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Oral Microbiology/Periodontology

Isolation and characterization of subgingival staphylococci from periodontitis patients and controls

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OBJECTIVES: To isolate and characterize subgingival staphylococci from patients with periodontal disease and from periodontally healthy controls, to evaluate the periodontal environment as a potential source for systemic staphylococcal infections.

METHODS: Periopaper™ strips were used to isolate subgingival staphylococci from 28 patients with chronic periodontitis and 28 periodontally healthy age and sex-matched controls. Staphylococci were identified by microbiological methods and antibiotic resistance profiles determined.

RESULTS: Staphylococci were isolated from 54% diseased subgingival and 43% healthy subgingival sites in over 50% periodontitis patients and from 29% healthy subgingival sites in 54% controls. No significant differences in the frequency of isolation or numbers of staphylococci isolated from diseased and healthy sites were noted. *Staphylococcus epidermidis* was the predominant oral species. Seventy per cent (115 of 165) of all isolates were penicillin-resistant.

CONCLUSIONS: Subgingival staphylococci are present in both periodontitis patients and controls. In periodontitis there is an increased risk of bacteraemia because of the increased dentogingival surface area. The dental and periodontal health of patients at risk from haematogenous infections should therefore be maintained at a high level. Antibiotic resistance profiles of the oral staphylococcal isolates suggest that amoxicillin may no longer be a suitable antibiotic for prophylaxis against systemic infections such as prosthetic valve endocarditis.

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Keywords: periodontal disease; staphylococci; subgingival; antibiotic resistance; prosthetic valve endocarditis

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Introduction

Staphylococcal species, in particular *Staphylococcus epidermidis* and *S. aureus*, dominate the microbial aetiology of prosthetic valve endocarditis (Bayliss *et al*, 1983; Fang *et al*, 1993; Chastre and Trouillet, 1995; Horstkotte *et al*, 1995; Gordon *et al*, 2000; Piper *et al*, 2001). Staphylococci are found in abundance on the skin and mucous membranes and, in some cases of prosthetic valve endocarditis (PVE), a skin-related origin has been identified, such as intravenous catheters, skin infections and skin wounds (Fang *et al*, 1993; Kloos, 1997). However, the oral cavity has been established as a source of the organisms for native valve endocarditis (NVE), where Viridans streptococci are responsible for 50% of cases (Debelian *et al*, 1994). Staphylococci have been isolated from the oral cavity (Sánchez-Cordero *et al*, 1979; Rams *et al*, 1990b; Slots *et al*, 1990; Jackson, 1998; Jackson *et al*, 1999), but they are not considered resident oral bacteria and are generally regarded as transient organisms (Dahlén *et al*, 1992; Dahlén and Wikström, 1995). However, periodontal pockets provide a site where non-specific bacterial adherence can occur and bacteria be retained within the oral cavity, in close proximity to the bloodstream. In periodontitis, the mean dentogingival surface area may be 8–20 cm^2 compared with 5 cm^2 in periodontally healthy persons (Slots, 2003). Moreover, the micro-ulceration of the sulcular and pocket lining epithelium facilitate bacteraemia and systemic spread of bacterial bi-products and immunocomplexes (Socransky and Manganiello, 1971; Debelian *et al*, 1994; Herzberg and Meyer, 1996).

Whilst it is not clear whether there is a causal relationship between staphylococci and chronic periodontal disease (Dahlén and Wikström, 1995), staphylococci have been isolated from subgingival sites within periodontitis patients (Rams *et al*, 1990a,b; Slots *et al*, 1990; Dahlén and Wikström, 1995). However, few subgingival plaque samples have been collected from non-diseased sites and consequently it has not been possible to determine if the isolation of staphylococci was because of the diseased state of the tissues or whether staphylococci are a feature of all subgingival sites. Moreover, little is

known of the prevalence of oral staphylococci in patients with periodontitis compared with healthy controls. The purpose of this study was to determine the prevalence of staphylococci from both healthy and diseased periodontal sites. This study aimed to:

- Isolate oral staphylococci from patients with periodontal disease and from periodontally healthy controls in order to determine how frequently they can be isolated from the periodontal environment.
- Characterize the isolated organisms with respect to species and biotype in order to investigate their diversity and persistence/transience within the oral cavity.
- Determine microbial resistance profiles, to assess the appropriateness of current prophylaxis regimes for oral and dental procedures.

Materials and methods

Patient selection

Twenty-eight previously untreated periodontitis patients (ages 32–59 years, mean age 45 years) were recruited to the study from those attending the Periodontal Department at Birmingham Dental Hospital. Ethical approval was obtained from the South Birmingham Local Research Ethics Committee (LREC number 0594). Patients were deemed suitable for inclusion in the study if they had chronic periodontitis and fitted the following criteria: subjects were in good general health with no sign of soft tissue pathology other than their periodontal inflammation; they had not received antibiotics for 3 months prior to the study or any medication that may have affected the study outcome; they had a minimum of 20 teeth with detectable subgingival deposits in all four quadrants of the mouth; a minimum bleeding score of 30% (dichotomous, % sites); a minimum plaque score of 50% (dichotomous, % sites); all subjects had a minimum of two pockets ≥5 mm, which bled upon probing, in each quadrant of the mouth; they had radiographic evidence of bone loss ≥30% at ≥2 sites per quadrant, which were non-first molar or incisor sites.

