



The association between oral bacteria, the cough reflex and pneumonia in patients with acute stroke and suspected dysphagia

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Abstract

Objective: To establish how oral bacteria are related to cough sensitivity and pneumonia in a clinical stroke population.

Background: Stroke patients are at risk of colonisation by respiratory pathogens due, in part, to sudden discontinuation of effective oral hygiene. When combined with reduced cough reflex sensitivity, aspiration of contaminated oropharyngeal contents and can lead to pneumonia. Relationships between oral bacteria, cough sensitivity and pneumonia have not been established.

Materials and methods: A total of 102 patients with acute stroke underwent saliva sampling and cough reflex testing at admission to hospital, discharge and one month. A qPCR assay compared levels of bacteria in saliva. Pneumonia events were recorded.

Results: Relative levels of bacteria were lowest at admission to hospital (6.04×10^{-6}). There was a slight (non-significant) increase in bacterial levels at discharge (1.69×10^{-2} , $P = .73$). By one month, bacterial levels had significantly increased (9.17×10^{-2}) relative to admission [$P < .001$] and discharge [$P < .001$]. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* are not typically found in healthy mouths, yet were detected in 22% of patients during hospitalisation. Combined bacterial levels measured at one month was associated with pneumonia ($P = .004$) but there was no relationship to cough sensitivity.

Conclusion: Acute stroke patients were at increased risk of colonisation from respiratory pathogens throughout their recovery. The presence of these pathogens in saliva at one month was associated with adverse respiratory events. Data support the development of protocols to explore risk factors and sequelae of microbiological changes in stroke.

KEYWORDS

Aspiration Pneumonia, CVA, oral hygiene, qPCR

1 | INTRODUCTION

Dysphagia, with poor pulmonary clearance, substantially increases the risk of aspiration pneumonia (AP) in patients following acute stroke due to an increase of potentially pathogenic bacteria in the oral cavity and upper airways.^{1,2} AP has high morbidity and mortality and carries a significant financial burden.³⁻⁶

The oral cavity is the principal source of respiratory pathogens responsible for AP. The bacteria from bronchoalveolar lavage in patients with ventilator-associated pneumonia are generally derived from their oral bacteria.^{7,8} Six species in particular are implicated in the pathogenesis of AP: *Streptococcus mitis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*.⁹⁻¹³

Patients with acute stroke are particularly susceptible to disruption of the oral microbial ecology due to decreased mastication, salivation, swallowing and oral hygiene. In this population, the presence of pathogenic oral bacteria is an indicator of a poor prognosis.¹⁴

Evidence from nursing home residents, ventilated and non-ventilated hospitalised patients suggests that interventions that improve oral hygiene reduce pneumonia rates by between 8% and 54%,¹⁵⁻¹⁷ with \$1.6 million in avoided pneumonia-related costs.¹⁶ However, there may be an additional benefit to improving oral hygiene. Watando et al¹⁸ reported improved cough reflex sensitivity among the elderly following one month of intensive professionally delivered oral hygiene, though the underlying reason for this effect remains unknown. Before further studies are undertaken, a comprehensive understanding of the relationships between bacteria and cough sensitivity will be informative because this population is likely to experience an acute detriment in oral hygiene and consequent increase in the oral bacterial load. Clarifying this relationship could guide treatment of patients who are at risk of decreased cough reflex sensitivity, AP and consequent morbidity.¹⁹ This study assessed how pathogenic respiratory bacteria were related to cough reflex sensitivity and AP in patients with acute stroke; hypothesising that patients with higher levels of pathogenic respiratory bacteria would have reduced cough reflex sensitivity and a predisposition to AP.

2 | METHODS

This study was approved by the Southern Health and Disability Ethics Committee (ref 13/STH/121). An a priori sample size of 102 participants was calculated for an estimated effect size of 0.4 and 90% statistical power ($P < .05$), based on the proportion of patients with AP caused by either *Strep mitis* or *Strep pneumoniae*¹³ for a 2×2 chi-squared test. Consecutive patients admitted to a metropolitan hospital with a diagnosis of stroke and referred for a swallowing assessment were recruited. Patients with suspected dysphagia were targeted as they were considered to be at increased risk for AP.^{20,21} Patients referred for palliative swallowing advice were excluded.

Informed consent or consent by proxy was obtained prior to participation. The study conformed to the requirements of the Declaration of Helsinki.

