

## ORIGINAL ARTICLE

# Oral carriage of yeasts and coliforms in stroke sufferers: a prospective longitudinal study

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**AIM:** To investigate prospectively the qualitative and quantitative changes in oral carriage of yeasts and coliforms in southern Chinese people suffering from stroke. **MATERIALS AND METHODS:** In 56 elderly people suffering from stroke in a rehabilitation unit of a general medical hospital in Hong Kong, oral microbiological sampling using a combined imprint culture, oral rinse approach and clinical assessment was made during the acute stroke phase, on hospital discharge and 6 months later.

**RESULTS:** The oral carriage of yeasts increased significantly during acute stroke ( $P < 0.05$ ), whereas coliform carriage did not. A reduction in oral carriage of yeasts was found on hospital discharge and 6 months later and in coliforms at the 6-month assessment ( $P < 0.05$ ). *Candida albicans* and *Klebsiella pneumoniae* were the predominant yeast and coliform respectively. Stroke-related difficulty in tooth brushing and denture wearing were associated with higher oral yeast carriage ( $P < 0.05$ ). We also report here for the first time that the use of aspirin was associated with lower oral yeast carriage in people suffering from stroke.

**CONCLUSION:** Oral yeast carriage was closely linked to the level of stroke-related functional disability that improved over time but had not totally resolved 6 months after hospital discharge. The oral reservoir of yeasts and coliforms in people suffering from stroke is noteworthy by care providers as *K. pneumoniae* may cause aspiration pneumonia.

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## Introduction

In healthy people, the oral microflora is in a state of equilibrium, but this condition can be altered in the face of serious systemic diseases and treatment for these conditions (Michalek *et al*, 2002). The carriage rate of oral yeasts and overt oral candidiasis generally increases with systemic diseases (Jones and Mason, 1990). The increased carriage of oral yeasts and coliforms has been reported in several groups of medically compromised patients including those on cytotoxic therapy (Samaranayake *et al*, 1984), patients with acute leukaemia (Wahlin and Holm, 1988) and those with burning mouth syndrome (Samaranayake *et al*, 1989). In contrast, whilst the carriage of yeasts was higher in human immunodeficiency virus (HIV)-infected individuals, the oral carriage rate of coliforms was observed to be lower (Tsang and Samaranayake, 2000).

Stroke is a major cause of functional disability in the elderly (van Exel *et al*, 2004). Depending on the severity and type, stroke can leave a person with residual impairment of physical, psychological and social functions (Kim *et al*, 1999). Oral function deficit depends on the severity and the duration of the stroke (Locker *et al*, 2002; McMillan *et al*, 2005). Stroke-related oral motor impairment is very disabling as speech, mastication and swallowing problems are common, and these functions do not always fully recover after rehabilitation (Smit-hard, 2002). After stroke, limb paralysis and manual dexterity are common and affect the stroke survivors' ability to clean their teeth and dentures and to maintain a healthy oral condition (Sztriha *et al*, 2005). There is, however, limited information on the oral carriage of yeasts and coliforms during acute stroke or the ensuing recovery phase. Millns *et al* (1997) reported that the carriage rates of Gram-negative bacilli (GNB) and *Candida* species tended to increase following stroke. Another study found that the oral carriage of GNB increased during the acute phase and then decreased as the patients recovered (Millns *et al*, 2003). Stroke-related dysphagia and antibiotic therapy are also risk

factors for the oral colonization of *Candida albicans* (Aizen *et al*, 2004). Studies of the oral prevalence of these opportunistic pathogens would lead to the elucidation of the aetiology and pathology of stroke-related illnesses such as aspiration pneumonia and overt mucosal candidiasis. Hence, the main aim of this prospective, longitudinal study was to investigate qualitative and quantitative changes in oral carriage of yeasts and coliforms in a group of southern Chinese people suffering from stroke in Hong Kong, during the acute stroke phase, on discharge from hospital and 6 months later. In addition, the association of demographic factors, oral health condition and medication, with the oral carriage rate of yeasts and coliforms was investigated.

