Age-related microbiological changes in the salivary and plaque microflora of healthy adults

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Summary. The effect of age on quantitative or qualitative differences in selected bacteria of dental significance and on the carriage of opportunistic pathogens and transient oral species was determined in 79 healthy, non-denture wearing individuals divided into four age groups: 20–39 years (group A), 40–59 years (group B), 60–79 years (group C) and ≥ 80 years (group D). Samples of dental plaque and whole saliva were cultured on appropriate selective and non-selective bacteriological media. The total numbers of viable bacteria in saliva, and the prevalence of mutans streptococci in plaque and saliva were similar in all age groups. Similarly, there was no correlation between the numbers of spirochaetes in plaque and age. In contrast, statistically significantly higher mean proportions ($p=0.004$), mean $\log_{10}$ viable counts ($p=0.001$) and isolation frequencies ($p<0.01$) of lactobacilli were found in the saliva of those aged ≥ 70 years compared to subjects in group A. The isolation frequency ($p<0.05$) and proportions ($p=0.056$) of staphylococci in saliva were also higher in those aged ≥ 70 years. Yeasts were isolated most often and in higher numbers from saliva in those aged ≥ 80 years and the proportion of yeasts was higher after 60 years of age, but these differences were not significant in comparison with results from individuals in group A. *Actinomyces* spp. were commonly isolated from plaque, but there was a change, with age, in the ratio of the proportions of *A. viscosus* and *A. naeslundii* so that *A. viscosus* predominated in elderly subjects (groups C and D). The results suggest that genuine age-related changes in the oral microflora can be detected, particularly after the age of 70 years, which are not related to denture-wearing or disease.

Introduction

The oral cavity comprises a series of different environments such as the tongue, cheek, palate, tooth surfaces and gingival crevice, which are colonised by different microfloras. These microfloras are influenced by host factors such as tooth eruption or loss of teeth, oral hygiene, and hormonal changes of the host.¹ The numbers and types of micro-organisms in the mouth also change with age, particularly in the early years during the development of the dentition.^{2,3} Similarly, the isolation frequency of black-pigmented gram-negative anaerobic bacilli,^{4,5} spirochaetes,^{6–8} and *Actinomyces viscosus*⁹ are reported to increase in adolescents and young adults. However, much less is known of the effect of subsequent natural ageing processes on the stability of the resident oral microflora.

The host is able to maintain microbial homeostasis in the mouth by means of specific and innate host defences.¹⁰ These defences, together with the commensal microflora, act to prevent colonisation by non-resident species and opportunist pathogens. Cell-

mediated immunity is reported to decline with age,¹¹ but little is known about the effect of age on the oral host defences,¹² nor how this may be reflected in changes in the oral microflora. Thus, the aim of the present study was to compare the resident oral microflora of healthy subjects in different age groups. Because of the complexity of the microflora and the known inter-subject variation, only selected species of dental significance were chosen for comparison, together with species considered normally to be present only transiently, thereby acting as indicators of the breakdown of homeostasis.¹⁰

Materials and methods

Study population

A total of 79 healthy subjects took part in the study. They were sub-divided into four groups: (A) 20–39 years, mean age 26.8 years, ± 1.03 , $n=30$; (B) 40–59 years, mean age 50.1 years, ± 1.1 , $n=23$; (C) 60–79 years, mean age 72.5 years, ± 1.4 , $n=16$; and (D) ≥ 80 years, mean age 83.8 years, ± 0.9 , $n=10$. The subjects in groups A and B included staff and patients

of Guy's Hospital, London, who were attending routine dental examinations, and groups C and D were elderly groups living mainly in sheltered residential accommodation comprising a mixture of houses, apartments and units with access to central dining facilities (Morden College, London). The study population satisfied the following requirements for inclusion within this project: (a) the presence of a minimum number of teeth (seven), including one molar; (b) the absence of active oral disease; (c) the absence of dentures; (d) no recent history of antimicrobial therapy or other drug therapy, including immunosuppressives; and (e) no history of diabetes. A haematological screen was performed on all subjects in groups C and D to exclude latent disease. These subjects also received regular medical checks.