Outcome measures were taken (a) within two days of stroke, (b) at discharge from the acute stroke ward and (c) at 30 days post-stroke. Measured outcomes were cough reflex sensitivity, AP, and occurrence of six target bacteria: *Strep mitis*, *Strep pneumoniae*, *Staph aureus*, *K pneumoniae*, *E coli* and *P aeruginosa*. Demographic information was collected from medical records.

2.1 | Cough reflex sensitivity

Participants underwent cough reflex threshold testing using citric acid (0-1.2 mol/L)²² in 0.9% sodium chloride and a tidal breathing method.^{22,23} Citric acid was delivered via a facemask (Hudson Micro Mist Nebuliser Model 41893; Standard Connector & Adult Mask, Hudson RCI) connected to a nebuliser (Turboneb 2 Nebuliser; Clement Clarke International Limited) with an obstructed flow rate of 6.6 L/min. As nebulised citric acid was presented for up to 15 seconds, participants were instructed to "breathe normally through your mouth. Try not to cough." The suppressed cough threshold (SCT) is considered a closer approximation to true reflexive cough than natural cough testing.²⁴⁻²⁷ Participants were blinded to the doses presented.

Initially, a placebo of 0.9% sodium chloride was presented to accustom participants to the test. Up to five citric acid doses were delivered in progressively higher concentrations, with placebo doses randomly interspersed to increase challenge blindness.²⁸ Each citric acid dose was presented two or three times, with at least 30 seconds between applications to prevent tachyphylaxis.²⁹ Cough response was considered positive when two or more consecutive coughs were triggered (C2 response threshold) on two of three exposures. The lowest concentration of citric acid to elicit a cough response was recorded as the SCT.

2.2 | Oral bacteria sampling

Saliva samples were obtained with sterile rayon swabs held in the sublingual cavity for 30 seconds.³⁰ Swabs were transferred to sterile tubes, kept cool and placed in a freezer (-70°C) within three hours. Samples were stored for up to one year until DNA extraction. The same person (S. P.) collected all saliva samples.

Bacteria were quantitated by qPCR. Type strains *Strep mitis* (American Type Culture Collection [ATCC] 903), *Strep pneumoniae* (ATCC 6303), *K pneumoniae* (ATCC 13 883), *Staph aureus* (New Zealand Reference Culture Collection [NZRM] 3409), *E coli* (DH5 α) and *P aeruginosa* (Otago University Department of Microbiology and Immunology Collection OT15) were used as positive controls. The primer sequences were adopted from published reports (Table 1). A *Streptococcus* primer set, rather than a *S mitis*-specific primer set, was included due to *S mitis* being the most dominant oral species of *Streptococcus*.^{31,32} Primers were synthesised by Sigma-Aldrich.

TABLE 1 Clinical and demographic information of participants

Clinical/Demographic variable	n
Age (years)	Mean = 76, SD = 12.5
Gender	
Male	61 (60%)
Female	41 (40%)
Ethnicity	
New Zealand European	85 (83%)
European	11 (11%)
Chinese	2 (2%)
New Zealand Maori	2 (2%)
Indian	1 (1%)
Niuean	1 (1%)
Comorbidities	
Previous stroke	27 (26%)
Respiratory illness	21 (21%)
Cardiac illness	58 (57%)
Dementia	4 (4%)
Diabetes Mellitus	19 (19%)
Type of stroke	
Ischaemic	89 (87%)
Haemorrhagic	11 (11%)
Ischaemic with haemorrhagic transformation	1 (1%)
Not reported	1 (1%)
Site of lesion	
Supratentorial	88 (86%)
Infratentorial	11 (11%)
Mixed	2 (2%)
Not reported	1 (1%)
Lesion laterality	
Left	42 (41%)
Right	56 (55%)
Bilateral	3 (3%)
Not reported	1 (1%)
Independent for oral hygiene at admission ^a	55 (54%)
Independent for oral hygiene at discharge ^a	74 (78%)
Independent for oral hygiene at one month ^a	78 (83%)
Days between admission to hospital and first data collection (median and range)	2, 33
Days between discharge from hospital and second data collection (median and range)	0, 11 ^c
Days between stroke and third data collection (median and range)	0, 11
Days on acute hospital ward ^b (median and range)	5, 12
More than 30 days in hospital ^{a,b}	30
Cough reflex threshold at admission (median)	0.4 mol/L
Cough reflex threshold at discharge (median)	0.4 mol/L

(Continues)

TABLE 1 (Continued)

Clinical/Demographic variable	n
Cough reflex threshold at one month (median)	0.4 mol/L
Suspected/diagnosed AP on admission ^b	6 (6%)
Developed AP during hospital admission ^b	11 (11%)
Incidence of AP over one-month period	11 (11%)
Mortality within one month of stroke	9 (9%)
Cause of death	
Pneumonia	5 (5%)
Other (stroke, cardiac disease)	4 (4%)

Abbreviations: AP, aspiration pneumonia; SD, standard deviation.

