K. L., & Wang, C. X. (2013). Analysis of the spectrum and antibiotic resistance of uropathogens in vitro: Results based on a retrospective study from a tertiary hospital. *American Journal of Infection Control, 41*(7), 601-606.

Mack, L. (2010). The philosophical underpinnings of educational research. *Polyglossia, 19*, 5-11.

Mackenzie, N., & Knipe, S. (2006). Research dilemmas: Paradigms, methods and methodology. *Issues in Educational Research, 16*(2), 193-205.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., . . . Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268-281.

Maraki, S., Mantadakis, E., Michailidis, L., & Samonis, G. (2013). Changing antibiotic susceptibilities of community-acquired uropathogens in Greece, 2005–2010. *Journal of Microbiology, Immunology and Infection, 46*(3), 202-209.

Maron, D. F., Smith, T. J., & Nachman, K. E. (2013). Restrictions on antimicrobial use in food animal production: An international regulatory and economic survey. *Globalization and Health, 9*(48). doi: 10.1186/1744-8603-9-48

Marques, L. P. J., Flores, J. T., Barros Junior, O., Rodrigues, G. B., Mourão, C., & Moreira, R. M. P. (2012). Epidemiological and clinical aspects of urinary tract infection in community-dwelling elderly women. *The Brazilian Journal of Infectious Diseases, 16*(5), 436-441.

Martin, C., Ofotokun, I., Rapp, R., Empey, K., Armitstead, J., Pomeroy, C., . . . Evans, M. (2005). Results of an antimicrobial control program at a university hospital. *American Journal of Health-System Pharmacy, 62*(7), 732-738.

Martinez, P., Garzon, D., & Mattar, S. (2012). CTX-M-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from community-acquired urinary tract infections in Valledupar, Colombia. *Brazilian Journal of Infectious Diseases, 16*(5), 420-425.

Masterton, R. (2008). The importance and future of antimicrobial surveillance studies. *Clinical Infectious Diseases, 47*(suppl 1), S21-S31.

Matuszkiewicz-Rowińska, J., Małyszko, J., & Wieliczko, M. (2015). Urinary tract infections in pregnancy: Old and new unresolved diagnostic and therapeutic problems. *Archives of Medical Science, 11*(1), 67-77.

Mazzulli, T. (2012). Diagnosis and management of simple and complicated urinary tract infections (UTIs). *The Canadian Journal of Urology, 19*(1), 42-48.

McGregor, J. C., Elman, M. R., Bearden, D. T., & Smith, D. H. (2013). Sex-and age-specific trends in antibiotic resistance patterns of *Escherichia coli* urinary isolates from outpatients. *BMC Family Practice, 14*(25). doi: 10.1186/1471-2296-14-25

McKenzie, D., Rawlins, M., & Del Mar, C. (2013). Antimicrobial stewardship: What's it all about? *Australian Prescriber, 36*(4), 116-120.

McNeil, V., Cruickshank, M., & Duguid, M. (2010). Safer use of antimicrobials in hospitals: The value of antimicrobial usage data. *Medical Journal of Australia, 193*(8), S114-S117.

Mcquiston, J., Rosborg, M., Sternhagen, A. B., Llor, C., & Bjerrum, L. (2013). Different recommendations for empiric first-choice antibiotic treatment of uncomplicated urinary tract infections in Europe. *Scandinavian Journal of Primary Health Care, 31*(4), 235-240.

Medicare Australia. (2016). *Medicare Australia statistics - Medicare item reports*. Retrieved from http://medicarestatistics.humanservices.gov.au/statistics/do.jsp?_PROGRAM=%2Fstatistics%2Fmbs_item_standard_report&DRILL=ag&group=69333&VAR=services&STAT=count&RPT_FMT=by+state&PTYPE=calyear&START_DT=200901&END_DT=201312

Mehnert-Kay, S. A. (2005). Diagnosis and management of uncomplicated urinary tract infections. *American Family Physician, 72*(3), 451-456.

Meier, S., Weber, R., Zbinden, R., Ruef, C., & Hasse, B. (2011). Extended-spectrum β-lactamase-producing Gram-negative pathogens in community-acquired urinary tract infections: An increasing challenge for antimicrobial therapy. *Infection, 39*(4), 333-340.

Meumann, E. M., Mitchell, B. G., McGregor, A., McBryde, E., & Cooley, L. (2015). Urinary *Escherichia coli* antimicrobial susceptibility profiles and their relationship with community antibiotic use in Tasmania, Australia. *International Journal of Antimicrobial Agents, 46*(4), 389-393.

Miragliotta, G., Di Pierro, M. N., Miragliotta, L., & Mosca, A. (2008). Antimicrobial resistance among uropathogens responsible for community-acquired urinary tract infections in an Italian community. *Journal of Chemotherapy, 20*(6), 721-727.

Mishra, S. K., & Agrawal, D. (2012). *Concise manual of pathogenic microbiology (1)*. Somerset, US: Wiley-Blackwell.

Mitchell, B. G., Collignon, P. J., McCann, R., Wilkinson, I. J., & Wells, A. (2014). Reply to Worth et al. *Clinical Infectious Diseases*, 59(12), 1809-1810.

Mitchell, B. G., Fasugba, O., Beckingham, W., Bennett, N., & Gardner, A. (2016). A point prevalence study of healthcare associated urinary tract infections in Australian acute and aged care facilities. *Infection, Disease & Health, 21*(1), 26-31.

Mitchell, B. G., Ferguson, J. K., Anderson, M., Sear, J., & Barnett, A. (2016). Length of stay and mortality associated with healthcare-associated urinary tract infections: a multi-state model. *Journal of Hospital Infection, 93*(1), 92-99.

Moellering, R. C. (2006). Antimicrobial resistance prevention initiative: Proceedings of an expert panel on resistance. *The American Journal of Medicine, 119*(6), S1-S2.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. *Annals of Internal Medicine, 151*(4), 264-269.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., . . . Stewart, L. A. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews, 4*(1). doi: 10.1186/2046-4053-4-1

Molina-López, J., Aparicio-Ozores, G., Ribas-Aparicio, R. M., Gavilanes-Parra, S., Chávez-Berrocal, M. E., Hernández-Castro, R., & Manjarrez-Hernández, H. Á. (2011). Drug resistance, serotypes, and phylogenetic groups among uropathogenic *Escherichia coli*

including O25-ST131 in Mexico City. *The Journal of Infection in Developing Countries, 5*(12), 840-849.

Monnet, D. L. (2007). Measuring antimicrobial use: The way forward. *Clinical Infectious Diseases, 44*(5), 671-673.

Moreira, E. D., De Siqueira, I. C., Alcantara, A. P., Guereiro De Moura, C. G., De Carvalho, W. A., & Riley, L. (2006). Antimicrobial resistance of *Escherichia coli* strains causing community-acquired urinary tract infections among insured and uninsured populations in a large urban center. *Journal of Chemotherapy, 18*(3), 255-260.

Morgan, D. J., Okeke, I. N., Laxminarayan, R., Perencevich, E. N., & Weisenberg, S. (2011). Non-prescription antimicrobial use worldwide: A systematic review. *The Lancet Infectious Diseases, 11*(9), 692-701.

Mukherji, P., & Albon, D. (2009). *Research methods in early childhood: An introductory guide*. London: Sage Publications Ltd.

Müller-Pebody, B., Muscat, M., Pelle, B., Klein, B. M., Brandt, C. T., & Monnet, D. L. (2004). Increase and change in pattern of hospital antimicrobial use, Denmark, 1997–2001. *Journal of Antimicrobial Chemotherapy, 54*(6), 1122-1126.

Murugan, K., Savitha, T., & Vasanthi, S. (2012). Retrospective study of antibiotic resistance among uropathogens from rural teaching hospital, Tamilnadu, India. *Asian Pacific Journal of Tropical Disease, 2*(5), 375-380.

Muvunyi, C. M., Masaisa, F., Bayingana, C., Mutesa, L., Musemakweri, A., Muhirwa, G., & Claeys, G. W. (2011). Decreased susceptibility to commonly used antimicrobial agents in bacterial pathogens isolated from urinary tract infections in Rwanda: Need for new antimicrobial guidelines. *American Journal of Tropical Medicine and Hygiene, 84*(6), 923-928.

Mwaka, A., Mayanja-Kizza, H., Kigonya, E., & Kaddu-Mulindwa, D. (2011). Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda. *African Health Sciences, 11*(2), 182-189.

Naber, K. G. (2000). Survey on antibiotic usage in the treatment of urinary tract infections. *Journal of Antimicrobial Chemotherapy, 46*(suppl 1), 49-52.

National Antimicrobial Utilisation Surveillance Program. (2015). *NAUSP contributor information: Data principles and definitions*. South Australia: SA Health.

National Audit Office. (2009). *Reducing healthcare associated infections in hospitals in England*. Retrieved from

https://www.nao.org.uk/wp-content/uploads/2009/06/0809560.pdf

National Health and Medical Research Council, Australian Research Council, & Australian Vice-Chancellors' Committee. (2007). *Australian Code for the Responsible Conduct of Research*. Retrieved from https://www.nhmrc.gov.au/_files_nhmrc/file/publications/r39_australian_code_responsible_conduct_research_150811.pdf

National Health and Medical Research Council, Australian Research Council, & Australian Vice-Chancellors' Committee. (2014). *National Statement on Ethical Conduct in Human Research.* Retrieved from https://msd.unimelb.edu.au/sites/default/files/docs/e72_national_statement_march_2014_140331.pdf

Neal, J., & Durwood, E. (2008). Complicated urinary tract infections. *Urologic Clinics of North America, 35*(1), 13-22.

Ni Chulain, M., Murray, A. M., Corbett-Feeney, G., & Cormican, M. (2005). Antimicrobial resistance in *E. coli* associated with urinary tract infection in the West of Ireland. *Irish Journal of Medical Science, 174*(4), 6-9.

Nickel, J. C. (2007). Urinary tract infections and resistant bacteria. *Reviews in urology, 9*(2), 78-80.

Nicolle, L. E. (2002). Epidemiology of urinary tract infections. *Clinical Microbiology Newsletter, 24*(18), 135-140.

Nicolle, L. E. (2005). Complicated urinary tract infection in adults. *Canadian Journal of Infectious Diseases and Medical Microbiology, 16*(6), 349-360.

Nicolle, L. E. (2008). Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. *Urologic Clinics of North America, 35*(1), 1-12.

Nicolle, L. E. (2013). Antimicrobial resistance in community-acquired *Escherichia coli* isolated from urinary infection: Good news or bad? *The Canadian Journal of Infectious Diseases & Medical Microbiology, 24*(3), 123-124.

Nielubowicz, G. R., & Mobley, H. L. (2010). Host–pathogen interactions in urinary tract infection. *Nature Reviews Urology, 7*(8), 430-441.

Nikaido, H. (2009). Multidrug resistance in bacteria. *Annual Review of Biochemistry, 78*, 119-146.

Nimmo, G. R., Bell, J. M., & Collignon, P. J. (2003). Fifteen years of surveillance by the Australian Group on Antimicrobial Resistance (AGAR). *Communicable Diseases Intelligence, 27*, S47-S54.

Noordzij, M., Tripepi, G., Dekker, F. W., Zoccali, C., Tanck, M. W., & Jager, K. J. (2010). Sample size calculations: Basic principles and common pitfalls. *Nephrology Dialysis Transplantation*, *25*(5), 1388-1393.

Nouvenne, A., Ticinesi, A., Lauretani, F., Maggio, M., Lippi, G., Guida, L., . . . Meschi, T. (2014). Comorbidities and disease severity as risk factors for carbapenem-resistant *Klebsiella pneumoniae* colonization: Report of an experience in an internal medicine unit. *PLoS ONE, 9*(10). doi: 10.1371/journal.pone.0110001

O'Brien, K., Edwards, A., Hood, K., & Butler, C. C. (2013). Prevalence of urinary tract infection in acutely unwell children in general practice: A prospective study with systematic urine sampling. *The British Journal of General Practice, 63*(607), e156-e164.

O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations.* Retrieved from https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

Okeke, I. N., Klugman, K. P., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., . . . Laxminarayan, R. (2005). Antimicrobial resistance in developing countries. Part II: strategies for containment. *The Lancet Infectious Diseases, 5*(9), 568-580.

Okeke, I. N., Lamikanra, A., & Edelman, R. (1999). Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases, 5*(1), 18-27.

Olson, R. P., & Haith, K. (2012). Antibiotic resistance in urinary tract infections in college students. *Journal of American College Health, 60*(6), 471-474.

Orenstein, R., & Wong, E. S. (1999). Urinary tract infections in adults. *American Family Physician, 59*(5), 1225-1234.

Otajevwo, F. (2013). Urinary tract infection among symptomatic outpatients visiting a tertiary hospital based in midwestern Nigeria. *Global Journal of Health Science, 5*(2), 187-199.

Owumi, W., Banaei, N., & Shortliffe, L. D. (2014). Adult and pediatric intra-institutional trends of ciprofloxacin susceptibility in *E. coli* positive urinary cultures. *Antibiotics, 3*(2), 163-173.

Paterson, D. L. (2006a). Resistance in Gram-negative bacteria: Enterobacteriaceae. *The American Journal of Medicine, 119*(6), S20-S28.

Paterson, D. L. (2006b). The role of antimicrobial management programs in optimizing antibiotic prescribing within hospitals. *Clinical Infectious Diseases, 42*(suppl 2), S90-S95.

Paul, M., Shani, V., Muchtar, E., Kariv, G., Robenshtok, E., & Leibovici, L. (2010). Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrobial Agents and Chemotherapy, 54*(11), 4851-4863.

Pépin, J., Plamondon, M., Lacroix, C., & Alarie, I. (2009). Emergence of and risk factors for ciprofloxacin-gentamicin-resistant *Escherichia coli* urinary tract infections in a region of Quebec. *The Canadian Journal of Infectious Diseases & Medical Microbiology, 20*(4), e163-e168.

Perencevich, E. N., McGregor, J. C., Shardell, M., Furuno, J. P., Harris, A. D., Morris, J. G., . . . Johnson, J. A. (2008). Summer peaks in the incidences of Gram-negative bacterial infection among hospitalized patients. *Infection Control & Hospital Epidemiology, 29*(12), 1124-1131.

Perrin, M., Donnio, P., Heurtin-Lecorre, C., Travert, M., & Avril, J. (1999). Comparative antimicrobial resistance and genomic diversity of *Escherichia coli* isolated from urinary tract infections in the community and in hospitals. *Journal of Hospital Infection, 41*(4), 273-279.

Piéboji, J. G., Koulla-Shiro, S., Ngassam, P., Adiogo, D., Njine, T., & Ndumbe, P. (2004). Antimicrobial resistance of Gram-negative bacilli isolates from inpatients and outpatients at Yaounde Central Hospital, Cameroon. *International Journal of Infectious Diseases, 8*(3), 147-154.

Pogorzelska-Maziarz, M., Herzig, C. T. A., Larson, E. L., Furuya, E. Y., Perencevich, E. N., & Stone, P. W. (2015). Implementation of antimicrobial stewardship policies in U.S. hospitals: Findings from a national survey. *Infection Control and Hospital Epidemiology, 36*(3), 261-264.

Polk, R. E., Fox, C., Mahoney, A., Letcavage, J., & MacDougall, C. (2007). Measurement of adult antibacterial drug use in 130 US hospitals: comparison of defined daily dose and days of therapy. *Clinical Infectious Diseases, 44*(5), 664-670.

Porta, M. (Ed.) (2014). *A Dictionary of Epidemiology* (5th Edition ed.). USA: Oxford University Press.

Power, E. (2006). Impact of antibiotic restrictions: the pharmaceutical perspective. *Clinical Microbiology and Infection, 12*(suppl 5), 25-34.

Powers, J. (2004). Antimicrobial drug development – the past, the present, and the future. *Clinical Microbiology and Infection, 10*(suppl 4), 23-31.

Prakash, D., & Saxena, R. (2013). Prevalence and antimicrobial susceptibility pattern of *Escherichia coli* in hospital-acquired and community-acquired patients related to urinary tract infection in India. *Journal of Applied Pharmaceutical Science, 3*(8), 124-132.

Randrianirina, F., Soares, J. L., Carod, J. F., Ratsima, E., Thonnier, V., Combe, P., . . . Talarmin, A. (2007). Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in Antananarivo, Madagascar. *Journal of Antimicrobial Chemotherapy, 59*(2), 309-312.

Rani, H., Kaistha, N., Gupta, V., & Chander, J. (2011). Choice of antibiotics in community acquired UTI due to *Escherichia coli* in adult age group. *Journal of Clinical and Diagnostic Research, 5*(3), 483-485.

Redgrave, L. S., Sutton, S. B., Webber, M. A., & Piddock, L. J. V. (2014). Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success. *Trends in Microbiology, 22*(8), 438-445.

Reller, L. B., Weinstein, M., Jorgensen, J. H., & Ferraro, M. J. (2009). Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases, 49*(11), 1749-1755.

Reller, L. B., Weinstein, M. P., Peterson, L. R., Hamilton, J. D., Baron, E. J., Tompkins, L. S., . . . Thomson, R. B. (2001). Role of clinical microbiology laboratories in the management and control of infectious diseases and the delivery of health care. *Clinical Infectious Diseases, 32*(4), 605-610.

Robinson, T., Bu, D., Carrique-Mas, J., Fèvre, E., Gilbert, M., Grace, D., . . . Woolhouse, M. (2016). Antibiotic resistance is the quintessential One Health issue. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *110*(7), 377-380.

Rodloff, A., Bauer, T., Ewig, S., Kujath, P., & Müller, E. (2008). Susceptible, intermediate, and resistant – the intensity of antibiotic action. *Deutsches Ärzteblatt International, 105*(39), 657-662.

Rodríguez, J. C., Sirvent, E., López-Lozano, J. M., & Royo, G. (2003). Criteria of time and antibiotic susceptibility in the elimination of duplicates when calculating resistance frequencies. *Journal of Antimicrobial Chemotherapy, 52*(1), 132-134.

Rogers, M. C., & Peterson, N. D. (2011). *E. coli infections : Causes, treatment and prevention*. New York, USA: Nova Science Publishers, Inc.

Ronald, A. (2002). The etiology of urinary tract infection: Traditional and emerging pathogens. *The American Journal of Medicine, 113*(1), 14-19.

Rothstein, H. R., Sutton, A. J., & Borenstein, M. (2006). *Publication bias in meta-analysis: Prevention, assessment and adjustments*. England: John Wiley & Sons.

SA Health. (2016). *National Antimicrobial Utilisation Surveillance Program (NAUSP)*. Retrieved from http://www.sahealth.sa.gov.au/wps/wcm/connect/public+content/sa+health+internet/clinical+resources/clinical+programs/antimicrobial+stewardship/national+antimicrobial+utilisation+surveillance+program+nausp

Sahm, D. F., Marsilio, M. K., & Piazza, G. (1999). Antimicrobial resistance in key bloodstream bacterial isolates: Electronic surveillance with the Surveillance Network Database—USA. *Clinical Infectious Diseases, 29*(2), 259-263.

Sahm, D. F., Thornsberry, C., Mayfield, D. C., Jones, M. E., & Karlowsky, J. A. (2001). Multidrug-resistant urinary tract isolates of *Escherichia coli*: Prevalence and patient demographics in the United States in 2000. *Antimicrobial Agents and Chemotherapy, 45*(5), 1402-1406.

Salisbury, C. (2000). The demand for out-of-hours care from GPs: A review. *Family Practice, 17*(4), 340-347.

Sanchez, G. V., Master, R. N., Karlowsky, J. A., & Bordon, J. M. (2012). In vitro antimicrobial resistance of urinary *Escherichia coli* isolates among US outpatients from 2000 to 2010. *Antimicrobial Agents and Chemotherapy, 56*(4), 2181-2183.

Sannes, M. R., Kuskowski, M. A., & Johnson, J. R. (2004). Geographical distribution of antimicrobial resistance among *Escherichia coli* causing acute uncomplicated pyelonephritis in the United States. *FEMS Immunology & Medical Microbiology, 42*(2), 213-218.

Schaeffer, A. J. (2002). The expanding role of fluoroquinolones. *The American Journal of Medicine, 113*(1), 45-54.

Schechner, V., Temkin, E., Harbarth, S., Carmeli, Y., & Schwaber, M. J. (2013). Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. *Clinical Microbiology Reviews, 26*(2), 289-307.

Schito, G. C., Naber, K. G., Botto, H., Palou, J., Mazzei, T., Gualco, L., & Marchese, A. (2009). The ARESC study: An international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *International Journal of Antimicrobial Agents, 34*(5), 407-413.

Schneeweiss, S., & Avorn, J. (2005). A review of uses of health care utilization databases for epidemiologic research on therapeutics. *Journal of Clinical Epidemiology, 58*(4), 323-337.

Schneider, P., & Riley, T. (1996). *Staphylococcus saprophyticus* urinary tract infections: Epidemiological data from Western Australia. *European Journal of Epidemiology, 12*(1), 51-54.

Schoen, C., Osborn, R., Huynh, P. T., Doty, M., Peugh, J., & Zapert, K. (2006). On the front lines of care: Primary care doctors' office systems, experiences, and views in seven countries. *Health Affairs, 25*(6), w555-w571.

Schreckenberger, P. C., & Binnicker, M. J. (2011). Optimizing antimicrobial susceptibility test reporting. *Journal of Clinical Microbiology, 49*(suppl 9), S15-S19.

Schuts, E. C., Hulscher, M. E., Mouton, J. W., Verduin, C. M., Stuart, J. W. C., Overdiek, H. W., . . . Wolfs, T. F. (2016). Current evidence on hospital antimicrobial stewardship objectives: A systematic review and meta-analysis. *The Lancet Infectious Diseases*, *16*(7), 847-856.

Scimago. (2016). *Scimago Journal & Country Rank*. Retrieved from http://www.scimagojr.com/

Scotland, J. (2012). Exploring the philosophical underpinnings of research: Relating ontology and epistemology to the methodology and methods of the scientific, interpretive, and critical research paradigms. *English Language Teaching, 5*(9), 9-16.

Sedgwick, P. (2013a). Logistic regression. *BMJ, 347*. doi:10.1136/bmj.f4488.

Sedgwick, P. (2013b). Prospective cohort studies: advantages and disadvantages. *BMJ, 347*. doi:10.1136/bmj.f6726.

Sedgwick, P. (2014). Retrospective cohort studies: advantages and disadvantages. *BMJ, 348*. doi:10.1136/bmj.g1072.

Sefton, A. M. (2002). Mechanisms of antimicrobial resistance. *Drugs, 62*(4), 557-566.

Seminerio, J. L., Aggarwal, G., & Sweetser, S. (2011). 26-year-old man with recurrent urinary tract infections. *Mayo Clinic Proceedings, 86*(6), 557–560.

Shaban, R., Cruickshank, M., & Christiansen, K. (2013). *National surveillance and reporting of antimicrobial resistance and antibiotic usage for human health in Australia*. Retrieved from https://www.safetyandquality.gov.au/wp-content/uploads/2014/02/National-surveillance-and-reporting-of-antimicrobial-resistance-and-antibiotic-usage-for-human-health-in-Australia.pdf

Shaifali, I., Gupta, U., Mahmood, S. E., & Ahmed, J. (2012). Antibiotic susceptibility patterns of urinary pathogens in female outpatients. *North American Journal of Medical Sciences, 4*(4), 163-169.

Shannon, K., & French, G. (2002a). Antibiotic resistance: Effect of different criteria for classifying isolates as duplicates on apparent resistance frequencies. *Journal of Antimicrobial Chemotherapy, 49*(1), 201-204.

Shannon, K., & French, G. (2002b). Validation of the NCCLS proposal to use results only from the first isolate of a species per patient in the calculation of susceptibility frequencies. *Journal of Antimicrobial Chemotherapy, 50*(6), 965-969.

Shariff, V., Shenoy, M., Yadav, T., & Radhakrishna, M. (2013). The antibiotic susceptibility patterns of uropathogenic *Escherichia coli*, with special reference to the fluoroquinolones. *Journal of Clinical and Diagnostic Research, 7*(6), 1027-1030.

Shrier, I., Boivin, J., Steele, R. J., Platt, R. W., Furlan, A., Kakuma, R., . . . Rossignol, M. (2007). Should meta-analyses of interventions include observational studies in addition to randomized controlled trials? A critical examination of underlying principles. *American Journal of Epidemiology, 166*(10), 1203-1209.

Sibthorpe, B., Kliewer, E., & Smith, L. (1995). Record linkage in Australian epidemiological research: Health benefits, privacy safeguards and future potential. *Australian Journal of Public Health, 19*(3), 250-256.

Sire, J., Nabeth, P., Perrier-Gros-Claude, J., Bahsoun, I., Siby, T., Macondo, E. A., . . . Breurec, S. (2007). Antimicrobial resistance in outpatient *Escherichia coli* urinary isolates in Dakar, Senegal. *The Journal of Infection in Developing Countries, 1*(3), 263-268.

Song, J. W., & Chung, K. C. (2010). Observational studies: Cohort and case-control studies. *Plastic and Reconstructive Surgery, 126*(6), 2234-2242.

Sood, S., & Gupta, R. (2012). Antibiotic resistance pattern of community-acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. *Indian Journal of Community Medicine, 37*(1), 39-44.

Sorenson, H. T., Sabroe, S., & Olsen, J. (1996). A framework for evaluation of secondary data sources for epidemiological research. *International Journal of Epidemiology, 25*(2), 435-442.

Sorlozano, A., Jimenez-Pacheco, A., del Castillo, J., Sampedro, A., Martinez-Brocal, A., Miranda-Casas, C., . . . Gutiérrez-Fernández, J. (2014). Evolution of the resistance to antibiotics of bacteria involved in urinary tract infections: A 7-year surveillance study. *American Journal of Infection Control, 42*(10), 1033-1038.

Sosa, A., Amábile-Cuevas, C. F., Byarugaba, D. K., Hsueh, P.-R., Kariuki, S., & Okeke, I. N. (2010). *Antimicrobial resistance in developing countries.* Retrieved from http://emerald.tufts.edu/med/apua/about_us/publications_21_3125925763.pdf

Stamm, W. E., Counts, G. W., Running, K. R., Fihn, S., Turck, M., & Holmes, K. K. (1982). Diagnosis of coliform infection in acutely dysuric women. *New England Journal of Medicine, 307*(8), 463-468.

Stamm, W. E., & Norrby, S. R. (2001). Urinary tract infections: Disease panorama and challenges. *Journal of Infectious Diseases, 183*(suppl 1), S1-S4.

Standing Medical Advisory Committee, & Sub-Group on Antimicrobial resistance. (1998). *The path of least resistance*. Retrieved from http://antibiotic-action.com/wp-content/uploads/2011/07/Standing-Medical-Advisory-Committee-The-path-of-least-resistance-1998.pdf

StataCorp. (2013). *Stata Statistical Software: Release 13.* College Station, TX: StataCorp LP.

Sterne, J. A. C., Egger, M., & Smith, G. D. (2008). Investigating and dealing with publication and other biases. In M. Egger, G. D. Smith & D. G. Altman (Eds.), *Systematic Reviews in Health Care* (pp. 189-208). London: BMJ Publishing Group.

Storby, K. A., Österlund, A., & Kahlmeter, G. (2004). Antimicrobial resistance in *Escherichia coli* in urine samples from children and adults: A 12 year analysis. *Acta Paediatrica, 93*(4), 487-491.

Stratchounski, L. S., & Rafalski, V. V. (2006). Antimicrobial susceptibility of pathogens isolated from adult patients with uncomplicated community-acquired urinary tract infections in the Russian Federation: two multicentre studies, UTIAP-1 and UTIAP-2. *International Journal of Antimicrobial Agents, 28*(suppl 1), 4-9.

Stroup, D. F., Berlin, J. A., Morton, S. C., Olkin, I., Williamson, G. D., Rennie, D., . . . Thacker, S. B. (2000). Meta-analysis of observational studies in epidemiology: A proposal for reporting. *Journal of the American Medical Association, 283*(15), 2008-2012.

Stuck, A. K., Täuber, M. G., Schabel, M., Lehmann, T., Suter, H., & Mühlemann, K. (2012). Determinants of quinolone versus trimethoprim-sulfamethoxazole use for outpatient urinary tract infection. *Antimicrobial Agents and Chemotherapy, 56*(3), 1359-1363.

Suda, K. J., Hicks, L. A., Roberts, R. M., Hunkler, R. J., & Taylor, T. H. (2014). Trends and Seasonal variation in outpatient antibiotic prescription rates in the United States, 2006 to 2010. *Antimicrobial Agents and Chemotherapy, 58*(5), 2763-2766.

Sun, L., Klein, E. Y., & Laxminarayan, R. (2012). Seasonality and temporal correlation between community antibiotic use and resistance in the United States. *Clinical Infectious Diseases*, *55*(5), 687-694.

