Oral flora in acute stroke patients: A prospective exploratory observational study

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Oral flora in acute stroke patients: A prospective exploratory observational study

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Objective: To describe the bacterial profile of the oral flora during the first 2 weeks following a stroke, examining changes in the condition of the oral cavity and infections.

Background: Dysphagia is common after a stroke and can lead to aspiration pneumonia. Oral flora changes associated with stroke have been implicated as a possible source of bacteria that can cause systemic infections.

Materials and methods: Seventy-seven participants were recruited over a period of 9 months. Fifty participants had a complete set of swabs from four different oral sites and a saliva sample taken at three time points over a 14-day period. Molecular identification of bacteria was performed on the pooled DNA extracted.

Results: A total of 103 bacterial phylotypes were identified, 29 of which were not in the Human Oral Microbiome Database (HOMD). Fourteen of the twenty most common bacterial phylotypes found in the oral cavity were Streptococcal species with *Streptococcus salivarius* being the most common. The condition of the oral cavity worsened during the study period. Fifteen (30%) patients had at least one infection.

Conclusions: There appears to be huge diversity of bacterial organisms in the oral cavity of stroke patients, and as most phylotypes identified were only found in one or two participants, no particular patterns linked to infection or the condition of the oral cavity could be discerned.

KEYWORDS
infection, oral care, oral hygiene, stroke

1 | INTRODUCTION

Stroke is the second most common cause of death and of adult disability worldwide.[1,2] In the UK, the overall number of strokes reported in 2013/14 was 233 261 with around 39 000 associated deaths including about 7500 premature deaths before the age of 75.[3]

Strokes account for about 1.1% of all hospital episodes and are a leading cause of moderate-to-severe disability.[3-5] Strokes place a significant burden on services costing the NHS about £2.8 billion a year in direct care costs with an additional £2.4 billion for informal care provided by patients' families.[6]

Eating, drinking and brushing teeth all contribute to the maintenance of a normal, balanced oral flora and good oral health.[7] After a stroke, dehydration is common and when coupled with changes in these activities can lead to disruption in the oral ecology and an overgrowth of pathogenic bacteria resulting in periodontal disease, abrasions, caries and systemic infections.[8-11] The cognitive and physical impairments that often occur after a stroke, combined with the disruption associated with being hospitalised, can make it difficult for stroke patients to attend to their own oral hygiene effectively.[12-14]

Cognitive impairment can lead to difficulties processing information and neglect, as well as an inability to recognise the need for oral care. Common stroke sequelae such as hemiplegia may weaken limb movement and motor control. Facial muscle weakness, a reduction in oral sensation and difficulties chewing, swallowing and coughing may also increase the risk of aspiration and precipitate pneumonia.[15,16]

Whilst not specific to stroke patients, a systematic review of 36 studies concluded that oral colonisation by respiratory pathogens, fostered by poor oral hygiene and periodontal disease, appears to be associated with the development of hospital-acquired pneumonia.[17]

In the first few weeks after a stroke, patients are at increased risk of acquiring a respiratory infection and those with higher levels of dependency have a greater risk of infection.[18,19] About 10% of patients develop pneumonia following a stroke, and this is significantly associated with increased risk of death (odds ratio 3.62 95% CI 2.80-4.68).[20]

Compared with healthy people, medically compromised patients such as those who have recently had a stroke tend to harbour a greater number of aerobic and facultative anaerobic gram-negative bacilli in their oral cavity, which appears to increase their risk of systemic infections.[21] In one study, aerobic gram-negative bacilli were found in 34% of acute stroke patients but were not found at all in healthy volunteers.[22] The same study found that presence of aerobic gram-negative bacilli was associated with an unsafe swallow and increased risk of mortality.[22] Sedgley found that potentially pathogenic Enterobacteriaceae such as *Escherichia coli* are frequently carried in the oral cavity and hospitalisation appears to create conditions that favour oropharyngeal colonisation.[23]

Little is known about the condition of the oral cavity, the oral flora and associated risk of infections in stroke patients. Advances in gene-sequencing technology have improved our ability to explore and understand the oral flora and have revealed a high level of diversity with about 10,000 microbial phylotypes identified in the human oral microflora in 2008.[24] This is considerably more than the 700 oral microbial phylotypes previously identified in 2000.[25]

The primary purpose of this exploratory observational study was to apply molecular barcoding technology to describe the bacterial profile of the oral cavity during the first 2 weeks following a stroke. A secondary goal was to explore changes in the condition of the oral cavity and associated clinical factors such as infection.

2 | MATERIALS AND METHODS

The study was undertaken in an acute stroke unit in a hospital in the north-west of England. Consecutively admitted patients to the stroke unit were recruited. Inclusion criteria were that patients had to be recruited within 48 hours of admission; were 18 years or older; and with a clinical diagnosis of acute stroke. Patients on an end-of-life pathway were excluded. Patients were screened for eligibility by the nursing staff and provided with an information sheet, and those wishing to participate gave written consent. A written consultee declaration was obtained for patients who lacked capacity, and witness consent was obtained for patients who had communication difficulties. All recruited patients were allocated a study number.

The study was approved by the National Research Ethics Service—Greater Manchester South Ethics Committee (Reference number 12/NW/0268); and by the NHS Trust's Research and Development Department.

Data were collected by one of five researchers, trained in all study procedures, at three time points: time point 1, <48 hours post-admission; time point 2, 48-72 hours after time point 1; and time point 3, 7-14 days post-admission.

The following data were collected from the case notes at time point 1: age; sex; previous stroke; dental state [edentulous, partially dentate or fully dentate]; smoking habits; side of stroke; stroke classification [total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), lacunar infarct (LACI), posterior circulation infarct (POCI)],[26] type of stroke [ischaemic or intracerebral haemorrhage]; functional dependence using the Barthel Index of Activities of Daily Living,[27] dependence for oral care; and swallowing difficulties. The condition of the oral cavity was assessed using The Holistic and Reliable Oral Assessment Tool (THROAT)[28] [a seven-item tool, which rates the condition of lips, teeth, gums, cheek, palate, tongue and saliva according to specified criteria; each item is scored: 0=normal; 1=mild problems; 2=moderate problems; 3=severe problems]. Each assessment was made blind to any previous ratings. The THROAT score for each item at each of the three time points was recorded. Although the THROAT is an ordinal scale, means for each item and time point were calculated so that differences between items and across time points could be explored.