Collection of saliva samples

Unstimulated whole saliva was collected from all subjects by direct expectoration into a sterile container during a 10-min period.

Collection of plaque samples

Supragingival molar plaque was collected above the gingiva on the lower right or left sixth tooth by running a standard dental probe along the buccal and mesio-buccal surfaces. Plaque samples were transferred into 1 ml of reduced transport fluid¹³ in sterile bottles containing 20–30 glass beads (3-mm diameter; Jencons Scientific Ltd, Bedfordshire). Both unstimulated whole saliva and plaque samples were processed within 2–4 h of collection.

Microbiological procedures

Saliva samples were dispersed by vortex mixing for 30 s and 10-fold dilutions were prepared in reduced transport fluid.¹³ Samples of 100 µl at dilutions from 10³ to 10⁷ were plated in duplicate on to Columbia blood agar and incubated aerobically and anaerobically in an atmosphere of CO₂ 10% in H₂ at 37°C for 4 days, to determine the total numbers of aerobic and anaerobic cfu. The saliva samples from neat to a dilution of 10⁴ were also plated on to a range of selective media to enable key micro-organisms to be enumerated. The media were Sabouraud Agar (Oxoid) for *Candida* spp., MacConkey Agar (Oxoid) for enteric bacteria, Rogosa Agar (Oxoid) for *Lactobacillus* spp., Mannitol-Salt Agar (Oxoid) for *Staphylococcus* spp. and TYC medium (Lab M, Bury) with sucrose 20% w/v and bacitracin 0.1 units/ml¹⁴ (TYCSB) for mutans streptococci. Sabouraud, MacConkey, and Mannitol-Salt media were incubated aerobically at 37°C for 4 days, TYCSB medium was incubated anaerobically at 37°C for 4 days, and Rogosa medium was incubated in CO₂ 5–10% in air at 37°C for 4 days.

Plaque samples were held under CO₂ and dispersed by vortex mixing for 1 min with 3-mm glass beads to

disaggregate any clumps of micro-organisms within the plaque. Plaque samples were treated in the same way as saliva samples except that, in addition to the above media, samples were plated on CFAT medium,¹⁵ which is selective for *A. viscosus* and *A. naeslundii*, and incubated anaerobically at 37°C for up to 10 days. Facultatively anaerobic, catalase-positive and catalase-negative isolates were identified as presumptive *A. viscosus* and *A. naeslundii*, respectively. Plaque samples were also plated on Columbia blood agar supplemented with vancomycin 2.5 µg/ml and incubated anaerobically at 37°C for up to 21 days for the isolation of black-pigmented gram-negative anaerobic bacilli. Yeasts were identified with API 20C commercial galleries (API, Basingstoke) according to the manufacturer's instructions. Coagulase-positive colonies on Mannitol-Salt agar were considered to be *S. aureus*.

Dark field microscopy was used to determine the presence and numbers of spirochaetes in dispersed plaque samples.¹⁶ For each sample, 60 fields were viewed and the number of spirochaetes in each counted and summated. Results were expressed as a percentage of the viable count.

The reproducibility of the bacterial isolation techniques was tested in six volunteers by dividing the saliva samples in two. Subsequently, these were treated as separate samples and serially diluted; inocula were spread on duplicate plates of Columbia blood agar and selective media for yeasts, enteric bacteria, lactobacilli, staphylococci and mutans streptococci.

Statistical procedures

Viable counts were transformed to log₁₀ cfu; results are presented as the mean of the log₁₀ cfu, and were compared by analysis of variance and Student's *t* test. Isolation frequencies were compared by χ^2 test, with Yates's correction for small numbers where appropriate. Spearman rank correlations were performed on the whole study population to determine significant correlations between age and percentage viable counts and age and log₁₀ viable counts. Where data were to be log-transformed, and micro-organisms were not detected, the baseline was set as half of the minimum levels of detection. The latter were 10 cfu/ml of saliva or plaque sample for yeasts, lactobacilli and staphylococci, and 10³ cfu per sample for mutans and total streptococci or total cfu. This represented a detection sensitivity of about 1 in 10⁸ cfu.