The control group consisted of 28 age and sex-matched volunteers without chronic periodontitis (ages 30–62 years, mean age 45 years).

Clinical protocol

Subgingival sampling techniques such as curettes and paper points have the disadvantage of causing gingival trauma and bleeding (Mullally *et al*, 1994). It has been demonstrated that sampling using Periopaper strips create negligible disturbances to the blood vessels during the first minute of sampling (Gustafsson *et al*, 1992), therefore Periopapers were selected as a minimally invasive sampling technique. Pilot studies (data not reported here: F. Murdoch PhD thesis, University of Birmingham, 2002), determined appropriate collection, transport, recovery and plating protocols using Periopaper strips and ascertained that mannitol salt broth (MSB) was a suitable transport medium for subgingival samples, provided processing was performed within 2 h of collection. Two trained operators recruited patients to the study according to the criteria outlined above. At the recruitment visit, the study was explained to each volunteer and informed consent was obtained. Those subjects fulfilling the recruitment criteria were enrolled into the study.

The sites chosen for sampling were isolated with sterile cotton rolls and gently air dried to remove any saliva. Subgingival bacteria were sampled by advancing a Periopaper™ strip (ORAFlow Inc., Babylon, NY, USA) into the gingival crevice/pocket until light resistance was felt. The strip was held in place for 30 s, then removed and placed in a sterile micro-centrifuge tube containing 200 μl of MSB and three glass beads (3.5 mm; PGT Scientifics, Bristol, UK). Three diseased and three healthy sites were sampled in each periodontal patient. In addition, sterile swabs (Probact, Heywood, UK) were used to sample bacteria from the anterior palate (PS) and the floor of the mouth (FOM). The process was repeated for the control subjects, but only three healthy subgingival sites were sampled. In addition, bacteria from the skin of the control group were sampled by pressing the volunteers' fingertips directly onto a mannitol salt agar plate and bacteria from the anterior nares were sampled using a sterile swab. All samples were maintained at room temperature and processed within 1 h of collection. A diseased site was defined as one that had > 5 mm pocketing, radiographic evidence of bone loss ≥30% and bleeding on probing (BOP) at the enrolment visit. Healthy sites had no BOP, probing pocket depths < 3 mm and no radiographic evidence of bone loss. All samples were collected within 10 days of the enrolment visit.

Care was taken to ensure that the paper strips were not contaminated by the patient's own flora prior to or after insertion into the crevice/pocket, by isolating the gingival margin with cotton rolls and discarding samples that touched the patients skin or lips. The patient's nostrils were shielded from the paper strips during insertion and removal, and saturated strips were also discarded, as this was a potential indication of saliva contamination. If a sample was discarded, an alternative site was selected and sampled.

To minimize contamination from the clinicians involved in sample collection, each wore a tight fitting surgical mask and gloves. In addition, all instruments and sampling materials were sterilized and only opened at the time of sampling, and all transport and isolation media were sterilized and rigorously quality controlled.

Culture and identification

Each sample was vortex-mixed for 10 s to disperse the bacteria from the Periopaper. In a Class II laminar flow hood the MSB from each tube was divided into two 100 μl aliquots and spread onto two MSA plates. The Periopaper strips were then removed with sterile forceps and streaked across an additional MSA plate eight times. Bacteria from the swabs were also inoculated onto MSA. All plates were incubated at 37°C in air for 48 h.

Staphylococci were identified by clonal morphology, a positive Gram stain and catalase reaction and a negative

oxidase reaction. Staphylococcal species were identified by a coagulase tube test and from the numerical profiles obtained from biochemical reactions in the API Staph system (bioMérieux (UK) Ltd., Basingstoke, UK), with reference to the API Staph Analytical Profile Index (bioMérieux). The same staphylococcal species may comprise several different biotypes, as identified by the numerical profile.

Repeat sampling

A longitudinal study was undertaken, whereby five patients undergoing regular treatment for periodontal disease were sampled repeatedly for oral staphylococci over a period of 2–25 weeks, depending on how often they attended the clinic, to establish if staphylococci with the same biotype could be repeatedly isolated from the same site.

Antibiotic sensitivity testing

Antibiotic sensitivity tests were performed by the disc diffusion method recommended by the British Society of Antimicrobial Chemotherapy (BSAC). In brief, a standard inoculum of the test organism with a density equal to that of a 0.5 McFarland standard was prepared in iso-sensitest broth (Oxoid Ltd., Basingstoke, UK), and then diluted 1:10 with distilled water. The prepared inoculum was evenly spread over the entire surface of iso-sensitest agar plates (ISTA; Oxoid) and the antimicrobial discs were firmly applied to each plate. The plates were then incubated at 37°C in air for 18–20 h, after which time the zones of inhibition were measured. To test for methicillin resistance, Columbia agar base (Oxoid) supplemented with 2% sodium chloride was used instead of ISTA, and the plates were incubated at 30°C for 18–20 h.

Resistance to the following antibiotics (Oxoid) was tested: Augmentin 3 μg, fusidic acid 10 μg, penicillin G 1 unit, tetracycline 10 μg, clindamycin 2 μg, gentamicin 10 μg, teicoplanin 30 μg and vancomycin 5 μg. Each test was performed in triplicate with control strains (*S. aureus* NCTC 6571 and methicillin resistant *S. aureus* NCTC 12493).