Data regarding infection and antibiotic use were recorded from the case notes. Participants were considered to have a community-acquired infection if they presented with, or developed clinical signs and symptoms of infection up to 48 hours from admission. Infection was considered as hospital-acquired if participants presented with clinical signs and symptoms of infection more than 48 hours post-admission. Infections were defined as per local hospital policies. Fungal infection in the oral cavity was identified from clinical description in the medical notes or if oral antifungal medication had been prescribed. Lower respiratory infection was identified through the presence of clinical signs and symptoms, such as productive cough, crackles on inspiration and either leucocytosis, temperature above 38°C or a positive radiograph. Urinary tract infection was identified through the presence of clinical signs and symptoms, such as dysuria and increased urinary frequency, temperature above 38°C, positive microbiological cultures or negative cultures with leucocytosis. An acute inflammation of the skin indicated cellulitis.[29] Sepsis was identified through the presence of a serious systemic infection.[30]

Age data were not normally distributed, so they were reported using medians and interquartile ranges. The Barthel Index was reported according to categories: 0-10, dependent; 11-17, moderate dependency; and 18-20, independent. Descriptive statistics were used to depict the distribution of the different bacterial species across time points.

A saliva sample and swabs from four different sites within the oral cavity were taken: the buccal mucosa, tongue, gingiva and hard palate. All samples were taken with plain plastic Sterilin swabs (Fisher Scientific UK Limited, F155CA). Each swab was labelled with the study number, swab site, time point and date. All swabs were transported in a cool box at 4°C to a biomedical research facility. On arrival, all specimens were checked for proof of receipt, logged electronically and then immediately stored at −80°C prior to subsequent analysis.

Laboratory-based molecular identification of bacteria was performed following genomic DNA isolation from all swabs and saliva samples in 20 mg mL^{-1} lysozyme in buffer (20 mmol L^{-1} Tris-HCl pH 8.0, 2 mmol L^{-1} EDTA, 1.2% Triton X-100). A Qiagen DNA easy blood & tissue kit 69504 was used (as per the manufacturer's instructions) to isolate and purify DNA. The purified DNA pellets were resuspended in 50 μL of AE buffer (from kit) and quantified using a Nanodrop spectrophotometer. Genomic DNA was pooled and then subjected to amplification as previously described by Paster et al.[31] using the universal 16S rRNA bacterial gene primers D88F and E94R. The amplification product was cleaned and cloned using the TA TOPO cloning kit (Invitrogen). Colonies were chosen and screened for having taken up the DNA of interest. Plasmid DNA was isolated from the colonies. Assembly of the data was carried out using Geneious Pro v6.1.5 (www.geneious.com). The sequences were submitted to the European Nucleotide Archive (ebi.ac.uk), and only those with more than 200 bases and 98%-100% alignment were considered sufficiently similar to confidently identify the phylotype.[32]

A phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis[33] software and the maximum likelihood method based on the Tamura-Nei model.[34] The phylogenetic tree with the highest log likelihood was obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log likelihood value. The analysis involved 95 nucleotide sequences. Codon positions included were 1st+2nd+3rd+non-coding. All positions with less than 95% site coverage were removed.

Associations between the phylotypes and various patient characteristics were explored using chi-squared test and *t* test as appropriate. All analyses were performed using Statistical Package for the Social Sciences software (SPSS) version 22.

3 | RESULTS

A total of 937 patients were admitted between July 2012 and April 2013, and of these, 390 had an initial clinical diagnosis of acute stroke. Seventy-seven (20%) participants were recruited into the study, and a complete data set was obtained for 50 participants (Figure 1).

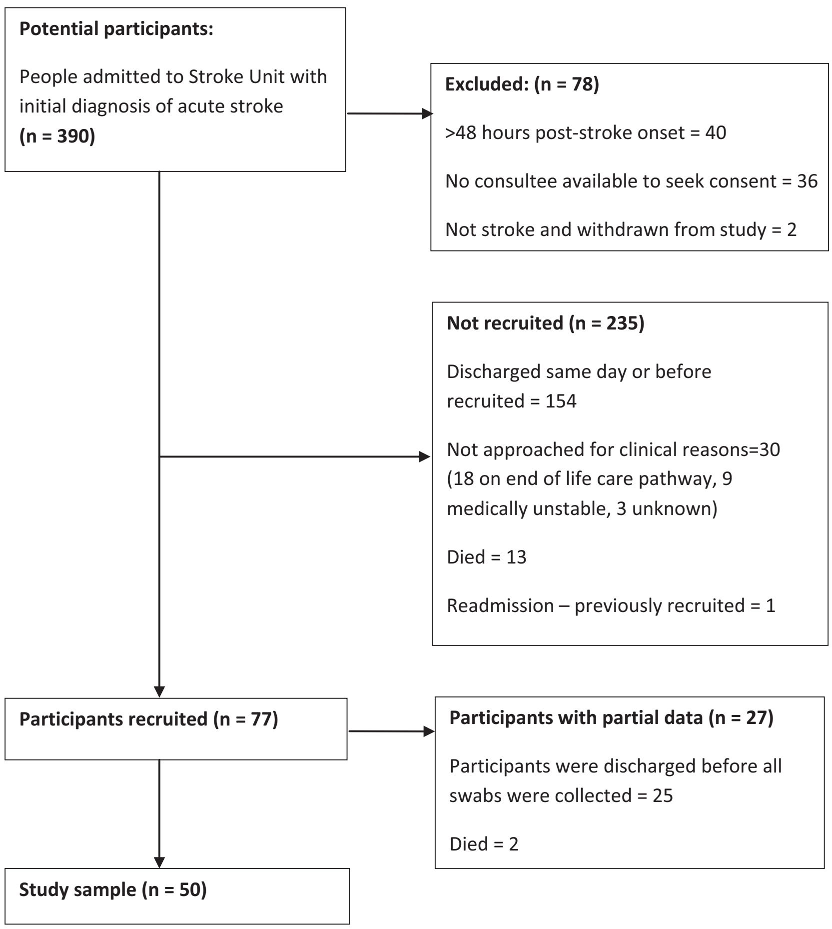

FIGURE 1 Recruitment flow chart of participants

On average, participants who provided complete data and were included in the full study sample were older and significantly more likely to be dependent and have swallowing difficulties than those who provided partial data (Table 1).