## Material and methods

### Study group

A total of 56 consecutive people suffering from stroke enrolled in the study between September 2003 and May 2005 were recruited at the Stroke Rehabilitation Unit, Tung Wah Hospital, Sheung Wan, Hong Kong. The hospital is a part of the public-funded Hospital Authority network throughout Hong Kong that provides in-patient medical services for the vast majority of the Hong Kong population. All participants were of southern Chinese ethnicity. The study was approved by the Institutional Review Board of The University of Hong Kong and the Hospital Authority, Hong Kong. The study was undertaken with the informed written consent of each subject.

All subjects participated in the study at three different stages: during the acute phase, on discharge from hospital and 6 months later. The selection criteria were unilateral paresis caused by hemispheric or sub-cortical stroke. All potential participants were screened by a specialist physician to ensure that they were in a suitable medical condition to participate.

### Procedure

After oral microbiological sampling, each subject suffering from stroke underwent a brief clinical oral assessment by the same clinician at the Tung Wah Hospital. The microbiological sampling and clinical assessment took place on three occasions: during the acute phase, on hospital discharge and at the 6-month follow-up.

### Microbiological sampling and laboratory techniques

Two techniques, the imprint culture and the oral rinse method, were used for microbiological sampling throughout.

### Imprint culture

The technique, described by Arendorf and Walker (1979), was used to determine candidal colonization in the oral mucosa. The dorsum of the tongue was chosen as the representative sampling site as this region is generally recognized as the most densely populated oral niche for both yeasts and coliforms (Arendorf and

Walker, 1979; Sedgley and Samaranayake, 1994a) and, also it was the simplest site to sample because of the debility of the patient. In brief, a sterile plastic foam pad ( $2.5 \times 2.5 \text{ cm}^2$ ) was dipped in Sabouraud's broth and placed on the dorsum of the patient's tongue for 60 s. The pad was then aseptically retrieved and pressed firmly onto a Sabouraud's dextrose agar plate (Oxoid Ltd, Hampshire, UK) and stored for *post hoc* microbiological analyses. In the laboratory, the plate was incubated aerobically at  $37^\circ\text{C}$  for 48 h. The candidal density was determined by the naked eye, visual counting and expressed as colony-forming units per square millimetre ( $\text{CFU mm}^{-2}$ ).

### Oral rinse technique

The technique, described by Samaranayake *et al* (1986), was used for the quantification of *Candida* species and Enterobacteriaceae. After the imprint was taken, the patient was supplied with 10 ml of sterile phosphate-buffered saline (PBS; 0.1 M, pH 7.2) in a universal container and was requested to rinse the mouth for 60 s. The rinse was then returned to the universal container and stored for *post hoc* microbiological analyses. The rinse sample was inoculated onto MacConkey's agar (Oxoid Ltd) and CHROMagar (Oxoid Ltd) using a spiral plater (Spiral Systems Marketing Ltd, Bethesda, MD, USA). The CHROMagar plates were incubated for 48 h while the MacConkey's plates were incubated for 24 h, both at  $37^\circ\text{C}$ . The number of yeasts in CHROMagar and coliforms in MacConkey's agar were quantified to yield the value ( $\text{CFU ml}^{-1}$ ) of the rinse sample.

### Identification of yeasts and coliforms

The yeasts obtained from imprint culture and oral rinse were identified and speciated based on colony morphology, cell morphology, Gram-staining reaction and a commercially available phenotyping system (API 32 C; bioMerieux, Inc, Marcy l'Etoile, France). The oral Enterobacteriaceae on MacConkey's agar were examined using the Gram staining reaction and identified using the commercially available API 20E system (BioMerieux, Inc). A single CFU of each of the colony types on Sabouraud's agar, CHROMagar and MacConkey's agar was selected through careful naked-eye examination, purified and speciated as above.

### Clinical assessment

The oral soft tissues were examined for clinical signs of candidal infection using the assessment procedure described in the WHO standard oral health assessment proforma (WHO, 1997). Oral hygiene was scored using the Bleeding Indices (Löe and Silness, 1963) and Plaque Indices (Silness and Löe, 1964). Tooth condition was assessed using the DMFT index, and prosthetic status was also determined (WHO, 1997).

