# Occurrence of staphylococci in oral cavities of healthy adults and nasal-oral trafficking of the bacteria

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# SUMMARY

To investigate a possible peroral route of infective endocarditis (IE), the occurrence of staphylococci in the oral cavity was examined using saliva and supragingival plaque specimens from 56 systemically and periodontally healthy adults aged 22-43 years old (27·1 ± 5·3). Nine *Staphylococcus* species and 334 isolates were identified. In saliva, the total occurrence rate was 83·9 % and total amount of bacteria was $10^2$-$10^4$ c.f.u. ml$^{-1}$. *S. aureus* was the most frequent species (46·4 %), followed by *S. epidermidis* (41·1 %) and others (*S. hominis, S. warneri, S. intermedius, S. capitis, S. haemolyticus, S. lugdunensis,* and *S. gallinarum*, isolation frequencies ranging in order from 12·5 % to 1·8 %). A similar isolation tendency was observed in supragingival plaque, with a total occurrence rate of 73·2 % and amounts of bacteria ranging from $10^2$-$10^5$ c.f.u. g$^{-1}$. Four common *Staphylococcus* species (*S. aureus, S. epidermidis, S. lugdunensis,* and *S. hominis*) were isolated from nasal swab samples taken from the oral staphylococci-positive subjects. Genotyping of all 18 combinations of oral- and nasal-derived isolates by PFGE indicated that identical clones or close relatives were commonly distributed in these two cavities. Since the provision of microorganisms from the nasal cavity was shown and occurrence rates in the oral cavity were adequate, these results suggest a possible peroral route of staphylococcal IE, as in cases of viridans streptococcal IE.

# INTRODUCTION

Infective endocarditis (IE) is an uncommon but life-threatening disease of the endocardium of the heart. The most common causative microorganisms are viridans streptococci, which have been isolated in the blood cultures taken from one third to one half of IE patients (Strom *et al.,* 1998; Nakatani *et al.,* 2003; Di Filippo *et al.,* 2006; Ferreiros *et al.,* 2006). Since viridans streptococci comprise a large proportion of resident oral microflora, their high frequency of isolation from IE patients indicates functional importance of the tooth-tissue interface as a unique site for these bacteria to enter the blood stream and potentially infect the heart. Thus, much attention has been given to the predisposing conditions of dental procedures and dental hygiene to prevent viridans

streptococcal IE, especially in patients with valvular abnormalities (Bayliss *et al.*, 1983; Durack 1995; Dajani *et al.*, 1997; Tomás Carmona *et al.*, 2002).   We in fact recently reported a case of IE caused by *Granulicatella elegans* originating from the oral cavity (Ohara-Nemoto *et al.*, 2005).

Following viridans streptococci, a high incidence of IE caused by *Staphylococcus* species has been reported.   Among those, *Staphylococcus aureus* was the most frequently isolated bacterial species, and 20-30 % of whole culture positive IE cases were due to *S. aureus* (Strom *et al.,* 1998; Hoen *et al.*, 2002; Nakatani *et al.*, 2003; Cecchi *et al.*, 2004; Ferreiros *et al.*, 2006).   Etiologic *Staphylococcus* spp. causing IE are assumed to be acquired via a percutaneous route, particularly in cases associated with nosocomial infection and intravenous drug use cases.   However, both of the predisposing conditions and infectious route related to staphylococcal IE are often not specified (Di Filippo *et al.,* 2006).   Nakatani *et al.* (2003) reported cases associated with vascular catheterization (17·9 %) and dental procedures (14·4 %), however, the main predisposing condition was not identified in more than half of the cases of staphylococcal IE.