^aRefers to the number of participants.^bExcluding participants who died while in hospital.^cRefers to the number of days prior to discharge from hospital.

Each qPCR assay included species-specific forward and reverse primers (0.4 µL, 20 µmol/L), PCR-grade water (4.2 µL) and 2× Fast SYBR® Green Master Mix (10 µL) (Thermo Fisher Scientific Incorporated). The combined reagents were dispensed (15 µL) into 96-well qPCR plates (MicroAmp® EnduraPlate Optical 96-Well Fast Green Reaction Plate; Applied Biosystems). Genomic DNA (gDNA) was recovered from each saliva sample using InstaGene™ Matrix (Bio-Rad Laboratories Incorporated, Hercules, California, USA) as per the manufacturer's instructions, and added (5 µL) to duplicate wells. qPCR using a universal bacterial primer set³³ and qPCR standards (0.2 ng/PCR well) were used to determine relative levels of gDNA extracted from twenty participant samples prior to the complete analysis. Relative gDNA levels in the participant subset were between 5.96×10^{-8} – 2.78×10^{-12} . Purified gDNA from type strain cultures (0.4 ng/µL) served as positive controls and ultra-pure water served as negative controls for each plate. Thermal cycling fluorescent measurement of double-stranded DNA was performed using the QuantStudio™ 6 Flex System (Thermo Fisher Scientific Incorporated).

The qPCR cycling parameters optimised for measuring *Staph aureus* and *K pneumoniae* were 20 seconds at 95°C followed by 40 cycles of three seconds at 95°C and 30 seconds at 60°C. The cycling parameters for *Streptococcus*, *Strep pneumoniae*, *E coli* and *P aeruginosa* were 20 seconds at 95°C followed by 40 cycles of 3 seconds at 95°C and 30 seconds at 63°C. Fluorescence was measured during each cycle and the quantitation cycle (Cq) calculated automatically by the QuantStudio™ 6 Flex System. Assays were calibrated with a dilution series of purified gDNA from each of the type strains and linear amplification was assessed over a range of 0.0002–2 ng/well.

2.3 | Aspiration pneumonia outcomes

From the participants' charts and general practitioner records, a diagnosis of AP was determined according to criteria proposed by Mann and colleagues.³⁴ Specifically, the presence of at least three of the following symptoms was required for a diagnosis of AP: fever (>38°C), abnormal respiratory examination (tachypnea > 22 breaths/

min, tachycardia, bronchial breathing, inspiratory crackles), productive cough with purulent sputum, abnormal chest x-ray, arterial hypoxaemia ($PO_2 < 70$ mm Hg) and isolation of a relevant pathogen from sputum (positive result on Gram stain and culture).

2.4 | Statistical analysis

All data were analysed with the SPSS software package (IBM Corp.). The raw data from the qPCR were transformed from Cq to relative numbers of bacteria (2^{-Cq})—a unitless measure that, in this context, refers to the number of bacteria in one sample relative to the next. This was done because it is not possible to convert directly from the amount of gDNA on a standard curve to a bacterial count.³⁵ Linear mixed modelling measured changes in the levels of each species, with time treated as a continuous variable.

One-way repeated-measures analysis of variance (RM-ANOVA) with Greenhouse-Geisser correction determined changes in combined bacteria levels (ie all six bacteria species together) over time, with age and gender as covariates. As nine participants died during hospitalisation and 14 participants were discharged within 24 hours of baseline testing, RM-ANOVA was not possible for describing changes in relative levels of individual species. Instead, linear mixed modelling was used to determine these changes.

Binary logistic regression predicted how the presence/absence of specific pathogenic bacterial species at admission to hospital, discharge from hospital and one-month post-stroke influenced patients' development of AP. A backwards-stepwise model was used, with age and gender included as covariates.