The Barthel Index (BI) (Barthel and Mahoney, 1965) is used by physicians at the Rehabilitation Unit in Tung Wah Hospital as an objective measure of physical function in all people suffering from stroke during rehabilitation. It is based on observed functions and assesses activities of daily living on a score 0–100, with a

higher score indicating better physical ability. The BI was assessed at the three stages as an objective indicator of physical disability. The ability to perform tooth brushing was also assessed objectively using a four-point Likert scale (1 = no difficulty, 2 = minor difficulty, 3 = moderate difficulty and 4 = major difficulty).

#### Statistical analyses

Data were coded and analysed using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA). The data were not normally distributed, therefore, percentiles and medians were used to describe the number and prevalence of *Candida* and coliform species. Nonparametric tests were used to test the changes over time and the difference between groups. The prevalence of yeasts and coliforms at the three stages was evaluated using the Cochran *Q*-test while the McNemar test was used to analyse differences between each of the stages. The number of yeasts at the three stages was evaluated by Friedman two-way ANOVA while Wilcoxon signed-rank test was used to analyse the difference between stages. The relationship of the oral carriage rate of yeasts and coliforms with age, gender, BI, Plaque Index, Bleeding Index, DMFT, denture wearing, dysphagia, aspirin use, smoking, diabetes and difficulty using a toothbrush was examined using a logistic regression model. The level of significance was set at 0.05.

## Results

Data were collected from 56 people suffering from stroke [41 males, 15 females, mean age 66.1 years (s.d. 12.3)] during the acute phase, on hospital discharge and at 6-month follow-up. Most of the subjects were retired males (75.0%), living with their family (81.4%), not receiving government social security assistance (83.9%) and were irregular dental attendees (78.6%). The mean time period when the acute-phase sample was taken was 2.6 days (s.d. 1.4) days after admission to the Stroke Rehabilitation Unit. The mean time from hospital admission to discharge was 22.5 days (s.d. 9.2).

#### Quantitative analysis of oral carriage of yeasts and coliforms

The oral carriage rates of oral yeasts and coliforms at the three sampling periods are shown in Table 1. Using the oral rinse technique, the oral carriage rate of yeasts at 6 months after hospital discharge was significantly lower than in the acute phase and at hospital discharge

( $P \leq 0.035$ ). There was a progressive reduction in the oral yeast carriage rate over time when the imprint culture technique was used ( $P \leq 0.031$ ). Among the 31 patients who tested positive for yeasts (using oral rinse) during the acute phase, more than half (64.5%) tested negative at subsequent sampling periods.

The oral carriage rate of coliforms was significantly lower at 6 months after hospital discharge ( $P \leq 0.016$ ). Among the 12 patients who tested positive for coliforms during the acute phase, most (91.7%) tested negative at subsequent sampling periods.

Data on yeast and coliform isolation are shown in Tables 2 and 3. Nine yeast species were isolated using the oral rinse technique and eight using the imprint culture technique. The predominant yeast species was *C. albicans*. *Candida glabrata* and *C. tropicalis* were also relatively common. In 18 (32.1%) of the subjects, *Candida* species were isolated at all three sampling periods. Seven coliform species were isolated with *Klebsiella pneumoniae* being the most common. None of the coliform species was isolated at all three sampling periods.

#### Comparison of oral rinse and imprint sampling techniques

A comparison of the oral yeast carriage rate detected by imprint culture and oral rinse technique is shown in Table 4. The sensitivity of the imprint culture technique was higher at all sampling periods. The oral carriage rate of yeasts detected using imprint culture technique was significantly higher at 6 months after hospital discharge ( $P = 0.02$ ).

#### Clinical findings

The DMFT score [mean (s.d.)] ranged from 19.5 (10.0) during acute stroke to 19.8 (10.0) at 6-month follow-up. There was no difference in the scores among the three assessments ( $P > 0.05$ ). Approximately 50% of the people suffering from stroke wore removable dentures, 25% of whom wore complete dentures. There was no significant difference in the prosthetic status between visits ( $P > 0.05$ ).