A total of 103 different bacterial phylotypes were identified with 98%-100% sequence similarity cut-off for defining a phylotype. The phylogenetic tree with the highest log likelihood (−51127.6140) is shown in Figure 2.

The Human Oral Microbiome Database (HOMD) was used as the benchmark for expected phylotypes present in the human oral cavity.[35] Twenty-nine bacterial phylotypes that were not mentioned in the HOMD were recovered. The number of participants found with these

TABLE 1 Participant characteristics

Demographics	Participants with full data (n=50)	Participants with partial data (n=27)	Participants recruited (n=77)
Median age [years] (Interquartile range)	80.5 (65-86)	75 (62-80)	76 (65-84.5)
Male sex	24 (48%)	17 (63%)	41 (53%)
Previous stroke	10 (20%)	2 (7%)	12 (16%)
Dentate	13 (26%)	9 (33%)	22 (29%)
Partial dentate	21 (42%)	7 (26%)	28 (36%)
Dentures	16 (32%)	11 (41%)	27 (35%)
Smoker	10 (20%)	5 (19%)	15 (20%)
Ex-smoker	13 (26%)	10 (37%)	23 (30%)
Non-smoker	25 (50%)	12 (44%)	37 (48%)
Not documented	2 (4%)	0	2 (2%)
Side affected by stroke			
Right	23 (46%)	9 (33%)	32 (42%)
Left	22 (44%)	12 (44%)	34 (44%)
Both	0	1 (4%)	1 (1%)
Neither	5 (10%)	5 (19%)	10 (13%)
Stroke classification			
TACI	14 (28%)	5 (19%)	19 (25%)
PACI	28 (56%)	11 (40%)	39 (51%)
LACI	5 (10%)	6 (22%)	11 (14%)
POCI	3 (6%)	5 (19%)	8 (10%)
Unconscious	1 (2%)	0	1 (2%)
Type of stroke			
Ischaemic	46 (92%)	25 (93%)	71 (92%)
Haemorrhagic	4 (8%)	2 (7%)	6 (8%)
Barthel Index			
0-10 dependent*	33 (66%)	6 (22%)	39 (50%)
11-17 moderate dependency	11 (22%)	8 (30%)	19 (25%)
18-20 independent*	6 (12%)	13 (48%)	19 (25%)
Oral care			
Independent oral care*	17 (34%)	20 (74%)	37 (48%)
Dependent for oral care*	33 (66%)	7 (26%)	40 (52%)
Swallowing			
Nil by Mouth	13 (26%)	3 (11%)	16 (21%)
Modified diet*	13 (26%)	1 (4%)	14 (18%)
Full oral intake*	24 (48%)	23 (85%)	47 (61%)

*indicates statistically significant difference (chi-square $P<.05$) between the group of participants with full data and the group with partial data.

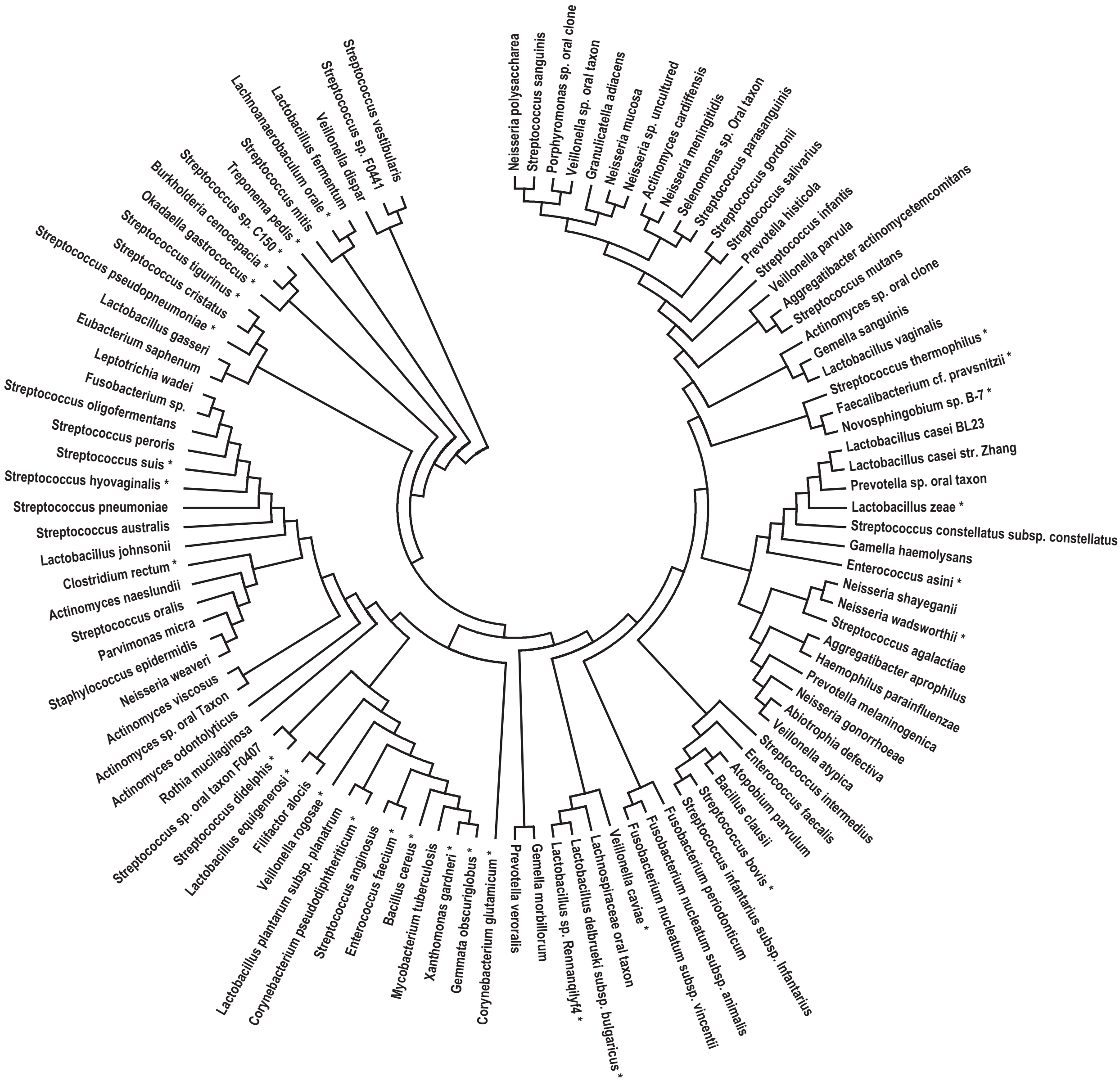

FIGURE 2 The Phylogenetic tree of the bacterial species found in the study sample

bacterial phylotypes in their oral flora at each time point is given in Table 2.