Swami, S. K., Liesinger, J. T., Shah, N., Baddour, L. M., & Banerjee, R. (2012). Incidence of antibiotic-resistant Escherichia coli bacteriuria according to age and location of onset: A population-based study from Olmsted County, Minnesota*. Mayo Clinic Proceedings, 87*(8), 753-759.

Sydnor, E. R., & Perl, T. M. (2011). Hospital epidemiology and infection control in acute-care settings. *Clinical Microbiology Reviews, 24*(1), 141-173.

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002. *Emerging Infectious Diseases, 18*(5), 741-749.

Tai, B. C., & Machin, D. (2013). *Regression methods for medical research (1)*. West Sussex, UK: John Wiley and Sons.

Talan, D. A., Takhar, S. S., Krishnadasan, A., Abrahamian, F. M., Mower, W. R., & Moran, G. J. (2016). Fluoroquinolone-resistant and extended-spectrum β-lactamase–producing *Escherichia coli* infections in patients with pyelonephritis, United States. *Emerging Infectious Diseases, 22*(9), 1594–1603.

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine, 119*(6), S3-S10.

Teoh, P., Basarab, A., Pickering, R., Ali, A., Hayes, M., & Somani, B. K. (2013). Changing trends in antibiotic resistance for urinary *E. coli* infections over five years in a university hospital. *Journal of Clinical Urology*. doi:10.1177/2051415813514578

Thompson, S. (2008). Why and how sources of heterogeneity should be investigated. In M. Egg er, G. D. Smith & D. G. Altman (Eds.), *Systematic Reviews in Health Care* (pp. 157-175). London: BMJ Publishing Group.

Thompson, S. G., & Higgins, J. (2002). How should meta-regression analyses be undertaken and interpreted? *Statistics in Medicine, 21*(11), 1559-1573.

Tolkoff-Rubin, N. E., & Rubin, R. H. (1997). Urinary tract infection in the immunocompromised host: Lessons from kidney transplantation and the AIDS epidemic. *Infectious Disease Clinics of North America, 11*(3), 707-717.

Trautner, B. W. (2010). Management of catheter-associated urinary tract infection (CAUTI). *Current Opinion in Infectious Diseases, 23*(1), 76-82.

Trutwein, B., Holman, C. A. J., & Rosman, D. L. (2006). Health data linkage conserves privacy in a research-rich environment. *Annals of Epidemiology, 16*(4), 279-280.

Tuli, F. (2010). The basis of distinction between qualitative and quantitative research in social science: Reflection on ontological, epistemological and methodological perspectives. *Ethiopian Journal of Education and Sciences, 6*(1), 97-108.

Turnidge, J., & Paterson, D. L. (2007). Setting and revising antibacterial susceptibility breakpoints. *Clinical Microbiology Reviews, 20*(3), 391-408.

United States Interagency Task Force on Antimicrobial Resistance. (2011). *A public health action plan to combat antimicrobial resistance*. Retrieved from http://www.cdc.gov/drugresistance/pdf/public-health-action-plan-combat-antimicrobial-resistance.pdf

Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A., & Laxminarayan, R. (2014). Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases, 14*(8), 742-750.

van der Donk, C. F., van de Bovenkamp, J. H., De Brauwer, E. I., De Mol, P., Feldhoff, K., Kalka-Moll, W. M., . . . Stobberingh, E. E. (2012). Antimicrobial resistance and spread of multi drug resistant *Escherichia coli* isolates collected from nine urology services in the Euregion Meuse-Rhine. *PLoS ONE, 7*(10). doi:10.1371/journal.pone.0047707

Van Nieuwkoop, C., Bonten, T. N., van't Wout, J. W., Becker, M. J., Groeneveld, G. H., Jansen, C. L., . . . TerMeer-Veringa, E. M. (2010). Risk factors for bacteremia with uropathogen not cultured from urine in adults with febrile urinary tract infection. *Clinical Infectious Diseases, 50*(11), e69-e72.

van Saene, R., Fairclough, S., & Petros, A. (1998). Broad- and narrow-spectrum antibiotics: A different approach. *Clinical Microbiology and Infection, 4*(1), 56-57.

Vandenbroucke, J. P., & Pearce, N. (2012). Incidence rates in dynamic populations. *International Journal of Epidemiology, 41*(5), 1472-1479.

Vandenbroucke, J. P., Von Elm, E., Altman, D. G., Gøtzsche, P. C., Mulrow, C. D., Pocock, S. J., . . . Egger, M. (2007). Strengthening the reporting of observational studies in epidemiology (STROBE): Explanation and elaboration. *Annals of Internal Medicine, 147*(8), W163-W194.

Vellinga, A., Murphy, A. W., Hanahoe, B., Bennett, K., & Cormican, M. (2010). A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic *Escherichia coli* in general practice. *Journal of Antimicrobial Chemotherapy, 65*(7), 1514-1520.

Vellinga, A., Cormican, M., Hanahoe, B., Bennett, K., & Murphy, A. W. (2011). Antimicrobial management and appropriateness of treatment of urinary tract infection in general practice in Ireland. *BMC Family Practice, 12*(1). doi:10.1186/1471-2296-12-108

Vellinga, A., Tansey, S., Hanahoe, B., Bennett, K., Murphy, A. W., & Cormican, M. (2012). Trimethoprim and ciprofloxacin resistance and prescribing in urinary tract infection associated with *Escherichia coli*: A multilevel model. *Journal of Antimicrobial Chemotherapy, 67*(10), 2523-2530.

Vergnes, J., Marchal-Sixou, C., Nabet, C., Maret, D., & Hamel, O. (2010). Ethics in systematic reviews. *Journal of Medical Ethics, 36*(12), 771-774.

Vernaz, N., Huttner, B., Muscionico, D., Salomon, J., Bonnabry, P., López-Lozano, J. M., . . . Harbarth, S. (2011). Modelling the impact of antibiotic use on antibiotic-resistant *Escherichia coli* using population-based data from a large hospital and its surrounding community. *Journal of Antimicrobial Chemotherapy, 66*(4), 928-935.

Versporten, A., Coenen, S., Adriaenssens, N., Muller, A., Minalu, G., Faes, C., . . . Goossens, H. (2011). European surveillance of antimicrobial consumption (ESAC): Outpatient penicillin use in Europe (1997–2009). *Journal of Antimicrobial Chemotherapy, 66*(suppl 6), vi13-vi23.

von Elrn, E. (2004). The scandal of poor epidemiological research. *BMJ, 329*, 868-869.

Waksman, S. A. (1973). History of the word 'antibiotic'. *Journal of the History of Medicine and Allied Sciences, 28*(3), 284-286.

Walker, R., & Hiller, J. (2005). The Index of Relative Socio-economic Disadvantage: general population views on indicators used to determine area-based disadvantage. *Australian and New Zealand Journal of Public Health, 29*(5), 442-447.

Wang, Y., Zhao, S., Han, L., Guo, X., Chen, M., Ni, Y., . . . He, P. (2014). Drug resistance and virulence of uropathogenic *Escherichia coli* from Shanghai, China. *The Journal of Antibiotics, 67*(12), 799-805.

Warren, J. W., Abrutyn, E., Hebel, J. R., Johnson, J. R., Schaeffer, A. J., & Stamm, W. E. (1999). Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. *Clinical Infectious Diseases, 29*(4), 745-759.

Wei, W. W. S. (2013). Time series analysis. In T. D. Little (Ed.), *The Oxford handbook of quantitative methods* (pp. 458-485). New York: Oxford University Press.

Wells, G., Shea, B., O'connell, D., Peterson, J., Welch, V., Losos, M., & Tugwell, P. (2014). *The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses*. Retrieved from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

Whitley, E., & Ball, J. (2002). Statistics review 4: Sample size calculations. *Critical Care, 6*(4), 335-341.

WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance. (2012). *Critically important antimicrobials for human medicine - 3rd revision*. Retrieved from http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf.

WHO Collaborating Centre for Drug Statistics Methodology. (2015). *Guidelines for ATC classification and DDD assignment 2015*. Retrieved from https://www.whocc.no/filearchive/publications/2016_guidelines_web.pdf

Wilson, M. L., & Gaido, L. (2004). Laboratory diagnosis of urinary tract infections in adult patients. *Clinical Infectious Diseases, 38*(8), 1150-1158.

Wong, P. H., von Krosigk, M., Roscoe, D. L., Lau, T. T., Yousefi, M., & Bowie, W. R. (2014). Antimicrobial co-resistance patterns of Gram-negative bacilli isolated from bloodstream infections: A longitudinal epidemiological study from 2002–2011. *BMC Infectious Diseases, 14*(393). doi:10.1186/1471-2334-14-393

World Bank Group. (2015). *Country and lending groups*. Retrieved from http://data.worldbank.org/about/country-and-lending-groups

World Health Organization. (2001). *WHO global strategy for containment of antimicrobial resistance*. Retrieved from http://apps.who.int/iris/bitstream/10665/66860/1/WHO_CDS_CSR_DRS_2001.2.pdf

World Health Organization. (2002). *Surveillance standards for antimicrobial resistance*. Retrieved from http://apps.who.int/iris/bitstream/10665/67426/1/WHO_CDS_CSR_DRS_2001.5.pdf

World Health Organization. (2012). *The evolving threat of antimicrobial resistance: options for action*. Retrieved from http://apps.who.int/iris/bitstream/10665/44812/1/9789241503181_eng.pdf

World Health Organization. (2014). *Antimicrobial resistance: global report on surveillance*. Retrieved from http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf

World Health Organization. (2015a). *Global action plan on antimicrobial resistance*. Retrieved from http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf

World Health Organization. (2015b). *Global antimicrobial resistance surveillance system: Manual for early implementation*. Retrieved from http://apps.who.int/iris/bitstream/10665/188783/1/9789241549400_eng.pdf

World Health Organization. (2016). *Health systems strengthening glossary*. Retrieved from http://www.who.int/healthsystems/hss_glossary/en/

Wu, C., Lee, H., Chen, C., Tuan, P., & Chiu, C. (2016). High prevalence and antimicrobial resistance of urinary tract infection isolates in febrile young children without localizing signs in Taiwan. *Journal of Microbiology, Immunology and Infection, 49*(2), 243-248.

Xafis, V. (2015). The acceptability of conducting data linkage research without obtaining consent: Lay people's views and justifications. *BMC Medical Ethics, 16*(79). doi: 10.1186/s12910-015-0070-4

Yadav, K. K., Adhikari, N., Khadka, R., Pant, A. D., & Shah, B. (2015). Multidrug resistant Enterobacteriaceae and extended spectrum β-lactamase producing *Escherichia coli*: A cross-sectional study in National Kidney Center, Nepal. *Antimicrobial Resistance and Infection Control, 4*(42). doi: 10.1186/s13756-015-0085-0

Yildirim, M., Sahin, I., Gulcan, A., Ozdemir, D., Kucukbayrak, A., Uzun, H., & Kaya, D. (2010). Antimicrobial susceptibility and uropathogens isolated from children and adults with community-acquired urinary tract infections. *Journal of Medical Sciences, 30*(2), 533-538.

Yolbas, I., Tekin, R., Kelekci, S., Tekin, A., Okur, M. H., Ece, A., . . . Sen, V. (2013). Community-acquired urinary tract infections in children: Pathogens, antibiotic susceptibility and seasonal changes. *European Review for Medical and Pharmacological Sciences, 17*(7), 971-976.

Zarb, P., Coignard, B., Griskeviciene, J., Muller, A., Vankerckhoven, V., Weist, K., . . . Catry, B. (2012). The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. *Euro Surveillance, 17*(46), 1-16.

Zervos, M. J., Hershberger, E., Nicolau, D. P., Ritchie, D. J., Blackner, L. K., Coyle, E. A., . . . Hiltz, A. (2003). Relationship between fluoroquinolone use and changes in susceptibility to fluoroquinolones of selected pathogens in 10 United States teaching hospitals, 1991–2000. *Clinical Infectious Diseases, 37*(12), 1643-1648.

Zorc, J. J., Levine, D. A., Platt, S. L., Dayan, P. S., Macias, C. G., Krief, W., . . . Kuppermann, N. (2005). Clinical and demographic factors associated with urinary tract infection in young febrile infants. *Pediatrics, 116*(3), 644-648.

Appendix A: Research Portfolio

A1: Thesis output – Papers included in the thesis

Study one (published)

1. **Fasugba, O.**, Gardner, A., Mitchell, B. G., & Mnatzaganian, G. (2015). Ciprofloxacin resistance in community-and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies. *BMC Infectious Diseases, 15*(545). doi:10.1186/s12879-015-1282-4. Scimago ranking: Q1

Study two (published)

2. **Fasugba, O.**, Mitchell, B. G., Mnatzaganian, G., Das, A., Collignon, P., & Gardner, A. (2016). Five-year antimicrobial resistance patterns of urinary *Escherichia coli* at an Australian tertiary hospital: time series analyses of prevalence data. *PLoS ONE, 11*(10), e0164306. doi:10.1371/journal.pone.0164306. Scimago ranking: Q1

Study three (submitted manuscript)

3. **Fasugba, O.**, Das, A., Mnatzaganian, G., Mitchell, B. G., Collignon, P., & Gardner, A. (2017). Incidence of single-drug-, multidrug-, and extensively drug-antimicrobial resistance of *Escherichia coli* urinary tract infections: an Australian laboratory-based retrospective cohort study. Submitted during thesis examination period for journal review with the *Medical Journal of Australia*. Scimago ranking: Q1

A2: Thesis output – Statement of contributions to jointly published work

Chapter 4 – Publication One

Fasugba, O., Gardner, A., Mitchell, B. G., & Mnatzaganian, G. (2015). Ciprofloxacin resistance in community-and hospital-acquired Escherichia coli urinary tract infections: a systematic review and meta-analysis of observational studies. B*MC Infectious Diseases, 15*(545), doi: 10.1186/s12879-015-1282-4

Oyebola Fasugba	Conception and design of the systematic review Wrote the protocol Performed literature search, selection of eligible papers and data extraction Analysis and interpretation of data Wrote the manuscript and revised it for critically important intellectual content
Anne Gardner	Advised on conception and design of study Supervised protocol writing, study selection, data extraction, data analysis and interpretation Critically revised the draft manuscript for important intellectual content
Brett Mitchell	Advised on conception and design of study Supervised protocol writing, study selection, data extraction, data analysis and interpretation Critically revised the draft manuscript for important intellectual content
George Mnatzaganian	Advised on conception and design of study Supervised protocol writing, study selection and data extraction Assisted with analysis and interpretation of data Critically revised the draft manuscript for important intellectual content

Mrs Oyebola Fasugba

Prof Anne Gardner

A/Prof Brett Mitchell

Dr George Mnatzaganian

A2: Thesis output – Statement of contributions to jointly published work

Chapter 5 – Publication Two

Fasugba, O., Mitchell, B. G., Mnatzaganian, G., Das, A., Collignon, P., & Gardner, A. (2016). Five-year antimicrobial resistance patterns of urinary *Escherichia coli* at an Australian tertiary hospital: time series analyses of prevalence data *PLoS ONE, 11*(10), e0164306. doi: 10.1371/journal.pone.0164306

Oyebola Fasugba	Conceptualisation of research aims Design of methodology Formal analysis of study data Writing original draft , review and editing Visualisation, preparation and presentation of published work Project administration of research activity planning and execution
Brett Mitchell	Conceptualisation of research aims Design of methodology Supervision of research activity planning and execution Writing review and editing
George Mnatzaganian	Conceptualisation of research aims Design of methodology Validation of results Supervision of research activity planning and execution Writing review and editing
Anindita Das	Design of methodology Provision of study resources Writing review and editing
Peter Collignon	Design of methodology Provision of study resources Writing review and editing
Anne Gardner	Conceptualisation of research aims Design of methodology Supervision of research activity planning and execution Writing review and editing

Mrs Oyebola Fasugba

A/Prof Brett Mitchell

Dr George Mnatzaganian

Dr Anindita Das

Prof Peter Collignon

Prof Anne Gardner

A3: Thesis output – Conference presentations during candidature

1. **Fasugba, O.**, Gardner, A., Mnatzaganian, G., Mitchell, B. & Das, Anindita. (2015, November). *Five-year antimicrobial resistance patterns of urinary Escherichia coli at an Australian tertiary hospital*. Oral presentation at the Australasian College of Infection Prevention and Control Conference, Hobart, Australia.

2. **Fasugba, O.**, Gardner, A., Mnatzaganian, G. & Mitchell, B. (2015, November). *A systematic review and meta-analysis of ciprofloxacin resistance in community- and hospital-acquired E. coli UTI*. Oral presentation at the Australasian College of Infection Prevention and Control Conference, Hobart, Australia.

3. **Fasugba, O.**, Gardner, A., Mnatzaganian, G. & Mitchell, B. (2014, November). *Antimicrobial resistance among urinary tract infection isolates of Escherichia coli in an Australian population-based sample*. Oral presentation at the Australasian College of Infection Prevention and Control Conference, Adelaide, Australia.

4. **Fasugba, O.**, Gardner, A., Mnatzaganian, G. & Mitchell, B. (2014, November). *Antimicrobial resistance among urinary tract infection isolates of Escherichia coli in an Australian population-based sample*. Poster presentation at the Nursing Research Institute Symposium, St Vincent's Clinic, New South Wales, Australia.

5. **Fasugba, O.**, Gardner, A., Mnatzaganian, G. & Mitchell, B. (2014, August). *Antimicrobial resistance among urinary tract infection isolates of Escherichia coli in an Australian population-based sample*. Poster presentation at the Canberra Health Annual Research Meeting, Canberra, Australia.

A4: Outputs external to candidature and relevant to thesis topic

Journal publications

1. **Fasugba, O.**, Mitchell, B, Beckingham, W. Bennett, N. & Gardner, A. (2016). Development, pilot testing and evaluation of a face-to-face and online educational training package for point prevalence surveys of healthcare associated urinary tract infections*. Infection, Disease and Health,* submitted 19 April 2017.

2. **Fasugba, O.** & Gardner, A. (2017) Catheter associated urinary tract infections (CAUTIs): A research update. *Australian Nursing and Midwifery Journal*, *24*(8), 43

3. **Fasugba, O.**, Koerner, J., Mitchell, B. & Gardner, A. (2017). A systematic review and meta-analysis of the effectiveness of antiseptics for meatal cleaning in the prevention of catheter associated urinary tract infections. *Journal of Hospital Infection*, *95*(3), 233-242.

4. Mitchell, B., **Fasugba, O.**, Beckingham, W., Bennett, N. & Gardner, A. (2016). A point prevalence study of healthcare associated urinary tract infections in Australian acute and aged care facilities. *Infection, Disease and Health,* 21 (1), 26-31.

5. **Fasugba, O.**, Gardner, A., Mitchell, B., Beckingham, W. & Bennett, N. (2014) Surveillance to reduce urinary tract infections: The STRUTI project. *Australian Nursing and Midwifery Journal*, *22*(3), 34.

6. Gardner, A., Mitchell, B., Beckingham, W. & **Fasugba, O.** (2014) A point prevalence study of healthcare associated urinary tract infections in six Australian hospitals. *BMJ Open*, 4(7). doi:10.1136/bmjopen-2014-005099

7. Mitchell, B., Gardner, A., Beckingham, W. & **Fasugba, O.** (2014). Healthcare associated urinary tract infections: a protocol for a national point prevalence study. *Healthcare Infection, 19*(1), 26-31.

Oral and poster presentations

1. **Fasugba, O.**, Koerner, J., Mitchell, B. & Gardner, A. (2016, November). *The effectiveness of meatal cleaning with antiseptics for the prevention of catheter associated urinary*

traction infection: Findings of a systematic review and meta-analysis. Australasian College of Infection Prevention and Control Conference, Melbourne, Australia. Awarded Elaine Graham Robertson Award for best oral (free paper) presentation.

2. Mitchell, B., **Fasugba, O.**, Beckingham, W., Bennett, N., & Gardner, A. (2016, November). *A point prevalence study of healthcare associated urinary tract infections in Australian acute and aged care facilities: Results of the STRUTI project*. Australasian College of Infection Prevention and Control Conference, Melbourne, Australia.

3. **Fasugba, O.**, Koerner, J., Mitchell, B. & Gardner, A. (2016, August). *Effectiveness of antiseptics for meatal cleaning in the prevention of catheter associated urinary tract infection*. Oral presentation at the Canberra Health Annual Research Meeting, Canberra, Australia. Awarded best oral nursing presentation.

4. Mitchell, B., **Fasugba, O.**, Beckingham, W., Bennett, N., & Gardner, A. (2016, July). *A point prevalence study of healthcare associated urinary tract infections in Australian acute and aged care facilities: Results of the STRUTI project*. Oral presentation at the Tissue Viability/Infection Prevention and Control seminar, Canberra, Australia.

5. Mitchell, B., **Fasugba, O.**, Beckingham, W., Bennett, N., & Gardner, A. (2016, March). *A point prevalence study of healthcare associated urinary tract infections in Australian acute and aged care facilities: Results of the STRUTI project*. Poster presentation at the Canberra Health Annual Research Meeting (CHARM), Canberra, Australia.

6. Mitchell, B., **Fasugba, O.**, Beckingham, W., Bennett, N., & Gardner, A. (2016, August). *A point prevalence study of healthcare associated urinary tract infections in Australian acute and aged care facilities: Results of the STRUTI project.* Oral presentation at the sixteenth Congress of the International Federation of Infection Control, Vienna, Austria.

7. Mitchell, B., **Fasugba, O.**, Beckingham, W., Bennett, N., & Gardner, A. (2016, February). *The Surveillance to Reduce Urinary Tract Infections (STRUTI) Project: results of a multisite point prevalence study in Australia* [Webinar]. Australasian College of Infection Prevention and Control.

8. Koerner, J., **Fasugba, O.**, Snijders, T. & Gardner, A. (2015, August). *The effectiveness of meatal cleaning with antiseptics for the prevention of urinary traction infection: Protocol for a systematic review and meta-analysis*. Poster presentation at the Canberra Health Annual Research Meeting, Canberra, Australia.

9. Gardner, A., Mitchell, B., Beckingham, W., Bennett, N., & **Fasugba, O.** (2014, November). *Reporting on a proof concept plan for national online surveillance of healthcare associated urinary tract infections.* Oral presentation at the Australasian College of Infection Prevention and Control Conference, Adelaide, Australia.

10. Gardner, A., Mitchell, B., Beckingham, W., Bennett, N. & **Fasugba, O.** (2014, October). *Reporting on a proof concept plan for national online surveillance of healthcare associated urinary tract infections*. Oral presentation at the 3rd Biennial Australian Capital Region Nursing and Midwifery Research Centre Conference, Canberra, Australia.

11. Gardner, A., Mitchell, B., Beckingham, W., Bennett, N. & **Fasugba, O.** (2014, August). *Reporting on a proof concept plan for national online surveillance of healthcare associated urinary tract infections*. Poster presentation at the Canberra Health Annual Research Meeting, Canberra, Australia

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RESEARCH ARTICLE Open Access

Ciprofloxacin resistance in community- and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies

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Abstract

Background: During the last decade the resistance rate of urinary *Escherichia coli* (*E. coli*) to fluoroquinolones such as ciprofloxacin has increased. Systematic reviews of studies investigating ciprofloxacin resistance in community- and hospital-acquired *E. coli* urinary tract infections (UTI) are absent. This study systematically reviewed the literature and where appropriate, meta-analysed studies investigating ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTIs.

Methods: Observational studies published between 2004 and 2014 were identified through Medline, PubMed, Embase, Cochrane, Scopus and Cinahl searches. Overall and sub-group pooled estimates of ciprofloxacin resistance were evaluated using DerSimonian-Laird random-effects models. The I^2 statistic was calculated to demonstrate the degree of heterogeneity. Risk of bias among included studies was also investigated.

Results: Of the identified 1134 papers, 53 were eligible for inclusion, providing 54 studies for analysis with one paper presenting both community and hospital studies. Compared to the community setting, resistance to ciprofloxacin was significantly higher in the hospital setting (pooled resistance 0.38, 95 % CI 0.36-0.41 versus 0.27, 95 % CI 0.24-0.31 in community-acquired UTIs, $P < 0.001$). Resistance significantly varied by region and country with the highest resistance observed in developing countries. Similarly, a significant rise in resistance over time was seen in studies reporting on community-acquired *E. coli* UTI.

Conclusions: Ciprofloxacin resistance in *E. coli* UTI is increasing and the use of this antimicrobial agent as empirical therapy for UTI should be reconsidered. Policy restrictions on ciprofloxacin use should be enhanced especially in developing countries without current regulations.

Keywords: Antimicrobial resistance, Escherichia coli, Urinary tract infection, Systematic review, Meta-analysis

Background

Urinary tract infections (UTI) are one of the most frequent bacterial infections affecting people both in the community and in hospitals [1]. It is estimated that about 150 million people per annum are diagnosed with UTI worldwide [2]. A recent World Health Organisation (WHO) report on antimicrobial resistance (AMR) surveillance specified nine bacteria of international concern which are responsible for some of the most common infections in community and hospital settings [3]. *Escherichia coli* (*E. coli*), the pathogen most often implicated in UTIs, is listed as one of the nine. In all six WHO regions (Africa, Americas, Eastern Mediterranean, European, South-East Asia and Western Pacific) high rates of antimicrobial resistance have been observed in this pathogen [3].

Ciprofloxacin is the most commonly prescribed fluoroquinolone for UTIs because it is available in oral and intravenous preparations [4]. It is well absorbed from the gastrointestinal tract after oral administration. It also has a documented safety profile, broad Gram

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negative organism coverage and high urinary excretion rate [4]. During the last decade the resistance rate of *E. coli* to fluoroquinolones such as ciprofloxacin has increased [5]. A 10 year analysis of urinary *E. coli* specimens in Switzerland, found an increasing trend in resistance to ciprofloxacin, from 1.8 to 15.9 % [6]. Fluoroquinolones are ranked as one of four of the highest priority critically important antimicrobials [7] as they have an important role in the treatment of more severe infections, such as septicaemia. Therefore resistance to fluoroquinolones can have serious clinical consequences. They are one of few available therapies for serious *Salmonella* spp. and *E.coli* infections [5]. Resistance to fluoroquinolones emerges quickly, and this is likely to be related to the biology of resistance as well as a direct response to drug pressure [8]. They should therefore be used with caution and reserved for severe infections, and preceded by antimicrobial susceptibility testing of the bacteria involved [5]. The most recent Infectious Diseases Society of America (IDSA) guidelines recommend that fluoroquinolones should be reserved for important uses due to their propensity for ecological unfavorable effects of antimicrobial therapy such as the selection of drug-resistant pathogens and colonisation or infection with multidrug-resistant organisms [9].

Recent prescribing guidelines recommend reserving ciprofloxacin use for more severe infections and resistance to this agent is increasing prompting further research in this area [6, 10, 11]. Published quantitative syntheses of overall ciprofloxacin-resistant *E. coli* UTI prevalence and incidence in hospital and community settings are absent. This systematic review of observational studies therefore aims to compare ciprofloxacin resistance in both settings. Knowledge about ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTIs will provide information for control of resistant pathogens. This review also has the potential to provide a basis for which future interventions can be evaluated. The findings will, in addition, make available information on ciprofloxacin resistance in various regions of the world providing some evidence for further regulatory control of ciprofloxacin use globally.

Methods

Protocol and registration

The protocol for conducting this review has been registered and can be accessed on the International prospective register of systematic reviews (PROSPERO) (available at http://www.crd.york.ac.uk/prospero/ with registration number: CRD42014014473). Prior to registration, the protocol was reviewed by a reviewer external to the study team. Ethics approval was not sought as this review synthesized data from published studies for which approval had already been obtained.

Search strategy

We conducted a systematic review of observational (cross sectional, cohort and case control) studies published in the last 11 years (2004–2014) reporting on ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTIs. This time limit is based on changes in the microbiology and epidemiology of antimicrobial resistant pathogens which occurred in the past decade with subsequent changes in treatment regimens and patient outcomes [12]. Reporting of this review complied with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) [13].

The electronic bibliographic databases MEDLINE/PubMed, EMBASE, Cochrane, CINAHL and Scopus were searched. Searches were conducted for words in the title or abstract or within the full text of the papers. These included both keywords only and keywords with medical subject headings (MeSH) using the search terms 'resistance','urinary tract infection' and 'Escherichia coli' from 1st January 2004 to 31st December 2014 (see Additional file 1). The reference lists of papers identified from the electronic databases were hand-searched for additional papers.