There was no significant difference in the highest Community Periodontal Index (CPI) scores (the periodontal condition) and the percentage of red/white oral soft tissue lesions between visits ( $P > 0.1$ ; Table 5). The mean percentage of tooth sites with moderate to abundant plaque and immediate to spontaneous bleeding was significantly different among the three sampling periods ( $P \leq 0.01$ ) with a reduction in the mean percentage of sites over time.

Investigation	n (%)			All 3 visits	1 vs 2	1 vs 3	2 vs 3
	Visit 1	Visit 2	Visit 3				
Oral rinse							
<i>Candida</i>	31 (55.4)	25 (44.6)	16 (28.6)	0.001	0.146	0.001	0.035
Coliforms	12 (21.4)	9 (16.1)	2 (3.6)	0.005	0.549	0.006	0.016
Imprint culture							
<i>Candida</i>	37 (66.1)	31 (55.4)	23 (41.1)	<0.001	0.031	0.001	0.039

**Table 1** Oral prevalence (%) of *Candida* species and coliforms in 56 people suffering from stroke during the acute phase (visit 1), on hospital discharge (visit 2) and 6 months later (visit 3); data from two sampling techniques

<sup>a</sup>Cochran *Q*-test; <sup>b</sup>McNemar test, level of significance 0.05.

**Table 2** The identity and frequency of yeasts isolated in 56 people suffering from stroke during the acute phase (visit 1), on hospital discharge (visit 2) and 6 months later (visit 3)

Technique	Species	Number of isolates (%)			
		Visit 1	Visit 2	Visit 3	Total
Oral rinse	<i>Candida albicans</i>	27 (48.2)	21 (37.5)	13 (23.2)	61 (58.6)
	<i>Candida tropicalis</i>	8 (14.3)	4 (7.1)		12 (11.5)
	<i>Candida glabrata</i>	10 (17.9)	8 (14.3)	4 (7.1)	22 (21.2)
	<i>Candida guilliermondii</i>	1 (1.8)			1 (1.0)
	<i>Candida kefyr</i>	1 (1.8)	1 (1.8)		2 (1.9)
	<i>Saccharomyces cerevisiae</i>	1 (1.8)	2 (3.6)		3 (2.9)
	<i>Candida dubliniensis</i>	1 (1.8)			1 (1.0)
	<i>Candida parapsilosis</i>		1 (1.8)		1 (1.0)
	<i>Candida inconspicua</i>			1 (1.8)	1 (1.0)
	<i>Candida albicans</i>	28 (50.0)	23 (41.1)	18 (32.1)	69 (71.1)
	<i>Candida tropicalis</i>	4 (7.1)	2 (3.6)	1 (1.8)	7 (7.7)
	<i>Candida glabrata</i>	3 (5.4)	2 (3.6)	3 (5.4)	8 (8.2)
	<i>Candida guilliermondii</i>	2 (3.6)	2 (3.6)	1 (1.8)	5 (5.2)
	<i>Saccharomyces cerevisiae</i>	1 (1.8)	1 (1.8)		2 (2.0)
Imprint culture	<i>Candida dubliniensis</i>	1 (1.8)			1 (1.0)
	<i>Candida parapsilosis</i>	1 (1.8)	3 (5.4)		4 (4.1)
	<i>Candida sake</i>	1 (1.8)			1 (1.0)

**Table 3** Identity and frequency of Enterobacteriaceae isolated in 56 people suffering from stroke during the acute phase (visit 1), on hospital discharge (visit 2) and 6 months later (visit 3)

Enterobacteriaceae	Number of isolates (%)			
	Visit 1	Visit 2	Visit 3	Total
<i>Klebsiella pneumoniae</i>	4 (7.1)	3 (5.4)	1 (1.8)	8 (34.8)
<i>Enterobacter gergoviae</i>	2 (3.6)	2 (3.6)		4 (17.4)
<i>Chryseomonas luteola</i>	2 (3.6)	3 (5.4)	1 (1.8)	6 (26.1)
<i>Klebsiella oxytoca</i>	2 (3.6)			2 (8.7)
<i>Enterobacter cloacae</i>	1 (1.8)			1 (4.3)
<i>Acinetobacter baumannii</i>	1 (1.8)			1 (4.3)
<i>Stenotrophomonas maltophilia</i>		1 (1.8)		1 (4.3)