Results

The mean haemoglobin concentrations of subjects in groups C and D were within the normal range and were not significantly different from those in the younger age groups, implying no latent disease in the elderly.

The mean variation in cfu (percentage varia-

Table I. Frequencies of isolation of selected micro-organisms from saliva (S) or plaque (P) as a function of age

Micro-organism	Percentage isolation frequency from group							
	A (n=30)		B (n=23)		C (n=16)		D (n=10)	
	S	P	S	P	S	P	S	P
Spirochaetes	...	43	...	30	...	44	...	50
Enterobacteria	0	0	0	0	0	0	0	0
Lactobacilli	30	10	43	9	81	13	60	0
Staphylococci	60	17	61	4	88	19	70	10
BPGNAB	...	17	...	4	...	6	...	10
Mutans streptococci	77	40	74	52	88	63	60	50
<i>A. viscosus</i>	...	23	...	17	...	44	...	30
<i>A. naeslundii</i>	...	50	...	65	...	31	...	60
Yeasts	23	10	39	22	19	6	50	30

A, 20–39 years; B, 40–59 years; C, 60–79 years; D, ≥80 years.
BPGNAB, black pigmented gram-negative anaerobic bacilli.
..., not measured.

tion = difference $\times$ 100/mean) between the total viable counts on duplicate agar plates of each saliva sample was 16.2%; the variation between repeat samples was 17.4%. The similarity between the duplicate and the replicate samples demonstrated satisfactory reproducibility in the techniques employed.

There were no significant differences in the total number of viable cells (cfu/ml) in saliva samples from individuals in the four age groups, although the lowest mean count was in those aged ≥80 years. The mean viable counts for age groups A, B, C and D were 9.22, 9.52, 9.51 and 8.60 log₁₀ cfu/ml. In contrast, statistically significant differences were found in the prevalence of certain groups of micro-organism in saliva. The isolation frequency (table I), mean log₁₀ viable counts (table II) and proportions (fig. 1) of lactobacilli in saliva were significantly higher ($p=0.01$, $p=0.002$, $p=0.006$, respectively) in those aged ≥60 years (groups C and D) than in group A. Subdivision of group C into persons aged 60–69 years (group C₁) or 70–79 years (group C₂) revealed that these increases occurred mainly in those aged ≥70 years ($p=0.01$, $p=0.001$, $p=0.004$, respectively). In the series as a whole there was a significant correlation between age and the presence of lactobacilli, expressed as either log₁₀ cfu or percentage viable count (table IV).

The isolation frequency of staphylococci from saliva

Fig. 1. Comparison of the mean percentage viable count (bar, SEM) of lactobacilli in saliva in each age group. A, 20–39 years; B, 40–59 years; C, 60–79 years; D, ≥80 years. Group C was subdivided into those aged 60–69 years (C₁), and those aged 70–79 years (C₂).

was also significantly higher ($p<0.05$) from those in groups C and D (table I) than from group A; again the increases occurred primarily in those aged ≥70 years ($p<0.05$). The mean log₁₀ viable count (table II) and proportions (fig. 2) of staphylococci were highest in groups C and D, and in group D, respectively, although these findings were not significantly different from those in group A. The higher proportions of staphylococci in saliva of those aged ≥70 years did, however, approach statistical significance ($p<0.056$). A mean of 49% of all staphylococcal isolates were coagulase positive and this proportion was similar in each age group. The isolation frequency (table I) and mean viable counts (table II) of yeasts in saliva were highest in those aged ≥80 years, and their proportions were higher in those aged ≥60 years (fig. 3), but these findings were not statistically significantly different from those in group A, mainly because of the large variation in counts between individuals. The majority of yeasts were identified as *Candida albicans* and this species was isolated from 19 (24%) of 79 subjects; 4 (5%) of 79 subjects had *C. tropicalis* and 3 (4%) of 79 subjects had *C. parapsilosis*. There was no discernible trend in the prevalence of mutans streptococci in saliva, although the highest proportions were found in those aged ≥80 years (group D) (fig. 4a). In 10 (13%) of 79 subjects salivary counts of mutans streptococci were $>10^6$ cfu/ml. No enteric bacteria were isolated from the saliva of any subject (table I).