Inclusion and exclusion criteria

Papers were included if they reported prevalence or incidence rates of ciprofloxacin resistance in community- or hospital-acquired *E. coli* UTIs. Papers reporting on urinary *E. coli* ciprofloxacin susceptibility in which resistance rate could be calculated were also included. We included papers involving adults and/or children. Only peer reviewed manuscripts were considered. Grey material which includes unpublished literature, conference abstracts, letters to editors, newsletters and reports were excluded. Non-peer reviewed literature were also excluded. Papers written in languages other than English were also excluded. In addition, papers not clearly specifying the setting (hospital-acquired or community-acquired); drug (ciprofloxacin) or sample (urine) were excluded. Papers that focused on specific sub-populations (e.g. diabetics and patients with recurrent UTI) were also excluded as these did not represent the general population. This review included only papers that used the Centers for Disease Control and Prevention (CDC) definition of microbiologically confirmed UTI ($\geq 10^5$ colony forming unit/ml) [14].

Definitions

For the purpose of this review, a study was defined as all data from a published paper with the only distinction being 'hospital' or 'community' setting. Therefore, if a single paper meeting the eligibility criteria reported data on both settings, they were included as two separate studies.

Community-acquired UTI was defined as positive samples obtained from (i) outpatient clinics; (ii) general practice (GP) clinics; (iii) emergency departments; (iv) within

48 h of hospital admission or (v) from nursing homes or residential aged care facilities [15–17].

Hospital-acquired UTI was defined as positive samples obtained (i) after 48 h of hospital admission or (ii) within 48 h of hospital discharge [15].

Important changes in healthcare delivery over the last few years have seen some usually inpatient procedures now more often than not performed on an outpatient basis [18]. Patients transition freely within sometimes loosely defined levels of the health care system, for example between long-term care or rehabilitation services, to acute-care centres [19, 20]. This study only considered hospital-acquired UTIs as opposed to a wider definition of healthcare associated UTIs, to avoid this confusion.

Study selection

The titles and abstracts of all papers identified in the electronic databases were examined and assessed for relevance and appropriateness to the principal objective of the systematic review. Irrelevant studies were excluded. Full texts of the potentially relevant papers were printed and carefully assessed against the systematic review inclusion and exclusion criteria. Those not meeting the criteria were excluded. The remaining papers deemed to have data relevant to the systematic review and meta-analysis were assessed for quality and risk of bias.

The study selection process and other stages of the review were performed by the lead author (OF). At each stage, 10 % of papers identified were also screened against the study criteria independently by other authors (AG, GM and BM). Discrepancies in either the application of inclusion or exclusion of papers, quality assessment or on data extraction were discussed among all authors to make the final decision.

Data extraction process

Data were extracted by one author (OF) and 10 % of papers eligible for data extraction were independently extracted by another author (AG). Data extraction was compared between AG and OF demonstrating 100 % agreement for all items except the study design. This variable was therefore assessed by all authors. Where there was missing information on the study design of papers to be included in the meta-analysis, attempts were made to contact the authors. When there was no response, consensus on the study design was reached by all authors. Agreement between authors was assessed using Kappa coefficient. The agreement between all authors in deciding on the study design was 71 % (Kappa (95 % CI) = 0.429 (0.154–0.703), P Value = 0.003). Papers for which no agreement could be reached on the design, based on insufficient information, were assigned as non-classifiable. Any other missing information in the included papers was recorded as 'not stated'.

The first author, year of study, country of study, study setting, age and sex distribution, co-morbidities, sample size, study design, study aim, antimicrobial susceptibility testing method, ciprofloxacin resistance rate, risk factors for ciprofloxacin resistance (i.e. previous antibiotic use) and mortality data (if reported) were extracted. Where the ciprofloxacin resistance rate was not available, the susceptibility rate was used to determine resistance.

Risk of bias in individual studies

Quality and risk of bias of the final papers included in the review was conducted using a modified version of the Newcastle-Ottawa Scale (NOS) which is a risk of bias assessment tool for observational studies recommended by the Cochrane Collaboration [21, 22]. Content validity and inter-rater reliability of this tool have been established [22]. Studies were rated by assigning a judgment of 'Low risk' of bias, 'High risk' of bias, or 'Unclear risk' of bias according to published criteria [21].

Statistical analysis

Pooled ciprofloxacin resistance proportions (with 95 % confidence intervals) in patients with *E. coli* UTI were separately calculated and compared between hospital and community settings using a random-effects meta-analysis model based on DerSimonian and Laird method [23, 24]. This method incorporates an estimate of the between-study variation into both the study weights and the standard error of the estimate of the common effect. The precision of an estimate from each included study was represented by the inverse of the variance of the outcome pooled across all studies. If the value of the pooled prevalence was within the 95 % CI, then the effect size was statistically significant at the 5 % level ($P < 0.05$). The heterogeneity among studies was assessed by using the I^2 statistic with a P value of <0.05 considered statistically significant, and I^2 values below 25 % indicating low heterogeneity, 25–75 % moderate heterogeneity and over 75 % high heterogeneity [25]. Subgroup analyses were done by risk of bias, study duration, age group, UTI symptoms, world region and economy of country (categorised as developed and developing using the World Bank classification [26]). A meta-regression analysis was used to determine the effect of measured covariates on the observed heterogeneity in resistance estimates across studies [23]. Assessment of publication bias was estimated using funnel plots. Further analysis was undertaken to examine pooled ciprofloxacin resistance over time using the median study year. For studies occurring over 2 years, the first year was used; for studies occurring over 4 years, the 2nd year was used; for those over 6 years, the 3rd year was used. The non-parametric Spearman's rho correlation coefficient was

calculated to determine significance in resistance trend over time. Statistical analyses were undertaken using Stata statistical softwareversion 13 [27].

Results

Study selection

Electronic database searches identified 15,062 potential studies and 31 additional studies were identified through hand searching. After 11,397 duplicates were removed, 3696 articles remained for title and abstract screening. We assessed 1134 as potentially eligible and retrieved the full text of these articles. After applying inclusion and exclusion criteria, 53 papers (5 %) were deemed to have data relevant to the systematic review and meta-analysis. These 53 papers consisted of 54 studies comprising three hospital-acquired *E. coli* UTI studies and 51 community-acquired *E. coli* UTI studies. There was one paper that compared resistance in both hospital and community settings hence reported as two studies [15]. The PRISMA flow chart describing the papers identified from the search strategy and reasons for exclusion is shown in Fig. 1.

Study characteristics

Geographically, 53 of the 54 studies were carried out in Asia (28 %; $n = 15$), Europe (24 %; $n = 13$), Middle East (15 %; $n = 8$), Africa (13 %; $n = 7$), North America (11 %; $n = 6$) and South America (7 %; $n = 4$). The remaining study was conducted in multiple countries [28]. There were 17 (31 %) studies conducted in developed countries and 36 (67 %) in developing countries. The majority of the studies (80 %) followed a cross sectional design. The duration of studies ranged from 2 months to 84 months (median = 15.5; IQR = 12.0-30.0). The mean age and sex proportion of patients with an *E. coli* UTI were stated in 13 % ($n = 7$) and 44 % ($n = 24$) of studies respectively. Most study populations included patients of both sexes although 19 % ($n = 10$) included only women. Antimicrobial susceptibility testing and interpretation was performed using the disk diffusion method (74 %) and Clinical and Laboratory Standards Institute (CLSI) criteria (83 %) respectively in most studies. Table 1 provides further details on the description of the included studies.

Pooled ciprofloxacin resistance

Figures 2 and 3 show the forest plots of studies reporting on ciprofloxacin resistance in community acquired *E. coli* UTI by region and economy, respectively. Figure 4 shows the forest plot of studies reporting on ciprofloxacin resistance in hospital acquired *E. coli* UTI. Compared with the community-setting, resistance to ciprofloxacin in *E coli* UTIs was significantly higher in the hospital-setting ($P < 0.001$). Overall, the pooled rate for ciprofloxacin resistance in patients with community-acquired *E. coli* UTIs was 0.27 (95 % CI: 0.240-0.310), compared with 0.38 (95 % CI: 0.360-0.410) in the hospital setting. There was substantial heterogeneity among the community-setting studies ($I^2 = 98.8$ %, $P < 0.0001$), but very little in the hospital ones ($I^2 = <0.010$ %, $P = 0.641$). Further analysis of studies reporting on community-acquired *E. coli* UTI by region (Fig. 3) showed that Asia had the highest pooled resistance. Analysis by economy based on the World Bank classification (Fig. 4) showed a higher pooled resistance in developing countries.

Resistance over time in community-acquired UTI studies

Figure 5 shows the scatter plot of ciprofloxacin resistance in 47 studies reporting on community-acquired UTI using the median study year for each study. Four studies did not provide data on the year(s) the study was conducted and were excluded from this analysis [29–32]. The results of the Spearman's rho correlation test showed a statistically significant rise in resistance over time ($n = 47$, $r_s = 0.431$, $P = 0.003$). Similar findings were observed for developing countries. There was no significant rise in resistance over time in developed countries.

Subgroup analyses

Sub-group analysis was conducted within each major setting. For community-acquired UTI studies (Table 2), there was a significant difference in the pooled resistance within each subgroup examined (risk of bias, study duration, economy, region, age group and UTI symptoms). The subgroup analyses results for studies reporting on hospital-acquired *E. coli* UTI (see Additional file 2) showed no difference in the pooled resistance within the subgroups examined (region, economy and UTI symptoms). When both settings were compared (see Additional file 3), there were significant differences noted for risk of bias (high), study duration (>12 months), economy (developed), region (Americas), age group (adults and children) and UTI symptoms ($P < 0.001$). There were no data available on mortality for comparison between settings.

Meta-regression analyses

Random effects meta-regression analyses of studies reporting on community-acquired *E. coli* UTI showed that country's economy ($P = 0.008$), Asia as a region ($P = 0.002$), high risk of bias ($P = 0.003$), year of study ($P = 0.020$) and studies using only children as the study population ($P = 0.030$) were the study factors significantly accounting for the observed heterogeneity, responsible for 61 % of the between study variance (Adjusted R^2) in ciprofloxacin resistance.

Risk of bias

When studies were assessed for risk of bias using the Newcastle-Ottawa scale, 30 % ($n = 16$) were assessed as

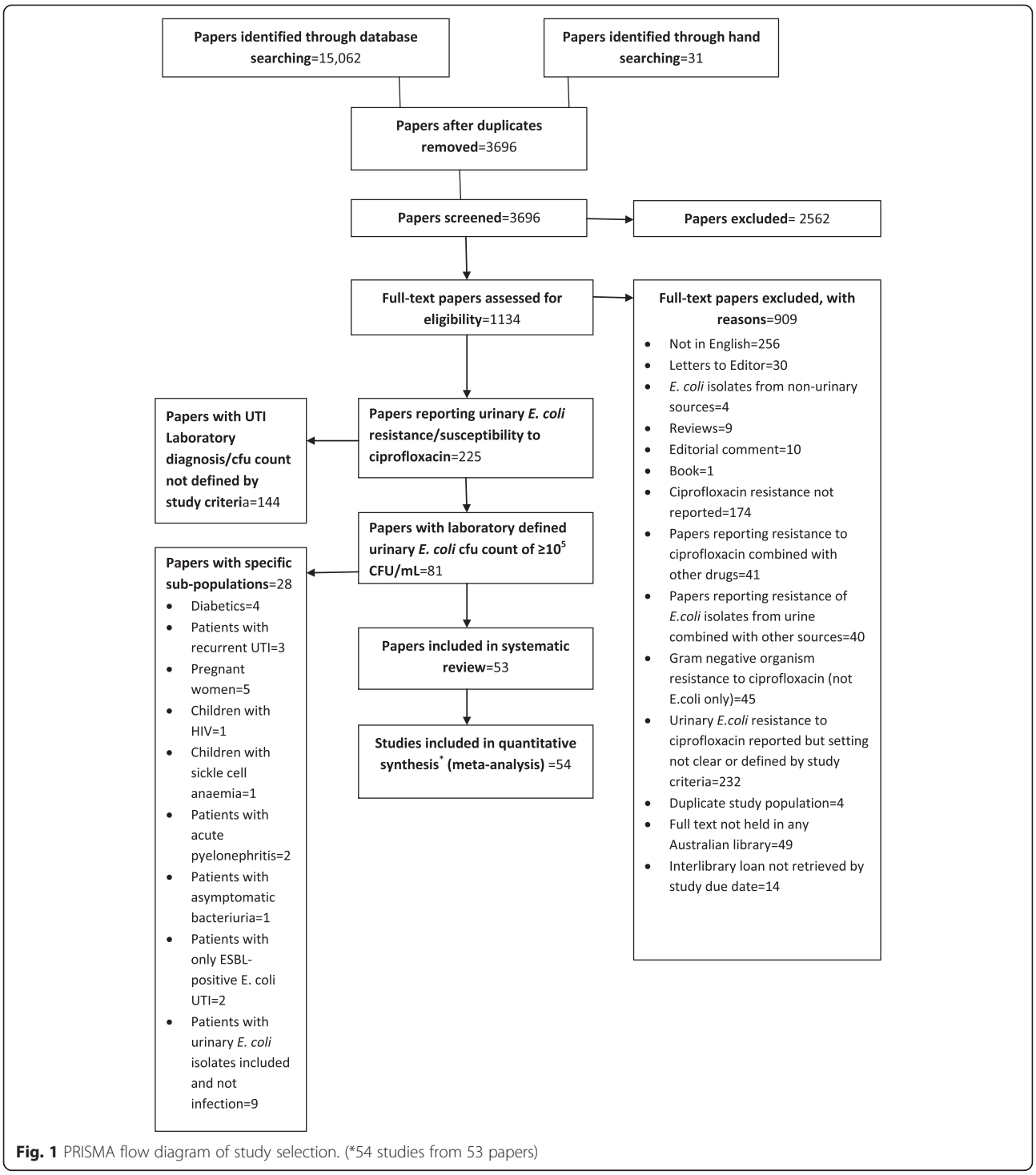

Fig. 1 PRISMA flow diagram of study selection. (*54 studies from 53 papers)

having a low risk of bias; 22 % ($n = 12$) unclear risk of bias and 48 % ($n = 26$) were deemed to have a high risk of bias. Further analysis of the 16 low risk studies only was consistent with findings reported from the analysis of all studies. An increasing resistance trend over time was also observed, however this increase did not reach statistical significance because of reduced statistical power.

Discussion

The findings of this systematic review and meta-analysis highlight the higher ciprofloxacin resistance in hospital-acquired *E.coli* UTI when compared to community-acquired UTI. There is also substantial evidence that ciprofloxacin resistance in community-acquired *E. coli* UTI has been increasing in recent years. Resistance was

Table 1 Description of studies included in meta-analysis

Study author	Country	Design[a]	Setting	Risk of bias	Study duration[b] (months)	Number of positive *E. coli* UTI samples[c]	Number of ciprofloxacin resistant *E. coli*	Proportion resistant (95 % CI)	Standard error	Weight[d] (%)
Ahmad, 2012	India	Cross sectional	Community	Unclear	24	318	48	0.15 (0.11, 0.19)	0.02	2.09
Akoachere et al., 2012	Cameroon	Cross sectional	Community	Low	12	43	11	0.26 (0.13, 0.39)	0.07	1.61
Akram et al., 2007	India	Cross sectional	Community	High	12	61	42	0.69 (0.57, 0.80)	0.06	1.70
AlSweih et al., 2005	Kuwait	Cross sectional	Community	High	12	1535	81	0.05 (0.04, 0.06)	0.01	2.15
Al-Tawfiq et al., 2009	Saudi Arabia	Cohort	Community	High	12	2281	592	0.26 (0.24, 0.28)	0.01	2.14
Ansbach et al., 2013	USA	Cross sectional	Community	High	7	98	2	0.02 (−0.01, 0.05)	0.01	2.12
Arabi et al., 2013	Iran	Cross sectional	Community	Low	33	103	23	0.22 (0.14, 0.30)	0.04	1.91
Araujo et al., 2011	Brazil	Cross sectional	Community	Unclear	24	391	36	0.09 (0.06, 0.12)	0.01	2.12
Arslan et al., 2005	Turkey	Cross sectional	Community	Low	5	514	135	0.26 (0.22, 0.30)	0.02	2.09
Astal, 2005	Palestine	Cross sectional	Community	High	6	252	30	0.12 (0.08, 0.16)	0.02	2.09
Azap et al., 2010	Turkey	Cohort	Community	Unclear	12	464	139	0.30 (0.26, 0.34)	0.02	2.08
Bahadin et al., 2011	Singapore	Cross sectional	Community	Unclear	12	90	22	0.24 (0.16, 0.33)	0.05	1.86
Biswas et al., 2006	India	Cross sectional	Community	High	36	354	124	0.35 (0.30, 0.40)	0.03	2.05
Bouchillon et al., 2013	USA	Cross sectional	Community	High	24	723	234	0.32 (0.29, 0.36)	0.02	2.10
Bouchillon et al., 2013	USA	Cross sectional	Hospital	High	24	253	103	0.41 (0.35, 0.47)	0.03	11.83
Dash et al., 2013	India	Cross sectional	Community	Low	30	397	212	0.53 (0.48, 0.58)	0.03	2.05
Dimitrov et al., 2004	Kuwait	Cross sectional	Community	High	84	780	92	0.12 (0.10, 0.14)	0.01	2.13
Farshad et al., 2011	Iran	Cross sectional	Community	Low	12	90	8	0.09 (0.03, 0.15)	0.03	2.01
Ghadiri et al., 2012	Iran	Cross sectional	Hospital	High	24	200	80	0.40 (0.33, 0.47)	0.03	9.41
Gobernado et al., 2007	Spain	Cross sectional	Community	Low	12	2292	418	0.18 (0.17, 0.20)	0.01	2.14
Ho et al., 2010	Hong Kong	Cross sectional	Community	Low	24	271	35	0.13 (0.09, 0.17)	0.02	2.09
Hoban et al., 2011	Multiple countries	Cross sectional	Hospital	High	24	1643	624	0.38 (0.36, 0.40)	0.01	78.76
Ismaili et al., 2011	Belgium	Cohort	Community	High	24	189	5	0.03 (0.00, 0.05)	0.01	2.13
Kashef et al., 2010	Iran	Cross sectional	Community	High	30	578	180	0.31 (0.27, 0.35)	0.02	2.09
Kiffer et al., 2007	Brazil	Cross sectional	Community	Unclear	48	22679	2699	0.12 (0.11, 0.12)	0.002	2.15
Killgore et al., 2004	USA	Case–control	Community	Low	12	120	40	0.33 (0.25, 0.42)	0.04	1.89
Kimando et al., 2010	Kenya	Cross sectional	Community	Unclear	6	92	6	0.07 (0.01, 0.12)	0.03	2.05
Kothari et al., 2008	India	Cross sectional	Community	High	6	361	260	0.72 (0.67, 0.77)	0.02	2.06
Kurutepe et al., 2005	Turkey	NC	Community	High	72	880	174	0.20 (0.17, 0.22)	0.01	2.12
Lau et al., 2004	Taiwan	Cross sectional	Community	Unclear	13	80	14	0.17 (0.09, 0.26)	0.04	1.89
Ljuca et al., 2010	Bosnia & Herzegovina	Cross sectional	Community	High	36	43	4	0.09 (0.01, 0.18)	0.04	1.87

Table 1 Description of studies included in meta-analysis *(Continued)*

Longhi et al., 2012	Italy	NC	Community	Low	6	154	36	0.23 (0.17, 0.30)	0.03	1.98
Martinez et al., 2012	Colombia	Cross sectional	Community	High	2	102	39	0.38 (0.29, 0.48)	0.05	1.83
Miragliotta et al., 2008	Italy	Cohort	Community	Low	60	2589	422	0.16 (0.15, 0.18)	0.01	2.14
Molina-Lopez et al., 2011	México	Cross sectional	Community	High	48	119	65	0.55 (0.46, 0.64)	0.05	1.86
Moreira et al., 2006	Brazil	Cross sectional	Community	Unclear	15	544	65	0.12 (0.09, 0.15)	0.01	2.12
Murugan et al., 2012	India	Cohort	Community	High	12	204	144	0.71 (0.64, 0.77)	0.03	2.00
Muvunyi et al., 2011	Rwanda	Cross sectional	Community	Low	6	72	23	0.32 (0.21, 0.43)	0.05	1.75
Mwaka et al., 2011	Uganda	Cross sectional	Community	High	NS	27	9	0.33 (0.16, 0.51)	0.09	1.32
Ni Chulain et al., 2005	Ireland	Cross sectional	Community	High	5	723	18	0.02 (0.01, 0.04)	0.01	2.15
Olson et al., 2012	USA	Cross sectional	Community	Unclear	16	95	4	0.04 (0.00, 0.08)	0.02	2.08
Otajevwo, 2013	Nigeria	Cross sectional	Community	High	6	5	4	0.80 (0.45, 1.15)	0.18	0.63
Prakash et al., 2013	India	Cross sectional	Community	Low	NS	23	16	0.70 (0.51, 0.88)	0.10	1.26
Randrianirina et al., 2007	Madagascar	Cross sectional	Community	Low	28	607	100	0.16 (0.14, 0.19)	0.02	2.12
Rani et al., 2011	India	Cross sectional	Community	Unclear	6	208	151	0.73 (0.67, 0.79)	0.03	2.01
Shaifali et al., 2012	India	Cross sectional	Community	Unclear	12	46	28	0.61 (0.47, 0.75)	0.07	1.54
Shariff et al., 2013	India	Cross sectional	Community	High	18	491	160	0.33 (0.28, 0.37)	0.02	2.08
Sire et al., 2007	Senegal	Cross sectional	Community	Low	33	1010	157	0.16 (0.13, 0.18)	0.01	2.13
Sood et al., 2012	India	NC	Community	High	30	214	160	0.75 (0.69, 0.81)	0.03	2.02
Stratchounski et al., 2006	Russia	NC	Community	Low	48	423	18	0.04 (0.02, 0.06)	0.01	2.14
Vellinga et al., 2012	Ireland	Case–control	Community	Low	9	633	78	0.12 (0.10, 0.15)	0.01	2.12
Wang et al., 2014	China	Cross sectional	Community	High	8	129	91	0.71 (0.63, 0.78)	0.04	1.92
Yildirim et al., 2010	Turkey	Cross sectional	Community	Unclear	24	450	85	0.19 (0.15, 0.23)	0.02	2.10
Yolbas et al., 2013	Turkey	Cross sectional	Community	High	12	113	24	0.21 (0.14, 0.29)	0.04	1.93

[a]Non-classifiable design
[b]Not stated
[c]Study denominator
[d]Weights are from random effects analysis using DerSimonian-Laird model

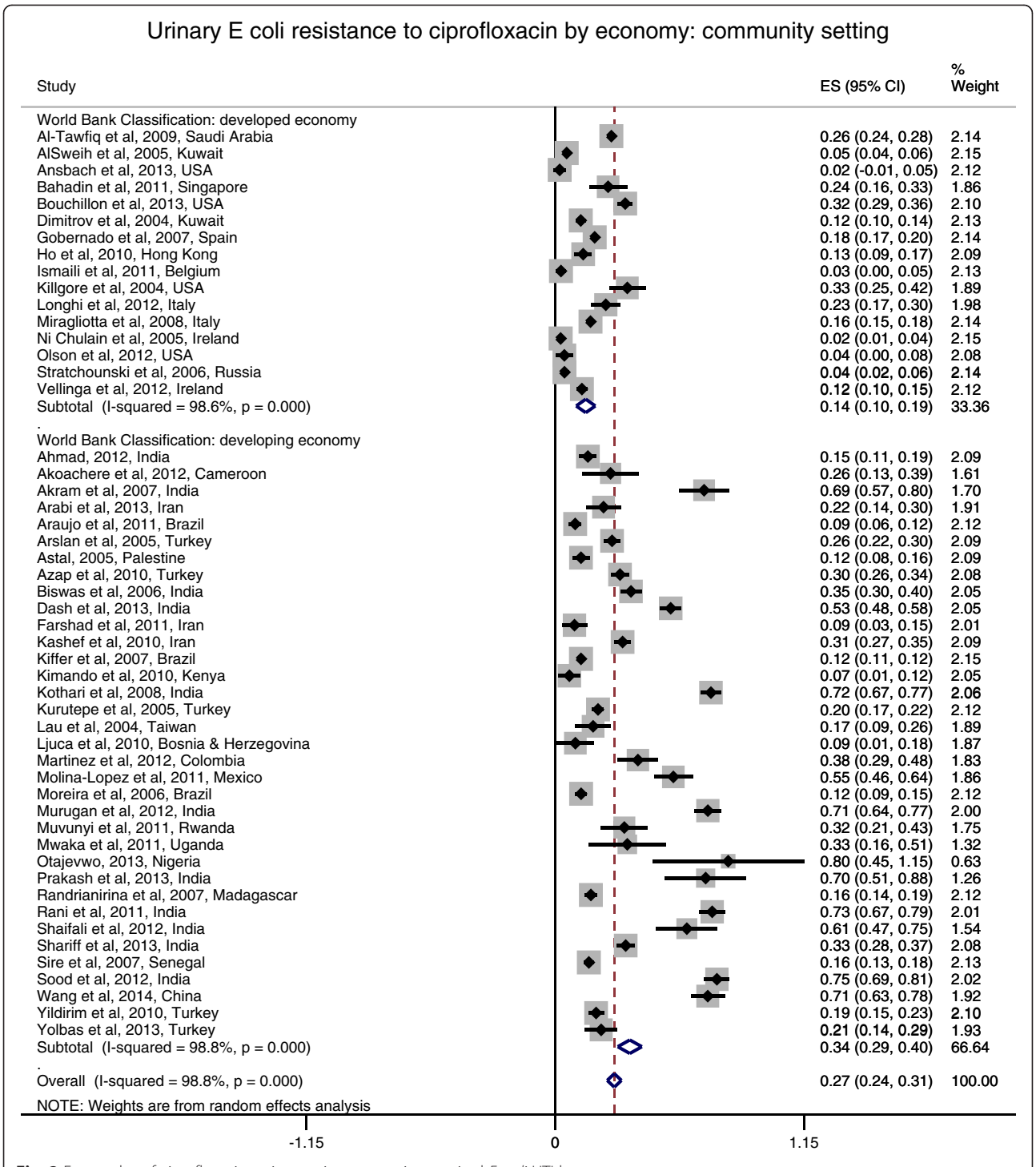

Fig. 2 Forest plot of ciprofloxacin resistance in community-acquired *E. coli* UTI by economy

also found to be significantly higher in developing countries reporting on *E. coli* UTI in community settings.