Statistical analysis

Differences in the numbers of colony forming units (cfu) isolated from the different paper types were analysed by analysis of variance (*post hoc* Tukey test). Where appropriate, differences between clinical groups were analysed by Fisher's exact test, except for differences in the numbers of cfu isolated, which were analysed using a Kolmogorov–Smirnov test. All tests were performed using SPSS software (version 10). A value of $P < 0.05$ was taken to represent statistical significance.

Results

Isolation of subgingival staphylococci using Periopaper™

No attempt was made to quantify numbers of staphylococci in comparison with other organisms as this study was intended for detection of staphylococci, and hence selective media for their transport and culture were used. The numbers of cfu of staphylococci isolated from the Periopaper strips were low, often below 10 (range 1 to >100), however, these isolates were included in the study because of the rigorous sampling and dispersal protocols used, and because no skin barriers are crossed when sampling from the oral cavity.

Staphylococci were isolated from both healthy and diseased subgingival sites, the palate and the FOM of both periodontitis patients and healthy controls. The percentages of patients positive for staphylococci are displayed in Figure 1. Twenty-nine per cent of periodontitis patients and 46% of control patients were negative for staphylococci. There was no significant difference in the frequency of isolation between either the diseased subgingival sites and the control sites, or the healthy subgingival sites (patients) and the control sites (control subjects), $P > 0.05$. There was also no significant difference in the numbers of cfu isolated from the periodontal patients compared with the control groups (periodontitis patients diseased sites and control $P = 0.45$, patient healthy sites and control $P = 0.94$). Within the periodontitis group, there was no significant difference ($P > 0.05$) in the frequency of isolation of staphylococci from the diseased subgingival sites compared with the healthy subgingival sites, or in the number of cfu isolated ($P = 0.99$).

The percentage of patients from which staphylococci were isolated from the palate and/or the FOM was the same for both the periodontal and control group and there was no significant difference in the frequency of isolation ($P > 0.05$). There was no significant difference in the frequency of isolation of subgingival staphylococci between male and female patients ($P > 0.05$).

Distribution of staphylococcal species within the oral cavity

The majority of staphylococcal species isolated from the oral cavity were coagulase negative (Table 1). The most frequently isolated species from each habitat sampled was *S. epidermidis*, which was isolated from 64.3% of periodontal patients and 42.9% of control subjects. *Staphylococcus aureus* was isolated from 7.1% of periodontal patients and 3.6% of control subjects. There was no significant difference in the isolation

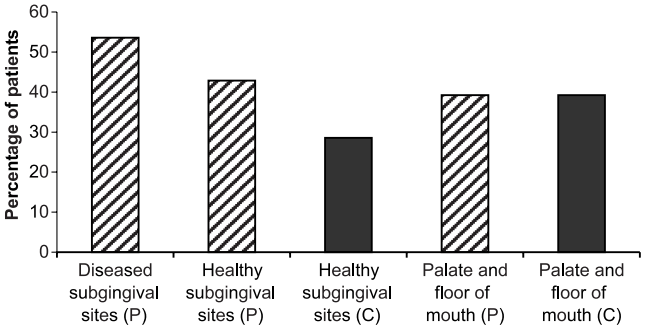

Figure 1 Species of staphylococci isolated from the oral cavity. Percentages of patients or controls positive for the isolation of staphylococci from at least one diseased or healthy subgingival site and from the mucosal surfaces. Isolates from the nares and fingertips of controls are also shown. P, periodontal patients; C, control subjects

Table 1 Species distribution of isolates from each habitat sampled

	Percentage of isolates						
	Periodontal patients n = 28			*Healthy controls n = 28*			
Species	*Diseased subgingival*	*Healthy subgingival*	*Palate and/or floor of mouth*	*Healthy subgingival*	*Palate and/or floor of mouth*	*Skin fingertips*	*Nares*
Isolates (patients)	41 (16)	33 (14)	31 (13)	18 (8)	14 (11)	34 (20)	39 (22)
S. epidermidis	80.5	69.7	74.2	66.7	71.4	41.2	64.1
S. capitis	9.8	3	3.2	5.5	7.1	23.5	
S. hominis	7.3	3	6.5	16.7	7.1	5.9	2.6
S. warneri	2.4	6.1	3.2	11.1	–	2.9	
S. aureus	–	15.2	9.7	–	14.3	11.8	30.8
S. cohnii	–	3	–	–	–		2.6
S. lugdunensis	–	–	3.2	–	–	2.9	
S. intermedius						2.9	
S. saprophyticus						5.9	
S. haemolyticus						2.9	

S., *Staphylococcus.*

frequency of either of these species from the two study groups ($P > 0.05$).

In both the diseased and healthy subgingival groups *S. epidermidis* accounted for 70–80% of the total number of isolates. *Staphylococcus capitis*, *S. hominis* and *S. warneri* were also present in all groups, albeit in much smaller numbers. There was no significant difference in the frequency of isolation of these species between the diseased sites and both sets of healthy sites ($P > 0.05$). However, the frequency of *S. aureus* isolation from the healthy subgingival sites in the periodontal patients was significantly greater than from the diseased subgingival sites ($P = 0.02$), but there was no significant difference between the healthy subgingival sites and the control subgingival sites ($P > 0.05$).

Staphylococcal isolates from the skin and nares

Staphylococci were isolated from the skin and the anterior nares of the control patients. The predominant species was *S. epidermidis*, as with the oral groups, but there was a much greater species variation on the skin. There was less species variation from the anterior nares and *S. aureus* accounted for approximately 30% of these isolates (Table 1).