Of the bacterial species found, 67 (65%) were gram-positive, 34 (33%) were gram-negative and two (2%) were gram-variable.

The proportion of participants with a gram-negative phylotype found in the oral cavity was 29.4%, 37.3% and 33.3% at time points 1, 2 and 3, respectively, and 60.8% of participants were harbouring at least one gram-negative phylotype at one or other time point.

Aerobic gram-negative bacteria were found in three participants at time point 1, an additional two participants at time point 2 and two more participants at time point 3. In total, seven participants (14%) were harbouring at least one aerobic gram-negative phylotype at one or other time point.

Figure 3 shows that 14 of the 20 most common bacterial phylotypes found in the oral cavity were Streptococcal species. Table 3 indicates that *Streptococcus salivarius* was the most commonly discovered phylotype, found in 36% of participants. Among the 20 most common bacterial phylotypes were also three different gram-negative Veillonella species, gram-positive *Rothia mucilaginosa*, gram-negative *Treponema pedis* and gram-positive *Lactobacillus fermentum*.

The average number of different bacterial phylotypes found in each participant was 2.72, 2.76 and 2.32 at time points 1, 2 and 3, respectively, and ranged from 0 to 11. There was no significant difference in the average number of different phylotypes found across all three time points. The relative ranking of each phylotypes remained fairly consistent over all three time points.

The mean THROAT scores (all items) at each time point are shown in Figure 4. The mean score at time point 2 was slightly lower than at time point 1, whilst the score at time point 3 was higher than at both previous time points.

TABLE 2 Occurrence of bacterial phylotypes not found in HOMD

Bacterial phylotypes		Number of participants			
		TP one	TP two	TP three	Total number of participants
1	*Bacillus cereus*	0	0	1	1
2	*Burkholderia cenocepacia*	0	0	2	2
3	*Clostridium rectum*	2	0	0	2
4	*Corynebacterium glutamicum*	0	0	1	2
5	*Corynebacterium pseudodiphtheriticum*	1	0	1	2
6	*Enterococcus asini*	0	2	2	4
7	*Enterococcus faecium*	0	1	1	2
8	*Faecalibacterium* cf. prausnitzii	0	2	0	2
9	*Gemmata obscuriglobus*	0	0	1	1
10	*Lachnoanaerobaculum orale*	1	0	0	1
11	*Lactobacillus delbrueckii* subspecies *bulgaricus*	0	0	1	1
12	*Lactobacillus equigenerosi*	0	1	0	1
13	*Lactobacillus zeae*	0	0	1	1
14	*Lactobacillus* sp. rennanqilyf4	0	0	1	1
15	*Neisseria wadsworthii*	1	0	0	1
16	Novosphingobium sp. B-7	0	1	0	1
17	*Okadaella gastrococcus*	1	0	0	1
18	*Streptococcus bovis*	0	0	2	2
19	*Streptococcus didelphis*	1	0	0	1
20	*Streptococcus hyovaginalis*	1	0	0	1
21	*Streptococcus pseudopneumoniae*	1	2	2	4
22	*Streptococcus* sp. C150	0	1	0	1
23	*Streptococcus suis*	3	3	4	9
24	*Streptococcus thermophilus*		3		3
25	*Streptococcus tigurinus*	0	1	0	1
26	*Treponema pedis*	1	4	3	7
27	*Veillonella caviae*	0	0	1	1
28	*Veillonella rogosae*	0	0	2	2
29	*Xanthomonas gardneri*	0	0	1	1

Shading indicates the relative frequency of the phylotype found at each time point (TP).

Average THROAT scores, where each area of the oral cavity is scored 0=normal; 1=mild problems; 2=moderate problems; 3=severe problems, are shown in Figure 5. The proportion of participants in each THROAT category for each area of the oral cavity is presented. A comparatively high proportion of participants had severe (40%, 32%, 40%) or moderately severe (38%, 14%, 20%) problems relating to their teeth over time points 1-3, respectively. Fewer participants had severe (2%, 14%, 10%) but a greater proportion had moderately severe (40%, 34%, 58%) problems with their tongue over time points 1=3, respectively. A considerable proportion of participants had severe (22%, 16%, 34%) or moderately severe (8%, 6%, 10%) problems associated with their saliva over time points 1-3, respectively. A large proportion of participants had mild (66%, 48%, 62%) problems with their lips over time points 1-3, respectively, but very few had moderate or severe problems in this area.

There were 32 participants (64%) taking medication associated with increased risk of xerostomia on admission. This reduced to 27 (54%) at time points 2 and 3.

The mean scores for the various items included in the THROAT oral cavity assessment are presented in Figure 6. In all areas of the oral cavity, except for the teeth, participants appeared to experience greater problems at time point 3 than at time point 1. For the teeth, gums, cheeks and saliva, fewer problems were evident at time point 2 than at time point 1 or 3.