Staphylococci are mainly harbored on the skin, as well as skin glands and mucous membranes in humans.   Although these microorganisms are considered to be transiently resident in the oral cavity (Percival *et al.,* 1991; Marsh & Martin 1999), during the course of our previous studies we have found that the occurrence of *Staphylococcus epidermidis* was significantly high in saliva from healthy adults (Ikeda *et al.,* 2004).   This finding led us to speculate that a portion of causative staphylococci in IE originated in the oral cavity.   In the present study, we examined the occurrence of *Staphylococcus* species in saliva and dental plaque specimens from healthy adults.   In addition, nasal-oral trafficking of these bacterial species was investigated by studying their genotypes using PFGE, to investigate a possible peroral route of IE.


**METHODS**


**Subjects and clinical specimens.**   Fifty-six subjects aged 22-43 years old (27·1 ± 5·3 years; 37 males, 19 females) provided informed consent to participate and were enrolled in this study. The subjects were pre-clinical students and laboratory research staff of Iwate Medical University School of Dentistry, and all were systemically healthy and showed no or negligible pathogenic signs in periodontal tissues or of dental caries.   None of the subjects received antibiotic medication

within the previous three months. This study received ethical approval from the Ethics Committees of Iwate Medical University School of Dentistry. Expectorated whole saliva was collected as described previously (Ohara-Nemoto *et al.,* 1997). A supragingival plaque sample was taken from the labial side of the gingival sulcus of the primary or permanently lower left canine with a sterile tooth pick, then weighed, and immediately suspended in 250 µl of sterile PBS. Nasal swabs were obtained from the anterior nares of the nose from 18 (32·7 ± 2·6 years, 12 males, 6 females) of 47 oral staphylococci-positive subjects. The swabs were immediately immersed in 250 µl of sterile PBS.

**Culture conditions and species identification.** Standard aseptic procedures for sampling and culturing were carefully performed to avoid contamination. Samples of dental plaque and nasal swab in PBS were dispersed by vortex mixing for 30 s, and an aliquot of samples (100 µl) at serial dilutions from $1 - 10^2$ was plated onto mannitol salt agar at least in duplicate. After 48 h at 35 ˚C in an aerobic atmosphere, c.f.u. were determined. For species identification, 3-6 mannitol-fermented colonies with yellow-zones and 6-10 non-fermented colonies with red-zones from each sample were subcultured on 5 % sheep-blood agar. Species identification was performed by gram-staining and a Staphyogram system (Wako Pure Chemical Industries). Colony-direct species-specific PCR was further performed for confirmation of *S. aureus* (Martineau *et al.,* 1998), and *S. epidermidis* isolates (Ikeda *et al.,* 2004), as described previously.

**PFGE.** PFGE was performed as described previously (Ohara-Nemoto *et al.,* 2005). Briefly, bacterial cells cultured in Todd Hewitt broth at 35 ˚C for 18 h were harvested and washed with TE (10 mM Tris-HCl, pH 8·0, 1 mM EDTA). Cells were re-suspended in Pett IV (1 M NaCl, 10 mM EDTA, pH 8·0) to give absorbance at 600 nm of 0·6. An aliquot of the suspension was mixed with an equal volume of 2 % low-melting agarose solution in TE, to prepare an agarose plug. Agarose plugs were incubated with gentle shaking in lysis solution [10 mM Tris-HCl, pH 8·0, 100 mM EDTA, and 25 % glucose] supplemented with 0·5 mg lysozyme ml$^{-1}$, 0·1 mg lysostaphin ml$^{-1}$, and 10 µg RNaseA ml$^{-1}$ at 37 ˚C for 16 h. The plugs were incubated in 0·5 M EDTA containing 100 µg protease K ml$^{-1}$ and 1 % SDS at 50 ˚C for 1 h, and then in TE containing 1 mM phenylmethyl sulfonyl fluoride at 25 ˚C for 1 h. After washing four times with TE, genomic DNA was digested

with 20 U of *Sma*I at 25 ˚C for 2 h, then separated with PFGE using a CHEF-DR III apparatus. The initial pulse of 1 s was increased linearly to 40 s over 12 h at 80 V at 10 ˚C. Gels were then stained with ethidium bromide and photographed under UV light. Bacterial strain typing to calculate relative coefficients was performed using a Fingerprinting II software (Bio-Rad Laboratories Hercules).

