High salivary *Staphylococcus aureus* carriage rate among healthy paedodontic patients

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**SUMMARY**

*Staphylococcus aureus* can be responsible for oral and dental healthcare-associated infections. Patients with high salivary *S. aureus* levels are potential sources of infection, because saliva is spread in the environment during dental therapy. This study assessed the salivary *S. aureus* carriage rate in 97 children (6-12 years) in good general health, attending a paedodontic department. Samples of unstimulated saliva were collected, *S. aureus* was presumptively identified. The salivary carriage rate was 43% (95% confidence interval, 33%-53%). 6.2% children harboured levels >10³ colony forming units/mL. These data suggest that the risk for environmental contamination and infection in dental healthcare settings could be high.

**KEY WORDS:** *Staphylococcus aureus*, Dentistry, Saliva, Infection control, Children.

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*Staphylococcus aureus* can be a putative pathogen of many oral diseases, such as oral mucositis (Gibson *et al.*, 2000), periodontitis (Passariello *et al.*, 2012), peri-implantitis (Heitz-Mayfield & Lang, 2010), endodontic infections (Poeschl *et al.*, 2011) and even dental caries (Kouidhi *et al.*, 2010). Although it is commonly detectable on skin and mucosae, the anterior nasal region is the primary ecological reservoir, followed by oro-pharynx and perineum. Persistent carriers are as many as 20% of the healthy population, while intermittent carriers are 30%. Carriage, infection and mortality rates are higher in the elderly, infancy and among immune-deficient individuals (van Belkum *et al.*, 2009; Grundmann *et al.*, 2010). Although it is commonly detectable on skin and mucosae, the anterior nasal region is the primary ecological reservoir, followed by oro-pharynx and perineum. Persistent carriers are as many as 20% of the healthy population, while intermittent carriers are 30%. Carriage, infection and mortality rates are higher in the elderly, infancy and among immune-deficient individuals (van Belkum *et al.*, 2009; Grundmann *et al.*, 2010). *S. aureus* carriers with detectable levels in dental plaque and saliva may play an important role as sources of HAIs in dental healthcare settings. Indeed, *S. aureus* is detected in saliva of patients who harbour these microorganisms in their oro-pharynx (Millar *et al.*, 2001), while dental plaque colonization generally occurs in denture-wearing elderly (Theilade *et al.*, 1983), immune-deficient subjects (Scannapieco *et al.*, 1992), patients with hyposalivation (Almståhl & Wikström, 1999) or with aggressive periodontal disease (Fritschi *et al.*, 2008). Oral *S. aureus* carriers may, therefore, be responsible for environmental contamination in dental healthcare settings during dental therapy (Rautemaa *et al.*, 2006; Szymańska & Dutkiewicz, 2008; Kim-
merle et al., 2012), thus increasing the chance of cross-infections.
The carriage rate in healthy children is poorly investigated (Millar et al., 2001), probably because healthy children are at low risk for S. aureus infections and complications (Grundmann et al., 2010). However, understanding whether young children are potential sources of infection is important from the point of view of infection control in dental healthcare settings, because it may help design evidence-based infection control guidelines (Petti & Polimeni, 2010). Therefore, the aim of this study was to assess salivary S. aureus carriage rate in healthy children undergoing dental therapy in a public paedodontic healthcare setting.

A consecutive sample was selected from children in mixed dentition aged 6-12 years attending the Paediatric Dentistry Section of the Department of Dental and Maxillofacial Sciences of the Sapienza University of Rome (Italy). The minimum sample size was estimated assuming an S. aureus carriage rate of 6.25%, based on the most recent survey on healthy children and adolescents visiting a Preventive Dentistry Clinic in Japan (Kitada et al., 2009). With the highest acceptable margin of error of 5% and confidence interval of 95%, the lowest sample size was set at 90 subjects.