There was a significant relationship between the dependency as indicated by the Barthel Index and THROAT scores at time points 1

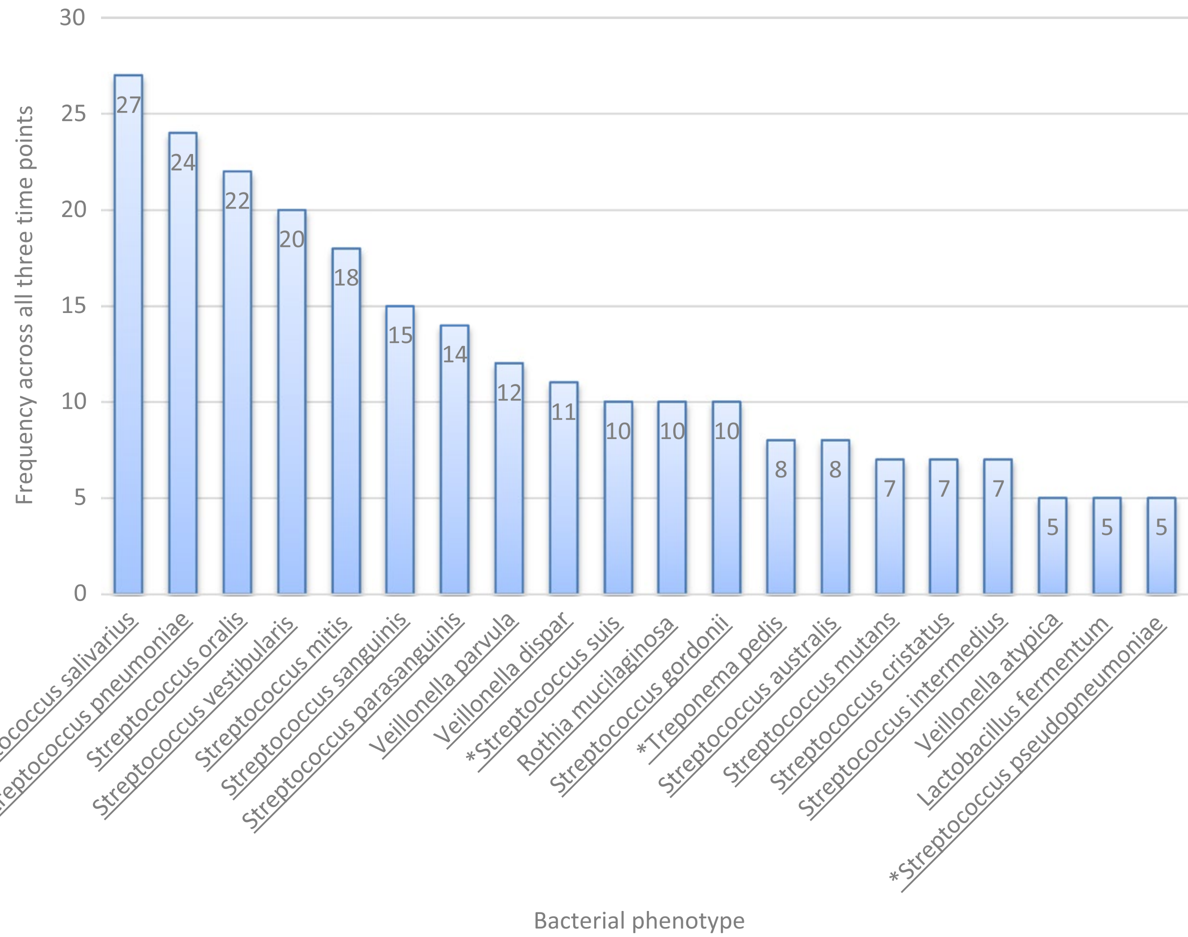

FIGURE 3 The twenty most common bacterial phenotypes found in the oral cavity [Colour figure can be viewed at wileyonlinelibrary.com]

and 3 (Spearman's correlation coefficient=−.437 and −.499, respectively, $P<.01$ at both time points).

A total of 15 (30%) participants had a documented clinical infection during the first 2 weeks of admission to the acute stroke unit. In total, nine participants were diagnosed with a respiratory infection, three had an oral infection, two had cellulitis, three had a urinary tract infection and one had septicaemia.

Seven participants were admitted with a community-acquired infection, one of whom had two infections.

Nine participants developed a hospital-acquired infection, and one of these had two infections. Four participants acquired an infection by time point 2, one was an oral fungal infection, another a respiratory infection, a third developed a urinary tract infection and a fourth developed cellulitis. Six participants acquired an infection by time point 3, which included respiratory infections (n=5) and a urinary tract infection (n=1) (Figure 7). No statistically significant associations could be found between those who developed an infection (whether community- or hospital-acquired) and a range of participant characteristics, including dental status and the presence or absence of specific bacterial phylotypes.

There were 18 clinical infections diagnosed, and in eight of these, the infection can be considered community-acquired as it was documented within the first 48 hours after admission. The remaining ten infections developed over the subsequent 2 weeks in hospital.

Table 4 shows the type of infection that was present in the participants with community- and hospital-acquired infections, and the bacterial phylotypes identified in their oral cavities at each of the three time points.

4 | DISCUSSION

The bacterial profile of the oral cavity during the first 2 weeks following a stroke was successfully described using TA TOPO cloning. There were 103 different bacterial phylotypes identified, including 29 not previously found by HOMD.[35]

The phylotypes found in the most common twenty, other than *Treponema pedis* and *Streptococcus suis*, are considered normal commensals of the oral cavity.[8,36-38]

The seven phylotypes found most frequently in the oral cavity were all Streptococcal species, with *S. salivarius* in 36% of the full study population discovered most often. *S. salivarius* can become pathogenic, but this is very rare and some strains are promoted as a probiotic, as they produce bacteriocins that inhibit other bacteria and

TABLE 3 Twenty most common bacterial phylotypes found in the oral cavity at each time point (TP)

Bacterial phenotypes	Rank overall	Rank at TP1	Rank at TP2	Rank at TP3
Streptococcus salivarius	1	2	1	1
Streptococcus pneumoniae	2	3	3	1
Streptococcus oralis	3	3	1	4
Streptococcus vestibularis	4	7	4	3
Streptococcus mitis	5	3	4	4
Streptococcus sanguinis	6	3	9	4
Streptococcus parasanguinis	7	1	13	13
Veillonella parvula	8	7	9	13
Veillonella dispar	9	9	6	20
[a]*Streptococcus suis*	10	12	13	4
Rothia mucilaginosa	10	9	18	8
Streptococcus gordonii	10	11	6	20
[a]*Treponema pedis*	13	22	9	8
Streptococcus australis	14	12	13	13
Streptococcus mutans	15	16	18	8
Streptococcus cristatus	15	16	18	8
Streptococcus intermedius	15	22	6	20
Veillonella atypica	18	0	18	8
Lactobacillus fermentum	19	16	23	13
[a]*Streptococcus pseudopneumoniae*	19	22	13	20

[a]Phylotypes not found in HOMD.