There were no clear age-related trends in the

Table II. Viable counts of selected micro-organisms in saliva as a function of age

Micro-organism	Mean log ₁₀ (SEM) viable count from group			
	A (n=30)	B (n=23)	C (n=16)	D (n=10)
Lactobacilli*	1.76 (0.30)	1.68 (0.33)	3.67 (0.43)	3.25 (0.72)
Staphylococci†	2.71 (0.34)	2.28 (0.33)	3.43 (0.31)	3.46 (0.47)
Mutans streptococci‡	4.48 (0.20)	4.67 (0.30)	4.92 (0.30)	4.45 (0.52)
Yeasts‡	1.40 (0.24)	1.56 (0.26)	1.40 (0.38)	2.40 (0.59)

*Group A vs D, NS ($p=0.06$); group A vs C, $p<0.001$.

†Group B vs C, $p<0.02$; group B vs D, $p<0.05$.

‡No significant differences.

Table III. Viable counts of selected micro-organisms in dental plaque as a function of age

Micro-organism	Mean percentage count (SEM) in group			
	A (n = 30)	B (n = 23)	C (n = 16)	D (n = 10)
Lactobacilli	0.01 (0.01)	2.51 (2.50)	0.003 (0.001)	0
Staphylococci	0.75 (0.47)	0	0.06 (0.03)	0.001
Yeasts	0.43 (1.55)	0.07 (0.05)	0.01	0.18 (0.13)
Spirochaetes ($\times 10^6$)	142 (97)	180 (165)	42 (27)	10.9 (8.6)

isolation frequency (table I) or proportions (table III) of yeasts, lactobacilli or staphylococci from dental plaque. The cell counts of spirochaetes appeared to decrease with age on a group basis (table III) but this was not statistically significant. However, on an individual basis in the whole series, there was a significant negative correlation between age and $\log_{10}$ percentage viable count ($r = -0.245$, $n = 79$, $p < 0.05$), although there was no obvious correlation between spirochaetes and the gingival index at the sample site or in the mouth as a whole. Black-pigmented gram-negative anaerobic bacilli were isolated infrequently (but more often from group A; table I) and in low numbers from dental plaque, irrespective of age. No enteric bacteria were isolated from plaque samples (table I). In contrast, *Actinomyces* spp. were commonly detected in plaque; the majority of isolates resembled *A. viscosus* and *A. naeslundii* (table I). In the younger

age groups (A and B), *A. naeslundii* was in the highest proportions whereas the situation was reversed in those aged ≥ 60 years, in whom *A. viscosus* predominated (fig. 5). The ratios of *A. viscosus* to *A. naeslundii* in age groups A, B, C and D were 0.76, 0.25, 2.04 and 2.46, respectively. There were also lower proportions of mutans streptococci in plaque from the most elderly subjects, but this trend was not statistically significant (fig. 4b).

Discussion

There is little information about the effect of natural ageing processes on the stability of the resident human microflora. Such information could be of value to determine the treatment needs for this increasing sector of the population, and may identify individuals at risk of certain infections.

There are several problems in the design of studies which attempt to correlate changes in the microflora with age. These include lack of a universally accepted definition of any age group; clearly, the chronological age of a person does not necessarily equate with their "physiological" age. Similarly, individuals in population groups generally have to satisfy certain minimum requirements for inclusion in a study. These may relate to the presence of a certain number of teeth, the absence of dentures or of active disease, or no recent history of medical treatment. Such requirements may be met easily by most of those in the younger age groups but a study population which involves the elderly may be skewed by the inclusion of a disproportionate number of so-defined "healthy" individuals

Fig. 2. Comparison of the mean percentage viable count (bar, SEM) of staphylococci in saliva in the four age groups. **Fig. 3.** Comparison of the mean percentage viable count (bar, SEM) of yeasts in saliva in the four age groups.**Table IV.** Correlation between age and percentage viable count and $\log_{10}$ viable count

Correlation	Spearman rank correlation (Z value) with	
	percentage viable count	$\log_{10}$ viable count
Age vs yeast	0.352	0.971
Age vs lactobacilli	1.968*	3.182†
Age vs staphylococci	0.428	0.733
Age vs mutans streptococci	0.899	1.212

* $p = 0.05$.† $p = 0.002$.