## RESULTS AND DISCUSSION

**Isolation frequencies of oral staphylococci.** Ninety-nine clinical isolates of *S. aureus* and 235 isolates of coagulase-negative staphylococci (CoNS) were identified from saliva and supragingival plaque samples. The isolation frequency of staphylococci was 83·9 % in saliva and 73·2 % in dental plaque, with a total of 9 different *Staphylococcus* species identified (Table 1). The amounts of cultivable staphylococci were $10^2$-$10^4$ c.f.u. $ml^{-1}$ of saliva and $10^3$-$10^5$ c.f.u. $g^{-1}$ of dental plaque. The most frequently isolated species in saliva were *S. aureus* (46·4 %) and *S. epidermidis* (41·1 %), followed in order by *S. hominis, S. warneri, S. intermedius, S. capitis,* and *S. haemolyticus* (12·5-7·1 %). *S. gallinarum* and *S. lugdunensis* were rarely isolated (1/56, 1·8 %). These results indicated that 9 of 15 *Staphylococcus* species known to be found in humans (Kloos & Bannerman 1999) were isolated from the oral cavity. Seven of these species, excluding *S. gallinarum* and *S. lugdunensis*, were cultured from the dental plaque samples. The isolation frequency of staphylococcal species from dental plaque was similar to that of those from saliva, though *S. epidermidis* (60·7 %) was more dominant than *S. aureus* (33·9 %). Staphylococci-positive subjects (n=47) harbored from 1 to 5 species (2·3 ± 1·0) in their samples (a portion of these results is shown in Table 2). These findings indicated that the occurrence of oral staphylococci was significantly high in systemically and periodontally healthy adults subjects.

The isolation frequencies in the present report were relatively high, as compared previous studies that reported 60 % in saliva and 17 % in dental plaque from healthy subjects aged 20-39 years old (Percival *et al.,* 1991), and 43 % in plaque from healthy subjects (Murdoch *et al.,* 2004), and 50 % in those with gingivitis and periodontitis (Rams *et al.,* 1990). Although the reason is not clear, larger amounts of oral specimens may be needed for accurate counting of viable staphylococcal cells on selective media. The amounts of cultivable staphylococci from our

staphylococci-positive subjects were significantly fewer compared to those of viridans streptococci by approximately $10^{-2}$ to $10^{-5}$.

**Genetic relatedness between oral and nasal staphylococci.**   Nasal swab specimens from 18 out of 47 oral staphylococci-positive subjects were cultured for isolation of staphylococci, to investigate nasal-oral trafficking of the bacteria.   Swab sampling and cultivation were performed at the same time (n=3) or within 2 weeks after the oral examination (n=15).   Consequently, a total of 7 different *Staphylococcus* species were identified in nasal swab specimens (Table 1).   Among them, *S. epidermidis* was predominant (72·2 %), followed by *S. aureus* (44·4 %).   In 13 of the subjects, *S. aureus, S. epidermis, S. hominis,* and *S. lugdunensis* were commonly isolated in both oral and nasal specimens (Table 2).   *S. capitis, S. haemolyticus, S. intermedius,* and *S. warneri* were found in either oral or nasal specimen, probably due to low bacterial numbers.