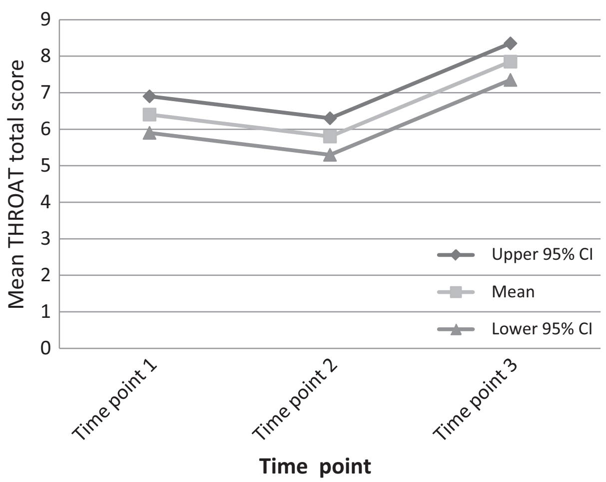

FIGURE 4 Mean THROAT scores with 95% confidence intervals (CI) over three time points

foster a healthy, balanced oral flora.[39-41] Participants who had *S. salivarius* in their oral cavity had greater diversity and significantly more phylotypes found across all three time points (average of 10.78 compared to 5.25, $P<.001$) than those without this bacterium. They also experienced fewer systemic infections, indicating a possible protective effect, but this was not statistically significant.

The second most common phylotype found in the oral cavity was *Streptococcus pneumoniae*. Although this is considered part of the normal oral flora, it can become pathogenic and in the literature is associated with the development of pneumonia.[42] However, no association between the presence of *S. pneumoniae* and the development of pneumonia was found in this study.

The tenth most commonly found phylotype in this study, and one not in HOMD, was *Streptococcus suis*. This bacterium is part of the normal oral flora in pigs and a recognised pig pathogen but is known to cause infections in people working with pigs.[43] Transmission in pigs is mainly through the respiratory route, and since it was first described in Denmark in 1968,[44] over 1600 human cases of *S. suis* infection have been reported.[43] Most sporadic cases of human infections appear to be due to occupational contact with pigs or pork products, but the number of human infections is increasing and two epidemics were reported in China in 1998 and in 2005.[45] *S. suis* is the most common cause of human meningitis in Vietnam and has been linked to the development of pneumonia in many parts of the world.[46]

Treponema pedis was not in HOMD, but was found eight times in six participants, making it the thirteenth most common phylotype found in this study. *T. pedis* is an anaerobic gram-negative bacterium associated with digital dermatitis in pigs, but it is very close phylogenetically to *T. denticola*, part of the known human oral flora associated with periodontitis.[47] To the best of our knowledge, *T. pedis* has not previously been found in the human oral flora.

Streptococcus pseudopneumoniae was also not in HOMD but has previously been isolated in people with gingivitis[48] and in this study was found comparatively frequently on five separate occasions in four

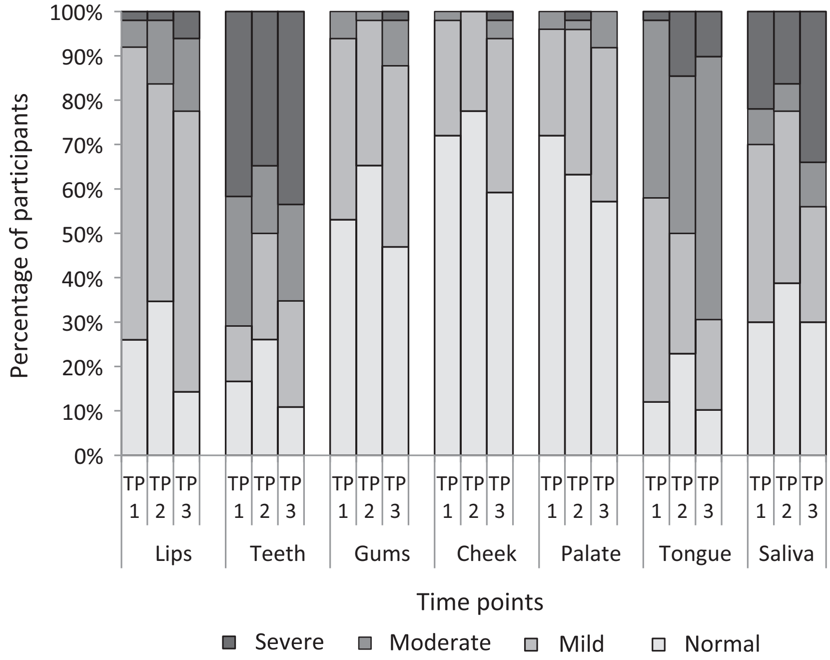

FIGURE 5 Condition of the oral cavity based on THROAT assessment over three time points (TP)

different participants. *S. pseudopneumoniae* is phylogenetically related to *S. pneumoniae* and *S. mitis* and appears to be a respiratory tract coloniser, with the potential to become pathogenic.[49] It is associated with chronic obstructive pulmonary disease and respiratory tract infections, particularly aspiration pneumonia.[49-51]

No associations between any particular phylotype and infection were found. On average, participants with an infection had fewer different phylotypes in the oral cavity compared with those without any infection recorded, but this was not statistically significant and possibly linked to antibiotic use. It may be that the balance of organisms is as important for containing risk of aspiration pneumonia and other infections as the presence or absence of any particular species or group of bacteria.

The condition of the oral cavity deteriorated in the first 2 weeks following admission to the acute stroke unit. The condition of the palate and the tongue indicated by the THROAT score progressively worsened over time. In all other areas of the oral cavity, THROAT scores improved at time point 2 compared with time point 1, but then worsened at time point 3 compared with time point 1 or 2. Participants with higher levels of dependency had significantly worse THROAT scores, suggesting that by 2 weeks after admission, oral care did not meet their needs. The worsening THROAT score could also be associated with dehydration that is common after a stroke.[52]

There did not appear to be any association between the condition of the oral cavity as indicated by the THROAT score and systemic infection. The mean overall THROAT score across all areas for participants with an infection (7.7) was higher than for those without an infection (6.1), but the difference was not statistically significant.