Antimicrobial resistance has been described as an international hazard to public health threatening the successful prevention and treatment of bacterial, viral, parasitic and fungal infections [3, 33]. As such, research into its prevention and reduction is very important. Our estimated pooled ciprofloxacin resistance of 27 and 38 % in community- and hospital-acquired *E. coli* UTI respectively could not be compared to any other systematic review findings because, to our knowledge, this is the first systematic review and meta-analysis comparing ciprofloxacin

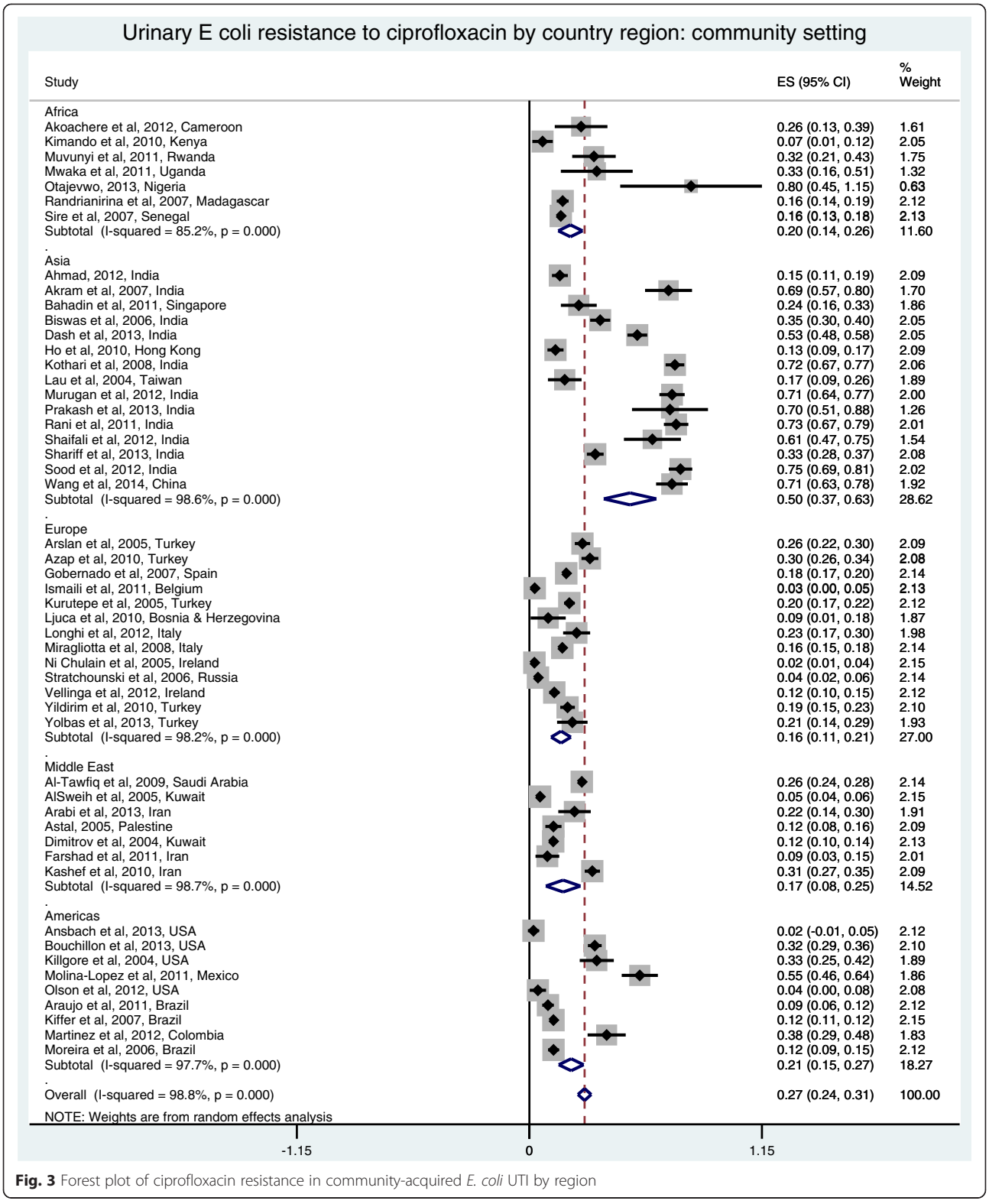

Fig. 3 Forest plot of ciprofloxacin resistance in community-acquired *E. coli* UTI by region

resistance in community- and hospital-acquired *E. coli* UTI. However, national data from five WHO regions show at least 50 % resistance to fluoroquinolones (ciprofloxacin, norfloxacin or ofloxacin) in *E. coli* [3]. Data on *E. coli* in the WHO report are from various settings and sources (including blood and urine) hence cannot be directly compared with the results from our systematic review. Another recent review on global fluoroquinolone resistance

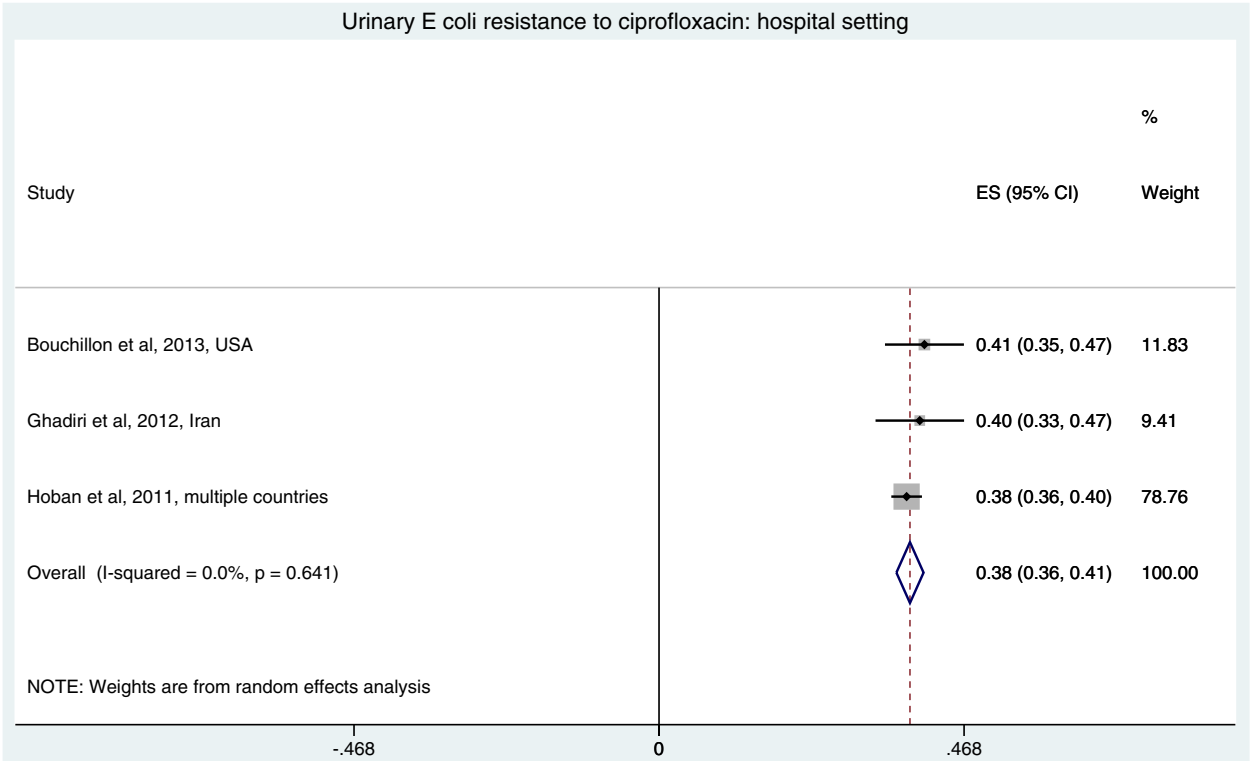

Fig. 4 Forest plot of ciprofloxacin resistance in hospital-acquired *E. coli* UTI

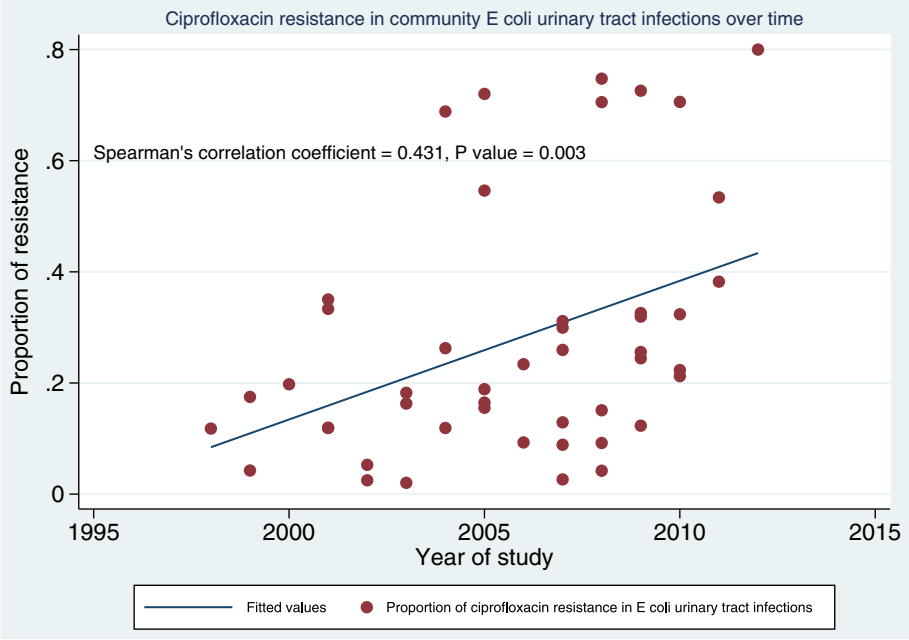

Fig. 5 Scatter plot of ciprofloxacin resistance in community-acquired UTI by year of study (1998–2012). *N* = 47 (4 studies excluded due to missing information on year study was conducted)

Table 2 Subgroup analyses of pooled ciprofloxacin resistance in community setting

Subgroup		Community Setting $N = 51$	P value*
		Pooled resistance	
Risk of bias	Low and unclear $n = 28$ studies	0.221	<0.0001
	High $n = 23$ studies	0.337	
Study duration[a]	≤12 months$n = 25$ studies	0.323	<0.0001
	>12 months$n = 24$ studies	0.219	
Economy	Developed$n = 16$ studies	0.141	<0.0001
	Developing$n = 35$ studies	0.345	
Region	Africa, Asia and Middle East$n = 29$ studies	0.361	<0.0001
	Europe, North and South American$n = 22$ studies	0.174	
Age group[a]	Adults and children [b]$n = 24$ studies	0.265	<0.0001
	Adults only$n = 19$ studies	0.302	
UTI symptoms	Symptomatic and asymptomatic patients $n = 11$ studies	0.185	<0.0001
	Symptomatic patients only $n = 40$ studies	0.295	

n = number of studies reporting on community acquired UTI
*Comparing pooled resistance for difference in subgroup in community setting
[a]Studies with missing information on this sub-analysis were not included
[b]Studies reporting resistance in adults and children or children only

epidemiology reported a range of 2 to 69 % for fluoroquinolone resistance in uncomplicated community-acquired UTI and up to 98 % in complicated cases, with fluoroquinolone resistance in healthcare associated UTIs ranging from 6 to 62 % [34]. The findings from our systematic review are within the above reported ranges. However, the latter ranges were wide and the data were from a number of different Gram negative uropathogens and not specifically *E. coli* accounting for the higher rates. Available published data show relatively high rates of urinary *E. coli* resistance to ciprofloxacin [35–41] prompting the need for a renewed effort in the further prevention of spread of resistance to this antimicrobial agent.

We found that urinary *E. coli* resistance to ciprofloxacin was higher in the hospital compared to the community setting. Our finding is comparable to individual studies which have assessed urinary *E.coli* resistance to ciprofloxacin in both, hospital and community settings [31, 41–45]. However, often studies do not apply the criterion of 48 h post admission used in our systematic review for identifying hospital acquired UTI [45, 46]. The Canadian national surveillance study (CANWARD), a large population-based study undertaken from 2007 to 2009, further confirms our finding of higher resistance in the hospital setting [47]. Inpatients had a significantly higher urinary *E. coli* resistance to ciprofloxacin. Similar findings were reported by Cullen et al. in Dublin [16]. This is not an unusual finding and may be attributed to the selective pressure resulting from antimicrobial use in hospital settings [47]. Patients in hospital, already acutely ill, become more at risk of developing a resistant infection because of potential immune deficiency and relative high exposure to antimicrobial agents [48]. Furthermore, hospitalized patients are more likely to be exposed to practices that result in cross infection or transmission of organisms. These and other risk factors enable the spread of resistance. This has significant implications for patient care as antimicrobial resistance may lead to treatment failure resulting in death.

The results of our systematic review showed a significant rise in resistance over time in the community setting. This finding is supported by a number of US-based studies investigating antimicrobial resistance trend in outpatients. A fivefold increase (from 3 to 17.1 %) in ciprofloxacin resistance was observed from 2000 to 2010 by Sanchez et al. [17] in comparison with other antibiotics investigated [49]. Our findings are also consistent with Blaettler et al. [6] who found that over a 10 year period (1997–2007), similar to the timeframe for our review, resistance increased significantly for ciprofloxacin from 1.8 to 15.9 % in Switzerland. This increase coincided with a rise in ciprofloxacin use in Switzerland [6]. These findings suggest that with increase in the use of fluoroquinolones generally over time, resistance ciprofloxacin is likely to further increase. It is now known that antimicrobial overuse or misuse is a risk factor for the development of AMR [50]. The specific effect of ciprofloxacin use on the development of its resistance in UTI pathogens is also clearly documented. A recent Irish study involving 72 general practices found higher ciprofloxacin resistance levels (5.5 %) in practices with 10 prescriptions per month compared with resistance levels of 3 % in practices with one prescription per month [51]. Wide spread use of this agent may have thus resulted in a rise in ciprofloxacin resistance. In the Netherlands and United States, an association has also been shown between high

fluoroquinolone prescriptions and a rise in bacterial resistance [52, 53]. Furthermore, changes in antimicrobial prescribing practices have been shown to precede changes in resistance rates. A study by Gottesman et al. [54] in Israel found a significant decrease in *E. coli* resistance to ciprofloxacin following a nationwide restriction on ciprofloxacin use. Resistance decreased from 12 % in the pre-intervention period to 9 % in the intervention period. Our results pose a strong argument for the development of more stringent criteria limiting ciprofloxacin use. In addition, other strategies such as adequate surveillance and monitoring, reinforcement of existing infection prevention and control measures as well as new technological advancement will help reduce the widespread problem of antimicrobial resistance [55–57] but these aspects are not within the scope of this paper.

Our finding of a significant rise in resistance over time also has implications for the development of treatment guidelines. The national recommendations for first-choice empiric antibiotic treatment of UTIs vary considerably [5]. In countries like Spain, Taiwan and Turkey, the treatment choice for uncomplicated UTIs are fluoroquinolones [5, 58, 59]. In 2000, fluoroquinolones were prescribed for treatment of uncomplicated UTIs in Switzerland in 64 % of cases [60]. There is concern that resistance to ciprofloxacin resulting from its first-line use may be associated with an increase in multidrug resistance [61]. The most recent IDSA guidelines [9] advise using nitrofurantoin, trimethoprim-sulphamethoxazole, fosfomycin or pivmecillinam for first-line treatment of acute uncomplicated cystitis. Fluoroquinolones should be reserved for important uses other than acute cystitis or used as an alternative only when these recommended agents cannot be used [9]. We recommend that ciprofloxacin should not be used as a first line treatment option for UTIs as continuous increases in resistance to ciprofloxacin further weaken the effectiveness of this drug.

Additional findings from the meta-analysis showed that resistance was significantly higher in developing countries compared to developed countries. A major factor accounting for this difference is the use of over the counter or non-prescription antibiotics which occur commonly in developing countries [62, 63]. Although this review did not directly consider antimicrobial resistance in relation to prescribing for the included studies, evidence shows that over the counter or non-prescription use results in unnecessary and excessive use of antibiotics. Some of the included studies in our review clearly state that there are no restrictions for over the counter prescribing of antimicrobials within their countries [29, 64–73]. A recent systematic review investigating global non-prescription antimicrobial use found that resistance was common in communities with frequent non-prescription antimicrobial use [74]. Non-prescription use was highest in Africa, Asia and Middle East at 100, 58 and 39 % respectively [74]. In our review, further analyses by region showed that Asia had the highest pooled resistance to ciprofloxacin with a significantly higher resistance in Africa, Asia and Middle East combined compared with Europe and the Americas. Our finding is supported by a recent paper by Dalhoff [75] reporting that fluoroquinolone resistance was highest in the Asia-Pacific region and moderate to low in Europe and North America. Furthermore, there is evidence to show that countries that have developed control policies to regulate non-prescription use have seen a decrease in antimicrobial use and resistance rates [74]. Based on our findings, we therefore emphasize the need for the development of policies restricting over the counter antimicrobial use in countries that do not have such policies thereby contributing to the prevention of patient morbidity and mortality associated with resistant infections. It is noteworthy to mention that another important factor contributing to antimicrobial resistance is the use of antibiotics in livestock for growth promotion [76]. Extensive antimicrobial use in food animal production has been associated with antimicrobial resistance globally [76]. This has considerable implications for human health with the need to protect the efficacy of these antimicrobials to ensure their effectiveness for the treatment of humans.

A large variation in ciprofloxacin resistance was found in studies reporting on community-acquired UTI. This variation highlights the significance of local resistance monitoring to guide the development of local antibiotic guidelines. The random effects meta-regression model confirmed that a number of factors significantly accounted for the variations in ciprofloxacin resistance. These include economy (developed and developing), Asia as a region, year of study, studies including only children and studies with a high risk of bias. The first three factors have been discussed in detail in the preceding paragraphs. We found that resistance was lower in studies involving only children. This finding is in line with a number of studies which have compared resistance in adults and children showing significantly higher ciprofloxacin resistance in adults compared to children [77, 78]. Increased age has also been shown to be significantly associated with ciprofloxacin resistance [6, 47]. Given that children are less exposed to antimicrobials with limited ciprofloxacin use in the paediatric age group, this finding is expected [77–79]. Although the importance of intrafamilial cross-infection of resistant pathogens is yet to be confirmed, it has been suggested that fluoroquinolone resistance may to some extent be dependent on cross-infection with transfer from adults to children [78]. Given this assumption, it is necessary to also monitor resistance levels in children to prevent further resistance development in this vulnerable age group. Other likely causes of higher resistance in adults may be the greater likelihood of comorbidities with more

frequent contact with healthcare settings [47]. The last factor found to account for heterogeneity between studies was high risk of bias. Most of the studies included in the review were found to have a high risk of bias as assessed using the NOS scale. These studies lacked methodological rigour including absence of the inclusion of possible confounding factors (such as age, sex and previous use of an antimicrobial) in the design and analysis of the studies. The poor reporting of observational studies poses limitations for conducting meta-analysis of these studies. Better presentation of definitions would enable inclusion in systematic reviews of some categories that had to be excluded in this review. Observational studies are more prone to confounding bias [80] further emphasizing the need for adherence to reporting guidelines such as such as that based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement [81] to ensure clear and comprehensive reporting prior to publication acceptance. The poor quality of many studies initially retrieved for this review resulted in a large number being excluded. Therefore the information provided in this systematic review and meta-analysis of 54 observational studies may not sufficiently address ciprofloxacin resistance globally but may provide satisfactory evidence to inform future interventions.

In addition, this systematic review highlights the weaknesses in the quality of antimicrobial resistance data that are being collected in various regions. These weaknesses have implications for development of effective surveillance systems to monitor resistance globally and strategies to prevent further resistance development. The need for the implementation of national and global surveillance systems to detect and continuously monitor AMR cannot be overemphasized. These systems would enable prospective studies to be conducted and would play a major role in curtailing the widespread effect of antimicrobial resistance and help healthcare providers in deciding on the most appropriate empirical therapy for UTI to ensure proper management of patients. Governments need to put in place policies to restrict over the counter use and inappropriate prescribing of ciprofloxacin and other antimicrobials to prevent further development of resistance.

Strengths and limitations

There are a number of notable strengths to our review. To our knowledge, this is the first systematic review to compare the overall prevalence of ciprofloxacin resistance in community- and hospital-acquired *E. coli* UTI. We undertook a comprehensive literature search process to identify and screen articles against eligibility criteria. Given that generic versions of ciprofloxacin were first marketed at different times in various countries, our choice of 2004 as the start date was therefore made on the basis of changes in the epidemiology of antimicrobial resistant pathogens which had resulted in changes to treatment regimens. A further strength of this systematic review is the development of a peer reviewed, registered protocol prior to undertaking the review. For studies to be included in the review, they were restricted to those that used a standard laboratory UTI criterion of $\geq 10^5$ cfu/mL as recommended by the CDC. Although applying the internationally recognised CDC criteria may definitely be considered a strength as it ensures the quality and uniformity of included studies, this criterion limited the number of hospital-acquired UTI studies included in our systematic review. Despite this, resistance was still found to be higher in the hospital setting compared to the community setting similar to published studies. While lower counts of uropathogens are relevant for acute episodes of uncomplicated cystitis, the use of different colony counts makes comparison of data between studies difficult. Including all urinary *E.coli* isolates was considered but not done because this existing surveillance criterion ($\geq 10^5$ cfu/mL and 48 h cut off) is usually applied to defining infections not isolates. Also, including all isolates carries the risk of including duplicates. This approach poses some degree of ascertainment bias as our systematic review focuses on laboratory identified UTIs which may not only underestimate the total number of UTIs but also lead to selection of samples from complicated cases thereby overestimating resistance. Another limitation is the wide variation of resistance estimates between studies and the inclusion of studies having substantial clinical and methodological heterogeneity. Visual inspection of the funnel plot (Fig. 6) showed asymmetry suggesting evidence of publication bias, with studies reporting high resistance rates being more likely to be published posing a limitation to this review. Also, the quality and risk of bias of some of the studies included in the review were assessed as high. These limitations were addressed by undertaking a random effects meta-analysis with subsequent subgroup analyses and random effects meta-regression to explain the sources of heterogeneity. For studies in which the design was not stated, the review authors faced difficulties in categorising such studies hence some of these studies were grouped as non-classifiable. These studies did not provide clear and explicit information on the methods used for conducting the studies. This emphasizes the need for implementation and adherence to clear reporting standards prior to publication of papers. Furthermore, in some included studies, adjustments were not made for important confounding factors relevant to antimicrobial resistance such as antibiotic use and patient demographics including age and sex. For this systematic review, studies on samples obtained from emergency department (ED) patients were classified as community-acquired samples. Included papers did not provide any information on whether some of these patients may have

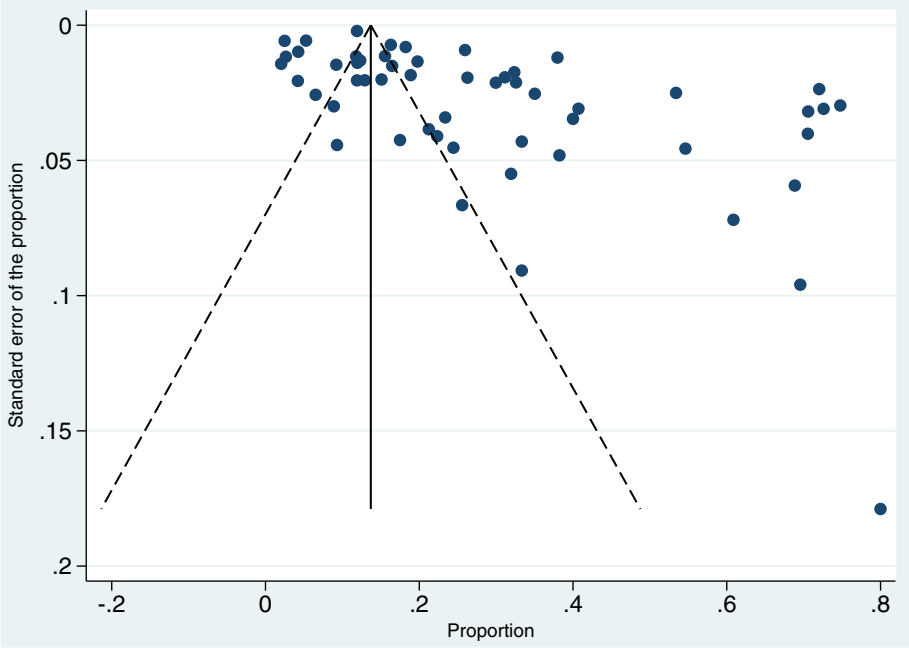

Fig. 6 Funnel plot of studies included in meta-analysis

returned from a recent hospitalisation and represented to the ED. Ideally, these should be considered as hospital-acquired infections as some of these patients may have been discharged in the previous 48 h. For the purpose of this review and to overcome inherent variations in how individual studies have defined these patients, we classified all papers reporting on ED patients as community-acquired UTI studies. It was not possible to determine the potential effect of samples obtained from nursing home or residential aged care studies on the pooled resistance because this participant group did not meet the inclusion criteria for analysis. Furthermore, classification of this setting as hospital or community remains controversial. Finally, validity issues may have arisen from the use of different antimicrobial susceptibility test and interpretation methods with differing breakpoints which tend to change over the years. To date, there is still no worldwide consensus on the most suitable antimicrobial susceptibility testing method with the fact that various countries and even laboratories within the same country use different tests and interpretative criteria. Subgroup analysis for AST method was considered but not done because almost all studies used the disk diffusion method and CLSI criteria.

Conclusions

Ciprofloxacin resistance in *E. coli* UTI is increasing. The use of this antimicrobial agent as empirical therapy for UTI should be reconsidered and efforts should be made to limit its use to clinical conditions for which there are clear therapeutic indications. Policy restrictions on ciprofloxacin use need to be developed and enforced especially in developing countries that are yet to have such policies put in place. Further research is needed to describe ciprofloxacin resistance in hospital-acquired *E. coli* UTI using widely accepted definitions.

Availability of Data and Materials

Data supporting the findings of this systematic review are available upon request from the corresponding author.

Additional files

Additional file 1: Search strategy by database. Search of EMBASE, CINHAL, Scopus, PubMed, MEDLINE and COCHRANE. (PDF 58 kb)

Additional file 2: Subgroup analyses of pooled ciprofloxacin resistance in hospital setting. Results of subgroup analyses for studies reporting on hospital acquired *E. coli* UTI. (PDF 12 kb)

Additional file 3: Subgroup analyses of pooled ciprofloxacin resistance by setting. Results of subgroup analyses comparing the pooled resistance within each subgroup examined for both community and hospital settings. (PDF 36 kb)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

OF conceived the idea for this study and took primary responsibility for writing the protocol. AG, GM and BM contributed to the development of the idea and supervised the writing of the protocol. OF searched the literature and made the primary selection of eligible papers including data extraction. AG, GM and BM supervised and checked the study selection process, data extraction and data analysis plan. OF and GM analysed the data. All authors contributed to interpretation of the analysis. OF wrote the manuscript. AG,

GM and BM critically reviewed and contributed to the manuscript. All authors have seen and approved the final version.