To compare strain similarities, genotypes of all 18 combinations of the *Staphylococcus* species (Table 2) consisting of 91 strains, commonly found in both the oral and nasal cavities were investigated using PFGE.   Representative results are presented for *S. epidermidis* (Fig. 1), and *S. aureus* and *S. lugdunensis* (Fig. 2).   PFGE patterns of the *S. epidermidis* isolates from the oral samples were nearly identical to those of the nasal-derived isolates.   For example, in the case of subject No. 1, the relative coefficient of 1 strain from saliva, 2 strains from dental plaque, and 4 strains from nasal swab was 76·5 %.   Further, the coefficient of 2 strains from the oral cavity and 2 from the nasal cavity was 82·7 % in subject No. 4.   As shown in Figure 2, the PFGE patterns of 1 of 3 *S. aureus* isolates from saliva and 2 plaque-derived isolates from subject No. 3 were apparently identical to their respective nasal-derived isolates (Fig. 2a).   In addition, genotypes of oral and nasal-derived *S. lugdunensis* isolates from subject No. 5 were indistinguishable, with the relative coefficient of 97·0 % (Fig. 2b).   The relative coefficients of all 18 cases ranged from 76·5 % to 100 %.   These results, according to the criteria of Tenover *et al.* (1995), indicates that identical clones or close relatives were commonly distributed in the oral and nasal cavities, and suggests nasal-oral trafficking of staphylococci.

Staphylococci are common residents of nasal flora (Wenzel & Perl 1995; Kluytmas *et al.,* 1997; Güçlü *et al.,* 2007), thus they may consistently pass into the mouth.   Contamination from skin flora is also possible, however, the PFGE genotyping provided evidence of nasal-oral

trafficking of the *Staphylococcus* species.   Alternatively, the number of indigenous staphylococcal strains may be limited in each individual.   In addition, a longitudinal sampling and cultivation of saliva, dental plaque and nasal swab were preliminarily performed with subjects No. 6 and No. 7 over a 2-month period after the first examination, and we found that *S. aureus* and *S. epidermidis* were continuously isolated from separately collected the cultures, respectively.   Together, these findings suggest that *Staphylococcus* species found in the oral cavity are capable of surviving in the oral flora, although these may be continuously provided from the nasal cavity.

*S. aureus* is the most frequently isolated bacterial species in whole culture positive IE cases (Hoen *et al.,* 2002; Niwa *et al.,* 2005), even when excluding nosocomial cases and drug users (Strom et al., 1998; Nakatani *et al.,* 2003).   *S. epidermidis* is also a predominant agent for IE (Nakatani *et al.,* 2003; Niwa *et al.,* 2005), and aggressive clinical courses with high mortality IE due to *S. lugdunensis* were reported (Auguera *et al*., 2005).   All these species were recovered from oral specimens.   According to another report (Percival *et al.,* 1994), the occurrence rates of staphylococci in saliva increase with age.   Therefore, considering that most patients suffering from IE are elderly (Hoen *et al.,* 2002; Ferreiros *et al.,* 2006), we think that it is important to assess the peroral route for staphylococcal IE, as in cases with viridans streptococcal IE.   In fact, cases of staphylococcal IE after dental extraction and treatments have been previously reported (Bayliss *et al.*, 1983; Etienne *et al.,* 1986).   The predisposing condition is occasionally not identified in cases of staphylococcal IE (Nakatani *et al.,* 2003), and thus peroral infection may be involved in those patients.

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**FIGURE LEGENDS**

**Fig. 1.** Genetic relatedness of oral and nasal *S. epidermidis* isolates. Chromosomal DNA of *S. epidermidis* from saliva, dental plaque, and nasal samples from subjects No. 1 (a) and No. 4 (b) were digested with *Sma*I, and separated using PFGE. The relative coefficients of the strains examined were 76.5 % and 82.7 % (a and b, respectively), as calculated with Fingerprinting II software. Lanes: M, yeast DNA PFGE markers.

**Fig. 2.** Genetic relatedness of oral and nasal *S. aureus* and *S. lugdunensis* isolates. Chromosomal DNA of *S. aureus* isolates from saliva, dental plaque, and nasal samples from subject No. 3 (a) and *S. lugdunensis* isolates from subject No. 5 (b) were digested with *Sma*I, then separated using PFGE. The relative coefficients of the strains examined were 100 % (lanes 3 to 7) and 97.0 % (a and b, respectively). Lanes: M, yeast DNA PFGE markers.