Pressure on staffing levels and an inability to recruit at weekends limited the researchers' ability to recruit consecutively admitted patients, and full data could only be obtained from participants who stayed on the unit for at least 2 weeks. A consequence of this was that participants included in this study were older, were more dependent and had more problems than the wider cohort of stroke patients originally recruited.

Another limitation was that this study adopted the partial community DNA analysis employing cloning of PCR product of interest, followed by characterising cloned sequences for assessment of the genetic diversity in the number of different taxa present. This meant that not every bacterial organism present within the clinical sample was identified and the relative abundance of the different species of bacteria in the given sample could not be measured.

The authors also acknowledge the limitation in the lack of controls from non-stroke cases which makes interpretation of the results more difficult. However, a similar approach has been taken in several other studies.[22,53-55] Criteria for determining the nature of an appropriate control group are problematic. Patients recently admitted to a medical ward with a condition other than stroke would allow the effect of hospitalisation to be taken into account, but there may be important differences in a range of risk factors and baseline characteristics between stroke survivors and other medical patients that could create bias. A control group drawn from healthy elderly people in the community who have not had a stroke could provide a pre-stroke comparator, but again is not ideal for similar reasons and because it would not allow for the effects of hospitalisation to be taken into account.

All participants who were diagnosed with an infection were given antibiotics or antifungal medication, so it was impossible to control for this as the potential impact of these two factors on the condition of the oral cavity and the oral flora could not be separated.

Although swabs were taken from different regions of the oral cavity, the contents were combined and the oral flora in each specific area was not identified.

These results concur with a study conducted in Hong Kong which found that periodontal health as measured by plaque and

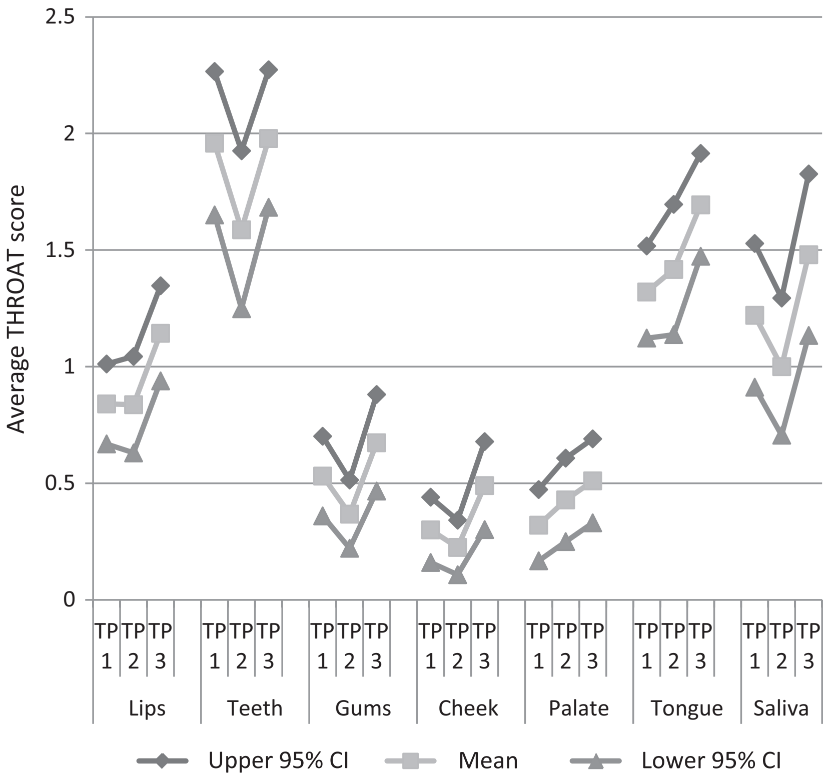

FIGURE 6 Mean THROAT scores with 95% confidence intervals (CI) over the three time points (TP)

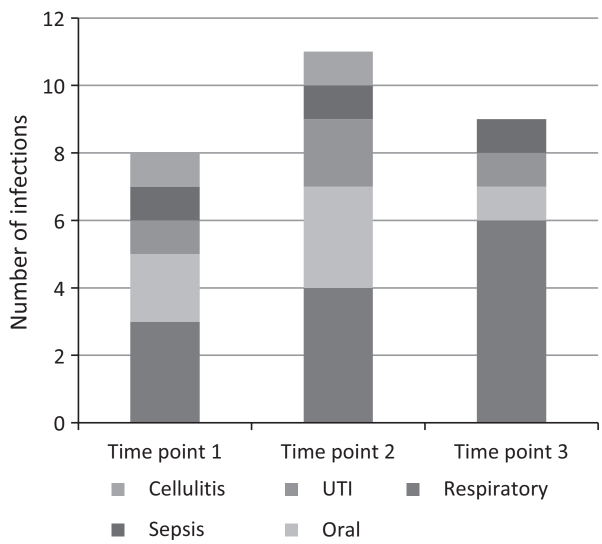

FIGURE 7 Number of infections at each time point

bleeding scores was worse in a group of elderly people who had experienced a mild-to-moderate stroke compared with a comparative group of community-dwelling elderly people who had not had a stroke.[56] The authors of the Hong Kong study attributed this to the protracted reduction in hand, arm and oral sensori-motor function experienced by people who have had a stroke. However, periodontal disease and stroke share a considerable number of risk factors with the inflammatory processes associated with the former increasing risk of the latter.[57,58] As the oral health status of stroke survivors was not measured before admission, this outcome could be attributed to the possibility that people who suffer a stroke have comparatively worse oral health than an equivalent group in the community.[59]

A limited number of phylotypes were found in the oral cavity in this study compared with those recovered in other similar studies.[8,35] Aas[8] took samples from nine different sites and found the greatest diversity of phylotypes in samples taken from the tonsils, tooth surface and the subgingival area, which were facets of the oral cavity not sampled in this study. We used TA TOPO cloning, which appears to be relatively restrictive in revealing the diversity of bacteria and a 98% sequence similarity as the cut-off for defining our phylotypes. It is possible that Aas[8] used a different technique and a lower threshold as it was not mentioned in their paper.