**Table 4** A comparison of oral rinse and imprint culture technique in detecting oral carriage of yeasts in people suffering from stroke ( $n = 56$ ) during the acute phase (visit 1), on hospital discharge (visit 2) and 6 months later (visit 3)

Investigation	Test results (%)			Sensitivity (%) <sup>a</sup>	P-value <sup>b</sup>
	Positive	Negative	False-negative		
Visit 1					
Imprint culture	66.1	33.9	3.6	94.9	0.109
Oral rinse	55.4	44.6	14.3	79.5	
Visit 2					
Imprint culture	55.4	44.6	1.8	96.9	0.07
Oral rinse	44.6	55.4	12.5	78.1	
Visit 3					
Imprint culture	41.1	58.9	0	100	0.016
Oral rinse	28.6	71.4	12.5	69.6	

<sup>a</sup>Sensitivity = Positive results/(positive results + false-negative results).

<sup>b</sup>McNemar test was used.

The BI [mean (s.d.)] during the acute phase, on hospital discharge and 6 months later were 58.1 (18.7), 78.5 (16.3) and 82.6 (21.5) respectively. There was a significant difference in the mean BI score over time ( $P < 0.01$ ). The majority of people suffering from

stroke had difficulty in tooth brushing during the acute phase (83.9%) and on hospital discharge (71.4%), and about half of them at the 6-month follow-up (51.8%). There was a statistically significant decrease over time ( $P \leq 0.016$ ).

#### Variables associated with oral carriage of yeasts and coliforms

Table 6 shows the regression analyses of the relationship of oral yeast carriage with age, gender, BI, Plaque Index, Bleeding Index, DMFT, denture wearing, dysphagia, aspirin use, smoking, diabetes and tooth brushing difficulty. The use of aspirin was associated with lower yeast carriage rate at all assessments ( $P < 0.05$ ). When imprint culture technique was used, denture wearers had a significantly higher yeast carriage rate at the three sampling periods ( $P \leq 0.024$ ). When using the oral rinse technique, denture wearers had a significantly higher oral carriage rate of yeasts on hospital discharge ( $P = 0.001$ ). Those with difficulty in tooth brushing during the acute phase and on hospital discharge had a significantly higher oral yeast carriage when imprint culture was used ( $P < 0.01$ ). There was no significant relationship between all the variables studied and the oral carriage rate of coliforms at any of the assessments ( $P > 0.05$ ).

#### Discussion

This was a prospective, longitudinal study that yielded comprehensive data on the oral carriage of yeasts and coliforms in elderly people following stroke. A notable finding was the significant increase in oral yeast carriage rate during the acute stage and a progressive decrease during the recovery period that was clearly linked to the level of functional disability, notably difficulty in tooth brushing. Stroke-related limb paralysis and reduced manual dexterity are known to affect a patient's ability to maintain a healthy oral condition (Lai et al, 2002). In addition, unilateral paresis of the lower face, tongue and

	Visit 1 (n = 46) <sup>a</sup> (%)	Visit 2 (n = 46) <sup>a</sup> (%)	Visit 3 (n = 46) <sup>a</sup> (%)
Highest CPI			
Healthy	0	0	0
Bleeding only	0	0	0
Calculus	20.9	21.2	15.3
4–5 mm pockets	60.5	60.6	64.4
≥6 mm pockets	18.6	18.2	20.2
Soft-tissue lesions			
Sites with moderate to abundant plaque [mean (s.d.)]	89.51 (18.73)	81.14 (22.32)	66.67 (32.58)*
Sites with immediate to spontaneous bleeding [mean (s.d.)]	85.07 (20.90)	76.41 (24.27)	60.96 (29.86)**
Red/white patches	32.1	26.8	23.2

\* $P < 0.01$ ; \*\* $P \leq 0.01$ ; no significant differences for the other comparisons between groups ( $P > 0.1$ ).

<sup>a</sup>10 subjects were excluded as they were edentulous.

<sup>b</sup>McNemar tests were used to compare the differences in the distribution of the highest CPI score and the prevalence of soft tissue lesion for the stroke survivors over time.