**Table 1.** Occurrence of staphylococci in saliva, dental plaque and nasal samples

| Species | Numbers of positive subjects (% isolation frequency) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Saliva (n = 56) | | Plaque (n = 56) | | Nasal swab[*] (n = 18) | |
| *S. aureus* | 26 | (46.4) | 19 | (33.9) | 8 | (44.4) |
| *S. intermedius* | 5 | (8.9) | 5 | (8.9) | 0 | (0) |
| *S. capitis* | 5 | (8.9) | 5 | (8.9) | 3 | (16.7) |
| *S. epidermidis* | 23 | (41.1) | 34 | (60.7) | 13 | (72.2) |
| *S. gallinarum* | 1 | (1.8) | 0 | (0) | 0 | (0) |
| *S. haemolyticus* | 4 | (7.1) | 3 | (5.4) | 1 | (5.6) |
| *S. hominis* | 7 | (12.5) | 7 | (12.5) | 4 | (22.2) |
| *S. lugdunensis* | 1 | (1.8) | 0 | (0) | 1 | (5.6) |
| *S. warneri* | 6 | (10.7) | 5 | (8.9) | 3 | (16.7) |
| total staphylococci | 47 | (83.9) | 41 | (73.2) | 17 | (94.4) |

[*]Nasal swab samples were taken from oral staphylococci-positive subjects.

**Table 2.** *Staphylococcal* species isolated from the oral and nasal cavities

| | *Staphylococcal* species[*] | | |
|---|---|---|---|
| **Subject No.** | **Saliva** | **Plaque** | **Nasal swab** |
| 1 | *S. aureus*, **S. epidermidis** | **S. epidermidis** | **S. epidermidis** |
| 2 | **S. aureus, S. epidermidis** | *S. intermedius* | **S. aureus, S. epidermidis** |
| 3 | **S. aureus**, *S. warneri* | **S. aureus, S. epidermidis** | **S. aureus**, *S. capitis*, **S. epidermidis** |
| 4 | **S. epidermidis** | **S. aureus, S. epidermidis** | **S. aureus, S. epidermidis**, *S. hominis* |
| 5 | *S. aureus*, **S. lugdunensis** | *S. aureus*, **S. epidermidis** *S. warneri* | **S. epidermidis, S. lugdunensis** |
| 6 | **S. aureus**, *S. intermedius* | **S. aureus** | **S. aureus**, *S. warneri* |
| 7 | **S. epidermidis**, *S. hominis*, *S. warneri* | **S. epidermidis**, *S. intermedius* | **S. epidermidis**, *S. capitis* *S. hominis* |
| 8 | *S. epidermidis* | *S. epidermidis* | *S. capitis*, *S. hominis* |
| 9 | *S. epidermidis* | *S. epidermidis* | ND |
| 10 | *S. intermedius*, *S. warneri* | *S. warneri* | *S. aureus*, *S. epidermidis* |
| 11 | *S. intermedius* | ND | *S. epidermidis* |
| 12 | *S. aureus*, *S. warneri* | **S. epidermidis** | **S. epidermidis** |
| 13 | *S. capitis* | ND | *S. aureus*, *S. epidermidis*, *S. warneri* |
| 14 | *S. capitis* | **S. hominis** | **S. hominis**, *S. warneri* |
| 15 | **S. epidermidis** | **S. epidermidis**, *S. capitis*, *S. haemolyticus* | **S. epidermidis** |
| 16 | **S. epidermidis** | **S. epidermidis** | **S. epidermidis** |
| 17 | **S. aureus**, *S. epidermidis* *S. hominis* | **S. aureus**, *S. epidermidis* *S. hominis* | **S. aureus** |
| 18 | **S. aureus** | **S. aureus** | **S. aureus**, *S. epidermidis*, *S. haemolyticus* |