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References

1. Laupland K, Ross T, Pitout J, Church D, Gregson D. Community-onset urinary tract infections: a population-based assessment. Infection. 2007;35:150–3.
2. Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann Intern Med. 2001;135:41–50.
3. World Health Organisation. Antimicrobial resistance: global report on surveillance. 2014. http://www.who.int/drugresistance/documents/surveillancereport/en/. Accessed 25 Oct 2014.
4. Schaeffer AJ. The expanding role of fluoroquinolones. Am J Med. 2002;113:45–54.
5. Mcquiston Haslund J, Rosborg Dinesen M, Sternhagen Nielsen AB, Llor C, Bjerrum L. Different recommendations for empiric first-choice antibiotic treatment of uncomplicated urinary tract infections in Europe. Scand J Prim Health. 2013;31:235–40.
6. Blaettler L, Mertz D, Frei R, Elzi L, Widmer A, Battegay M, et al. Secular trend and risk factors for antimicrobial resistance in Escherichia coli isolates in Switzerland 1997–2007. Infection. 2009;37:534–9.
7. World Health Organisation Advisory Group on Integrated Surveillance of Antimicrobial Resistance. Critically Important Antimicrobials for Human Medicine-3rd Revision. 2012. http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf. Accessed 5 Dec 2014.
8. Redgrave LS, Sutton SB, Webber MA, Piddock LJV. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol. 2014;22:438–45.
9. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011;52:e103–e20.
10. Linhares I, Raposo T, Rodrigues A, Almeida A. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a 10-year surveillance study (2000–2009). BMC Infect Dis. 2013;13:19.
11. Ma KL, Wang CX. Analysis of the spectrum and antibiotic resistance of uropathogens in vitro: Results based on a retrospective study from a tertiary hospital. Am J Infect Control. 2013;41:601–6.
12. United States Interagency Task Force on Antimicrobial Resistance. A Public Health Action Plan to Combat Antimicrobial Resistance 2012 Update. 2012. http://www.cdc.gov/drugresistance/pdf/action-plan-2012.pdf. Accessed 5 Dec 2014.
13. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Ann Intern Med. 2009;151:W-65–94.
14. Centers for Disease Control and Prevention. Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) and Other Urinary System Infection [USI]) Events. 2015. http://www.cdc.gov/nhsn/PDFs/pscManual/7pscCAUTIcurrent.pdf. Accessed 10 Feb 2015.
15. Bouchillon SK, Badal RE, Hoban DJ, Hawser SP. Antimicrobial Susceptibility of Inpatient Urinary Tract Isolates of Gram-Negative Bacilli in the United States: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) Program: 2009–2011. Clin Ther. 2013;35:872–7.
16. Cullen IM, Manecksha RP, McCullagh E, Ahmad S, O'Kelly F, Flynn RJ, et al. The changing pattern of antimicrobial resistance within 42,033 Escherichia coli isolates from nosocomial, community and urology patient-specific urinary tract infections, Dublin, 1999–2009. BJUI. 2012;109:1198–206.
17. Sanchez GV, Master RN, Karlowsky JA, Bordon JM. In vitro antimicrobial resistance of urinary Escherichia coli isolates among US outpatients from 2000 to 2010. Antimicrob Agents Chemother. 2012;56:2181–3.
18. Horcajada JP, Shaw E, Padilla B, Pintado V, Calbo E, Benito N, et al. Healthcare-associated, community-acquired and hospital-acquired bacteraemic urinary tract infections in hospitalized patients: a prospective multicentre cohort study in the era of antimicrobial resistance. Clin Microbiol Infec. 2013;19:962–8.
19. Henderson KL, Muller-Pebody B, Johnson AP, Wade A, Sharland M, Gilbert R. Community-acquired, healthcare-associated and hospital-acquired bloodstream infection definitions in children: a systematic review demonstrating inconsistent criteria. J Hosp Infect. 2013;85:94–105.
20. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev. 2011;24:141–73.
21. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration. 2011. http://www.cochrane-handbook.org. Accessed 10 Nov 2014.
22. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2014. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 10 Nov 2014.
23. Cooper H, Hedges LV, Valentine JC. The handbook of research synthesis and meta-analysis. New York, USA: Russell Sage Foundation; 2009.
24. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177–88.
25. Higgins J, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557–60.
26. The World Bank Group. Country and Lending Groups. 2015. http://data.worldbank.org/about/country-and-lending-groups. Accessed 4 Mar 2015.
27. StataCorp. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP; 2013.
28. Hoban DJ, Nicolle LE, Hawser S, Bouchillon S, Badal R. Antimicrobial susceptibility of global inpatient urinary tract isolates of Escherichia coli: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program: 2009–2010. Diagn Micr Infec Dis. 2011;70:507–11.
29. Kimando JM, Okemo PO, Njagi EN. Resistance to antibiotics in urinopathogenic bacteria isolated in patients attending Kenyatta University Health Clinic, Nairobi. E Afr Med J. 2010;87:115–9.
30. Mwaka AD, Mayanja-Kizza H, Kigonya E, Kaddu-Mulindwa D. Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda. Afr Health Sci. 2011;11:182–9.
31. Prakash D, Saxena RS. Prevalence and antimicrobial susceptibility pattern of Escherichia coli in hospital acquired and community acquired patients related to urinary tract infection in India. J Appl Pharm Sci. 2013;3:124–32.
32. Shaifali I, Gupta U, Mahmood SE, Ahmed J. Antibiotic susceptibility patterns of urinary pathogens in female outpatients. N Am J Med Sci. 2012;4:163–9.
33. World Health Organisation. The evolving threat of antimicrobial resistance: options for action. 2012. http://whqlibdoc.who.int/publications/2012/9789241503181_eng.pdf. Accessed 25 Oct 2014.
34. Dalhoff A. Global fluoroquinolone resistance epidemiology and implications for their clinical use. Interdiscip Perspect Infect Dis. 2012; doi:10.1155/2012/976273.
35. Azap OK, Arslan H, Serefhanoglu K, Colakoglu S, Erdogan H, Timurkaynak F, et al. Risk factors for extended-spectrum beta-lactamase positivity in uropathogenic Escherichia coli isolated from community-acquired urinary tract infections. Clin Microbiol Infect. 2010;16:147–51.
36. Hassanzadeh P, Motamedifar M. The prevalence of asymptomatic bacteriuria in long term care facility residents in Shiraz, Southwest Iran: A cross-sectional study. Pak J Biol Sci. 2007;10:3890–4.
37. Killgore KM, March KL, Guglielmo BJ. Risk factors for community-acquired ciprofloxacin-resistant Escherichia coli urinary tract infection. Ann Pharmacother. 2004;38:1148–52.
38. Molina-Lopez J, Aparicio-Ozores G, Ribas-Aparicio RM, Gavilanes-Parra S, Chavez-Berrocal ME, Hernandez-Castro R, et al. Drug resistance, serotypes,

and phylogenetic groups among uropathogenic Escherichia coli including O25-ST131 in Mexico City. J Infect Dec Ctries. 2011;5:840–9.
39. Otajevwo FD. Urinary tract infection among symptomatic outpatients visiting a tertiary hospital based in midwestern Nigeria. Glob J Health Sci. 2013;5:187–99.
40. Sood S, Gupta R. Antibiotic resistance pattern of community acquired uropathogens at a Tertiary Care Hospital in Jaipur, Rajasthan. Indian J Commun Med. 2012;37:39–44.
41. Wang Y, Zhao S, Han L, Guo X, Chen M, Ni Y, et al. Drug resistance and virulence of uropathogenic Escherichia coli from Shanghai. Chin J Antibiot. 2014;67:799–805.
42. Ljuca S, Zvizdic S, Hamzic S, Kalajdzija M, Ljuca A. Antimicrobial susceptibility of bacteria isolated from inpatients and outpatients urine samples. Med Arh. 2010;64:161–4.
43. Longhi C, Conte MP, Marazzato M, Iebba V, Totino V, Santangelo F, et al. Plasmid-mediated fluoroquinolone resistance determinants in Escherichia coli from community uncomplicated urinary tract infection in an area of high prevalence of quinolone resistance. Eur J Clin Microbiol Infect Dis. 2012;31:1917–21.
44. Ni Chulain M, Murray AM, Corbett-Feeney G, Cormican M. Antimicrobial resistance in E. Coli associated with urinary tract infection in the West of Ireland. Ir J Med Sci. 2005;174:6–9.
45. Shariff VAA, Shenoy MS, Yadav T, M R. The antibiotic susceptibility patterns of uropathogenic Escherichia coli, with special reference to the fluoroquinolones. J Clin Diagn Res. 2013;7:1027–30.
46. Al Sweih N, Jamal W, Rotimi VO. Spectrum and antibiotic resistance of uropathogens isolated from hospital and community patients with urinary tract infections in two large hospitals in Kuwait. Med Princ Pract. 2005;14:401–7.
47. Karlowsky JA, Lagacé-Wiens PR, Simner PJ, DeCorby MR, Adam HJ, Walkty A, et al. Antimicrobial resistance in urinary tract pathogens in Canada from 2007 to 2009: CANWARD surveillance study. Antimicrob Agents Chemother. 2011;55:3169–75.
48. Tenover FC. Mechanisms of Antimicrobial Resistance in Bacteria. Am J Med. 2006;119:S3–S10.
49. Maraki S, Mantadakis E, Michailidis L, Samonis G. Changing antibiotic susceptibilities of community-acquired uropathogens in Greece, 2005–2010. J Microbiol Immunol Infect. 2013;46:202–9.
50. Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. BMJ. 2010;340:c2096.
51. Vellinga A, Murphy AW, Hanahoe B, Bennett K, Cormican M. A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic Escherichia coli in general practice. J Antimicrob Chemother. 2010;65:1514–20.
52. Goettsch W, Van Pelt W, Nagelkerke N, Hendrix M, Buiting A, Petit P, et al. Increasing resistance to fluoroquinolones in Escherichia coli from urinary tract infections in the Netherlands. J Antimicrob Chemother. 2000;46:223–8.
53. Zervos MJ, Hershberger E, Nicolau DP, Ritchie DJ, Blackner LK, Coyle EA, et al. Relationship between fluoroquinolone use and changes in susceptibility to fluoroquinolones of selected pathogens in 10 United States teaching hospitals, 1991–2000. Clin Infect Dis. 2003;37:1643–8.
54. Gottesman BS, Carmeli Y, Shitrit P, Chowers M. Impact of quinolone restriction on resistance patterns of Escherichia coli isolated from urine by culture in a community setting. Clin Infect Dis. 2009;49:869–75.
55. Australian Commission on Safety and Quality in Healthcare. A Report of the Australian One Health Antimicrobial Resistance Colloquium. 2013. https://www.health.gov.au/internet/main/publishing.nsf/Content/1803C433C71415CACA257C8400121B1F/$File/Colloquium-Report-Final-Feb2014.pdf. Accessed 13 Oct 2014.
56. Dancer SJ. Infection control in the post-antibiotic era. Healthc Infect. 2013;18:51–60.
57. Australian Government Department of Health. National Antimicrobial Resistance Strategy 2015–2019. 2015. http://www.health.gov.au/internet/main/publishing.nsf/Content/1803C433C71415CACA257C8400121B1F/$File/amr-strategy-2015-2019.pdf. Accessed 17 Jun 2015.
58. Infectious Diseases Society of the Republic of China. Guidelines for antimicrobial therapy of urinary tract infections in Taiwan. J Microbiol Immunol Infect. 2000;33:271–2.
59. Karaca Y, Coplu N, Gozalan A, Oncul O, Citil BE, Esen B. Co-trimoxazole and quinolone resistance in *Escherichia coli* isolated from urinary tract infections over the last 10 years. Int J Antimicrob Agents. 2005;26:75–7.
60. Naber KG. Survey on antibiotic usage in the treatment of urinary tract infections. J Antimicrob Chemother. 2000;46:49–52.
61. Olson RP, Haith K. Antibiotic resistance in urinary tract infections in college students. J Am Coll Health. 2012;60:471–4.
62. Okeke IN, Klugman KP, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part II: strategies for containment. Lancet Infect Dis. 2005;5:568–80.
63. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis. 1999;5:18.
64. Ahmad S. Pattern of urinary tract infection in Kashmir and antimicrobial susceptibility. Bangladesh Med Res Counc Bull. 2012;38:79–83.
65. Akoachere JF, Yvonne S, Akum NH, Seraphine EN. Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. BMC Res Notes. 2012;5:219.
66. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann Clin Microbiol Antimicrob. 2007; doi:http://dx.doi.org/10.1186/1476-0711-6-4.
67. Arabi FMZ, Banazadehi A. Prevalence and antimicrobial susceptibility patterns of uropathogens among patients referring to valieasr laboratory in Najafabad, Isfahan. Iran Mid East J Sci Res. 2013;13:85–90.
68. Astal ZE. Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in the Gaza Strip. Singapore Med J. 2005;46:457–60.
69. Dash M, Padhi S, Mohanty I, Panda P, Parida B. Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha. India J Family Community Med. 2013;20:20–6.
70. Kashef N, Djavid GE, Shahbazi S. Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran. Iran J Infect Dev Ctries. 2010;4:202–6.
71. Kothari A, Sagar V. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: a multicenter study. J Infect Dev Ctries. 2008;2:354–8.
72. Murugan K, Savitha T, Vasanthi S. Retrospective study of antibiotic resistance among uropathogens from rural teaching hospital, Tamilnadu. India Asian Pac J Trop Dis. 2012;2:375–80.
73. Sire JM, Nabeth P, Perrier-Gros-Claude JD, Bahsoun I, Siby T, Macondo EA, et al. Antimicrobial resistance in outpatient Escherichia coli urinary isolates in Dakar. Senegal J Infect Dev Ctries. 2007;1:263–8.
74. Morgan DJ, Okeke IN, Laxminarayan R, Perencevich EN, Weisenberg S. Non-prescription antimicrobial use worldwide: a systematic review. Lancet Infect Dis. 2011;11:692–701.
75. Dalhoff A. Resistance surveillance studies: a multifaceted problem—the fluoroquinolone example. Infection. 2012;40:239–62.
76. Maron DF, Smith TJ, Nachman KE. Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Global Health. 2013;9:48.
77. Owumi W, Banaei N, Shortliffe LD. Adult and Pediatric Intra-Institutional Trends of Ciprofloxacin Susceptibility in E. coli Positive Urinary Cultures. Antibiotics. 2014;3:163–73.
78. Storby KA, Österlund A, Kahlmeter G. Antimicrobial resistance in Escherichia coli in urine samples from children and adults: a 12 year analysis. Acta Paediatr. 2004;93:487–91.
79. Adefurin A, Sammons H, Jacqz-Aigrain E, Choonara I. Ciprofloxacin safety in paediatrics: a systematic review. Arch Dis Child. 2011;96:874–80.
80. Von Elm E, Egger M. The scandal of poor epidemiological research: reporting guidelines are needed for observational epidemiology. BMJ. 2004;329:868.
81. Vandenbroucke JP, Von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Ann Intern Med. 2007;147:W-163–W-94.

PLOS ONE

RESEARCH ARTICLE

Five-Year Antimicrobial Resistance Patterns of Urinary *Escherichia coli* at an Australian Tertiary Hospital: Time Series Analyses of Prevalence Data

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Abstract

This study describes the antimicrobial resistance temporal trends and seasonal variation of *Escherichia coli* (*E. coli)* urinary tract infections (UTIs) over five years, from 2009 to 2013, and compares prevalence of resistance in hospital- and community-acquired *E. coli* UTI. A cross sectional study of *E. coli* UTIs from patients attending a tertiary referral hospital in Canberra, Australia was undertaken. Time series analysis was performed to illustrate resistance trends. Only the first positive *E. coli* UTI per patient per year was included in the analysis. A total of 15,022 positive cultures from 8724 patients were identified. Results are based on 5333 first *E. coli* UTIs, from 4732 patients, of which 84.2% were community-acquired. Five-year hospital and community resistance rates were highest for ampicillin (41.9%) and trimethoprim (20.7%). Resistance was lowest for meropenem (0.0%), nitrofurantoin (2.7%), piperacillin-tazobactam (2.9%) and ciprofloxacin (6.5%). Resistance to amoxycillin-clavulanate, cefazolin, gentamicin and piperacillin-tazobactam were significantly higher in hospital- compared to community-acquired UTIs (9.3% versus 6.2%; 15.4% versus 9.7%; 5.2% versus 3.7% and 5.2% versus 2.5%, respectively). Trend analysis showed significant increases in resistance over five years for amoxycillin-clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin, trimethoprim-sulphamethoxazole, cefazolin, ceftriaxone and gentamicin ($P<0.05$, for all) with seasonal pattern observed for trimethoprim resistance (augmented Dickey-Fuller statistic = 4.136; $P = 0.006$). An association between ciprofloxacin resistance, cefazolin resistance and ceftriaxone resistance with older age was noted. Given the relatively high resistance rates for ampicillin and trimethoprim, these antimicrobials should be reconsidered for empirical treatment of UTIs in this patient population. Our findings have important implications for UTI treatment based on setting of acquisition.

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Introduction

Urinary tract infections (UTIs) are predominantly bacterial infections affecting people both in the community and in hospitals [1]. Over 80% are caused by *Escherichia coli (E. coli)*, a Gram negative bacillus [2]. Data from the combined National Ambulatory Health Care Surveys in the United States (US) for 2009–2010 showed that UTIs accounted for approximately 9.8 million visits to ambulatory care settings such as primary care, outpatient and emergency departments [3]. Visits due to UTI were estimated to be 0.8% of all ambulatory care visits [3]. In Australia, national data on UTI are unavailable but recent estimates from 82 hospitals and 17 aged care facilities reported a point prevalence of 1.4% and 1.5% respectively for healthcare associated UTIs [4].

While UTIs are a major infection burden globally, the growing problem of antimicrobial resistance (AMR) can result in treatment failures and increased cost of healthcare [5]. There is evidence to show that the AMR pattern of urinary *E. coli* is increasing [6]. In Switzerland, an analysis of urinary *E. coli* specimens obtained from a university hospital from 1997 to 2007 found an increasing trend in resistance to trimethoprim/sulfamethoxazole, ciprofloxacin and amoxycillin/clavulanic acid (from 17.4% to 21.3%, 1.8% to 15.9%, and 9.5% to 14.5%, respectively) [6]. The Australian Group on Antimicrobial Resistance (AGAR) which undertakes AMR prevalence surveys within Australia also noted a gradual rise in overall percentage of *E. coli* strains resistant to beta-lactam antibiotics and ciprofloxacin [7]. From 2009 to 2011, resistance of hospital-onset *E. coli* isolates to ampicillin and ciprofloxacin increased from 48% to 51% and 8% to 11% respectively [7]. Furthermore, the resistance rates of urinary *E. coli* to various antimicrobials show large inter-country variability [8]. Only a few studies have shown that *E. coli* resistance rates differ for hospital-acquired and community-acquired UTIs [9–11]. Measuring and comparing the levels of AMR in both hospital- and community-acquired UTIs is essential because although effects of AMR are mainly felt in healthcare facilities, the greatest use of antimicrobials occurs in the community [12]. Comparing resistance rates in hospital- and community-acquired UTIs may influence therapeutic recommendations for UTIs based on setting of acquisition.

The prevalence of AMR including hospital and community urinary *E. coli* resistance levels is not completely known in Australia. Obtaining this information is important because it not only provides knowledge about the health status of a population, but also contributes to disease management decisions [13]. This study describes the AMR temporal trends and seasonal variation of *E. coli* UTI over five years at an Australian tertiary hospital. The study also compares the prevalence of resistance between hospital- and community-acquired *E. coli* UTIs.

Materials and Methods

Study design and setting

A retrospective cross sectional design was used. The study was conducted with data from ACT Pathology which is based at a tertiary referral hospital, the Canberra Hospital and Health Services. This is Australian Capital Territory's (ACT) main hospital which provides acute and specialist care services to over 600,000 people in the surrounding region. The 600 bed publicly-funded hospital which includes an emergency department and intensive care unit, offers a comprehensive range of health services such as acute inpatient and day services, outpatient services, women's and children's services and pathology services. Solid organ transplant services are not offered in Canberra.

Human research ethics approval was granted by ACT Health Human Research Ethics Committee's Low Risk Sub-Committee and Australian Catholic University Human Research Ethics

Committee. Consent from patients was not obtained as a waiver of consent was granted by the ethics committees.

Urine sample and data collection

The microbiology records of inpatients and those attending Canberra Hospital who had urine samples processed at ACT Pathology from January 2009 to December 2013 were retrospectively reviewed. Demographic data and clinical information such as date of birth, gender, admission date, specimen collection date and antimicrobial susceptibility test result were obtained from the microbiology laboratory database and administrative record system.

Bacterial isolation and identification

Urine samples were analysed and processed based on the microbiology laboratory standards [14]. For this study, a culture with presence of $\geq 10^7$ colony forming unit (cfu) per litre of urine was considered positive for UTI based on the laboratory recommendations. This 10^7 cfu/L cut-off is commonly used as it increases the sensitivity of the urine culture test making it a practical threshold [15]. The criterion has also been used by several studies reporting on antimicrobial resistance of urinary *E. coli* [1,16,17]. Cultures with three or more bacterial species isolated were considered contaminated and excluded. Only the first positive *E. coli* UTI per patient per year was included in the final analysis.

Definitions

Urine cultures were classified based on the setting of acquisition of infection (hospital-acquired and community-acquired, also known as hospital-onset and community-onset) using criteria from the Centers for Disease Control and Prevention definitions [18]. Positive *E. coli* urine cultures obtained within the first 48 hours of admission (including cultures from non-admissions such as outpatient clinics) were defined as community-acquired UTIs. Positive cultures obtained more than 48 hours after admission and within 48 hours of discharge were defined as hospital-acquired UTIs.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed by a disc diffusion method and the automated minimum inhibitory concentration (MIC) method using Vitek2 (Biomerieux Diagnostics). Interpretation was based on Clinical Laboratory Standard Institute (CLSI, formerly NCCLS) criteria [19]. Based on a stepwise laboratory testing protocol used during the study period, all significant *E. coli* ($>10^7$cfu/L) isolated after overnight incubation on culture had disc susceptibility testing done. The antibiotic discs used for these tests were ampicillin (10μg), amoxycillin-clavulanate (augmentin) (30μg), cephalexin/cefazolin (30 μg), trimethoprim (5μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), nitrofurantoin (300 μg) and gentamicin (10μg). The isolates which were found to be resistant to at least three of the routinely tested antibiotics were then sent for Vitek2 testing to determine the MICs for ceftriaxone, trimethoprim-sulphamethoxazole, meropenem and piperacillin-tazobactam in addition to the routinely tested antibiotics. Direct susceptibility testing method on urine specimens for *E. coli* has been validated at ACT Pathology and is comparable to the CLSI recommended methods.

The quality control strains used for disc diffusion tests were *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and for Vitek *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213.

Extended spectrum beta lactamase (ESBL) confirmation

Detection of ESBL-producing isolates was performed with combination discs of cefotaxime (30μg), cefotaxime/clavulanic acid (30/10μg), ceftazidime (30μg) and ceftazidime/clavulanic acid (30/10μg) whenever required according to CLSI guidelines [15]. Extended spectrum beta lactamase production was inferred when the zone diameter of the disc with clavulanate was ≥5mm larger than the disc without clavulanate for the same antibiotic. *K. pneumoniae* ATCC 700603 was used as the quality control strain.

Statistical analysis

The overall 5-year and yearly resistance rates of *E. coli* to the routinely tested first-line antimicrobials on over 4,000 isolates (ampicillin, amoxycillin-clavulanate, cephalexin/cefazolin, ciprofloxacin, gentamicin, nalidixic acid, and trimethoprim) were calculated by dividing the number of urinary *E. coli* isolates resistant to each antimicrobial by the number of isolates tested against an individual antimicrobial agent. For the isolates which were sent for further susceptibility testing on Vitek2 against second-line antimicrobials (ceftriaxone, trimethoprim-sulphamethoxazole, meropenem, piperacillin-tazobactam and nitrofurantoin), the denominator used in calculating the resistance rates was the total number of isolates included in the study. This denominator was used based on the assumption that isolates were initially not tested for the Vitek2 antibiotics because they were considered highly unlikely to be resistant to these antibiotics. Hence in order not to overestimate the resistance rates of these isolates the denominator included all isolates tested on both antibiotic discs and Vitek2. The binomial exact 95% confidence intervals (CI) of the resistance percentages were calculated. The 5-year resistance rates were compared for community- and hospital-acquired isolates. The chi-square test was used to check for statistically significant differences in AMR between both groups. Mean differences in age between the two groups were tested using Student's t-tests. A time series analysis was performed separately for all antimicrobials tested to identify patterns in resistance (trends and seasonal variation) over the five year period. Seasonality is a pattern that shows periodic repetitive fluctuations over time. An autoregressive (AR) model was constructed to assess time-varying resistance patterns (i.e., resistance is non-stationary, or changing, over time) and multiple time series models were fitted to also account for age and sex. The analysis on age and sex followed an ecological study design because these variables were aggregated for each season. The Dickey-Fuller (DF) and the augmented Dickey-Fuller (ADF) tests were used to assess a unit root in the time series data. Both DF and ADF statistics are negative numbers; the more negative, the stronger the rejection of the null hypothesis (that there is unit root at some level of confidence). These unit root tests investigate whether a time series variable (e.g., resistance) is non-stationary using the AR model [20]. Urinary *E. coli* isolates for which the antimicrobial showed an intermediate susceptibility category (amoxycillin-clavulanate, trimethoprim, and ciprofloxacin) were excluded from the final analysis. A significance level of $P < 0.05$ was used. Data were analysed using STATA statistical software (version 13, StataCorp).

Results

A total of 106,512 urine samples from 47,727 patients attending Canberra Hospital from 2009 to 2013 were processed by ACT Pathology. Of these, 14.1% (n = 15,022) had positive cultures with *E. coli* being the most common organism isolated in 7670 (51.1%) samples. The distribution of samples by study year is shown in S1 Table.

Of the 7670 *E. coli* cultures, most (7103 isolates) could be further classified as community- or hospital-acquired UTI based on available data. The data were then restricted to the first positive *E. coli* UTI per patient per year of which there were 5346 positive *E. coli* UTIs but only

5333 had susceptibility test results. Hence 5333 *E. coli* UTIs belonging to 4732 patients in the 5-year period were included in the final analysis. The majority (84.2%, n = 4492) of UTIs were classified as community-acquired and 15.8% (n = 841) as hospital-acquired. The mean age of all patients was 57.0 years (SD = 27.5) and patients were mostly female (80.2%, n = 3795). There was a significant difference in age between patients with hospital- and community-acquired *E. coli* UTI (mean age 67.2 years versus 55.1 years, P<0.001) but no significant differences in gender.

Antimicrobial resistance

All 5333 isolates had routine susceptibility testing performed against first-line antimicrobials and the overall 5-year and stratified (hospital- and community-acquired) AMR rates are summarised in Table 1. Of the 5333 isolates, 1599 (29.9%) were sent for further antimicrobial susceptibility testing for second-line antimicrobials on Vitek2. The overall 5-year resistance rates to these second-line antimicrobials are reported in Table 2.

The highest overall 5-year resistance rates to urinary *E. coli* for both hospital and community isolates combined were seen for ampicillin (41.9%; 95% CI = 40.6–43.3) and trimethoprim (20.7%; 95% CI = 19.6–21.8). The lowest resistance rates were for meropenem (0.0%), nitrofurantoin (2.7%; 95% CI = 2.3–3.2) and piperacillin-tazobactam (2.9%; 95% CI = 2.5–3.4). Resistance to amoxycillin-clavulanate, cephalexin/cefazolin, gentamicin and piperacillin-tazobactam was significantly higher in hospital- compared to community-acquired UTIs (P<0.001, P<0.001, P = 0.043 and P = 0.002, respectively). For ampicillin, trimethoprim, nalidixic acid, ciprofloxacin, ceftriaxone and trimethoprim-sulphamethoxazole, resistance rates were also higher for hospital- compared with community-acquired UTI but this did not reach statistical significance (Fig 1).

Trend analysis showed a significant increase in resistance to amoxycillin-clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin, trimethoprim-sulphamethoxazole, cefazolin, ceftriaxone and gentamicin over the five year period (Fig 2). There was no significant increase in resistance for ampicillin, nalidixic acid, meropenem and piperacillin-tazobactam. A seasonal pattern was only observed for trimethoprim (ADF statistic = -4.136; P = 0.006) with higher resistance rates for this antimicrobial seen in the summer months. Regression analysis indicated an association between increasing age and resistance to ciprofloxacin (regression coefficient = 0.01; P = 0.004), cefazolin (regression coefficient = 0.004; P = 0.038) and ceftriaxone (regression coefficient = 0.01; P = 0.002).

ESBL production

Overall 5-year prevalence of ESBL-producing *E. coli* isolates was 1.9% (95% CI = 1.5–2.3; n = 100). Extended spectrum beta-lactamase production was low by international standards but was significantly higher in hospital-acquired (3.0%; 95% CI = 1.9–4.4; n = 25) compared with community-acquired UTIs (1.7%; 95% CI = 1.3–2.1; n = 75, P = 0.01). The levels of ESBL-producing *E. coli* increased from 0.7% (95% CI = 0.0–3.8) in hospital-acquired UTIs in 2009 to 6.5% (95% CI = 3.2–11.6) in 2013. An increase was also noted for community-acquired UTIs (0.6%; 95% CI = 0.2–1.4 in 2009 to 3.7%; 95% CI = 2.5–5.3 in 2013). The increasing trend in ESBL production over the five years was statistically significant for both hospital (P = 0.035) and community-acquired UTIs (P<0.001).

Discussion

This study provides information about the AMR pattern of *E. coli* UTIs in an Australian tertiary hospital. To our knowledge this is the first Australian study to compare AMR in

Table 1. Resistance profile of urinary *E. coli* isolates sent for routine susceptibility testing from 2009 to 2013 by setting.