Linear mixed modelling determined changes in cough reflex sensitivity thresholds over time. Following this, cough reflex sensitivity thresholds were re-coded as either normal (ie ≥ 0.8 mol/L) or abnormal (ie > 0.8 mol/L) based on previous work in healthy adults²² and people with acute stroke,³⁶ and target bacteria were re-coded as either present or absent. Binomial logistic regression determined the influence of the presence of target bacteria on participants' response to the cough reflex test at admission to hospital, discharge from hospital and one-month post-stroke, with age and gender as covariates. Ordinal regression determined the influence of relative levels of bacteria (combined and individual species) on cough reflex sensitivity thresholds at admission to hospital, discharge from hospital and one-month post-stroke, with age and gender as covariates. Cough reflex sensitivity thresholds were treated as ordinal variables, and relative bacterial levels were treated as continuous variables. Statistical significance was set at $P \leq .05$ (one-tailed).

3 | RESULTS

Clinical and demographic participant information is detailed in Table 2. Of the 102 participants (61 males and 41 females) recruited, the majority were of New Zealand European descent. The mean age

of participants was 76 years. Most participants presented with a unilateral (96%), supratentorial (86%) and ischaemic (87%) lesion. The median number of hospitalised days prior to the study was two.

3.1 | Bacteriology

Relative levels of bacteria changed over time [$F(1.66, 163.99) = 18.05, P < .001$]. Levels were lowest at admission to hospital (6.04×10^{-6}), and there was a slight (non-significant) increase in levels at discharge ($1.69 \times 10^{-2}, P = .37$). One month following the stroke, bacterial levels had increased (9.17×10^{-2}), relative to admission levels [$P < .001$] and discharge levels [$P < .001$]. These represented medium-sized effects [$r = .41, r = .53$, respectively]. Older participants demonstrated higher relative levels of bacteria [$r = .30$] [$F(1.66, 163.99) = 7.21, P = .001$]. Bacterial levels were unrelated to gender [$P = .11$].

As expected, *Streptococcus* was detected in almost all saliva samples at all time points (Figure 1). The proportions of patients with detectable *Strep pneumoniae* and *Staph aureus* were highest at admission (67% and 13%, respectively) but had decreased after one month (41% and 3%, respectively). In contrast, the proportions of patients with detectable levels of *E coli*, *P aeruginosa* and *K pneumoniae* were lowest at admission (1%, 3% and 2%, respectively), peaked at discharge (7%, 21% and 10%, respectively), and lowered to approximate admission levels after one month (1%, 6% and 7%, respectively).

Relative levels of *Streptococcus* spp. and *Strep pneumoniae* decreased between admission and discharge [$P < .001$], but rose above admission levels one month after the stroke [$P < .001$] (Figure 2). On the other hand, relative levels of *K pneumoniae* and *P aeruginosa* significantly increased between admission and discharge [$P = .02, P = .01$, respectively]. After one month, relative levels of these bacteria had reduced, but continued to remain above admission levels [$P = .02, P = .005$, respectively]. Relative levels of *E coli* were too low for inferential statistics. However, levels of this species were highest at admission (6.38×10^{-8}), lower at discharge (2.23×10^{-11}) and lowest at 1 month (1.71×10^{-12}). Relative levels of *Staph aureus* did not differ between admission and discharge [$P = .07$], or between discharge and one month [$P = .07$].

3.2 | Relationships between bacteria and aspiration pneumonia

Six participants (6%) presented with suspected pneumonia, eleven (11%) developed AP during hospitalisation with one positive for influenza. There were no instances of AP following discharge. At admission, combined (ie all six species together) relative levels of bacteria were not significantly associated with the development of AP [$P = .31$]. However, the presence of *Strep pneumoniae* specifically was associated with the development of AP [$P = .04, OR = 3.56$]. In addition, the absence of *Staph aureus* was associated with the development of AP

TABLE 2 Prevalence and relative level of the six target bacteria at the three measurement times determined by qPCR