Biotype profiles and investigation into the transient nature of oral staphylococci

A combined total of 20 different biotypes (as determined by the API Staph profiles) were observed for *S. epidermidis* species isolated from subgingival sites and from the palate and FOM of both periodontal patients and healthy controls. Isolates with the same API Staph profiles were recovered from both subgingival sites and from either/or the palate and FOM, of seven periodontitis patients (25%), and from multiple subgingival sites in four of the periodontitis patients (14%). These trends were not observed in the healthy controls, except for one subject, who had isolates with the same API profile recovered from two subgingival sites.

The longitudinal study undertaken with five periodontal patients showed that although in some patients the same staphylococcal species, (*S. epidermidis*), could be repeatedly isolated from an individual subgingival site, in none of the patients was a single biotype repeatedly isolated from the same subgingival site over a period of weeks. In two patients, *S. epidermidis* strains with a single biotype (different for each patient) were isolated from the palate on consecutive occasions, 2 and 9 weeks apart respectively. Staphylococci were isolated from the FOM less frequently and no patterns of isolation were observed. If the oral cavity was taken as a whole, three patients had *S. epidermidis* strains with the same biotype repeatedly isolated over a period of weeks, but from different sites on each occasion.

Antibiotic sensitivity tests

The percentage of resistant isolates from each oral habitat tested is displayed in Figure 2. Seventy per cent (115 of 165) of all oral staphylococcal isolates were resistant to penicillin. There was no significant difference in the observed frequencies of antibiotic resistance between the diseased and healthy subgingival sites. Eighteen per cent of isolates were resistant to fusidic

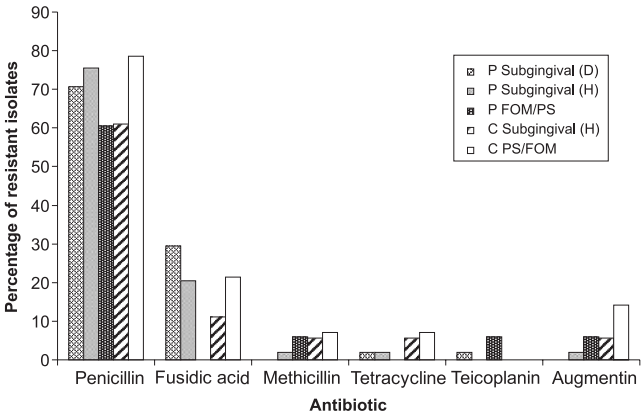

Figure 2 Percentage of staphylococci from each oral habitat sampled displaying antibiotic resistance. P, periodontal; C, control; D, diseased; H, healthy

acid. Again, there was no significant difference in the frequency of resistance between the diseased and healthy subgingival sites. However, there was a significantly greater frequency of fusidic acid resistance in the diseased subgingival sites ($P < 0.01$), and the healthy subgingival sites ($P = 0.05$) compared with the mucosal surfaces (PS and FOM) of the periodontitis patients. This was not observed in the control patients. Three per cent of isolates were resistant to methicillin. Less than 2.5% were resistant to tetracycline, teicoplanin and Augmentin. No isolates were resistant to clindamycin, gentamicin or vancomycin.

Discussion

This study aimed to isolate and characterize subgingival staphylococci from patients with periodontal disease and from periodontally healthy controls, to examine the prevalence of oral staphylococci in both health and disease and to evaluate the periodontal environment as a potential source of systemic staphylococcal infections. Prosthetic valve endocarditis was highlighted here as an example of a systemic infection in which staphylococci are the major causative organisms, and because an oral origin has been established in the case of NVE. However, staphylococci dominate the aetiology of many other biomaterials-related infections (Stickler and Maclean, 1995; Gristina, 1994). These include infections associated with materials and devices that come into direct and constant contact with blood, such as vascular graft materials, and others such as artificial joints, which may nevertheless be susceptible to haemtogenous infections (Bartzokas *et al*, 1994; LaPorte *et al*, 1999; Waldman *et al*, 1997; Smith *et al*, 2001). An oral route has not been established for such infections, but must be considered a possibility.

Staphylococci were isolated from at least one diseased site in 54% of periodontal patients. Whilst there are some discrepancies within the literature regarding the prevalence of subgingival staphylococcal species, this figure is comparable with those of Rams *et al* (1990b) who isolated staphylococci from 50.4% of patients with advanced adult periodontitis and Dahlén and Wikström (1995), who isolated *S. epidermidis* from 54.4% of patients. In both these studies the patient cohort was >500 patients. In other studies the prevalence of staphylococci was recorded as both higher, 75% (Rawlinson *et al*, 1993, Tanner *et al*, 1979) and lower, 28% (Slots *et al*, 1990) and 5.6% (Listgarten *et al*, 1993), than the two former studies.

Unlike diseased subgingival sites (Sánchez-Cordero *et al*, 1979; Moore *et al*, 1985; Rams *et al*, 1990b; Rawlinson *et al*, 1993; Dahlén and Wikström, 1995; Kamma *et al*, 1999) very few studies have attempted to isolate staphylococci from healthy subgingival sites, and none have directly compared the prevalence of staphylococci in both healthy and diseased sites within the same patient. In the current study, there was no significant difference in the frequency of isolation of staphylococci or numbers of cfu isolated from the diseased subgingival sites and the healthy subgingival sites of the periodontitis patients or the frequency of isolation from healthy sites in periodontitis and the control subjects. This suggests that staphylococci can be isolated from the subgingival environment irrespective of disease status. In addition, no correlation between gender and the isolation of subgingival staphylococci was observed in either subject group; this is in agreement with Dahlén and Wikström (1995).