[*]Clinical isolates commonly isolated from oral and nasal cavities are written in bold, and these strains were subjected to PFGE to compare genotypes.
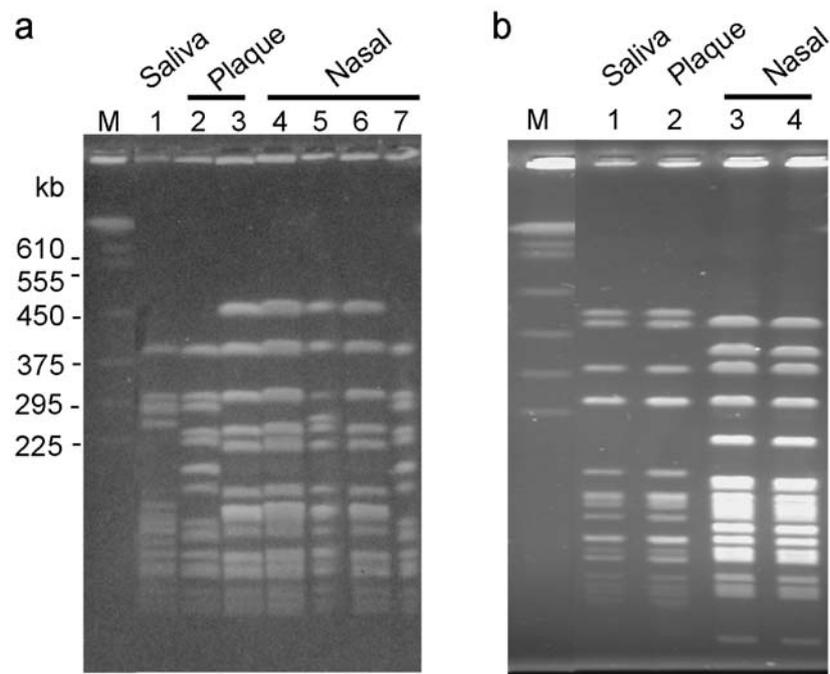
ND, not detected.

Fig. 1    Ohara-Nemoto Y *et al*.

a

Saliva  Plaque  Nasal
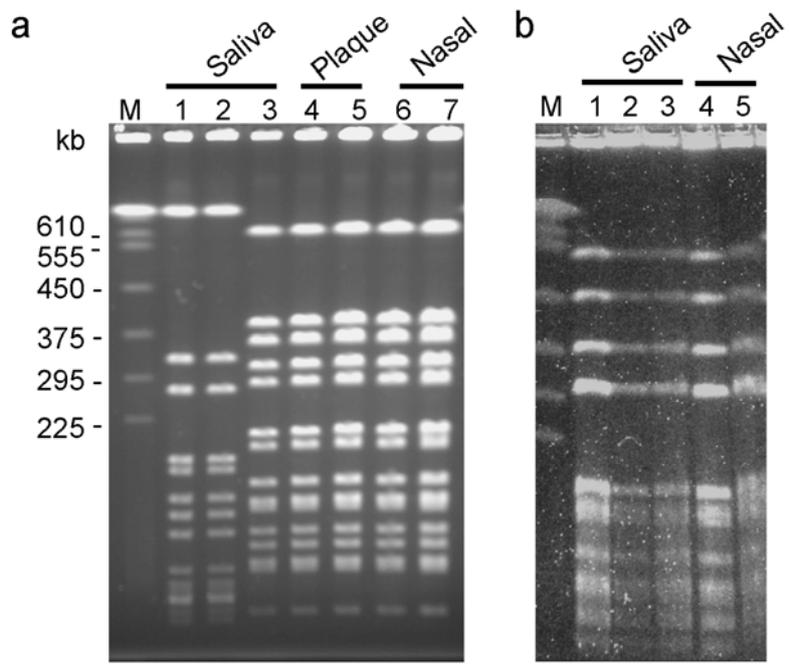
M  1  2  3  4  5  6  7

kb

610
555
450
375
295
225

b

Saliva  Nasal

M  1  2  3  4  5

Fig. 2    Ohara-Nemoto Y *et al.*