Wilcoxon signed rank tests were used to compare the differences in the median scores for the stroke survivors over time.

**Table 5** Percentage distribution of highest CPI score, mean percentages of tooth sites with moderate to abundant plaque and immediate to spontaneous bleeding, soft tissue lesions in people suffering from stroke during the acute phase (visit 1), on hospital discharge (visit 2) and 6 months later (visit 3)

**Table 6** Logistic regression analysis of the oral carriage of yeasts in people suffering from stroke on nine explanatory variables during the acute stroke phase (visit 1), on hospital discharge (visit 2) and 6 months later (visit 3)

Variable	Oral rinse technique (n = 56)						Imprint culture technique (n = 56)					
	Visit 1		Visit 2		Visit 3		Visit 1		Visit 2		Visit 3	
	P-value	Odds ratio	P-value	Odds ratio	P-value	Odds ratio	P-value	Odds ratio	P-value	Odds ratio	P-value	Odds ratio
Age	0.057		0.178		0.659		0.719		0.474		0.653	
Gender	0.405		0.618		0.293		0.445		0.067		0.96	
Barthel index	0.901		0.482		0.896		0.714		0.984		0.212	
Denture	0.24		0.001*	12.28	0.054		0.001*	70.511	0.003*	20.536	0.024*	4.348
Dysphagia	0.607		0.447		0.96		0.513		0.071		0.325	
Aspirin	0.001*	0.103	0.007*	0.128	0.041*	0.237	0.021*	0.109	0.004*	0.04	0.043*	0.274
Smoking	0.411		0.117		0.999		0.335		0.06		0.999	
Diabetes	0.688		0.723		0.522		0.575		0.933		0.341	
Plaque index	0.732		0.665		0.315		0.299		0.203		0.379	
Bleeding index	0.738		0.552		0.263		0.287		0.204		0.388	
DMFT	0.660		0.457		0.712		0.271		0.651		0.627	
Difficulty tooth brushing	0.171		0.144		0.873		0.002*	77.047	0.004*	24.534	0.552	

\*Odds ratios are shown when  $P < 0.05$

palate leads to impaired ability to clear the mouth of food, with the attendant risk of oral infections including yeasts (Rose *et al*, 2002; Samaranayake *et al*, 2002).

Functional status is closely associated with the capacity for oral self-care in the elderly (Ruiz-Medina *et al*, 2005). Functional ability, as measured by the BI, was severely impaired during acute stroke, then improved over time although there was still significant impairment 6 months after hospital discharge. Thus, residual functional disability probably accounted for the sustained higher incidence of oral yeast carriage. Difficulty holding and manipulating a toothbrush would also account for the higher incidence of gingival bleeding and plaque accumulation compared with healthy community-dwelling elderly people of similar age and gender (Pow *et al*, 2005).

The oral yeast carriage rate in patients suffering from stroke (41–66%) was higher than in healthy volunteers

mainly from the West (34%) (Samaranayake and MacFarlane, 1990) and elderly community-dwelling Hong Kong Chinese (30–35%) (Sedgley and Samaranayake, 1994b; Pow *et al*, 2005). Although there was a decrease in oral yeast carriage over time, it was still significantly higher than in medically healthy people. Thus, there appeared to be persistent colonization in some people suffering from stroke (35%) whereas in the others the colonization was transient. The findings support previous observations by Millns *et al* (1997, 2003). However, Pow *et al* (2005) found a marginally lower oral yeast carriage rate in Chinese stroke survivors on hospital discharge (40%), although the nature of their study population (mild to moderate stroke) could account for the difference. The current findings, support the general view that *C. glabrata* and *C. tropicalis* are the commonest oral yeast species isolated after *C. albicans* (Samaranayake and MacFarlane, 1990).