With the exception of the tongue there is minimal literature available on the isolation of staphylococci from mucosal surfaces. In the current study, staphylococci were isolated from the palate and/or floor of the mouth of approximately 40% of both the periodontitis and control subject groups. Rams *et al* (1990a) noted that subjects harbouring subgingival staphylococci also tended to harbour staphylococci on the anterior palate and the dorsum of the tongue, which was observed in the present study in approximately 25% of patients.

The predominant staphylococcal species isolated from the oral cavity was *S. epidermidis*, which is in agreement with other studies (Rams *et al*, 1990b; Dahlén and Wikström, 1995; Jackson *et al*, 1999). *Staphylococcus epidermidis* is also the most frequently isolated species in cases of PVE (Calderwood *et al*, 1985; Chastre and Trouillet, 1995; Horstkotte *et al*, 1995; Dyson *et al*, 1999; Gordon *et al*, 2000; Piper *et al*, 2001). *Staphylococcus aureus* was also isolated from the oral cavity, albeit in smaller numbers than *S. epidermidis*, but it is also considered a major causative organism of PVE (Chastre and Trouillet, 1995; Horstkotte *et al*, 1995; Gordon *et al*, 2000; Piper *et al*, 2001), and *S. aureus* PVE carries a high mortality rate (Wolff *et al*, 1995; Tornos *et al*, 1997). Interestingly, the frequency of *S. aureus* isolation from the healthy subgingival sites was significantly greater than from the diseased subgingival sites ($P = 0.02$), but there was no significant difference between the healthy subgingival sites in periodontal patients and the control subgingival sites ($P > 0.05$). As 60% of *S. aureus* isolates from the healthy subgingival sites came from a single patient, and there was no significant difference between the healthy subgingival sites and the control sites, it is possible that the data has been skewed by the results from this patient. If the study was repeated with a larger patient group, it is likely that the difference in *S. aureus* isolation would not be significant.

Staphylococcus capitis, *S. hominis* and *S. warneri* were also isolated from each type of subgingival site. These have previously been isolated from diseased subgingival sites (Moore *et al*, 1985; Rams *et al*, 1990b; Colombo *et al*, 1998). In the present study, there was no significant difference in the frequency of isolation of these species from diseased and healthy sites, and each species was isolated in very low numbers. By comparison Moore *et al* (1985) noted that in 21 patients with juvenile periodontitis (ages 10–28), *S. hominis* and *S. capitis* were isolated in higher numbers from diseased sites than healthy sites. However Moore *et al* (1985) used a combination of paper points and curettes to sample the subgingival bacteria, therefore a larger proportion of the subgingival flora was being sampled.

Alternately it may be characteristic of the flora associated with juvenile periodontitis relative to that of the chronic periodontitis subjects in this study.

Staphylococcus capitis and *S. hominis* were also isolated from the mucosal surfaces of both patient groups. Both these species have been responsible for several documented cases of PVE (Fleurette *et al*, 1987; McCartney *et al*, 1987; Bandres and Darouiche, 1992; Terada *et al*, 1996; Rodriguez *et al*, 1999).

The finding that *S. epidermidis* was the predominant species isolated from both the skin and the nares (Table 1) is in agreement with several studies (Kloos, 1997; Kloos and Musselwhite, 1975). *Staphylococcus capitis* is not normally a predominant organism on the skin, but the high percentages shown in Table 1 may reflect the site of isolation. *Staphylococcus capitis* reaches climax populations in the scalp following puberty (Kloos, 1997; Kloos and Musselwhite, 1975), and as the fingertips are often in contact with the hair and scalp, this is the most likely reason for the elevated proportions of this species.

To investigate whether staphylococci are transient organisms or part of the resident flora within the oral cavity, a longitudinal study was undertaken in a subgroup of patients to determine if the same staphylococcal species could be repeatedly isolated from the same site. The results of the study are in agreement with the current opinion that staphylococci are transient organisms (Dahlén *et al*, 1992; Dahlén and Wikström, 1995; Marsh and Martin, 1999), as in none of the oral habitats studied were staphylococci with the same phenotypic characteristics regularly isolated in high numbers. During the 5-week course of treatment the periodontal condition of patients improved (data not shown) but there was no change in the number of cfu or staphylococci isolated from the subgingival environment during this period, in any patients. These results agree with those of Rams *et al* (1990b) and Tanner *et al* (1979).

Numbers of cfu isolated were low but this was not unexpected as staphylococci have been reported to make up a very small proportion of the total cultivable subgingival flora, in many cases $<1\%$ (Rams *et al*, 1990b, 1996; Rawlinson *et al*, 1993; Dahlén and Wikström, 1995; Edwardsson *et al*, 1999). It would have been interesting in this study to have compared staphylococcal cfu ml^{-1} in relation to total numbers of bacteria cfu ml^{-1} recovered, to obtain a quantitative estimate of the proportion of staphylococci in the total subgingival bacterial populations. Mullally *et al* (1994) investigated percentage recovery of subgingival organisms from periodontitis patients by Periopaper sampling, in comparison with curettes. Periopapers recovered 18, 7 and 36% cfu ml^{-1} of total cfu ml^{-1} recovered by curettes in periodontitis, gingivitis and healthy sites, respectively. In this study, the sampling time was 30 rather than 5 s, as used by Mullaly *et al*, but the recovery is likely to be similar and hence numbers of staphylococci isolated may be an underestimate of the actual numbers present.