Bacteria	Prevalence (%)	Mean relative level of bacteria (2^{-Cq})	b	SE b	P	95% CI
<i>Streptococcus</i>						
Admission	97	6.00×10^{-6}				
Discharge ^{a,b}	100	7.00×10^{-6}	-0.15	0.00	<.001	-0.16 to -0.14
One month ^{a,b}	100	9.82×10^{-2}	0.05	0.00	<.001	0.05 to 0.05
<i>Streptococcus Pneumoniae</i>						
Admission	67	6.00×10^{-6}				
Discharge ^{a,b}	44	7.00×10^{-6}	-0.15	0.00	<.001	-0.16 to -0.15
One month ^{a,b}	41	1.02×10^{-1}	0.05	0.00	<.001	0.05 to 0.05
<i>Staphylococcus Aureus</i>						
Admission	13	4.41×10^{-11}				
Discharge ^{a,b}	10	1.33×10^{-10}	0.00	0.00	.13	0.00 to 0.00
One month ^{a,b}	3	4.94×10^{-11}	0.00	0.00	.13	0.00 to 0.00
<i>Escherichia coli</i>						
Admission	1	6.38×10^{-8}				
Discharge ^{a,b}	7	2.23×10^{-11}	0.00	0.00	c	c
One month ^{a,b}	1	1.71×10^{-12}	0.00	0.00	c	c
<i>Klebsiella pneumoniae</i>						
Admission	2	7.00×10^{-12}				
Discharge ^{a,b}	10	4.14×10^{-11}	0.00	0.00	.03	0.00 to 0.00
One month ^{a,b}	7	8.46×10^{-12}	0.00	0.00	.03	0.00 to 0.00
<i>Pseudomonas aeruginosa</i>						
Admission	3	7.00×10^{-9}				
Discharge ^{a,b}	21	9.58×10^{-2}	0.33	0.09	.02	0.09 to 0.56
One month ^{a,b}	6	6.48×10^{-2}	-0.08	0.02	.01	-0.14 to -0.02

Abbreviation: CI, confidence interval.

^aParticipants who discharged from hospital on the same day as admission, or at one month, did not contribute data.

^bParticipants who died before this outcome measurement did not contribute data.

^cInsufficient data for inferential statistics.

[$P = .01$, $OR = 0.17$]. At discharge, combined relative levels of bacteria were associated with AP [$P = .03$]. None of the species measured at this time point individually emerged as a predictor of AP. At one month, the combined relative levels of bacteria were associated with recent AP, with higher levels of bacteria associated with disease occurrence [$P = .002$]. None of the species measured at this time point emerged as a predictor of AP in the regression model.

3.3 | Relationships between bacteria and cough reflex sensitivity

Although median cough reflex thresholds were unchanged across admission, discharge and at one month [median = 0.4 mol/L, $P = .18$], the proportion of participants with an abnormal response to the test changed over time. At admission to hospital, 84% of participants had a normal response to the test. This decreased to 74% at discharge and remained (75%) at 1 month (Figure 3).

Although the multiple regression model predicted cough reflex thresholds at admission [$F(12, 100) = 2.52$, $P = .004$, $1-\beta = 0.78$], none of the measured bacterial species was significantly associated with cough reflex sensitivity. Similarly, the models did not predict cough reflex thresholds either at discharge [$F(13, 92) = 1.39$, $P = .09$, $1-\beta = 0.72$] or 1-month post-stroke [$F(12, 89) = 1.45$, $P = .08$, $1-\beta = 0.14$].

4 | DISCUSSION

This study investigated relationships between six oral bacteria associated with AP, cough reflex sensitivity and the development of AP in an acute stroke population. There was no relationship between oral bacteria and cough reflex sensitivity, and the null hypothesis was retained. However, for the association between these bacteria and the development of AP post-stroke, the null hypothesis was rejected. Further examination revealed that, in the month following