		COMMUNITY			HOSPITAL			TOTAL		
Antibiotic	**Year**	**Number of community isolates tested**	**R n (%)**	**95% CI of resistance percentage**	**Number of hospital isolates tested**	**R n (%)**	**95% CI of resistance percentage**	**Total number of isolates tested***	**R n (%)**	**95% CI of resistance percentage**
Ampicillin	2009	835	331 (39.6)	36.3–43.1	143	71 (49.7)	41.2–58.1	978	402 (41.1)	38.0–44.3
	2010	897	358 (39.9)	36.7–43.2	182	70 (38.5)	31.4–45.9	1079	428 (39.7)	36.7–42.7
	2011	1037	443 (42.7)	39.7–45.8	189	91 (48.2)	40.8–55.5	1226	534 (43.6)	40.8–46.4
	2012	939	412 (43.9)	40.7–47.1	173	74 (42.8)	35.3–50.5	1112	486 (43.7)	40.8–46.7
	2013	784	315 (40.2)	36.7–43.7	154	71 (46.1)	38.1–54.3	938	386 (41.2)	38.0–44.4
	Total	4492	1859 (41.4)	39.9–42.8	841	377 (44.8)	41.4–48.3	5333	2236 (41.9)	40.6–43.3
AMC	2009	785	24 (3.1)	2.0–4.5	133	6 (4.5)	1.7–9.6	918	30 (3.3)	2.2–4.6
	2010	832	49 (5.9)	4.4–7.7	172	11 (6.4)	3.2–11.2	1004	60 (6.0)	4.6–7.6
	2011	981	61 (6.2)	4.8–7.9	170	17 (10.0)	5.9–15.5	1151	78 (6.8)	5.4–8.4
	2012	895	71 (7.9)	6.2–9.8	161	19 (11.8)	7.3–17.8	1055	89 (8.4)	6.8–10.3
	2013	754	58 (7.7)	5.9–9.8	145	23 (15.9)	10.3–22.8	899	81 (9.0)	7.2–11.1
	Total	4247	263 (6.2)	5.5–6.9	781	76 (9.3)	7.7–12.0	5027	338 (6.7)	6.0–7.5
Cefazolin	2009	821	60 (7.3)	5.6–9.3	129	14 (10.9)	6.1–17.5	950	74 (7.8)	6.2–9.7
	2010	885	96 (10.9)	8.9–13.1	179	24 (13.4)	8.8–19.3	1064	120 (11.3)	9.4–13.3
	2011	1019	103 (10.1)	8.3–12.1	178	30 (16.9)	11.7–23.2	1197	133 (11.1)	9.4–13.0
	2012	917	82 (8.9)	7.2–11.0	168	26 (15.5)	10.4–21.8	1085	108 (10.0)	8.2–11.9
	2013	776	89 (11.5)	9.3–13.9	151	30 (19.9)	13.8–27.1	927	119 (12.8)	10.8–15.2
	Total	4418	430 (9.7)	8.9–10.6	805	124 (15.4)	13.0–18.1	5223	554 (10.6)	9.8–11.5
Trimethoprim	2009	830	153 (18.4)	15.9–21.2	143	28 (19.6)	13.4–27.0	973	181 (18.6)	16.2–21.2
	2010	897	172 (19.2)	16.6–21.9	181	33 (18.2)	12.9–24.6	1078	205 (19.0)	16.7–21.5
	2011	1036	217 (20.9)	18.5–23.6	189	42 (22.2)	16.5–28.8	1225	259 (21.1)	18.9–23.5
	2012	939	200 (21.3)	18.7–24.1	173	40 (23.1)	17.1–30.1	1112	240 (21.6)	19.2–24.1
	2013	784	181 (23.1)	20.2–26.2	154	36 (23.4)	16.9–30.9	938	217 (23.1)	20.5–26.0
	Total	4486	923 (20.6)	19.4–21.8	840	179 (21.3)	18.6–24.2	5326	1102 (20.7)	19.6–21.8
Nalidixic acid	2009	826	63 (7.6)	5.9–9.7	143	12 (8.4)	4.4–14.2	969	75 (7.7)	6.1–9.6
	2010	892	73 (8.2)	6.5–10.2	182	12 (6.6)	3.5–11.2	1074	85 (7.9)	6.4–9.7
	2011	1034	109 (10.5)	8.7–12.6	188	22 (11.7)	7.5–17.2	1222	131 (10.7)	9.0–12.6
	2012	755	56 (7.4)	5.7–9.5	140	17 (12.1)	7.2–18.7	895	73 (8.2)	6.4–10.1
	2013	585	33 (5.6)	3.9–7.8	103	11 (10.7)	5.5–18.3	688	44 (6.4)	4.7–8.5
	Total	4092	334 (8.2)	7.3–9.0	756	74 (9.8)	7.8–12.1	4848	408 (8.4)	7.6–9.2
Ciprofloxacin	2009	808	33 (4.1)	2.8–5.7	139	7 (5.0)	2.0–10.1	947	40 (4.2)	3.0–5.7
	2010	701	35 (5.0)	3.5–6.9	150	4 (2.7)	0.7–6.7	851	39 (4.6)	3.3–6.2
	2011	795	52 (6.5)	4.9–8.5	156	10 (6.4)	3.1–11.5	951	62 (6.5)	5.0–8.3
	2012	749	56 (7.5)	5.7–9.6	143	11 (7.7)	3.9–13.3	892	67 (7.5)	5.9–9.4
	2013	631	60 (9.5)	7.3–12.1	135	17 (12.6)	7.5–19.4	766	77 (10.1)	8.0–12.4
	Total	3684	236 (6.4)	5.6–7.2	723	49 (6.8)	5.1–8.9	4407	285 (6.5)	5.8–7.2
Gentamicin	2009	514	17 (3.3)	1.9–5.2	85	5 (5.9)	1.9–13.2	599	22 (3.7)	2.3–5.5
	2010	893	23 (2.6)	1.6–3.8	182	2 (1.1)	0.1–3.9	1075	25 (2.3)	1.5–3.4
	2011	1036	38 (3.7)	2.6–5.0	189	12 (6.4)	3.3–10.8	1225	50 (4.1)	3.0–5.3
	2012	931	40 (4.3)	3.1–5.8	172	12 (7.0)	3.7–11.9	1102	52 (4.7)	3.5–6.1
	2013	783	36 (4.6)	3.2–6.3	154	10 (6.5)	3.2–11.6	937	46 (4.9)	3.6–6.5

(*Continued*)

Table 1. (*Continued*)

		COMMUNITY			HOSPITAL			TOTAL		
Antibiotic	**Year**	**Number of community isolates tested**	**R n (%)**	**95% CI of resistance percentage**	**Number of hospital isolates tested**	**R n (%)**	**95% CI of resistance percentage**	**Total number of isolates tested***	**R n (%)**	**95% CI of resistance percentage**
	Total	4157	154 (3.7)	3.2–4.3	782	41 (5.2)	3.8–7.0	4938	195 (3.9)	3.4–4.5

*Note that not all 5333 isolates were tested against each antimicrobial. Isolates not tested: AMC = 3; Cephazolin = 110; Trimethoprim = 3; Nalidixic acid = 485; Ciprofloxacin = 893; Gentamicin = 395

Number of isolates with intermediate susceptibility to an antimicrobial: AMC = 303; Trimethoprim = 4; Ciprofloxacin = 33

R = Resistant

n = Number of isolates

AMC = Amoxycillin-clavulanate; TMP-SMX = Trimethoprim-sulphamethoxazole

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hospital- and community-acquired *E. coli* UTI and assess AMR temporal trends and seasonal variation of *E. coli* UTI over time. Our results showed that overall resistance was highest for ampicillin and trimethoprim. We also found significantly higher resistance rates in hospital-compared to community-acquired UTIs for amoxycillin-clavulanate, cephalexin/cefazolin, gentamicin and piperacillin-tazobactam with an increasing resistance trend for eight of the twelve antimicrobials tested which include the four commonly used antimicrobials for first line treatment of UTI in Australia.

In Australia, trimethoprim, cephalexin, amoxycillin-clavulanate or nitrofurantoin are recommended for first line treatment of UTI [21]. The Infectious Diseases Society of America (IDSA) and European Society for Microbiology and Infectious Diseases recommend trimethoprim-sulphamethoxazole as an appropriate treatment choice if local resistance rates do not exceed 20%. The IDSA guidelines also recommend that amoxycillin or ampicillin should not be used alone for empirical treatment because of the relatively poor efficacy and the relatively high prevalence of AMR to these agents worldwide [22]. Given the high levels of resistance to ampicillin and trimethoprim identified in this study, the appropriateness of these antimicrobials in the management of UTI in this patient population should be assessed. The IDSA suggests that beta-lactam agents, including amoxycillin-clavulanate are appropriate choices for therapy when other recommended agents cannot be used [22]. Based on our findings, the majority of UTIs have very low resistance to amoxycillin-clavulanate and nitrofurantoin which are commonly used for UTI treatment in Canberra. Ciprofloxacin, which is recommended in Australia for complicated UTIs, was also found to have a low resistance rate. Through the national pharmaceutical subsidy scheme, the use of quinolones in humans has been restricted in Australia. Quinolone use in food-producing animals is also not permitted. Therefore, fluoroquinolone resistance in the community has been slow to emerge and has remained at low levels in important pathogens such as *E. coli* compared to most countries [23]. Our overall resistance rates are also generally lower than reported for other single site studies [9,24], demonstrating that resistance may vary geographically, as shown in a recent meta-analysis [25]. The explanation for the varying resistance rates is not clearly understood but possible reasons have been postulated. A study conducted in the United States demonstrated a geographic gradient in resistance with the highest resistance rates noted in the Pacific region and lowest rates in the South Atlantic region [26]. It was suggested that geographic clustering of resistance phenotypes may have accounted for the geographic differences in resistance. It is therefore possible that the lower rates we found in comparison to those reported for other single site studies may be due to lower levels of bacteria with resistance phenotypes in our locality. Another possible suggestion for geographic variation

Table 2. Resistance profile of urinary *E. coli* isolates sent for further testing on Vitek2 from 2009 to 2013 by setting.

Year	Setting	N	Antibiotic									
			Ceftriaxone		TMP-SMX		MER		PIT		NIT	
			R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage	R n (%)	95% CI of resistance percentage
2009	CA	835	12 (1.4)	0.7–2.5	58 (6.9)	5.3–8.9	0 (0.0)	-	2 (0.2)	0.0–0.9	14 (1.7)	0.9–2.8
	HA	143	2 (1.4)	0.2–5.0	12 (8.4)	4.4–14.2	0 (0.0)	-	0 (0.0)	-	4 (2.8)	0.8–7.0
	Total	978	14 (1.4)	0.8–2.4	70 (7.2)	5.6–9.0	0 (0.0)	-	2 (0.2)	0.0–0.7	18 (1.8)	1.1–2.9
2010	CA	897	22 (2.5)	1.5–3.7	76 (8.5)	6.7–10.5	0 (0.0)	-	27 (3.0)	2.0–4.3	15 (1.7)	0.9–2.7
	HA	182	4 (2.2)	0.6–5.5	11 (6.0)	3.1–10.6	0 (0.0)	-	4 (2.2)	0.6–5.5	5 (2.7)	0.9–6.3
	Total	1079	26 (2.4)	1.6–3.5	87 (8.1)	6.5–9.9	0 (0.0)	-	31 (2.9)	2.0–4.1	20 (1.9)	1.1–2.8
2011	CA	1037	46 (4.4)	3.3–5.9	99 (9.5)	7.8–11.5	0 (0.0)	-	19 (1.8)	1.1–2.8	17 (1.6)	1.0–2.6
	HA	189	13 (6.9)	3.7–11.5	30 (15.9)	11.0–21.9	0 (0.0)	-	9 (4.8)	2.2–8.8	3 (1.6)	0.3–4.6
	Total	1226	59 (4.8)	3.7–6.2	129 (10.5)	8.9–12.4	0 (0.0)	-	28 (2.3)	1.5–3.3	20 (1.6)	1.0–2.5
2012	CA	939	43 (4.6)	3.3–6.1	102 (10.9)	8.9–13.0	1 (0.1)	0.0–0.6	33 (3.5)	2.4–4.9	35 (3.7)	2.6–5.1
	HA	173	13 (7.5)	4.1–12.5	22 (12.7)	8.1–18.6	0 (0.0)	-	15 (8.7)	4.9–13.9	5 (2.9)	0.9–6.6
	Total	1112	56 (5.0)	3.8–6.5	124 (11.1)	9.4–13.1	1 (0.1)	0.0–0.5	48 (4.3)	3.2–5.7	40 (3.6)	2.6–4.9
2013	CA	784	45 (5.7)	4.2–7.6	87 (11.1)	9.0–13.5	0 (0.0)	-	30 (3.8)	2.6–5.4	42 (5.4)	3.9–7.2
	HA	154	15 (9.7)	5.6–15.6	25 (16.2)	10.8–23.0	0 (0.0)	-	16 (10.4)	6.1–16.3	4 (2.6)	0.7–6.5
	Total	938	60 (6.4)	4.9–8.2	112 (11.9)	9.9–14.2	0 (0.0)	-	46 (4.9)	3.6–6.5	46 (4.9)	3.6–6.5
Total	CA	4492	168 (3.7)	3.2–4.3	422 (9.4)	8.6–10.3	1 (0.0)	0.0–0.1	111 (2.5)	2.0–3.0	123 (2.7)	2.3–3.3
	HA	841	47 (5.6)	4.1–7.4	100 (11.9)	9.8–14.3	0 (0.0)	-	44 (5.2)	3.8–7.0	21 (2.5)	1.6–3.8
	Total	5333	215 (4.0)	3.5–4.6	522 (9.8)	9.0–10.6	1 (0.0)	0.0–0.1	155 (2.9)	2.5–3.4	144 (2.7)	2.3–3.2

R = Resistant

N = Number of isolates tested

CA = Community isolates; HA = Hospital isolates

TMP-SMX = Trimethoprim-sulphamethoxazole; MER = Meropenem; PIT = Piperacillin-tazobactam; NIT = Nitrofurantoin

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in resistance is the differences in antimicrobial use [26,27]. Several studies have demonstrated an association between antimicrobial use and resistance [28–30]. Hence it is probable that the lower resistance noted may be as a result of lower antimicrobial use resulting in lower antimicrobial selection pressure. This emphasizes the need for continuous local monitoring of resistance patterns to ensure appropriate treatment for people in the locality.

The sample size of the data was able to detect some significant differences between community- and hospital-acquired UTI resistance rates but for some antimicrobials the differences observed could not be confirmed statistically, possibly due to an insufficient sample size. Overall, we found lower rates of antibiotic resistance for community- compared with

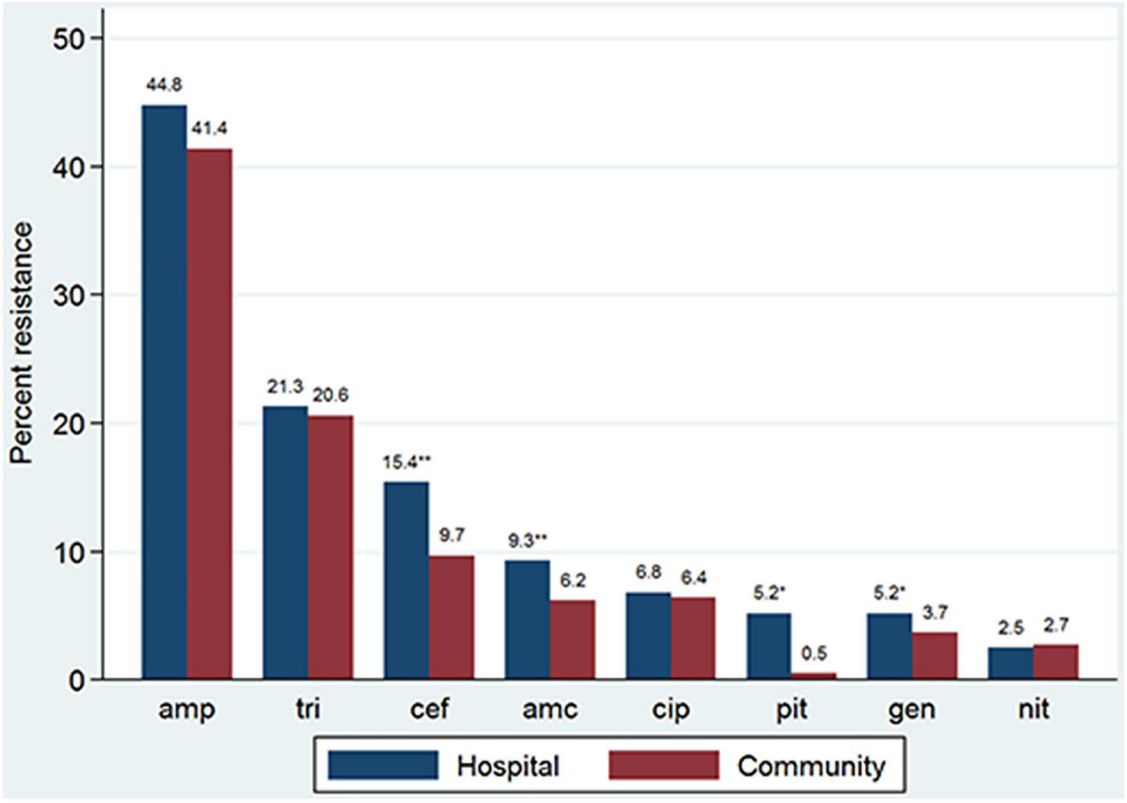

Fig 1. Five-year resistance rates of hospital- and community-acquired *E. coli* UTIs by selected antibiotics. amp = ampicillin; tri = trimethoprim; cef = cefazolin; amc = amoxycillin-clavulanate; cip = ciprofloxacin; pit = piperacillin-tazobactam; gen = gentamicin; nit = nitrofurantoin. ** 0.001 < p value < 0.05. ** p < 0.001

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hospital-acquired *E. coli* UTIs, consistent with other studies [9,31]. The difference in resistance rates is however only small and supports the view that *E. coli*, a bacterium carried in the bowel and acquired in the community, is brought into hospital usually by patients themselves rather than being hospital-acquired. This finding may also have been partially dictated by our methodology from using the first positive UTI per person per year. The different resistance rates for hospital- and community-acquired urinary *E. coli* isolates seen in this study are comparable with findings reported previously [10,11]. Similar results have been seen in blood culture isolates of *E. coli* in Canberra [32]. While the difference in resistance rates was not large and most antimicrobial use occurs in the community, the proportion of patients receiving antimicrobials is much higher in the hospital and hence explains the difference seen [33]. We agree with recommendations that to accurately represent *E. coli* resistance rates, antibiograms should be stratified by setting of infection onset [34].

The increasing resistance trend noted in our study for the eight antimicrobials is consistent with previously reported Australian data and published studies from other developed countries [6,7,9,35,36]. The increasing trend may be attributable to antimicrobial overuse or misuse which is a known risk factor for the development of AMR [37]. However, clinical data on hospital antimicrobial use at the study location showed stable rates for most antimicrobials tested (data not shown). We also found seasonal increases in trimethoprim resistance especially in summer months. The literature suggests a possible seasonality with UTI incidence [38,39] but this was not demonstrated in our study. It is possible that seasonality in UTI may lead to seasonal variation in antimicrobial use with subsequent seasonal resistance patterns although to

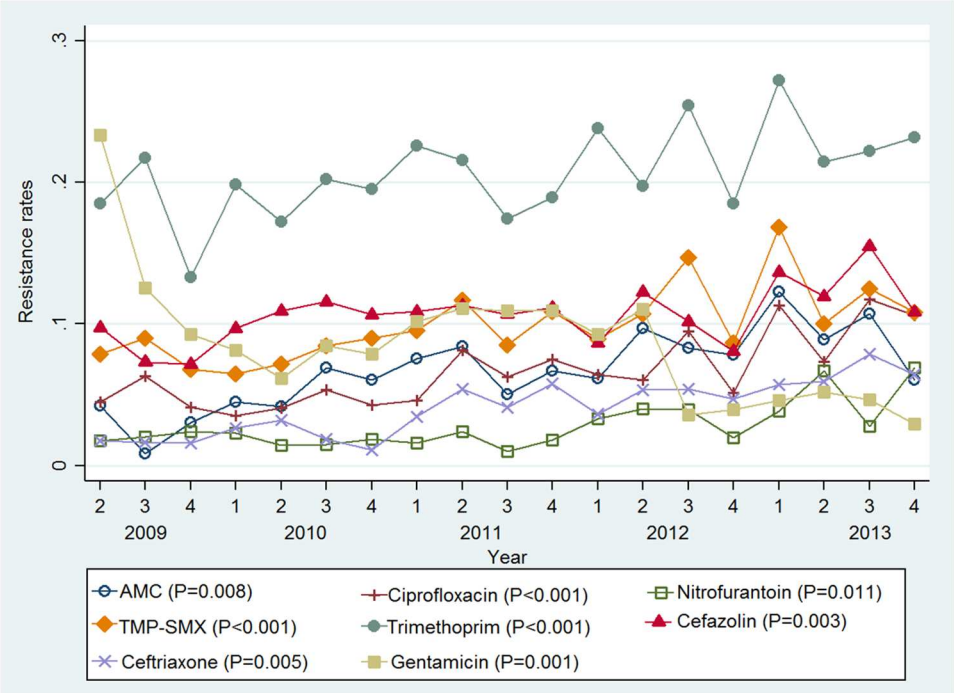

Fig 2. Seasonal antimicrobial resistance rates for *E. coli* UTIs. 1 = Summer; 2 = Autumn; 3 = Winter; 4 = Spring. P = significance level for an increasing trend. AMC = Amoxycillin-clavulanate; TMP-SMX = Trimethoprim-sulphamethoxazole

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our knowledge, this is yet to be demonstrated in published studies. Evidence currently exists to show higher use of antimicrobials in winter months which is likely related to the increased incidence of respiratory tract infections during that period with consequent increases in resistance during winter [40,41]. Therefore the seasonal trimethoprim resistance is a potentially important finding which should be explored in future studies especially in relation to antimicrobial use. The ecological analysis conducted in this study showed an association between older age and resistance to ciprofloxacin, cefazolin and ceftriaxone consistent with published studies [6,34,42]. The association between increasing age and increased resistance is not surprising given that the physiological changes caused by aging and increased comorbidities predispose to a higher risk of infection leading to more contact with healthcare settings and hence more frequent exposure to antibiotics [42].

It is worth emphasizing that our overall ESBL rate of 1.9% was low compared to most other published studies [31]. Results from the 2009–2011 SMART study in the United States reported an ESBL rate of 6.8% for *E. coli* UTI [31]. Although our reported ESBL rate is relatively low, the presence and increasing trend of ESBL-producing *E. coli* in both hospital and community-acquired UTIs pose considerable public health concern. This is because this organism renders many of the conventional empirical treatment options for UTI ineffective especially in community-acquired UTI where options for oral antibiotic therapy appear to be limited [43]. For hospital-acquired UTI caused by ESBL-producing *E. coli*, carbapenems are considered the treatment of choice [43]. In our study, the lowest resistance rate reported was for meropenem, a carbapenem.

This study has some limitations. As most UTIs are treated empirically, it is possible that samples submitted to the laboratory included patients with recurrent UTIs and asymptomatic

bacteriuria thereby overestimating the resistance rates. In addition, inclusion of the first positive *E. coli* UTI per person per year may have underestimated the resistance rates reported in our study. Evidence suggests that analysis of antimicrobial resistance data should include each individual positive isolate in order to ensure sensitivity, but this positive isolate should only be included once to guarantee specificity [44]. This approach of using only the first positive isolate per patient per year is also consistent with published studies on resistance in UTI pathogens including *E. coli* [17,34]. It is unlikely that repeated isolates are correlated but there is a small possibility that this could occur although it was not accounted for in the analysis. The 5-year period prevalence study could therefore have overestimated the resistance. The use of routinely collected microbiology data also posed some limitations as clinical information on patients including comorbidities and presence of indwelling urethral catheters was often missing. The incompleteness of this information prevented its inclusion in the analysis. This study was based on retrospective antimicrobial susceptibility data from a National Association of Testing Authorities, Australia (NATA) accredited clinical microbiology laboratory. The stepwise laboratory testing protocol involved routine first-line antibiotic sensitivity testing followed by more extensive testing with second-line antibiotics only for isolates resistant to at least three of the routine antibiotics. Although this laboratory approach is widely used [44] there is the potential for testing bias and/or selection bias with consequent overestimation of resistance rates. Given the lack of consensus on an appropriate denominator using this testing approach and to prevent possible overestimation of the resistance rates against second-line antibiotics, the denominator therefore included all isolates tested, which, in turn, may have under-estimated resistance rates of broad spectrum antimicrobials. Determining the resistance rate can be influenced by the extent of laboratory testing which in turn influences the selection of the denominator. Using the total number of isolates tested or the number of isolates tested against second-line antibiotics alone as the denominator will either underestimate or overestimate the resistance rates respectively. Although using all isolates for calculating resistance rates for second-line antibiotics has its limitations, this was an appropriate denominator choice to make the findings relevant for use in the clinical setting. For ideal comparison of susceptibility patterns, all isolates would need to be tested against the extended panel of antibiotics in a properly designed prospective study. Regardless of these limitations, our reported resistance rates are low compared to other studies. The use of ecological data to account for the effects of age and sex on resistance also poses limitations to interpretation of these results at the individual patient level. Although our data are from a single tertiary hospital and may not be generalisable to other populations, the data were reported by a NATA accredited laboratory and are therefore satisfactory to provide recommendations to guide local empirical therapy.

Conclusions

Antimicrobial resistance poses grave concerns for antimicrobial effectiveness in treating infections such as UTI. This study demonstrates the increasing resistance of urinary *E. coli* to commonly prescribed antimicrobials. Amoxycillin-clavulanate and nitrofurantoin are still effective for empirical treatment of UTI in this population. Overuse of ampicillin and trimethoprim should be avoided given the high resistance rates reported. In developing local antimicrobial prescribing guidelines, the choice of antimicrobial in the treatment of UTI should be based on setting (community or hospital) of acquisition.

Supporting Information

S1 Table. Distribution of all Canberra Hospital urine samples from 2009 to 2013.
(DOC)

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Author Contributions

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Formal analysis: OF.

Methodology: OF GM BM AG PC AD.

Project administration: OF.

Resources: PC AD.

Supervision: OF AG GM BM.

Validation: GM.

Visualization: OF.

Writing – original draft: OF.

Writing – review & editing: OF BM GM AD PC AG.

References

1. Laupland K, Ross T, Pitout J, Church D, Gregson D. Community-onset urinary tract infections: a population-based assessment. Infection 2007; 35:150–3. doi: 10.1007/s15010-007-6180-2 PMID: 17565455
2. Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urologic Clinics of North America 2008; 35:1–12. doi: 10.1016/j.ucl.2007.09.004 PMID: 18061019
3. Centers for Disease Control and Prevention, National Center for Health Statistics. 2009–2010 Combined Year Tables-Combined NAMCS and NHAMCS. USA: Centers for Disease Control and Prevention 2015. Available: http://www.cdc.gov/nchs/data/ahcd/combined_tables/2009-2010_combined_web_table01.pdf
4. Mitchell BG, Fasugba O, Beckingham W, Bennett N, Gardner A. A point prevalence study of healthcare associated urinary tract infections in Australian acute and aged care facilities. Infection, Disease & Health 2016; 21:26–31. doi: 10.1016/j.idh.2016.03.001
5. Howard DH, Scott RD, Packard R, Jones D. The global impact of drug resistance. Clinical Infectious Diseases 2003; 36:S4–10. doi: 10.1086/344656 PMID: 12516025
6. Blaettler L, Mertz D, Frei R, Elzi L, Widmer A, Battegay M, et al. Secular trend and risk factors for antimicrobial resistance in *Escherichia coli* isolates in Switzerland 1997–2007. Infection 2009; 37:534–9. doi: 10.1007/s15010-009-8457-0 PMID: 20013094
7. Australian Group for Antimicrobial Resistance. 2011 Antimicrobial susceptibility report: Gram-negative Survey. 2012. Australian Group for Antimicrobial resistance, Australia.
8. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. Antimicrobial Agents and Chemotherapy 2002; 46:2540–5. doi: 10.1128/AAC.46.8.2540-2545.2002 PMID: 12121930
9. Cullen IM, Manecksha RP, McCullagh E, Ahmad S, O'Kelly F, Flynn RJ, et al. The changing pattern of antimicrobial resistance within 42,033 *Escherichia coli* isolates from nosocomial, community and urology patient-specific urinary tract infections, Dublin, 1999–2009. BJU International 2012; 109:1198–206. doi: 10.1111/j.1464-410X.2011.10528.x PMID: 21883861
10. Ma KL, Wang CX. Analysis of the spectrum and antibiotic resistance of uropathogens in vitro: Results based on a retrospective study from a tertiary hospital. American Journal of Infection Control 2013; 41:601–6. doi: 10.1016/j.ajic.2012.09.015 PMID: 23352074

11. Perrin M, Donnio P, Heurtin-Lecorre C, Travert M, Avril J-L. Comparative antimicrobial resistance and genomic diversity of *Escherichia coli* isolated from urinary tract infections in the community and in hospitals. Journal of Hospital Infection 1999; 41:273–9. doi: 10.1053/jhin.1998.0521 PMID: 10392333
12. Coxeter P, Looke D, Hoffmann T, Lowe J, Del Mar C. The antibiotic crisis: charting Australia's path towards least resistance. Australian and New Zealand Journal of Public Health 2013; 37:403–4. doi: 10.1111/1753-6405.12137 PMID: 24090320
13. Buttner P, Muller R. Epidemiology. Victoria, Australia: Oxford University Press; 2011.
14. Australian Capital Territory Pathology. Microbiology Department Urine Manual. Canberra, Australia; 2013.
15. Wilson ML, Gaido L. Laboratory Diagnosis of Urinary Tract Infections in Adult Patients. Clinical infectious diseases 2004; 38:1150–58. doi: 10.1086/383029 PMID: 15095222
16. Linhares I, Raposo T, Rodrigues A, Almeida A. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000–2009). BMC Infectious Diseases 2013; 13. doi: 10.1186/1471-2334-13-19 PMID: 23327474
17. McGregor JC, Elman MR, Bearden DT, Smith DH. Sex-and age-specific trends in antibiotic resistance patterns of *Escherichia coli* urinary isolates from outpatients. BMC family practice 2013; 14. doi: 10.1186/1471-2296-14-25 PMID: 23433241
18. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care—associated infection and criteria for specific types of infections in the acute care setting. American Journal of Infection Control 2008; 36:309–32. doi: 10.1016/j.ajic.2008.03.002 PMID: 18538699
19. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24.Pennsylvania, USA; 2014.
20. Dickey DA, Fuller WA. Distribution of the estimators for autoregressive time series with a unit root. Journal of the American Statistical Association 1979; 74:427–31. doi: 10.1080/01621459.1979.10482531
21. Antibiotic Expert Groups. Therapeutic guidelines: antibiotic. Version 15. Melbourne: Therapeutic Guidelines Limited; 2014.
22. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical Infectious Diseases 2011; 52:e103–e20. doi: 10.1093/cid/ciq257 PMID: 21292654
23. Cheng AC, Turnidge J, Collignon P, Looke D, Barton M, Gottlieb T. Control of fluoroquinolone resistance through successful regulation, Australia. Emerging Infectious Diseases 2012; 18:1453–60. doi: 10.3201/eid1809.111515 PMID: 22932272
24. Wang Y, Zhao S, Han L, Guo X, Chen M, Ni Y, et al. Drug resistance and virulence of uropathogenic *Escherichia coli* from Shanghai, China. The Journal of Antibiotics 2014; 67:799–805. doi: 10.1038/ja.2014.72 PMID: 24984795
25. Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxacin resistance in community-and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies. BMC Infectious Diseases 2015; 15:1. doi: 10.1186/s12879-015-1282-4 PMID: 26607324
26. Sannes MR, Kuskowski MA, Johnson JR. Geographical distribution of antimicrobial resistance among *Escherichia coli* causing acute uncomplicated pyelonephritis in the United States. FEMS Immunology & Medical Microbiology 2004; 42:213–18. doi: 10.1016/j.femsim.2004.05.004 PMID: 15364106
27. Gupta K, Sahm DF, Mayfield D, Stamm WE. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. Clinical infectious diseases 2001; 33:89–94. doi: 10.1086/320880 PMID: 11389500
28. Bergman M, Nyberg ST, Huovinen P, Paakkari P, Hakanen AJ, et al. Association between Antimicrobial Consumption and Resistance in *Escherichia coli*. Antimicrobial Agents and Chemotherapy 2009; 53:912–917. doi: 10.1128/AAC.00856-08 PMID: 19104012
29. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. The Lancet 2005; 365:579–87. doi: 10.1016/S0140-6736(05)17907-0 PMID: 15708101
30. Vellinga A, Murphy AW, Hanahoe B, Bennett K, Cormican M. A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic *Escherichia coli* in general practice. Journal of Antimicrobial Chemotherapy 2010; 65:1514–20. doi: 10.1093/jac/dkq149 PMID: 20457673