Whilst staphylococci may be considered transient organisms, their frequent presence in the oral cavity must be considered as a potential source of infection (Smith and MacFarlane, 1999). Systemic diseases from oral bacteria are mostly caused by transient bacteraemias, which can occur spontaneously from mastication, toothbrushing, flossing or from dental surgical procedures (Slots, 2003). The risk of infection is enhanced in patients with periodontal disease because of the increased risk of bacteraemia due to the proximity of the organisms to the bloodstream via ulcerations within the pocket or crevice epithelium (Herzberg and Meyer, 1996; Slots, 2003). In a recent article on the general health risk of periodontal disease, Slots (2003) concluded 'maintenance of a healthy dentition and periodontium by means of daily oral hygiene and professional care is the most effective way of preventing systemic diseases from oral infections'. Given the frequency with which staphylococci can be isolated from the periodontal tissues, this advice would seem to be especially pertinent, not only to immuno-compromised patients, but also to those at risk from staphylococcal infections, including the increasing numbers of elderly patients with prosthetic heart valves and joint replacements.

Staphylococci must also be considered in antibiotic administration, because of the risk of infection and positive selection. The current prophylactic antibiotic regime recommended by both the working party of the BSAC (Seymour *et al*, 2000) and the American Heart Association (AHA, Dajani *et al*, 1997) for 'at risk' patients undergoing periodontal procedures such as scaling and root surface debridement, and who are not allergic to penicillin, is oral amoxycillin, although the dosage recommended by the two governing bodies is different (BSAC 3 g; AHA 2 g). Clindamycin is recommended for those allergic to penicillin.

This regime is based on the premise that the majority of cases of infective endocarditis are caused by streptococci. Whilst this is true for NVE (Bayliss *et al*, 1983; Fiehn *et al*, 1995), the majority of cases of PVE are caused by staphylococcal species, and *S. aureus* is now considered the most common cause of infective endocarditis (Lamas and Eykyn, 1997; Mylonakis and Calderwood, 2001). Under normal conditions, limitation of the available nutrients by the host defences, phagocytosis and the immune response would prevent the growth and spread of staphylococci, however valvular biomaterial surfaces disrupt the normal defence mechanisms, providing conditions favouring biofilm formation, bacterial growth and dissemination (Gristina, 1994; Gristina and Naylor, 1996). The presence of such biomaterials also allows infection by smaller inoculi of bacteria (Gristina, 1994). The current chemoprophylactic regime of amoxycillin is not directed towards these pathogens.

In the current study, amoxycillin was not specifically tested for, as the BSAC does not provide guidelines for testing the resistance of staphylococci to amoxycillin as its activity is judged by methicillin testing. If an organism is resistant to methicillin it is considered to be resistant to amoxycillin and co-amoxyclav (Augmentin). Three per cent of the oral staphylococcal isolates were resistant to methicillin and 70% were resistant to penicillin. Like

penicillin, amoxycillin is susceptible to the action of β-lactamases, produced by many staphylococcal species, and which are also frequently found in periodontal pockets (Walker *et al*, 1987), as they are produced by periodontal pathogens such as *Bacteroides* species (Legg and Wilson, 1990). Therefore, it is likely that the large percentage of oral staphylococci resistant to penicillin will also be resistant to amoxycillin, because of the action of β-lactamases. A study by Packer *et al* (1999) suggested that amoxycillin-resistant organisms are frequently, although transiently, present in low numbers in the plaque of individuals who have not received antibiotics. In their study 19% of the resistant organisms were staphylococci. Consequently, using amoxycillin for prophylaxis may not be effective against staphylococcal infection and may even encourage an overgrowth of staphylococci, potentially increasing the numbers able to enter the bloodstream. This could have particularly serious implications for patients with periodontitis, where micro-ulceration of the pocket lining epithelium could allow staphylococci to enter the bloodstream (Herzberg and Meyer, 1996) and spread haematogenously. As there is also mounting evidence to suggest that streptococcal species have developed resistance to amoxycillin, making prophylaxis less effective against these strains (Hess *et al*, 1983; Doern *et al*, 1996), it would appear that the use of amoxycillin for prophylaxis against PVE requires careful and comprehensive review.

Conclusion

The results of this study demonstrate that staphylococci can be isolated from both healthy and diseased subgingival sites. The presence of low numbers of staphylococci subgingivally and adjacent to significant areas of sulcular micro-ulceration in sites of active periodontitis, albeit transient, may be a source of systemic infection and suggests that the dental and periodontal health of patients at risk of haematogenous infection should be maintained. Current British National Formulary (Version 41; 2001) advice for risk procedures performed under general anaesthesia is that intra-venous amoxycillin and gentamicin are indicated, whereas for the same procedures performed under local anaesthesia is that oral amoxycillin is the antibiotic of choice. This advice may need to be reviewed if, as this and other studies suggest, staphylococcal isolates are as frequently present in the periodontal environment and are becoming resistant to amoxycillin.

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References

Bandres JC, Darouiche RO (1992). *Staphylococcus capitis* endocarditis: a new cause of an old disease. *Clin Infect Dis* **14:** 366–367.