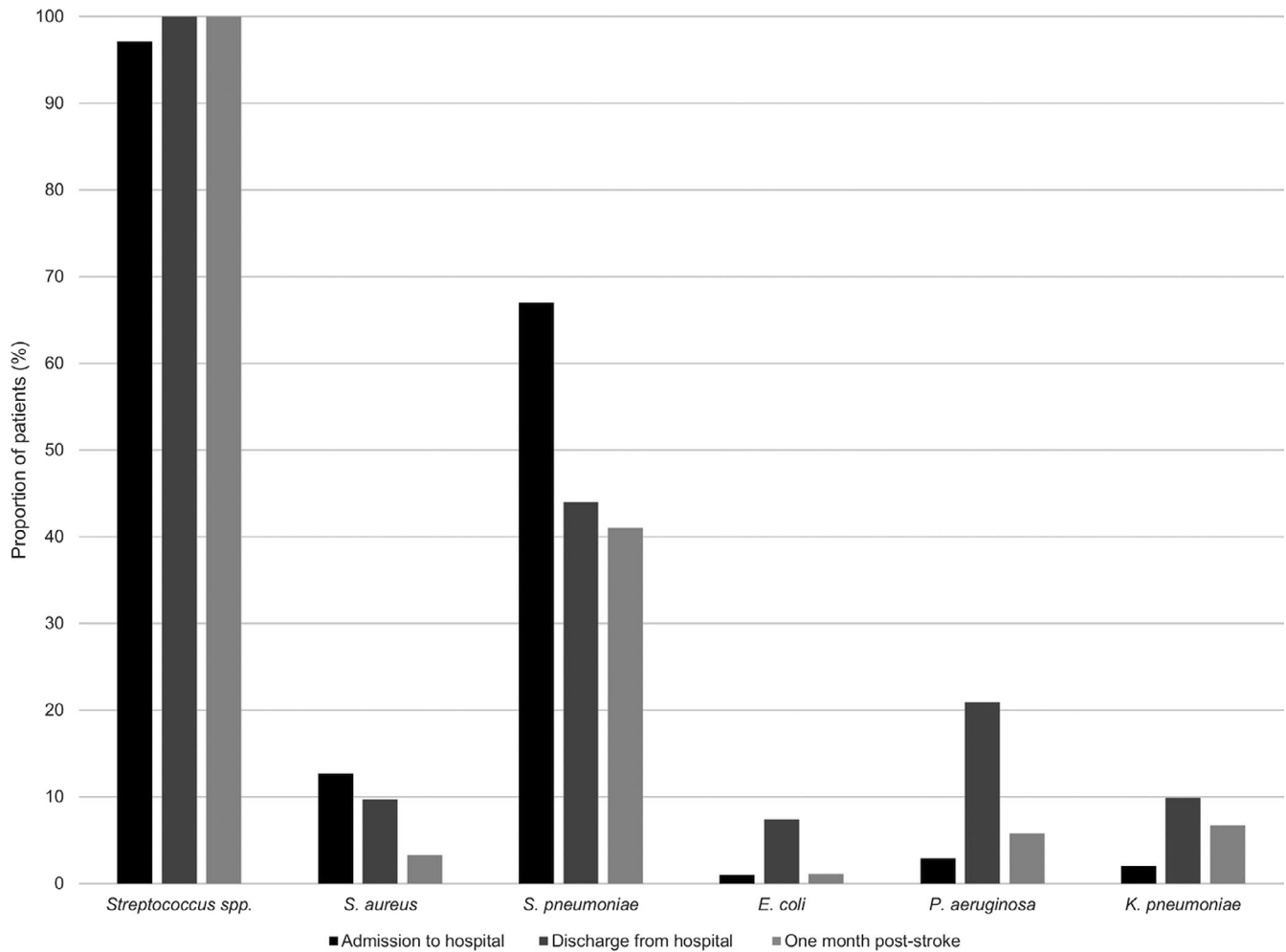


FIGURE 1 Proportions of patients with detectable levels of oral pathogens at admission to hospital, discharge from hospital and 1-month post-stroke

stroke, relative levels of potentially pathogenic oral bacteria significantly increased and did not return to baseline levels, suggesting that stroke influences patients' abilities to maintain/receive oral hygiene. As noted by Kowk et al³⁷ in their extensive review of oral care post-stroke, neglected or sub-optimal oral health may be a result of patient-specific factors such as physical limitations (hemiparesis, hemiplegia and dyscoordination), cognitive changes, xerostomic changes resulting from anticoagulant therapy, and change to denture fit, or carer-specific factors such as insufficient knowledge and/or experience providing oral care, delegation or low prioritisation of oral care routines.

Pseudomonas aeruginosa, *K pneumoniae* and *E coli* are not typically found in healthy mouths, yet were detected in up to 3% of this stroke patient cohort within 48 hours of admission to hospital, suggesting a critical window for preventing colonisation by respiratory pathogens in patient with stroke. These findings support previous reports of increased Gram-negative bacteria in the acute phase of stroke.¹⁴ However, contrary to Millns et al,¹⁴ there was no decrease in overall bacterial levels during stroke recovery. Due to methodological differences, it is not possible to determine whether this difference is due to the increased sensitivity of qPCR or to other reasons.