31. Bouchillon SK, Badal RE, Hoban DJ, Hawser SP. Antimicrobial susceptibility of inpatient urinary tract isolates of Gram-negative bacilli in the United States: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) Program: 2009− 2011. Clinical Therapeutics 2013; 35:872–7. doi: 10.1016/j.clinthera.2013.03.022 PMID: 23623624

32. Kennedy KJ, Roberts JL, Collignon PJ. *Escherichia coli* bacteraemia in Canberra: incidence and clinical features. Medical Journal of Australia 2008; 188:209–13. PMID: 18279126

33. Zarb P, Coignard B, Griskeviciene J, Muller A, Vankerckhoven V, Weist K, et al. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Eurosurveillance 2012; 17: pii = 20316. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20316 PMID: 23171822

34. Swami SK, Liesinger JT, Shah N, Baddour LM, Banerjee R. Incidence of antibiotic-resistant *Escherichia coli* bacteriuria according to age and location of onset: a population-based study from Olmsted County, Minnesota. Mayo Clinic Proceedings 2012; 87:753–9. doi: 10.1016/j.mayocp.2012.02.025 PMID: 22795635

35. Hsu L-Y, Tan T-Y, Tam VH, Kwa A, Fisher DA, et al. Surveillance and correlation of antibiotic prescription and resistance of Gram-negative bacteria in Singaporean hospitals. Antimicrobial Agents and Chemotherapy 2010; 54:1173–78. doi: 10.1128/AAC.01076-09 PMID: 20065055

36. Wong PH, von Krosigk M, Roscoe DL, Lau TT, Yousefi M, et al. Antimicrobial co-resistance patterns of gram-negative bacilli isolated from bloodstream infections: a longitudinal epidemiological study from 2002–2011. BMC infectious diseases 2014; 14. doi: 10.1186/1471-2334-14-393 PMID: 25308184

37. Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. BMJ 2010; 340: c2096. doi: 10.1136/bmj.c2096 PMID: 20483949

38. Freeman J, Anderson D, Sexton D. Seasonal peaks in *Escherichia coli* infections: possible explanations and implications. Clinical Microbiology and Infection 2009; 15:951–3. doi: 10.1111/j.1469-0691.2009.02866.x PMID: 19845705

39. Perencevich EN, McGregor JC, Shardell M, Furuno JP, Harris AD, et al. Summer peaks in the incidences of gram-negative bacterial infection among hospitalized patients. Infection Control & Hospital Epidemiology 2008; 29:1124–31. doi: 10.1086/592698 PMID: 19031546

40. Dagan R, Barkai G, Givon-Lavi N, Sharf AZ, Vardy D, et al. Seasonality of Antibiotic-Resistant Streptococcus pneumoniae That Causes Acute Otitis Media: A Clue for an Antibiotic-Restriction Policy? The Journal of infectious diseases 2008; 197:1094–1102. doi: 10.1086/528995 PMID: 18419528

41. Sun L, Klein EY, Laxminarayan R. Seasonality and Temporal Correlation between Community Antibiotic Use and Resistance in the United States. Clinical infectious diseases 2012; 55:687–94. doi: 10.1093/cid/cis509 PMID: 22752512

42. Adam HJ, Baxter MR, Davidson RJ, Rubinstein E, Fanella S, Karlowsky JA, et al. Comparison of pathogens and their antimicrobial resistance patterns in paediatric, adult and elderly patients in Canadian hospitals. Journal of Antimicrobial Chemotherapy 2013; 68:i31–i7. doi: 10.1093/jac/dkt024 PMID: 23587776

43. Falagas M, Karageorgopoulos D. Extended-spectrum β-lactamase-producing organisms. Journal of Hospital infection 2009; 73:345–54. doi: 10.1016/j.jhin.2009.02.021 PMID: 19596491

44. Cornaglia G, Hryniewicz W, Jarlier V, Kahlmeter G, Mittermayer H, Stratchounski L, et al. European recommendations for antimicrobial resistance surveillance. Clinical Microbiology and Infection 2004; 10:349–83. doi: 10.1111/j.1198-743X.2004.00887.x PMID: 15059129

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Review title and timescale

1 Review title

Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.

Comparing the prevalence of ciprofloxacin resistance in community-acquired versus hospital-acquired urinary E. coli isolates: systematic review and meta-analysis

2 Original language title

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3 Anticipated or actual start date

Give the date when the systematic review commenced, or is expected to commence.

04/08/2014

4 Anticipated completion date

Give the date by which the review is expected to be completed.

02/02/2015

5 Stage of review at time of this submission

Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	No	Yes
Piloting of the study selection process	No	Yes
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

Oyebola Fasugba

7 Named contact email

Enter the electronic mail address of the named contact.

oyebola.fasugba@acu.edu.au

8 Named contact address

Enter the full postal address for the named contact.

School of Nursing, Midwifery and Paramedicine (Signadou Campus) Australian Catholic University PO Box 256 Dickson ACT 2602 Australia

9 Named contact phone number

Enter the telephone number for the named contact, including international dialing code.

+61262091325

10 Organisational affiliation of the review

Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

Australian Catholic University

Website address:

http://www.acu.edu.au/

11 Review team members and their organisational affiliations

Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Mrs	Oyebola	Fasugba	Australian Catholic University
Professor	Anne	Gardner	Australian Catholic University
Dr	George	Mnatzaganian	Australian Catholic University
Professor	Brett	Mitchell	Avondale College of Higher Education

12 Funding sources/sponsors

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.
Not Applicable

13 Conflicts of interest

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.
Are there any actual or potential conflicts of interest?
None known

14 Collaborators

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title First name Last name Organisation details

Review methods

15 Review question(s)

State the question(s) to be addressed / review objectives. Please complete a separate box for each question.
Does the prevalence of ciprofloxacin resistance in people with community-acquired E. coli urinary tract infections differ from those with hospital-acquired infections?

16 Searches

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.
The electronic bibliographic databases MEDLINE/PubMed, EMBASE, Cochrane, CINAHL and Scopus will be searched to cover a ten year time period between 1st January 2004 and the present date of conducting the review. This time limit is based on changes in the microbiology and epidemiology of antimicrobial resistant pathogens which occurred in the past decade with subsequent changes in treatment and patient outcomes (United States Interagency Task Force on Antimicrobial Resistance, 2011). Searches will be conducted for words in the title or abstract or within the full text of the articles. The dates of the last search for each database, the period searched, and the number of records retrieved for each database searched will be recorded and all searches saved. Search filters to be used include the ten year publication time period. There will be no language restrictions when conducting the searches. The full search strategies for each database searched will be included in an appendix of the review paper. The search strategies will be copied and pasted exactly as run and included in full together with the line numbers for each search set. The reference lists of studies identified from the electronic databases will be hand-searched for additional studies. Only peer reviewed material will be considered. All grey material and all non-peer reviewed literature (e.g., conference abstract, letters to editors, etc.) will be excluded. The supervisory team has extensively discussed this point. The exclusion of such literature is often more tolerated than their inclusion.

17 URL to search strategy

If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

I give permission for this file to be made publicly available
No

18 Condition or domain being studied

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.
Antimicrobial resistance (AMR) is a global threat to public health threatening the effective prevention and treatment of a wide range of bacterial, viral parasitic and fungal infections (World Health Organisation, 2012, 2014). Antimicrobial resistant infections may be acquired in hospitals, in the community, and through the food supply, both domestically and overseas (United States Interagency Task Force on Antimicrobial Resistance, 2011). According to the most recent World Health Organisation (WHO) report on AMR surveillance, Escherichia coli (E. coli), the pathogen most frequently implicated in UTIs, is reported as one of the nine bacteria of international concern which are responsible for some of the most common infections in community and hospital settings (World Health Organisation, 2014). In all six WHO regions (Africa, Americas, Eastern Mediterranean, European, South-East Asia and Western Pacific), high rates of resistance have been observed in bacteria that cause common infections such as urinary tract infection (UTI) and pneumonia (World Health Organisation, 2014). Fluoroquinolones are recommended as the drugs of choice for UTIs in regions where the level of resistance to other antimicrobials namely co-trimoxazole is high (Rafalsky, Andreeva, & Rjabkova, 2006). Ciprofloxacin is the most frequently prescribed fluoroquinolone for UTIs because of its availability in oral and intravenous formulations. This antimicrobial agent has shown an excellent activity against pathogens commonly encountered in complicated UTIs. It is well absorbed in oral doses and is rapidly excreted from the body (El Astal, 2005). Use of fluoroquinolones has been linked to infection with methicillin-resistant S. aureus and with increasing fluoroquinolone resistance in gram-negative bacilli, such as Pseudomonas Aeruginosa (Gupta et al., 2011). Resistance to fluoroquinolones such as ciprofloxacin has been on the increase since their introduction for UTI treatment as reported in a number of studies worldwide (El Astal, 2005; Karlowsky, Kelly, Thornsberry, Jones, &

Sahm, 2002). Quantitative syntheses of overall ciprofloxacin-resistant E. coli UTI prevalence are absent and the increases in resistance to this antimicrobial agent have highlighted the need to assess the overall prevalence of urinary E. coli resistance to ciprofloxacin in both hospital and community settings to ensure adequate and appropriate therapy for patients with UTIs. In the era of increasing antimicrobial resistance (Schito et al., 2009), it is necessary to undertake continuous research in this area to ensure patients are effectively treated resulting in good clinical outcomes. References: 1) Centers for Disease Control and Prevention. (2014). CDC/NHSN Surveillance Definitions for Specific Types of Infections. Retrieved from http://www.cdc.gov/nhsn/pdfs/pscmanual/17pscnosinfdef_current.pdf. 2) El Astal, Z. (2005). Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Gaza Strip, Palestine. BioMed Research International, 2005(3), 238-241. 3) Gupta, K., Hooton, T. M., Naber, K. G., Wullt, B., Colgan, R., Miller, L. G., . . . Schaeffer, A. J. (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical infectious diseases, 52(5), e103-e120. 4) Karlowsky, J. A., Kelly, L. J., Thornsberry, C., Jones, M. E., & Sahm, D. F. (2002). Trends in antimicrobial resistance among urinary tract infection isolates of Escherichia coli from female outpatients in the United States. Antimicrobial Agents and Chemotherapy, 46(8), 2540-2545. 5) Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., . . . Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Annals of internal medicine, 151(4), W-65-W-94. 6) Rafalsky, V., Andreeva, I., & Rjabkova, E. (2006). Quinolones for uncomplicated acute cystitis in women. Cochrane Database Syst Rev, 3. 7) Schito, G. C., Naber, K. G., Botto, H., Palou, J., Mazzei, T., Gualco, L., & Marchese, A. (2009). The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. International Journal of Antimicrobial Agents, 34(5), 407-413. doi: http://dx.doi.org/10.1016/j.ijantimicag.2009.04.012 8) United States Interagency Task Force on Antimicrobial Resistance. (2011). A Public Health Action Plan to Combat Antimicrobial Resistance: Interagency Task Force on Antimicrobial Resistance. 9) World Health Organisation. (2012). The evolving threat of antimicrobial resistance: options for action. GPS Publishing, France. 10) World Health Organisation. (2014). Antimicrobial resistance: global report on surveillance.

19 Participants/population

Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.

Inclusion criteria: •studies reporting E. coli UTI •studies reporting prevalence rates of ciprofloxacin resistance in E. coli UTI •studies of hospital populations •studies of community populations •studies of outpatient populations •studies of general practice (GP) populations •studies of nursing home or residential aged care populations •studies involving adults and/or children •peer reviewed letters with data Exclusion criteria: •articles published over 10 years ago •articles not relevant to principal study objective or review question •non-peer reviewed literature •studies not reporting prevalence of ciprofloxacin resistance or data from which prevalence rates cannot be calculated •Papers written in languages other than English will first be considered for translation and if this is not possible, they will be excluded

20 Intervention(s), exposure(s)

Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed

Exposure (1): Being in a community setting OR Exposure (2): Being in a hospital setting

21 Comparator(s)/control

Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).

Not Applicable

22 Types of study to be included initially

Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.

Observational (cross sectional; prospective and retrospective cohort; case-control) studies

23 Context

Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

•Community: studies using -Samples obtained from outpatient clinics -Samples obtained from general practice (GP) clinics -Samples obtained within 48 hours of hospital admission -Samples from nursing homes or residential aged care facilities (RACFs) •Hospital: studies using -Samples obtained after 48 hours of hospital admission

24 Primary outcome(s)

Give the most important outcomes.

Prevalence of ciprofloxacin resistant E. coli UTI in both settings

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes

List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.

Mortality associated with resistance in both settings

Give information on timing and effect measures, as appropriate.

26 Data extraction, (selection and coding)

Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

Selection of studies Stage 1: The titles and abstracts of all publications identified on the electronic databases will be examined and assessed for relevance and appropriateness to the principal study objective or review question. Those that are clearly not relevant will be excluded. Stage 2: Full texts of the potentially relevant papers will be printed and carefully assessed against the criteria. Assessment will be performed independently by the reviewers and a consensus made on those that meet all the criteria. After applying the inclusion and exclusion criteria, articles not meeting the criteria will be excluded. Stage 3: The remaining articles will be deemed to have data relevant to the systematic review and meta-analysis and will be assessed for quality and risk of bias. This assessment will be performed by four reviewers. OF, who is a student will be the lead author. Her three supervisors will act as mentors and will be involved in all stages including the literature search, data extraction, analysis and report writing. Data extraction and management: A paper-based data extraction form has been designed for the purpose of extracting data for the systematic review and meta-analysis. For all eligible studies meeting the inclusion and exclusion criteria, the following data will be extracted: first author; year of publication; country/place of study; year study was conducted; study population; study setting (Hospital setting/Community setting/Hospital with RACFs/Community with RACFs); age and sex distribution; sample size; study design; Antimicrobial susceptibility testing method; prevalence of ciprofloxacin resistance; numerator; denominator; 95% confidence intervals; standard error; mortality data (if reported) The discrepancies in either the decision on inclusion or exclusion of studies, quality assessment or on data extraction will be discussed with supervisors to make the final decision.

27 Risk of bias (quality) assessment

State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.

Quality and risk of bias assessment of the final papers included in the review will be conducted using a validated risk of bias tool for observational studies or separate tools depending on the specific type of observational study design.

28 Strategy for data synthesis

Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.

Based on the data obtained from eligible studies, the following forms of analysis will be used – tabulation, meta-analysis and narrative (descriptive). Measures of effect: The pooled proportions or pooled odds ratio will be calculated and compared across both hospital settings and community settings. The level of resistance will be evaluated using a random-effects meta-analysis model using DerSimonian and Laird method. This method incorporates an estimate of the between-study variation into both the study weights and the standard error of the estimate of the common effect. The precision of an estimate from each included study will be represented by the inverse of the variance of the outcome pooled across all studies. The pooled effect size (ES) (estimated by the pooled proportion) with 95% CI will be calculated. If the value of the pooled proportion ‘zero’ is not within the 95% CI, then the ES is statistically significant at the 5% level (P

29 Analysis of subgroups or subsets

Give any planned exploration of subgroups or subsets within the review. ‘None planned’ is a valid response if no subgroup analyses are planned.

Subgroup analyses will be done by sex, age, time period, and region. Meta-analyses using Mantel-Haenszel fixed-effect models may also be considered in the sub-analyses.

Review general information

30 Type of review

Select the type of review from the drop down list.

Epidemiologic

31 Language

Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.

English

Will a summary/abstract be made available in English?

Yes

32 Country

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.

Australia

33 Other registration details

List places where the systematic review title or protocol is registered (such as with he Campbell Collaboration, or The Joanna Briggs Institute). The name of the organisation and any unique identification number assigned to the review by that organization should be included.

Not Applicable

34 Reference and/or URL for published protocol

Give the citation for the published protocol, if there is one.
Not Applicable
Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

I give permission for this file to be made publicly available
Yes

35 Dissemination plans

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.
-Conference presentation -Publication submission to a high ranking peer reviewed journal (Q1)
Do you intend to publish the review on completion?
Yes

36 Keywords

Give words or phrases that best describe the review. (One word per box, create a new box for each term)
ciprofloxacin resistance
urinary tract infection
escherichia coli
hospital
community

37 Details of any existing review of the same topic by the same authors

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.
Not Applicable

38 Current review status

Review status should be updated when the review is completed and when it is published.
Ongoing

39 Any additional information

Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)

This field should be left empty until details of the completed review are available.
Give the full citation for the final report or publication of the systematic review.
Give the URL where available.

Appendix E: Data extraction form with quality and risk of bias assessment tool

Review title	Comparing the prevalence of ciprofloxacin resistance in community-acquired versus hospital-acquired *E. coli* UTIs : systematic review and meta-analysis
Study ID ***(year first full report of study was published, surname of first author and study setting e.g. 2001SmithHospital)***	
Name of publishing journal	
Notes:	

1. General information

Date form completed *(dd/mm/yyyy)*	
Name/initials of person extracting data	
Study author email address	
Notes:	

2. **Characteristics of included studies**

Participants

	Description	Location in text or source *(pg & ¶/fig/table)*
Sample size (total number of study participants)	☐ Not stated	
Number of participants on whom resistance was reported	☐ Not stated	
Country/place of study	☐ Not stated	
Informed consent obtained	☐ Yes ☐ No ☐ Unclear/not stated	
Age distribution of all participants	Mean…….. SD…… Median……. IQR…… ☐ Not stated	
Age distribution of participants with positive UTIs	Mean…….. SD…… Median……. IQR…… ☐ Not stated	
Age distribution of participants with *E. coli* UTIs	Mean…….. SD…… Median……. IQR…… ☐ Not stated	
Age distribution of participants with resistant UTIs	Mean…….. SD…… Median……. IQR…… ☐ Not stated	
Sex distribution of all participants	Male (n; %)…… Female (n; %)…….. ☐ Not stated	

Sex distribution of participants with positive UTIs	Male (n; %)…… Female (n; %)…….. ☐ Not stated	
Sex distribution of participants with *E. coli* UTIs	Male (n; %)…… Female (n; %)…….. ☐ Not stated	
Sex distribution of participants with resistant UTIs	Male (n; %)…… Female (n; %)…….. ☐ Not stated	
Study setting	☐ Hospital ☐ After 48 hours of hospital admission ☐ Within 48 hours of hospital discharge ☐ Community ☐ Outpatients ☐ General practice clinic ☐ Emergency department ☐ Within 48 hours of admission ☐ Nursing home/RACF	
Co-morbidities	List all co-morbidities if present and state proportion ☐ Diabetes mellitus_____ n; % ☐ Cancer_____ n; % ☐ Spinal cord injury_____ n; % ☐ Other_____ n; % ☐ Not stated	
Notes:		

Methods

		Descriptions as stated in report/paper	Location in text *(pg & ¶/fig/table)*
Aim/ Objective of study	Primary aim		
	Secondary aim		
Study duration		Start date: End date: Total duration: ☐ Not stated	
Study design		☐ Cross sectional ☐ Retrospective cohort ☐ Prospective cohort ☐ Case control ☐ Nested case control ☐ Not stated	
Antimicrobial susceptibility testing method		☐ Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) ☐ European Committee on Antimicrobial Susceptibility testing (EUCAST) ☐ Other_____ ☐ Not stated	
Notes:			

Results

		Description	**Location in text or source** *(pg & ¶/fig/table)*
Total number of urine samples		☐ Not stated	
Number of positive UTI samples		☐ Not stated	
Number of positive *E.coli* UTI samples		☐ Not stated	
Prevalence of ciprofloxacin susceptible *E. coli* strains		☐ Not stated	
Prevalence of ciprofloxacin intermediate *E. coli* strains		☐ Not stated	
Prevalence of ciprofloxacin resistant *E. coli* strains		☐ Not stated	
95% confidence interval of resistance prevalence		☐ Not stated	
Standard error		☐ Not stated	
Mortality rate of participants with resistant infections		Crude/adjusted mortality rate_____% In-hospital mortality rate_____ % 30-day mortality rate_____% 90-day mortality rate_____%	
For studies reporting resistance in two or more of the defined settings: State p-value if differences noted ☐ Not stated	Age	Settings_____ p value_____	
	Gender	Settings_____ p value_____	
	Ciproflox-acin resistance	Settings_____ p value_____	
	Other	Settings_____ p value_____	

Risk factors for ciprofloxac-in resistance ☐ Not stated	Age	OR_____ 95% CI_____ p value_____	
	Gender	OR_____ 95% CI_____ p value_____	
	Prior use of antibiotic	OR_____ 95% CI_____ p value_____	
	Other	OR_____ 95% CI_____ p value_____	
Number of missing participants		☐ Not stated	
Reasons missing		☐ Not stated	
Notes:			

Study quality and risk of bias assessment: The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses GA Wells, B Shea, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell, http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

Cohort Studies				
Bias		High quality*		
Selection (max 4*)	Representativeness of exposed cohort	☐ Truly representative of the target population ☐ Somewhat representative of target population	☐ Selected group of participants	☐ No description of derivation of cohort
	Selection of non-exposed cohort	☐ Drawn from the same community as the exposed cohort	☐ Drawn from a different source	☐ No description of derivation of non-exposed cohort
	Ascertainment of exposure (UTI measurement)	☐ Confirmed laboratory diagnosis	☐ Self-report of UTI	☐ No description
	Demonstration that outcome of interest was not present at start of study	☐ Yes	☐ No	
Comparability (max 2*)	Comparability of cohorts on basis of design or analysis	☐ Study controls for important factor (antibiotic use)	☐ Fails to control for an important factor	
		☐ Study controls for any additional factor (age, gender etc)	☐ Does not control for any factors	

Outcome (max 3*)	Assessment of outcome (Ciprofloxacin resistant *E. coli* UTI)	☐Standard laboratory test (e.g. CLSI or EUCAST) ☐Record linkage (e.g. identified through ICD codes on database records)	☐Self-report	☐No description
	Was follow-up long enough for outcome to occur	☐Yes (>=12 months)	☐No (<12 months)	
	Adequacy of follow up of cohorts	☐Complete follow up-all subjects accounted ☐Subjects lost to follow up unlikely to introduce bias – small number lost (>80% follow up), or description provided of those lost	☐Follow up rate <20% and no description of those lost	☐No statement

Cross-sectional Studies				
Bias		High quality*		
Selection (max 2*)	Representativeness of sample	☐ Truly representative of the target population ☐ Somewhat representative of target population	☐ Selected group of participants	☐ No description of sample
	Sampling strategy	☐ Probability sampling	☐ Non-probability sampling	☐ No description of sampling strategy
	Ascertainment of exposure (UTI measurement)	☐ Confirmed laboratory diagnosis	☐ Self-report of UTI	☐ No description
Comparability (max 2*)	Comparability of participants on basis of design or analysis	☐ Study controls for important factor (antibiotic use)	☐ Fails to control for an important factor	
		☐ Study controls for any additional factor (age, gender etc)	☐ Does not control for any factors	
Outcome (max 1*)	Assessment of outcome (Ciprofloxacin resistant *E. coli* UTI)	☐ Standard laboratory test (e.g. CLSI or EUCAST) ☐ Record linkage (e.g. identified through ICD codes on database records)	☐ Self-report	☐ No description

Case-control Studies				
Bias		High quality*		
Selection (max 4*)	Is the case definition adequate?	☐ Yes, with independent validation	☐ Yes, e.g. record linkage or based on self-report	☐ No description
	Representativeness of the cases	☐ Consecutive or obviously representative series of cases	☐ Potential for selection bias or not stated	
	Selection of controls	☐ Community controls	☐ Hospital controls	☐ No description
	Definition of controls	☐ No history of disease (endpoint)	☐ No description of source	
Comparability (max 2*)	Cases and controls on the basis of the design or analysis	☐ Study controls for important factor (antibiotic use)	☐ Fails to control for an important factor	
		☐ Study controls for any additional factor (age, gender etc)	☐ Does not control for any factors	
Exposure (max 3*)	Ascertainment of exposure	☐ Secure record ☐ Structured interview where blind to case/control status	☐ Interview not blinded to case/control status	☐ Written self-report or medical record only ☐ No description
	Same method of ascertainment for cases and controls	☐ Yes	☐ No	
	Non-response rate	☐ Same rate for both groups	☐ Non respondents described	☐ Rate different and no designation

Other

Study funding sources *(including role of funders)*		
Possible conflicts of interest *(for study authors)*		
Is there any additional study in the reference list of this article that might be included in the review?	☐ Yes ☐ No If yes, state reference(s):	
Notes:		

Appendix F: Approvals for studies two and three

F1: ACT Health Human Research Ethics Committee Approval

ACT Government
Health

ACT Health
Human Research Ethics Committee
Low Risk Sub-Committee

Ms Oyebola Fasugba
School of Nursing, Midwifery and Paramedicine
Signadou Building, Level 1
Australian Catholic University
Canberra Campus
223 Antill St
Watson ACT 2602

Dear Ms Fasugba

ETHLR.14.223

The ACT Health Human Research Ethics Committee's Low Risk Sub-Committee received notification of the proposed study:

Antimicrobial resistance and urinary tract infections in an Australian population-based sample at its meeting of 27 August 2014.

I am pleased to inform you that, following further correspondence, your application has been approved.

The Sub-Committee agreed that the application is for low risk research and determined that the research meets the requirements of the National Statement on Ethical Conduct in Human Research and is ethically acceptable.

I attach for your records an Outcome of Consideration of Protocol form.

I confirm that the ACT Health Human Research Ethics Committee is constituted according to the National Statement on Ethical Conduct in Human Research 2007 and is certified for single review of multi-centre clinical trials. ACT Health HREC operates in compliance with applicable regulatory requirements and the International Conference on Harmonization Guidelines on Good Clinical Practice.

Yours sincerely

Talaulikar

Dr Dipti Talaulikar
MBBS PhD FRCPA FRACP Grad Cert HE
A/g Chair, Low Risk Sub-Committee
ACT Health Human Research Ethics Committee

12 September 2014

ACT HEALTH HUMAN RESEARCH ETHICS COMMITTEE

Outcome of Consideration of Protocol

Submission No: ETHLR.14.223 **Date of Approval:** 12 **September 2014**

Project Title: Antimicrobial resistance and urinary tract infections in an Australian population-based sample

Submitted by: Ms Oyebola Fasugba

Your project was considered by the ACT Health Human Research Ethics Committee and Approved for a period of 3 years.

First Annual Review due: September 2015

The Ethics Committee require as part of the review process that:

- At regular periods, and not less frequently than annually, Principal Investigators are to provide reports on matters including:
  - security of records
  - compliance with approved consent procedures and documentation
  - compliance with other approved procedures.
  - as a condition of approval of the protocol, that Investigators report immediately:
  - adverse affects on subjects
  - proposed changes in the protocol
  - unforeseen events that might affect continued ethical acceptability of the project.
- All published reports to carry an acknowledgement stating:
  - Approved on September 2014 by the ACT Health Human Research Ethics Committee's Low Risk Sub-Committee.