Fig. 4. Comparison of the mean percentage viable count of mutans streptococci (bar, SEM) in (a) saliva, and (b) plaque in the four age groups.

who are regarded as "normal" but may not be representative of the elderly. Finally, even if quantitative differences in the microflora do occur with age, the known wide inter-site and inter-subject variations can make it difficult to establish these changes in a cross-sectional survey. Our inclusion of only healthy individuals, even in the most elderly age groups, was intended to prevent any confusion with changes in the oral microflora which might be due to disease or to any indirect age-associated factors, such as medication or denture-wearing, and is a unique feature of this study.

In the young, changes in the oral microflora that have been related directly to age are reasonably well

Fig. 5. Mean percentage viable count (bar, SEM) of *A. viscosus* (cross-hatched) and *A. naeslundii* (diagonal lines) in dental plaque in the four age groups.

established and are usually associated with major perturbations to the habitat (e.g., tooth eruption, hormonal changes, etc.).²⁻⁸ In adults, the resident microflora is believed to remain relatively stable unless the environment is disturbed, as occurs during disease, but there have been few studies of the oral microflora of healthy elderly subjects. Qualitative differences in the microbial composition of plaque between young and elderly individuals were inferred from the biochemical composition and enzyme activity of pooled samples,¹⁷ but a detailed bacteriological analysis was not reported.

The isolation frequencies of *A. viscosus*,⁹ black-pigmented gram-negative anaerobic bacilli^{4,5} and spirochaetes⁶⁻⁸ are reported to increase with age in adolescents and young adults. In the present study, no significant age-associated changes in both the prevalence or proportions of spirochaetes or black-pigmented gram-negative anaerobic bacilli were found; the presence of both groups of organism appeared to be independent of gingival inflammation. *Actinomyces* spp. were commonly isolated, but there was a marked shift in the ratio of the predominant species associated with ageing. *A. naeslundii* was in the highest proportions in the younger groups (A and B) whereas this was reversed in those aged ≥ 60 years (groups C and D) in whom *A. viscosus* predominated.

There were no clear age-related differences in either the isolation frequency or in the proportions of mutans streptococci from saliva or plaque of subjects in our study; these organisms were isolated commonly and sometimes in high numbers, even in the most elderly age group. In contrast, the recovery, absolute viable counts, and proportions of lactobacilli in saliva increased with age, particularly in those aged ≥ 70 years. A high carriage rate for cariogenic bacteria was also reported in a study of elderly Swedish individuals (mean age 85 years)¹⁸ and elderly Americans (range 60-87 years, median 68 years).¹⁹ In one of these studies, analyses revealed that lactobacilli were more prevalent in individuals wearing dentures,¹⁸ whereas our results showed higher levels of these bacteria in elderly dentate subjects proving this to be a genuine age-related observation. The combined findings from these studies suggest that those who retain natural teeth remain at risk of both enamel and root surface caries with increasing age. Many of the elderly people from these studies had $>10^6$ cfu of mutans streptococci/ml of saliva and could also act, therefore, as vectors in the transmission of these potentially cariogenic bacteria.^{18,19}

The prevalence of yeasts in saliva also increased with age, with greater isolation frequencies and viable counts in those aged ≥ 80 years, and higher proportions in those aged ≥ 60 years; although, due to the wide inter-subject variation in counts between subjects, these differences were not statistically significant. Denture-wearers were excluded from our study so that the increased prevalence of *Candida* spp. in the elderly was probably due to ageing and may reflect changes

in the activity of the host defences or a decrease in saliva flow. Enhanced oral colonisation rates with yeasts have also been found following irradiation or cytotoxic therapy. Such treatments reduce saliva flow rates which might also affect IgA levels; the latter can inhibit the adhesion of candida to oral surfaces. Thus, although total IgA levels have been reported to be similar in old (70–91 years) and in young adults, the older adults had far lower levels of specific antibodies reacting with two common mucosal antigens (*Str. mutans* glucosyl transferase and killed poliovirus).²⁰ Also, opsonic activity against a standard preparation of yeast cells was impaired in saliva samples from elderly subjects (mean age 72 years) compared with younger control individuals (mean age 33 years).²¹