Bartzokas CA, Johnson R, Jane M, Martin MV, Pearce PK, Saw Y (1994). Relation between mouth and hematogenous infection in total joint replacements. *BMJ* **309:** 506–508.

Bayliss R, Clarke C, Oakley C *et al* (1983). The microbiology and pathogenesis of infective endocarditis. *Br Heart J* **50:** 513–519.

Calderwood SB, Swinski LA, Waternaux CH *et al* (1985). Risk factors for the development of prosthetic valve endocarditis. *Circulation* **72:** 31–37.

Chastre J, Trouillet JL (1995). Early infective endocarditis on prosthetic valves. *Eur Heart J* **16** (Supplement B)**:** 32–38.

Colombo AP, Haffajee AD, Dewhirst FE *et al* (1998). Clinical and microbiological features of refractory periodontitis subjects. *J Clin Periodontol* **25:** 169–180.

Dahlén G, Wikström M (1995). Occurrence of enteric rods, staphylococci and *Candida* in subgingival samples. *Oral Microbiol Immunol* **10:** 42–46.

Dahlén G, Jonsson R, Öhman S-C *et al* (1992). Infections of oral mucosa and submucosa. In: Slots J, Taubman MA, eds. *Contemporary oral microbiology and immunology*. Mosby Year Book Inc: USA, pp. 476–477.

Dajani AS, Taubert KA, Wilson W *et al* (1997). Prevention of bacterial endocarditis: recommendations of the American Heart Association. *J Am Dent Assoc* **128:** 1142–1151.

Debelian G, Olsen I, Tronstad L. (1994). Systemic diseases caused by oral microorganisms. *Endodontics and Dental Traumatology* **10:** 57–65.

Doern GV, Ferraro MJ, Brueggemann AB *et al* (1996). Emergence of high rates of antimicrobial resistance among Viridans group streptococci in the United States. *Antimicrob Agents Chemother* **40:** 891–894.

Dyson C, Barnes RA, Harrison GAJ (1999). Infective endocarditis: an epidemiological review of 128 episodes. *J Infect* **38:** 87–93.

Edwardsson S, Bing M, Axtelius B, Lindberg B, Söderfeldt B, Attström R. (1999). The microbiota of periodontal pockets with different depths in therapy-resistant periodontitis. *J Clin Periodontol* **26:** 143–152.

Fang G, Keys T, Gentry L *et al* (1993). Prosthetic valve endocarditis resulting from nosocomial bacteremia. A prospective, multicenter study. *Ann Intern Med* **119:** 560–567.

Fiehn N-E, Gutschik E, Larsen T *et al* (1995). Identity of Streptococcal blood isolates and oral isolates from two patients with infective endocarditis. *J Clin Microbiol* **33:** 1399–1401.

Fleurette J, Brun Y, Bes M *et al* (1987). Infections caused by coagulase-negative staphylococci other than *S. epidermidis* and *S. saprophyticus*. In: Pulverer G, Quie PG, Peters G, eds. *Pathogenicity and clinical significance of coagulase-negative staphylococci*. Gustav Fischer Verlag: Stuttgart, pp. 195–208.

Gordon SM, Serkey JM, Longworth DL *et al* (2000). Early onset prosthetic valve endocarditis: the Cleveland Clinic experience 1992–1997. *Ann Thorac Surg* **69:** 1388–1392.

Gristina AG (1994). Implant failure and the immune-incompetent fibro-inflammatory zone. *Clin Orthop* **298:** 106–118.

Gristina AG, Naylor PT (1996). Implant-associated infection. In: Ratner BD, Hofmann AS, Schoen FJ, Lemons JE, eds. *Biomaterials science. An introduction to materials in medicine*. Academic Press: USA, pp. 205–214.

Gustafsson A, Åsman B, Bergström K *et al* (1992). Granulocyte elastase in gingival crevicular fluid. A possible discriminator between gingivitis and periodontitis. *J Clin Periodontol* **19:** 535–540.

Herzberg M, Meyer M (1996). Effects of oral flora on platelets: possible consequences in cardiovascular disease. *J Periodontol* **67:** 1138–1142.

Hess J, Dankert J, Durack D (1983). Significance of penicillin tolerance *in vivo*: prevention of experimental *Streptococcus sanguis* endocarditis. *J Antimicrob Chemother* **11:** 555–564.

Horstkotte D, Piper C, Niehues R *et al* (1995). Late prosthetic valve endocarditis. *Eur Heart J* **16** (Supplement B)**:** 39–47.

Jackson MS (1998). *Oral staphylococci in health and disease*. FIBMS Thesis. University of Glasgow: Institute of Biomedical Science.

Jackson M, Bagg J, Gupta M *et al* (1999). Oral carriage of staphylococci in patients with rheumatoid arthritis. *Rheumatology (Oxford)* **38:** 572–575.

Kamma J, Nakou M, Baehni P (1999). Clinical and microbiological characteristics of smokers with early onset periodontitis. *J Periodontal Res* **34:** 25–33.

Kloos WE, Musselwhite MS (1975). Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Appl Microbiol* **30:** 381–395.

Kloos W (1997). Taxonomy and systematics of staphylococci indigenous to humans. In: Crossley KB, Archer GL, eds. *The staphylococci in human disease*. Churchill Livingston: New York, pp. 113–137.