Dr Dipti Talaulikar
MBBS PhD FRCPA FRACP Grad Cert HE
A/g Chair, Low Risk Sub-Committee
ACT Health Human Research Ethics Committee

12 September 2014

F2: Australian Catholic University Human Research Ethics Committee Approval

From:	Kylie Pashley on behalf of Res Ethics
To:	Anne Gardner; Oyebola Fasugba
Cc:	Res Ethics
Subject:	2014 276N Registration of External Ethics Approval
Date:	Friday, 17 October 2014 12:05:17 PM

Dear Pamela,

Principal Investigator: Prof Anne Gardner
Student Researcher: Ms Oyebola Fasugba
Ethics Register Number: 2014 276N
Project Title: Antimicrobial resistance and urinary tract infections in an Australian population-based sample
Risk Level: Multi Site
Date Approved: 17/10/2014
Ethics Clearance End Date: 30/09/2015

The Australian Catholic University Human Research Ethics Committee has considered your application for registration of an externally approved ethics protocol and notes that this application has received ethics approval from ACT Health [Reference: ETHLR.14.223].

The ACU HREC accepts the ethics approval with no additional requirements, save that ACU HREC is informed of any modifications of the research proposal and that copies of all progress reports and any other documents be forwarded to it. Any complaints involving ACU staff must also be notified to ACU HREC (National Statement 5.3.3)

We wish you well in this research project.

Regards,

Kylie Pashley
on behalf of ACU HREC Chair, Dr Nadia Crittenden
Ethics Officer | Research Services
Office of the Deputy Vice Chancellor (Research)
res.ethics@acu.edu.au

ACT Government
Health

ACT Health
Human Research Ethics Committee
Low Risk Sub-Committee

Ms Oyebola Fasugba
School of Nursing, Midwifery and Paramedicine
Signadou Building, Level 1
Australian Catholic University
Canberra Campus
223 Antill St
Watson ACT 2602

Dear Ms Fasugba

ETHLR.14.223

Thank you for your recent letter, requesting amendments relating to:

Antimicrobial resistance and urinary tract infections in an Australian population-based sample

At its meeting of 8 September 2015, the Committee approved:

- Obtaining antimicrobial use data at Canberra Hospital for the period 2009 to 2013 from the National Antimicrobial Utilisation Surveillance Program

This information is now recorded on the Committee's files

Yours sincerely,

Louise Morauta

Louise Morauta PSM PhD
Chair
ACT Health Human Research Ethics Committee
Low Risk Sub-Committee

8 September 2015

F4: Approval from Executive Director Performance Information Branch, ACT Health

From: Cohen, Sarit (Health)
To: Oyebola Fasugba
Cc: Bailey, Andrew (Health); Anne Gardner
Subject: RE: ETHLR.14.223 ACT Health ethics approval to obtain antimicrobial use data
Date: Monday, 4 January 2016 11:38:42 AM

Dear Bola,

An apology for not getting back to you sooner. Hope you had a good break.

I have a note here from Phil Ghirardello accepting and okay to release the data.

Kind regards
Sarit

Sarit Cohen

Personal Assistant to Phil Ghirardello | Executive Director Performance Information Branch
Level 3, 11 Moore Street Canberra City
Phone: 6205 0549 | Email: sarit.cohen@act.gov.au

Care Excellence Collaboration Integrity

ACT Government Health

From: Oyebola Fasugba [mailto:oyebola.fasugba@myacu.edu.au]
Sent: Thursday, 17 December 2015 2:49 PM
To: Cohen, Sarit (Health)
Cc: Bailey, Andrew (Health); Anne Gardner
Subject: RE: ETHLR.14.223 ACT Health ethics approval to obtain antimicrobial use data

Dear Sarit,

Please find attached the signed data release form for processing. Can you kindly notify me of when most likely I will get final approval/confirmation from your office?

Thank you for your assistance.

Kind regards,
Bola

Oyebola Fasugba MPHTM MBBS
PhD Candidate & Research Associate | School of Nursing, Midwifery and Paramedicine (Signadou Campus)
Australian Catholic University
223 Antill Street, Watson, ACT 2602
PO Box 256, Dickson ACT 2602

APPENDIX G: Additional/Supplementary files

Study one: Systematic review of ciprofloxacin resistance in *E. coli* UTI

Additional file 1: Search strategy by database

EMBASE Search strategy 1: Keywords only

#	Searches	Results
1	resistance.mp.	798263
2	urinary tract infection.mp.	80151
3	Escherichia coli.mp.	358062
4	1 and 2 and 3	3709
5	4 and 2004:2014.(sa_year).	2423

EMBASE Search strategy 2: Keywords and subject headings

#	Searches	Results
1	antibiotic resistan*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	123069
2	antimicrobial resistan*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	12903
3	drug resistan*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	114373
4	bacterial resistan*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	5524
5	antibiotic resistance/	114397
6	drug resistance/	58485
7	1 or 2 or 3 or 4 or 5 or 6	225773
8	urinary tract infection*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	83408
9	uti.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	9689
10	bacteriuria.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	10917
11	pyuria.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	3890
12	urinary tract infection/	75810
13	bacteriuria/	8487
14	pyuria/	3150
15	8 or 9 or 10 or 11 or 12 or 13 or 14	91929
16	Escherichia coli.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	358062

17	e coli.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	125639
18	Escherichia coli/	294320
19	16 or 17 or 18	371005
20	7 and 15 and 19	3284
21	20 and 2004:2014.(sa_year).	2160

CINAHL Search strategy 1: Keywords only

#	Searches	Results
1	resistance	55753
2	urinary tract infection	7192
3	escherichia coli	4970
4	1 AND 2 AND 3	235
5	4 Limiters-Published date 20040101-20141231	208

CINAHL Search strategy 2: Keywords and subject headings

#	Searches	Results
1	antibiotic resistan*	4759
2	antimicrobial resistan*	3316
3	drug resistan*	33393
4	bacterial resistan*	6765
5	(MH “Drug Resistance, Microbial”)	12164
6	(MH “Drug Resistance”)	4838
7	1 OR 2 OR 3 OR 4 OR 5 OR 6	36416
8	urinary tract infection*	7781
9	uti	1081
10	bacteriuria	740
11	pyuria	129
12	(MH “Urinary Tract Infections”)	5924
13	(MH “Bacteriuria”)	518
14	8 OR 9 OR 10 OR 11 OR 12 OR 13	8169
15	escherichia coli	4970
16	e coli	1428
17	(MH “Escherichia Coli”)	2722
18	15 OR 16 OR 17	5191
19	7 AND 14 AND 18	270
20	19; Limiters – Published Date: 20040101-20141231	236

SCOPUS Search strategy 1: Keywords only

#	Searches	Results
1	TITLE-ABS-KEY (resistance)	1,406,458
2	TITLE-ABS-KEY (urinary tract infection)	87,237
3	TITLE-ABS-KEY (escherichia coli)	411,648
4	(TITLE-ABS-KEY (resistance)) AND (TITLE-ABS-KEY (urinary tract infections)) AND (TITLE-ABS-KEY (escherichia coli))	4,353
5	(TITLE-ABS-KEY (resistance)) AND (TITLE-ABS-KEY (urinary tract infections)) AND (TITLE-ABS-KEY (	2472

	escherichia coli)) AND (LIMIT-TO (PUBYEAR , 2014-2004)	

SCOPUS Search strategy 2: Keywords and subject headings

#	Searches	Results
1	TITLE-ABS-KEY (antibiotic resistan*)	186424
2	TITLE-ABS-KEY (antimicrobial resistan*)	56777
3	TITLE-ABS-KEY (drug resistan*)	507251
4	TITLE-ABS-KEY (bacterial resistan*)	181814
5	1 OR 2 OR 3 OR 4	597338
6	TITLE-ABS-KEY (urinary tract infection)	87237
7	TITLE-ABS-KEY (uti)	7656
8	TITLE-ABS-KEY (bacteriuria)	11141
9	TITLE-ABS-KEY (pyuria)	3187
10	6 OR 7 OR 8 OR 9	94035
11	TITLE-ABS-KEY (escherichia coli)	411648
12	TITLE-ABS-KEY (e coli)	168002
13	11 OR 12	429893
14	5 AND 10 AND 13	5184
15	14 AND (LIMIT-TO (PUBYEAR , 2014-2004)	2777

PubMed Search strategy 1: Keywords only

#	Searches	Results
1	Search resistance	610335
2	Search urinary tract infection	54,476
3	Search escherichia coli	318047
4	Search ((resistance) AND urinary tract infection) AND escherichia coli	2,416
5	4 AND Filters: Publication date from 2004/01/01 to 2014/12/31	1243

PubMed Search strategy 2: Keywords and subject headings

#	Searches	Results
1	Search antibiotic resistance	146589
2	Search antimicrobial resistance	165154
3	Search bacterial resistance	116736
4	Search drug resistance	375235
5	Search "Drug Resistance, Microbial"[Mesh]	125068
6	Search "Drug Resistance, Bacterial"[Mesh]	60535
7	1 OR 2 OR 3 OR 4 OR 5 OR 6	409413
8	Search urinary tract infection	54469
9	Search uti	5705
10	Search bacteriuria	8749
11	Search pyuria	1976
12	Search "Urinary Tract Infections"[Mesh]	38550
13	Search "Bacteriuria"[Mesh]	6931
14	Search "Pyuria"[Mesh]	906

15	8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14	56345
16	Search escherichia coli	318008
17	Search e coli	333732
18	Search "Escherichia coli"[Mesh]	232783
19	16 OR 17 OR 18	333732
20	7 AND 15 AND 19	2623
21	20 AND Filters: Publication date from 2004/01/01 to 2014/12/31	1301

MEDLINE Search strategy 1: Keywords only

#	Searches	Results
1	resistance.mp.	574974
2	urinary tract infections.mp.	37313
3	Escherichia coli.mp.	311661
4	1 and 2 and 3	1923
5	4 and 2004:2014. (sa_year)	964

MEDLINE Search strategy 2: Keywords and subject headings

#	Searches	Results
1	antibiotic resistan*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	19772
2	antimicrobial resistan*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	8841
3	drug resistan*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	197925
4	bacterial resistan*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	3438
5	Drug Resistance, Microbial/	54415
6	Drug Resistance/	39696
7	1 or 2 or 3 or 4 or 5 or 6	208986
8	urinary tract infection*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	43114
9	uti.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	5172
10	bacteriuria.mp. [mp=title, abstract, original title, name of	8646

	substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	
11	pyuria.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	1890
12	Urinary Tract Infections/	32461
13	Bacteriuria/	7048
14	Pyuria/	925
15	8 or 9 or 10 or 11 or 12 or 13 or 14	49102
16	Escherichia coli.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	311661
17	e coli.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	120235
18	Escherichia coli/	227408
19	16 or 17 or 18	324796
20	7 and 15 and 19	1886
21	20 and 2004:2014.(sa_year).	933

COCHRANE Search strategy
Cochrane Database of Systematic Reviews

#	Searches	Cochrane reviews
1	resistance in Title, Abstract, Keywords (Word variations have been searched)	263
2	urinary tract infection in Title, Abstract, Keywords (Word variations have been searched)	77
3	Escherichia coli in Title, Abstract, Keywords (Word variations have been searched)	3
4	1 and 2 and 3	1
5	4 and Publication Year from 2004 to 2014 (Word variations have been searched)	1

Additional file 2: Subgroup analyses of pooled ciprofloxacin resistance in hospital setting

Subgroup		Hospital setting N=3	*P* value*
		Pooled resistance	
Region	Middle East n=1 study	0.400**	0.880
	North America n=1 study	0.407**	
Economy	Developed n=1 study	0.407	0.880
	Developing n=1 study	0.400	
UTI symptoms	Symptomatic and asymptomatic patients n=1 study	0.380	0.356
	Symptomatic patients only n=2 studies	0.404	

*Comparing pooled resistance for difference in subgroup in hospital setting

n=number of studies reporting on hospital acquired UTI

**Middle East only; North America only

Additional file 3: Subgroup analyses of pooled ciprofloxacin resistance by setting

Subgroup		Community Setting N=51	Hospital setting N=3	*P* value*
		Pooled resistance	Pooled resistance	
Risk of bias	Low and unclear C=28 studies H= 0 study	0.221	-	-
	High C=23 studies H=3 studies	0.337 (10262)	0.385	<0.0001
Study duration	≤12 months C=25 studies H=0 study	0.323	-	-
	>12months C=24 studies H=3 studies	0.219 (34328)	0.385	<0.0001
Study design	Cross sectional C=40 studies H=3 studies	0.271 (36909)	0.385	<0.0001
	Cohort C=5 studies H=0 study	0.287	-	-
	Case control C=2 studies H=0 study	0.224	-	-
Economy	Developed C=16 studies H=1 study	0.141 (12996)	0.407	<0.0001
	Developing C=35 studies H=1 study	0.345 (32064)	0.400	0.103
Region	Africa C=7 studies H=0 study	0.203	-	-
	Asia and Middle East C=22 studies H=1 study	0.390 (8866)	0.400**	0.774
	Europe C=13 studies H=0 study	0.156	-	-

	North and South America C=9 studies H=1 study	0.207 (24871)	0.407**	<0.0001
Age group	Adults and children ǂ C=24 studies H=2 studies	0.265 (33090)	0.382	<0.0001
	Adults only C=19 studies H=0 study	0.302	-	-
UTI symptoms	Symptomatic and asymptomatic patients C=11 studies H=1 study	0.185 (27984)	0.380	<0.0001
	Symptomatic patients only C=40 studies H=2 studies	0.295 (17076)	0.404	<0.0001
Overall		0.27	0.38	<0.0001

*****Comparing pooled resistance for difference in subgroup in community and hospital settings

ǂ Studies reporting resistance in adults and children or children only

C= number of studies reporting on community acquired UTI

H= number of studies reporting on hospital acquired UTI

** Middle East only; North America only

Study two: Prevalence of antimicrobial resistance in *E. coli* UTI

S1 Table. Distribution of all Canberra Hospital urine samples from 2009 to 2013

	2009	2010	2011	2012	2013	Overall five year period
Total number of urine samples	19,455	20,005	22,070	22,496	22,486	106,512
Proportion of positive urine samples	14.2% (n=2,767)	14.8% (n=2,951)	14.3% (n=3,167)	13.7% (n=3,075)	13.6% (n=3,062)	14.1% (n=15,022)
Proportion of positive samples where *E. coli* was isolated	49.5% (n=1,370)	50.6% (n=1,494)	53.6% (n=1,699)	49.6% (n=1,526)	51.6% (n=1,581)	51.1% (n=7,670)

Study three: Incidence of antimicrobial resistance in *E. coli* UTI

Supplementary Table S1 Multivariate logistic regression models for the effects of age, gender and socioeconomic status on antimicrobial resistant *E. coli* UTI*

Antibiotic	Variable	Categories	Odds ratio	95% CI
Ampicillin	Age (years)	≤23	1.3	1.2-1.5
		38-56	1.3	1.1-1.4
		57-73	1.9	1.7-2.1
		≥74	2.8	2.5-3.1
		24-37 (reference)	1.0	
	Gender	Female	3.1	2.8-3.4
		Male (reference)	1.0	
	SES	Middle	1.0	0.9-1.1
		High	1.0	0.9-1.0
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	0.7	0.6-0.9
		GP clinics	1.1	1.0-1.2
		After-hours GP clinics	2.5	2.1-3.0
		Community health services	1.5	1.2-1.8
		Specialist health services	1.0	0.7-1.5
		Others[#]	1.4	0.9-2.0
		Public acute hospitals (reference)	1.0	
AMC	Age	≤23	1.2	0.9-1.7
		38-56	1.5	1.1-2.0
		57-73	2.9	2.2-3.8
		≥74	4.2	3.2-5.4
		24-37 (reference)	1.0	
	Gender	Female	2.8	2.3-3.5
		Male (reference)	1.0	
	SES	Middle	1.0	0.8-1.2
		High	1.1	0.9-1.3
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	0.8	0.5-1.3
		GP clinics	0.9	0.7-1.1
		After-hours GP clinics	2.1	1.3-3.1
		Community health services	1.3	0.8-2.1
		Specialist health services	2.2	1.3-3.8
		Others[#]	1.7	0.8-3.7
		Public acute hospitals (reference)	1.0	
Cefazolin	Age	≤23	1.5	1.2-1.8
		38-56	1.4	1.1-1.7
		57-73	2.6	2.1-3.2
		≥74	3.8	3.1-4.6
		24-37 (reference)	1.0	
	Gender	Female	2.6	2.3-3.0
		Male (reference)	1.0	

	SES	Middle	1.0	0.9-1.1
		High	0.9	0.8-1.1
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	0.9	0.6-1.3
		GP clinics	0.9	0.8-1.1
		After-hours GP clinics	1.7	1.2-2.5
		Community health services	1.6	1.2-2.3
		Specialist health services	1.4	0.8-2.3
		Others[#]	1.6	0.9-3.0
		Public acute hospitals (reference)	1.0	
Ceftriaxone	Age	≤23	1.1	0.7-1.6
		38-56	1.1	0.8-1.7
		57-73	2.2	1.6-3.1
		≥74	3.4	2.5-4.7
		24-37 (reference)	1.0	
	Gender	Female	2.7	2.1-3.5
		Male (reference)	1.0	
	SES	Middle	0.9	0.7-1.2
		High	1.1	0.9-1.4
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	1.1	0.6-1.9
		GP clinics	0.9	0.7-1.2
		After-hours GP clinics	1.1	0.5-2.3
		Community health services	1.8	1.0-3.0
		Specialist health services	2.7	1.5-5.1
		Others[#]	2.0	0.8-5.0
		Public acute hospitals (reference)	1.0	
Trimethoprim	Age	≤23	1.2	1.1-1.5
		38-56	1.2	1.0-1.4
		57-73	2.0	1.7-2.3
		≥74	2.8	2.4-3.2
		24-37 (reference)	1.0	
	Gender	Female	3.4	3.0-3.9
		Male (reference)	1.0	
	SES	Middle	1.1	1.0-1.2
		High	1.1	0.9-1.2
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	0.7	0.5-0.9
		GP clinics	1.1	1.0-1.3
		After-hours GP clinics	2.2	1.7-2.8
		Community health services	1.5	1.1-1.9
		Specialist health services	1.3	0.9-2.0
		Others[#]	1.7	1.0-2.7
		Public acute hospitals (reference)	1.0	

TMP-SMX	Age	≤23	1.3	1.1-1.7
		38-56	1.1	0.9-1.4
		57-73	2.2	1.8-2.7
		≥74	2.7	2.2-3.4
		24-37 (reference)	1.0	
	Gender	Female	2.8	2.3-3.2
		Male (reference)	1.0	
	SES	Middle	1.1	0.9-1.2
		High	1.1	0.9-1.2
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	0.8	0.6-1.3
		GP clinics	1.0	0.9-1.2
		After-hours GP clinics	1.9	1.3-2.7
		Community health services	1.7	1.2-2.4
		Specialist health services	1.7	1.0-2.8
		Others[#]	1.7	0.9-3.3
		Public acute hospitals (reference)	1.0	
Nalidixic acid	Age	≤23	0.9	0.7-1.1
		38-56	0.8	0.7-1.1
		57-73	1.7	1.4-2.1
		≥74	1.9	1.5-2.4
		24-37 (reference)	1.0	
	Gender	Female	2.9	2.4-3.5
		Male (reference)	1.0	
	SES	Middle	1.2	1.0-1.4
		High	1.2	1.0-1.5
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	1.0	0.7-1.6
		GP clinics	1.0	0.9-1.2
		After-hours GP clinics	1.7	1.1-2.5
		Community health services	1.8	1.2-2.6
		Specialist health services	1.5	0.8-2.7
		Others[#]	1.4	0.6-3.2
		Public acute hospitals (reference)	1.0	
Ciprofloxacin	Age	≤23	1.1	0.8-1.5
		38-56	1.1	0.8-1.5
		57-73	2.5	1.9-3.4
		≥74	2.7	2.1-3.8
		24-37 (reference)	1.0	
	Gender	Female	2.4	1.9-3.0
		Male (reference)	1.0	
	SES	Middle	1.1	0.9-1.4
		High	1.3	1.0-1.6
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	1.3	0.8-2.2
		GP clinics	1.0	0.8-1.3

		After-hours GP clinics	1.1	0.6-2.1
		Community health services	1.8	1.1-3.0
		Specialist health services	1.5	0.7-3.2
		Others[#]	2.2	1.0-5.0
		Public acute hospitals (reference)	1.0	
Norfloxacin	Age	≤23	1.1	0.8-1.6
		38-56	1.1	0.8-1.6
		57-73	2.7	1.9-3.6
		≥74	2.9	2.1-3.9
		24-37 (reference)	1.0	
	Gender	Female	2.4	1.9-3.1
		Male (reference)	1.0	
	SES	Middle	1.1	0.9-1.4
		High	1.3	1.0-1.6
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	1.3	0.8-2.2
		GP clinics	1.0	0.8-1.3
		After-hours GP clinics	1.0	0.5-2.0
		Community health services	1.9	1.1-3.1
		Specialist health services	1.5	0.7-3.3
		Others[#]	2.3	1.0-5.2
		Public acute hospitals (reference)	1.0	
Gentamicin	Age	≤23	1.1	0.7-1.6
		38-56	1.3	0.9-1.9
		57-73	2.3	1.6-3.2
		≥74	3.0	2.1-4.2
		24-37 (reference)	1.0	
	Gender	Female	2.3	1.8-2.9
		Male (reference)	1.0	
	SES	Middle	1.1	0.8-1.4
		High	1.2	0.9-1.6
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	1.5	0.8-2.5
		GP clinics	1.1	0.8-1.4
		After-hours GP clinics	0.3	0.1-1.3
		Community health services	1.5	0.8-2.8
		Specialist health services	3.2	1.7-6.0
		Others[#]	1.5	0.5-4.6
		Public acute hospitals (reference)	1.0	
PIP	Age	≤23	1.5	0.9-2.4
		38-56	1.7	1.0-2.7
		57-73	3.7	2.4-5.7
		≥74	4.9	3.3-7.6
		24-37 (reference)	1.0	
	Gender	Female	2.4	1.9-3.2

		Male (reference)	1.0	
	SES	Middle	1.0	0.7-1.2
		High	0.9	0.6-1.2
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	0.8	0.4-1.7
		GP clinics	0.9	0.7-1.3
		After-hours GP clinics	1.8	0.9-3.5
		Community health services	1.6	0.8-3.0
		Specialist health services	2.7	1.3-5.4
		Others[#]	0.5	0.1-3.8
		Public acute hospitals (reference)	1.0	
Nitrofurantoin	Age	≤23	1.0	0.6-1.8
		38-56	1.8	1.2-2.9
		57-73	3.2	2.1-4.9
		≥74	4.7	3.1-7.0
		24-37 (reference)	1.0	
	Gender	Female	3.8	2.7-5.2
		Male (reference)	1.0	
	SES	Middle	0.9	0.7-1.2
		High	0.9	0.7-1.2
		Low (reference)	1.0	
	Sample origin	Private acute hospitals	1.5	0.9-2.7
		GP clinics	0.7	0.5-1.0
		After-hours GP clinics	2.6	1.5-4.6
		Community health services	1.3	0.6-2.6
		Specialist health services	2.0	0.9-4.6
		Others[#]	1.2	0.3-4.8
		Public acute hospitals (reference)	1.0	

*****A separate model was run for each antibiotic

AMC=Amoxycillin-clavulanate; TMP-SMX=Trimethoprim-sulphamethoxazole; PIP=Piperacillin-tazobactam

[#]non-acute hospitals, correctional services, dialysis clinics, dental clinics, hospice, ambulance services and a life insurance organisation

SES=Socioeconomic status; GP=General practice

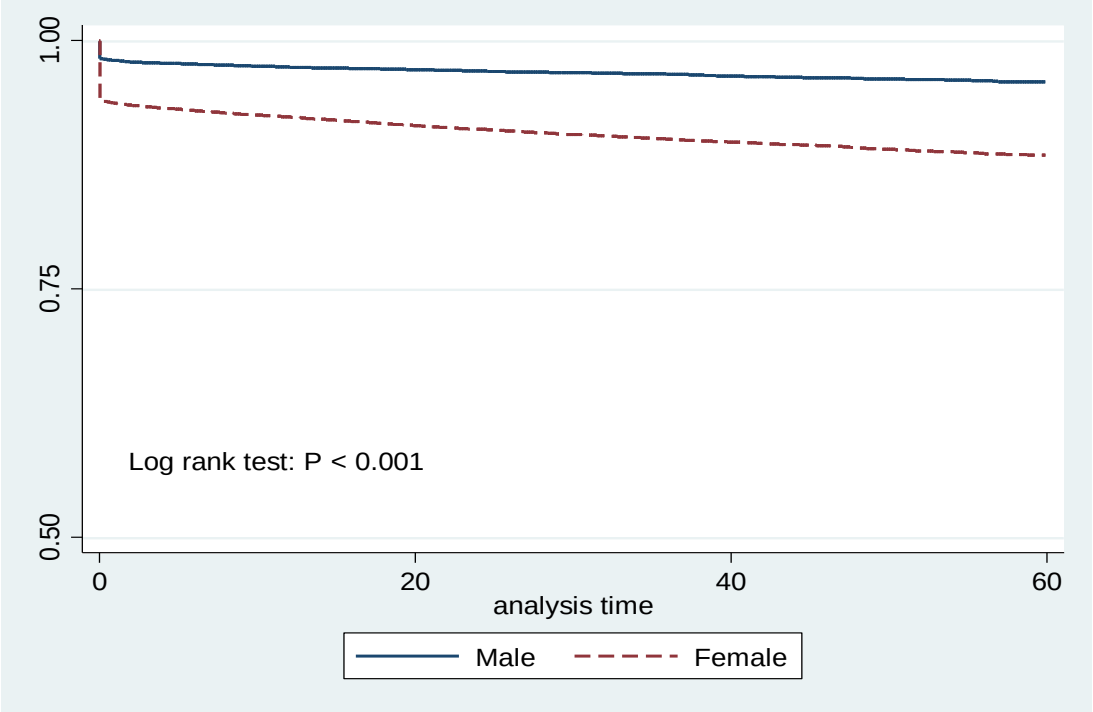

Figure (a) Kaplan-Meier curves of incidence of resistance to any antimicrobial agent by sex

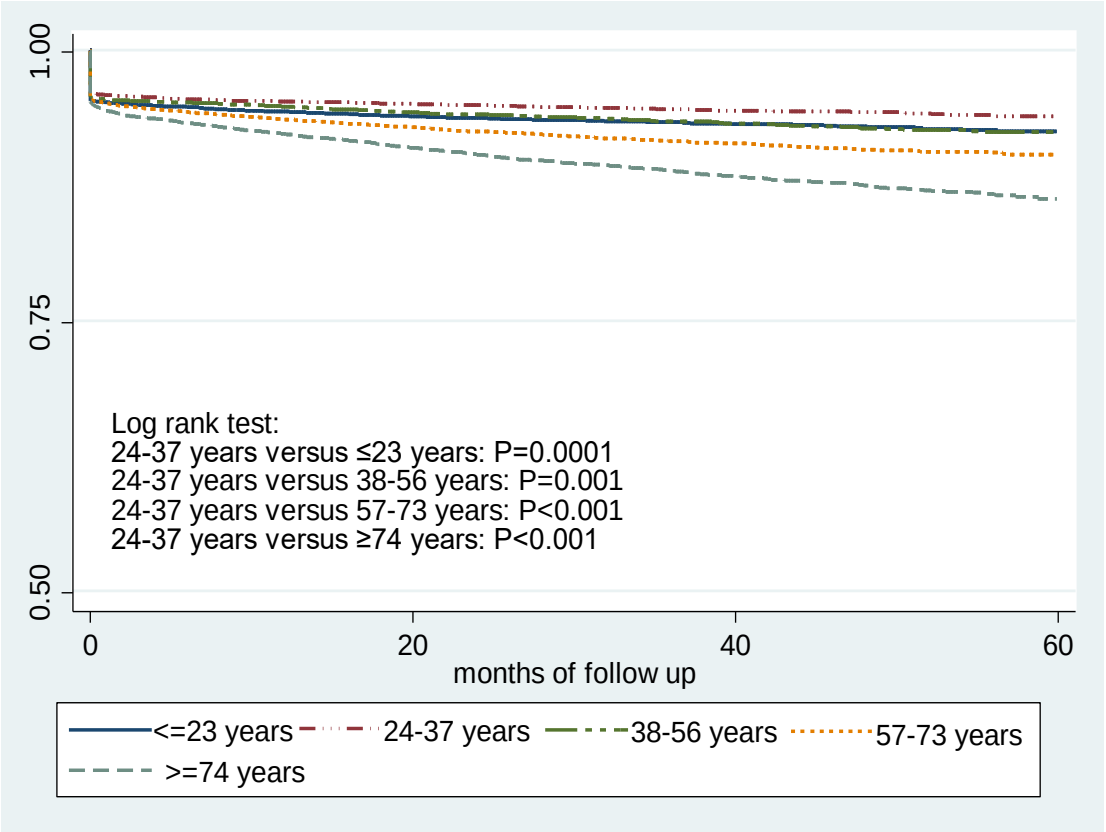

Figure (b) Kaplan-Meier curves of incidence of resistance to any antimicrobial agent by age group