Work is in progress to compare the host defences in the different age groups in our study. Preliminary results indicate a reduced whole salivary flow but not parotid salivary flow. The elderly can also suffer from various nutritional deficiencies (e.g., trace elements, vitamins) and it has been claimed that these could be predisposing factors in candidiasis,^{22,23} and hence to an increased carriage of yeasts.

In our study, enterobacteria were never isolated, even from the most elderly participants. Previously, although pseudomonads had been isolated from saliva, no trends with age had emerged.²⁴ In other studies, a range of enterobacteria (e.g., *Klebsiella* spp., *Escherichia coli*, *Proteus* spp.) have been isolated from the oropharynx of the elderly²⁵ but, perhaps significantly, the isolation rates for these organisms increased as the individuals became more debilitated and hospitalised. The prevalence of gram-negative bacilli among the oropharyngeal microflora was also low in another study of physiologically normal subjects but rose

markedly in elderly moribund patients (mean age 60 years).²⁶ The oral carriage of staphylococci is also low in healthy subjects but is higher in immunodeficient²⁷ and myelosuppressed subjects,^{28,29} and in patients with severe Sjögren's syndrome.³⁰ In our study, the isolation of staphylococci from saliva was significantly higher in those aged ≥ 70 years; their proportions and viable counts in saliva were also greater in this age group. As it has been reported that cell mediated immunity declines with age,¹¹ it is possible that homeostatic mechanisms that normally serve to maintain the balance of the resident oral microflora may become impaired in the elderly.

In summary, the isolation, viable counts and proportions of lactobacilli in saliva increased with age, as did the isolation of potential opportunist pathogens and other non-resident oral micro-organisms (yeasts and staphylococci). The ratio of the prevalence of *Actinomyces* spp. also shifted with age. Future work will attempt to determine the levels of the innate and specific host defences in the healthy subjects in different age groups described here, and then compare them with those of patients of a similar age but with various diseases. In certain individuals, alterations in the integrity of the host defences may perturb the stability of the resident oral microflora and lead to the increased likelihood of colonisation by potentially pathogenic species. These studies may help in the recognition of at-risk elderly subjects.

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References

- van Winkelhoff AJ, van Steenberghe TJM, de Graaff J. The role of black-pigmented *Bacteroides* in human oral infections. *J Clin Periodontol* 1988; **15**: 145–155.
- Socransky SS, Manganelli SD. The oral microbiota of man from birth to senility. *J Periodontol* 1971; **42**: 485–496.
- Kornman KS. Age, supragingival plaque and steroid hormones as ecological determinants of the sub-gingival flora. In: Genco RJ, Mergenhagen SE (eds) Host-parasite interactions in periodontal disease. Washington DC, American Society for Microbiology. 1982: 132–138.
- Bailit HL, Baldwin DC, Hunt EE. The increasing prevalence of gingival *Bacteroides melaninogenicus* with age in children. *Arch Oral Biol* 1964; **9**: 435–438.
- Kelstrup J. The incidence of *Bacteroides melaninogenicus* in human gingival sulci, and its prevalence in the oral cavity at different stages. *Periodontics* 1966; **4**: 14–18.
- Socransky SS, Gibbons RJ, Dale AC, Bortnick L, Rosenthal E, MacDonald JB. The microbiota of the gingival crevice area of man. I. Total microscopic and viable counts and counts of specific organisms. *Arch Oral Biol* 1963; **8**: 275–280.
- de Araujo WC, MacDonald JB. Gingival crevice microbiota of five preschool children. *J Periodontol* 1964; **35**: 285–289.
- Mikx FHM, Matee MI, Schaeken MJM. The prevalence of spirochetes in the subgingival microbiota of Tanzanian and Dutch children. *J Clin Periodontol* 1986; **13**: 289–293.
- Ellen RP. Establishment and distribution of *Actinomyces viscosus* and *Actinomyces naeslundii* in the human oral cavity. *Infect Immun* 1976; **14**: 1119–1124.
- Marsh PD. Host defenses and microbial homeostasis: role of microbial interactions. *J Dent Res* 1989; **68**: 1567–1575.
- Czonkowska A, Korlak J. The immune response during ageing. *J Gerontol* 1979; **34**: 9–14.
- Aldred MJ. Immunological changes in relation to age. *Microb Ecol Hlth Dis* 1988; **1**: 275–277.
- Hoover CI, Newbrun E. Survival of bacteria from human dental plaque under various transport conditions. *J Clin Microbiol* 1977; **6**: 212–218.
- van Palenstein Helderman WH, Ijsseldijk M, Huis in't Veld JHJ. A selective medium for the two major subgroups of the bacterium *Streptococcus mutans* isolated from human dental plaque and saliva. *Arch Oral Biol* 1983; **28**: 599–603.
- Zylber LJ, Jordan HV. Development of a selective medium for detection and enumeration of *Actinomyces viscosus* and *Actinomyces naeslundii* in dental plaque. *J Clin Microbiol* 1982; **15**: 253–259.
- Africa CW, Parker JR, Reddy J. Bacteriological studies of subgingival plaque in a periodontitis-resistant population. 1. Darkfield microscopic studies. *J Periodont Res* 1985; **20**: 1–7.
- Holm-Pedersen P, Folke LEA, Gawronski TH. Composition and metabolic activity of dental plaque from healthy young and elderly individuals. *J Dent Res* 1980; **59**: 771–776.