There were other potential reasons for the high prevalence of oral yeasts including denture wearing, nutritional factors and drug therapy. Denture wearing was clearly related to increased *Candida* carriage in the present study and has been observed previously especially when oral hygiene is poor (Oksala, 1990). People with stroke are medically and nutritionally more vulnerable because of decreased dietary intake due to dysphagia and other neurological deficits (Finestone and Greene-Finestone, 2002). Nutritional factors acting locally or via systemic mechanisms are implicated in the pathogenesis of oral candidiasis (Samaranayake, 1986). Some patients with stroke complained of drug therapy-related dry mouth. Although drugs with xerostomic side-effects predispose to oral candidiasis (Torres *et al*, 2003), other studies do not consider xerostomia to be a major risk factor for oral yeast carriage (Yamanaka *et al*, 2005). It is noteworthy that aspirin, a widely used medication in the treatment and prevention of thrombotic stroke (Sztriha *et al*, 2005), was associated with lower oral yeast carriage. Aspirin is thought to possess a potent anti-biofilm activity, which inhibits the growth of oral *Candida* (Alem and Douglas, 2004). As far as we are aware, there are no previous reports on the relationship between aspirin intake and oral yeast carriage in stroke patients. Further studies are warranted to confirm or refute our findings.

Coliforms are generally considered to be transient oral colonizers or non-resident members of the oral cavity, and wide ethnic variations in oral carriage have been reported (Jobbins *et al*, 1992). The transient, unstable coliform colonization observed supports previous findings. Global data on oral coliform carriage rate indicate a range of 10–26% in healthy individuals although an exceptionally higher prevalence (32%) has been reported in community-dwelling Hong Kong Chinese (Sedgley and Samaranayake, 1994b). An increased oral carriage of coliforms is generally seen in medically compromised people (Millns *et al*, 2003). Indeed, the presence of GNB is thought to be a prognostic indicator in acute stroke patients (Millns *et al*, 2003). However, in the current study coliform prevalence was lower than might have been expected. This disparity may be due to the variety of sampling methods used in different studies and/or a true reflection of the transient nature of oral coliform carriage.

In the regression model, no association was found between oral carriage of coliforms and other variables. It is nonetheless noteworthy that *K. pneumoniae* was by far the most prevalent coliform. As this organism is often involved in bronchial pneumonia, the most common cause of death from bacterial infection in the frail elderly (Schneider, 1983), their presence in the oral cavity as a putative reservoir of infection should be noted by care providers. Further research is warranted to investigate the role of professional cleaning of the oral cavity and its impact on oral coliform carriage, especially during the acute stroke phase.

Two oral sampling techniques were used in the present study. The sensitivity of the imprint culture appeared to be higher than the oral rinse technique in

detecting oral yeast carriage. This observation supports previous findings that the imprint culture technique was the most sensitive method for detecting oral yeast carriage in humans (Samaranayake *et al*, 1986). However, a limitation of the imprint technique is that enumeration of CFU obtained tends to be inaccurate when the yeast density exceeds 50 CFU cm<sup>-2</sup> because of confluent growth of colonies (Arendorf and Walker, 1980). Colony growth is less problematic in the rinse technique because the rinse can be diluted, the yeasts and coliform CFU quantified and then corrected to indicate CFU in the original rinse. Furthermore, the oral rinse is simple to perform and does not involve the clinician's subjective choice of sampling site. Nonetheless, it should be used with caution in stroke patients with dysphagia, especially in the acute stage when 29–64% of patients may have this condition (Gordon *et al*, 1987; Mann *et al*, 1999). In addition, facial weakness is common after stroke and makes the oral rinse more cumbersome. It seems that the optimal sampling protocol in patients suffering from stroke would be to perform the imprint culture to detect the oral carriage of yeasts, and the oral rinse technique to quantify the oral carriage of both yeasts and coliforms.

In conclusion, oral yeast carriage increased significantly during acute stroke and decreased significantly thereafter. Oral yeast carriage was still higher in people suffering from stroke 6 months after hospital discharge compared with community-dwelling elderly people without stroke. Protracted recovery of manual dexterity and oral motor function appears to be the most probable cause of sustained higher oral yeast carriage and also poor gingival health. Oral carriage of coliforms did not appear to be affected by stroke. However, *K. pneumoniae*, that causes significant morbidity in the medically compromised elderly, was identified as the predominant coliform in our cohort. A combination of imprint culture and the oral rinse technique appeared to be the optimum approach for detecting and quantifying oral yeasts and coliforms in patients suffering from stroke.

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