The main advantage of PCR over other methods of analysis is that bacteria which are unable to be cultured can be detected at the molecular level, reducing the risk of false negatives. Comparisons of culture methods and molecular methods such as PCR suggest higher bacterial detection rates using PCR³⁸⁻⁴⁰ even when antibiotics have been taken.⁴¹ The current study applied this molecular approach to patients with acute stroke. The findings accord with Ewan et al,⁴² who reported that the oral carriage of *E coli*, *S aureus* and *P aeruginosa* are associated with hospital-acquired pneumonia in elderly patients with lower limb fracture. In the present study, when relative levels of these bacteria and *Streptococcus spp.* were combined, a significant association with AP was apparent. Unlike Ewan et al, no single species was identified as predictive of AP, but this may be due to the comparatively fewer number of data collection days in the present study. Ewan et al followed their cohort for 90 days post-discharge, noting that 50% of pneumonias occurred after 25 days. It is conceivable that, in an extended study, relationships between individual species and AP in acute stroke patients would become evident.

Contrary to the a priori hypothesis, relationships between various bacteria and cough sensitivity were not apparent, though such associations have been suggested with lower concentrations of citric acid.¹⁸

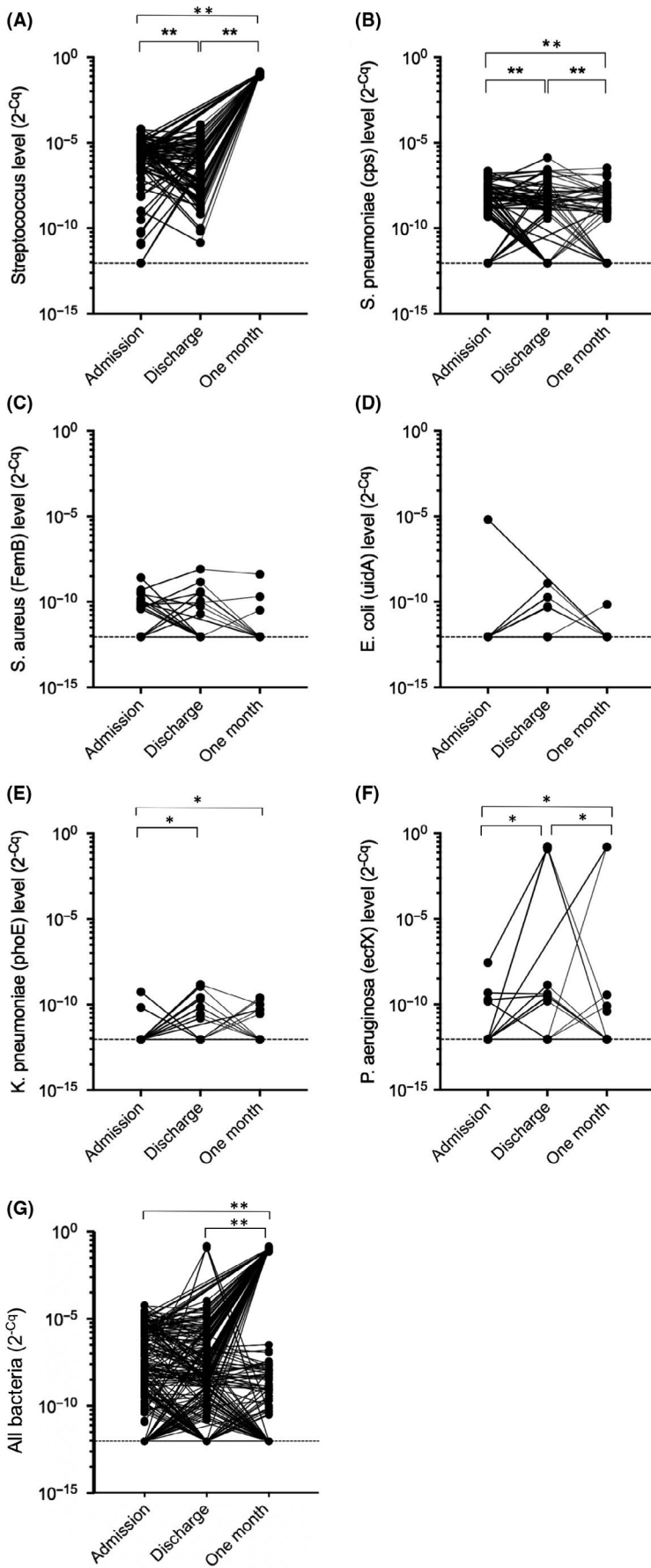
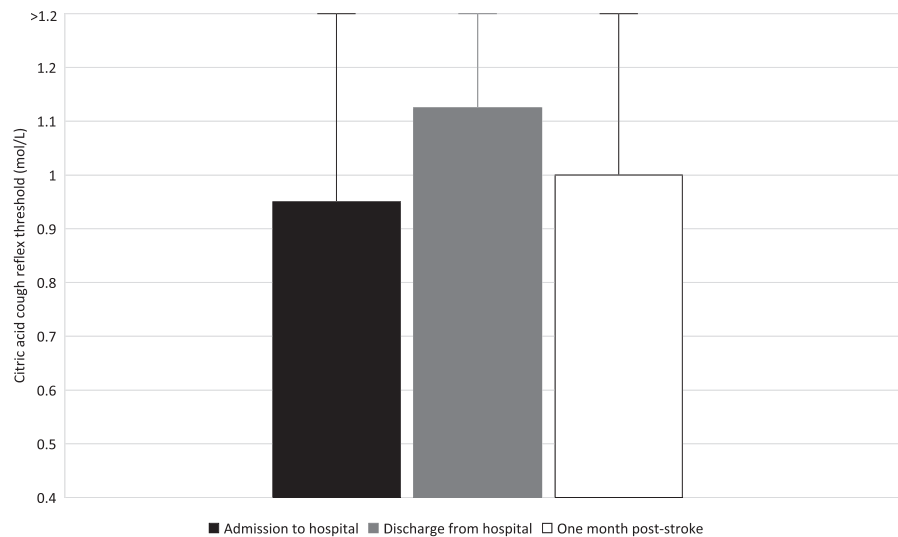