The aerobic gram-negative bacterial carriage rates identified in this study were lower than those found through more traditional culture techniques described in previous studies.[22,60] In these earlier studies, *E. coli* was one of the most common aerobic gram-negative bacteria found in the oral cavity after a stroke.[22,60] The TA TOPO cloning kit employed in this study uses the chemically treated *E. coli* DH5-α strain to take up the cloned vector which means that any sequencing data that identify *E. coli* are excluded. It might be worth considering using a mixture of methods including high-throughput sequencing to mitigate this limitation, so that this group of bacteria are not excluded in future studies.

Oral care following a stroke is difficult, and dehydration is a common complication that can exacerbate oral problems.[11] There is limited specialised care, inadequate knowledge among nurses and few established protocols for the oral health care of patients who have had a stroke.[61]

TABLE 4 Participants with an infection and bacterial phylotypes found in their oral cavity

Participant	Infection	Phylotypes found: Time point 1	Time point 2	Time point 3
1	Oral fungal infection (TP1) Septicaemia (TP1)	*S. oligofermentans* *S. parasanguinis* *S. salivarius* *S. suis* *S. vestibularis* *Rothia mucilaginosa* *Veillonella dispar*	[a]*Treponema pedis* *S. gordonii* *S. salivarius* *S. vestibularis*	*S. infantis* *S. mitis* *S. salivarius* *S. vestibularis*
2	Oral fungal infection (TP1)	None	*Enterococcus faecalis* *Haemophilus parainfluenzae*	*Haemophilus parainfluenzae*
3	Oral fungal infection (TP2) Respiratory infection (TP3)	*S. gordonii* *S. parasanguinis* *S. pneumoniae*	*Staph. epidermidis* *S. intermedius* *S. mitis* *S. parasanguinis* *S. pneumoniae* *S. salivarius* *S. vestibularis*	[a]*S. pseudopneumoniae* *Veillonella parvula*
4	Respiratory infection (TP1)	*Lachnospiraceae* oral taxon *Fusobacterium nucleatum* subsp. *animalis* *Fusobacterium nucleatum* subsp. *vincentii* *Fusobacterium peridonticum*	*Veillonella parvula*	[a]*Prevotella* species oral taxon *S. agalactiae* *S. australis* *S. infantarius* subsp. *infantarius* *S. suis* *Neisseria meningitides* *Neisseria mucosa*
5	Respiratory infection (TP1)	*Enterococcus faecalis*	*Bacillus clausii*	None
6	Respiratory infection (TP1)	*Lactobacillus gasseri* *Lactobacillus johnsonii*	None	None
7	Respiratory infection (TP2)	None	*S. pneumoniae*	*S. pneumoniae*
8	Respiratory infection (TP3)	*Lactobacillus fermentum* *Lactobacillus gasseri*	*Lactobacillus gasseri* *Lactobacillus johnsonii* *S. agalactiae* *S. australis* *S. infantarius* subsp. *infantarius* *Veillonella atypical* *Veillonella dispar* *Veillonella* oral taxon	[a]*Veillonella rogosae*
9	Respiratory infection (TP3)	*Veillonella parvula*	None	None
10	Respiratory infection (TP3) Urinary tract infection (TP1)	*Rothia mucilaginosa*	*S. pneumoniae*	None
11	Respiratory infection (TP3)	None	None	None
12	Urinary tract infection (TP2)	*S. parasanguinis*	None	None
13	Urinary tract infection (TP3)	*S.* species F0441	*S. pneumoniae*	*Lactobacillus fermentum* *Lactobacillus* sp. *rennanqilyf4*
14	Cellulitis (left forearm) (TP2)	*Neisseria* species uncultured *S. oralis* *S. sanguinis* *Gemella sanguinis* *Veillonella parvula*	*Abiotrophia defectiva*	*Staph. epidermidis*

(Continues)

TABLE 4 Continued

Participant	Infection	Phylotypes found		
		Time point 1	Time point 2	Time point 3
15	Cellulitis (right leg) (TP1)	None	*Lactobacillus casei* *Lactobacillus vaginalis*	[a]*Lactobacillus delbrueckii* subspecies *bulgaricus* *Lactobacillus casei* str. Zhang *Lactobacillus zeae* *Bacillus clausii*

[a]Phylotypes not found in HOMD; a shaded cell shows the time point at which the infection was first identified.

Guidelines for the oral health care of stroke survivors recommend the use of mechanical methods of oral care for stroke patients.[62-64] Antimicrobial paste appears to reduce the risk of aspiration pneumonia following a stroke, but does not have any impact on mortality.[60]

Training health workers on how to use the THROAT effectively may help identify stroke patients with oral health problems who need greater care and attention with their oral hygiene.

There is a need for improved research about how best to maintain oral health in patients who have had a stroke including the benefits of maintaining a good diversity of bacteria in the oral cavity.

It is not known whether the discovery of 29 bacterial phylotypes that were not in the HOMD is due to the different techniques employed to identify phylotypes or the incomplete nature of the HOMD, or whether the oral flora is altered either before or immediately following a stroke and this has not been captured in the HOMD. However, this study provides a baseline that will be useful for future research in this area.

Differences in the phylotypes found between this and other studies could be due to variations in cloning, number of colonies analysed and amplification and sequencing primers used. Should any further work be undertaken in this area, it would be worth amending protocols so that they are better able to identify a greater variety of phylotypes.

This description of the bacteria found in the oral cavity adds to our knowledge and understanding of the oral flora and how it changes in the 2 weeks after a stroke. It provides data to support the development of larger observational or interventional studies that could explore the impact of mechanical or other interventions on the oral flora and associated risk of pneumonia after a stroke.

Avenues for future studies might also include the impact of probiotic oral bacteria on the diversity of phylotypes, risk of pneumonia and oral health in people who have had a stroke.[65,66]

5 | CONCLUSIONS

There appears to be huge diversity of bacterial organisms in the oral cavity of stroke patients, and as most phylotypes identified were only found in one or two participants, no particular patterns linked to infection or the condition of the oral cavity could be discerned.

Risk of infection did not appear to be related to the presence or absence of any particular bacterial phylotype in the oral cavity.

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