18. Emilson CG, Thorselius I. Prevalence of mutans streptococci and lactobacilli in elderly Swedish individuals. *Scand J Dent Res* 1988; **96**: 14–21.
19. Fitzgerald DB, Fitzgerald RJ, Adams BO, Morhart RE. Prevalence, distribution of serotypes, and cariogenic potential in hamsters of mutans streptococci from elderly individuals. *Infect Immun* 1983; **41**: 691–697.
20. Smith DJ, Taubman MA, Ebersole JL. Ontogeny and senescence of salivary immunity. *J Dent Res* 1987; **66**: 451–456.
21. Ganguly R, Stablein J, Lockey RF, Shamblin P, Vargas L. Defective antimicrobial functions of oral secretions in the elderly. *J Infect Dis* 1986; **153**: 163–164.
22. Challacombe SJ. Haematological abnormalities in oral lichen planus, candidiasis, leukoplakia and non-specific stomatitis. *Int J Oral Maxillofac Surg* 1986; **15**: 72–80.
23. Samaranayake LP. Nutritional factors and oral candidosis. *J Oral Pathol* 1986; **15**: 61–65.
24. Sutter VL, Hurst V, Landucci AO. Pseudomonads in human saliva. *J Dent Res* 1966; **45**: 1800–1803.
25. Valenti WM, Trudell RG, Bentley DW. Factors predisposing to oropharyngeal colonization with gram-negative bacilli in the aged. *New Engl J Med* 1978; **298**: 1108–1111.
26. Tillotson JR, Finland M. Bacterial colonization and clinical superinfection of the respiratory tract complicating antibiotic treatment of pneumonia. *J Infect Dis* 1969; **119**: 597–624.
27. Brown LR, Mackler BF, Levy BM *et al.* Comparison of the plaque microflora in immunodeficient and immunocompetent dental patients. *J Dent Res* 1979; **58**: 2344–2353.
28. Minah GE, Rednor JL, Peterson DE, Overholser CD, Depaola LG, Suzuki JB. Oral succession of gram-negative bacilli in myelosuppressed cancer patients. *J Clin Microbiol* 1986; **24**: 210–213.
29. Bergmann OJ, Kilian M, Ellegaard J. Potentially pathogenic microorganisms in the oral cavity during febrile episodes in immunocompromised patients with haematologic malignancies. *Scand J Infect Dis* 1989; **21**: 43–51.
30. MacFarlane TW. The oral ecology of patients with severe Sjögren's syndrome. *Microbios* 1984; **41**: 99–106.