FIGURE 2 Bacterial levels at admission, discharge and one-month following stroke. A, *Streptococcus* (B) *Streptococcus Pneumoniae* (C) *Staphylococcus aureus* (D) *Escherichia coli* (E) *Klebsiella pneumoniae* (F) *Pseudomonas aeruginosa* (G) combined bacteria. Data expressed as average 2^{-Cq} for each sample. Dotted lines represent limits of detection. * $P < .05$, ** $P < .001$

FIGURE 3 Cough reflex thresholds (ie minimum concentration of citric acid required to elicit a C2 response) measured at three time points



Unexpectedly, the majority of participants (84%) in the present study exhibited baseline cough reflex sensitivity thresholds that were within the normal range for elderly people,²² suggesting a flooring effect. Note that, as a group, patients were variable in their response to the cough test over time. The proportion of patients with a normal cough reflex threshold decreased by 10% between admission and discharge from hospital, corresponding with a rise in overall bacterial levels. Given previous findings of improved (ie lower) cough sensitivity thresholds in the elderly during periods of intensive oral hygiene,¹⁸ it may be important to investigate this relationship further in the acute stroke population using lower concentrations of citric acid, as these concentrations may be more sensitive at detecting subtle changes in cough response.

4.1 | Limitations

A low recruitment rate (66%) of eligible patients and relatively normal cough reflex thresholds suggest a bias towards patients with milder strokes; thus, we may not have included those most prone to compromised oral hygiene. Difficulties in obtaining saliva samples and cough reflex thresholds at admission to hospital may have also contributed to the lack of changes in cough reflex sensitivity thresholds. When a patient is admitted to hospital with an acute stroke, stabilisation and medical treatment are prioritised over research participation. To account for this, a 48-hour window was allowed for “admission” measurements, which may permit bacterial numbers to increase in the absence of routine oral hygiene.⁴³ In addition, the opportunity to measure cough reflex sensitivity at its most impaired is lost. Regarding the bacterial measures, it is possible that freezing the saliva samples contributed to DNA degradation. However, sources generally agree that high quantities of DNA can be reliably extracted from saliva after long periods of time.⁴⁴ It is also acknowledged that variables such as the vigour and timing of toothbrushing may have affected the type and quantity of bacteria present in saliva samples. Future work in this area should aim to control this by collecting samples immediately prior to toothbrushing.

5 | CONCLUSION

This study adds to the limited evidence relating clinical, microbiological and pneumonia-related outcomes for patients with acute stroke. The presence of recognised respiratory pathogens in the mouths of patients within 48 hours of a stroke is clinically relevant. Such patients may be an ideal target population to receive benefit from interventions which prevent colonisation by respiratory pathogens. The findings justify larger-scale investigations validating oral hygiene interventions as a means of reducing AP.

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CONFLICT OF INTEREST

The authors whose names are listed above certify that they have no affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements) or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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