Lamas CC, Eykyn SJ (1997). Suggested modifications to the Duke criteria for the clinical diagnosis of native and prosthetic valve endocarditis: analysis of 118 pathologically proven cases. *Clin Infect Dis* **25:** 713–719.

LaPorte DM, Waldman BJ, Mont MA, Hungerford DS (1999). Infections associated with dental procedures in total hip arthroplasty. *J Bone Joint Surg Br* **81:** 56–59.

Legg JA, Wilson M (1990). The prevalence of beta-lactamase producing bacteria in subgingival plaque and their sensitivity to Augmentin. *Br J Oral Maxillofac Surg* **28:** 180–184.

Listgarten MA, Lai C, Young V (1993). Microbial composition and pattern of antibiotic resistance in subgingival microbial samples from patients with refractory periodontitis. *J Periodontol* **64:** 155–161.

McCartney AC, McGovern T, Cobb S *et al* (1987). The measurement of C-reactive protein and immune complexes in endocarditis caused by coagulase-negative staphylococci. *J Infect* **15:** 213–219.

Marsh P, Martin MV (1999). The resident oral microflora. In: *Oral Microbiology*. Wright, Oxford, pp. 17–33.

Moore L, Holdeman L, Cato E *et al* (1985). Comparative bacteriology of juvenile periodontitis. *Infect Immun* **48:** 507–519.

Mullally B, Wolff L, Hardie N *et al* (1994). Effect of gingival fluid collection on subgingival plaque sampling. *J Dent* **22:** 223–228.

Mylonakis E, Calderwood SB (2001). Infective endocarditis in adults. *N Engl J Med* **345:** 1318–1330.

Packer S, Woodley N, Wilson M, Mullany P (1999). Prevalence and persistence of amoxycillin-resistant bacteria in the dental plaques of adults. *Microbios* **100:** 135–144.

Piper C, Körfer R, Horstkotte D (2001). Prosthetic valve endocarditis. *Heart* **85:** 590–593.

Rams TE, Babalola OO, Slots J (1990a). Subgingival occurrence of enteric rods, yeasts and staphylococci after systemic doxycycline therapy. *Oral Microbiol Immunol* **5:** 166–168.

Rams TE, Feik D, Slots J (1990b). Staphylococci in human periodontal diseases. *Oral Microbiol Immunol* **5:** 29–32.

Rams TE, Listgarten MA, Slots J (1996). Utility of 5 major putative periodontal pathogens and selected clinical parameters to predict periodontal breakdown in patients on maintenance care. *J Clin Periodontol* **23:** 346–354.

Rawlinson A, Duerden BI, Goodwin L (1993). New findings on the microbial flora associated with adult periodontitis. *J Dent* **21:** 179–184.

Rodriguez AD, González MJ, Padrón AL *et al* (1999). Infectious endocarditis in bioprosthetic valves caused by *Staphylococcus capitis*: a new case. *Revista Española de Cardiologia* **52:** 277–278.

Sánchez-Cordero S, Hoffman H, Stahl SS (1979). Occurrence of staphylococci in periodontal pockets of diabetic and non-diabetic adults. *J Periodontol* **50:** 109–113.

Seymour RA, Lowry R, Whitworth JM, Martin MV (2000). Infective endocarditis, dentistry and antibiotic prophylaxis; time for a rethink? *Br Dent J* **189:** 610–616.

Slots J (2003). Update of general health risk of periodontal disease. *Int Dent J* **53**(Suppl. 3)**:** 200–207.

Slots J, Feik D, Rams TE (1990). Age and sex relationships of superinfecting microorganisms in periodontitis patients. *Oral Microbiol Immunol* **5:** 305–308.

Smith A, MacFarlane WT (1999). Fungi and other opportunistic pathogens in plaque. In: Newman HN, Wilson M, eds. *Dental plaque revisited bioline*. Cardiff: UK, pp. 387–417.

Smith AJ, Jackson MS, Bagg J (2001). The ecology of *Staphylococcus* species in the oral cavity. *J Med Microbiol* **50:** 940–946.

Socransky SS, Manganiello S (1971). The oral microbiota of man from birth to senility. *J Periodontol* **42:** 485–494.

Stickler DJ, Maclean RJC (1995). Biomaterials-associated infections: the scale of the problem. *Cells and Materials* **5:** 167–182.

Tanner ACR, Haffer C, Bratthall GT *et al* (1979). A study of the bacteria associated with advancing periodontitis in man. *J Clin Periodontol* **6:** 278–307.

Terada Y, Mitsui T, Enomoto Y (1996). Prosthetic valve endocarditis caused by *Staphylococcus capitis*. *Ann Thorac Surg* **62:** 324.

Tornos P, Almirante B, Olona M *et al* (1997). Clinical outcome and long-term prognosis of late prosthetic valve endocarditis: a 20 year experience. *Clin Infect Dis* **24:** 381–386.

Waldman BJ, Mont MA, Hungerford DS (1997). Total knee arthroplasty infections associated with dental procedures. *Clin Orthop* **343:** 164–172.

Walker CB, Tyler KT, Low SB (1987). Penicillin-degrading enzymes in sites associated with adult periodontitis. *Oral Microbiology Immunology* **2:** 129–131.

Wolff M, Witchitz S, Chastang C (1995). Prosthetic valve endocarditis in the ICU. Prognosis factors of overall survival in a series of 122 cases and consequences for treatment decision. *Chest* **108:** 688–694.