The study protocol was approved by the ethical committee of the Sapienza University and written informed consent to nasal swabbing and saliva collection was obtained from children’s parents or guardians. Eligible children had to be in good general health status, guaranteed by parents or guardians; they must not be affected by respiratory-tract infections or have taken antibiotics or other antimicrobials during the month before sampling, while on the day of the sample they had not to drink or eat anything after breakfast. The morning of the appointment and before the dental treatment, children underwent a nasal sample which was collected by two authors specifically trained for this purpose (G.A.M. and C.P.). A sterile swab was inserted in both nares and gently rotated on the nasal mucosa for at least five seconds. The swab was immediately passed on a plate containing Mannitol Salt Agar (MSA - Becton Dickinson Italia, Bucinasso, Italy) selective for staphylococci.

The aim of this study was to investigate oral S. aureus in saliva originated from dental plaque. Saliva was therefore collected with a technique previously validated and used to collect salivary samples which replicated dental plaque composition (Petti & Tarsitani, 1998; Petti & Hauser, 2006): namely, unstimulated whole saliva (approximately 2 mL) was collected in a sterile tube, inviting children to spit without expectorating, thus avoiding the collection of oropharyngeal secretion instead of or in addition to saliva. This sampling method was preferred to the more conventional mouth-washing technique, which is designed to collect microorganisms from oral and oropharyngeal mucosae. Indeed, the mouth-washing technique yields similar S. aureus recovery rates and counts as the anterior nares swabbing technique (Millar et al., 2001). For the same reason, tongue swabbing, another conventional sampling technique (Jackson et al., 2000), was not chosen.

The sample was maintained at 4°C during transport to the Dentistry Section of the Department of Public Health and Infectious Diseases, and was processed within one hour. The tube was vortexed for 3 min, 1:10 and 1:100 dilutions in a pre-reduced NaCl solution (9 g/L) were made. Aliquots of 0.1 mL of the two dilutions were inoculated on MSA plates. The plates were incubated aerobically at 37°C for 48 h. For every plate, colonies with different morphologies grown on MSA were counted, Gram stained and sub-cultured on Tryptic Soy Agar (Becton Dickinson Italia). After incubation (37°C for 48 h), microorganisms were tested for coagulase and catalase and presumptively identified using VITEK® 2 Gram Positive card (BioMérieux, Italia; Bagno a Ripoli, Italy). Since further identification tests were not made microorganisms were presumptively identified and counts were made on the basis of morphology of the colonies grown on MSA (Petti et al., 2013).

For each child, the levels of the various staphylococcal species detected in the nasal mucosa were assessed and were classified into undetected, low (1-9 Colony Forming Units -CFU), medium (10-99 CFU), and high (>100 CFU). The levels of the various staphylococcal species detected in saliva were expressed as CFU/mL and were classified into undetected, low (100-999 CFU/mL), medium (1,000-9,999 CFU/mL),
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and high (≥10,000 CFU/mL). The lowest limit of detection in saliva was 100 CFU/mL corresponding to 1 CFU detected on the plate where an aliquot of 0.1 mL from the 1:10 dilution was inoculated. Children with at least one *S. aureus* (CFU) were considered carriers. *S. aureus* carriage rates were assessed with 95% confidence intervals (95CIs).

In order to predict the potential status of salivary *S. aureus* carrier, which could promote environmental contamination and infection for dental staff and patients, the variables associated with *S. aureus* detection in saliva were investigated. Therefore, a logistic regression analysis was modelled. Explanatory variables were dichotomized to increase the power of the statistical test. They were *S. aureus* detection in the nasal mucosa (reference group, undetected), gender (reference group, male) and age, dichotomized into 6-9 years (reference group) and 10-12 years. The odds ratios (ORs) with 95CI estimates were assessed for the various explanatory variables. The robustness of risk estimates was investigated through pseudo-$R^2$ and likelihood ratio test ($\chi^2_{3df}$ test), with a level of significance of 95%.

Ninety-seven children participated in the study, 38.1% were males (n=37) and 61.9% females (n=60). Mean age was 8.4±1.8 years, males were younger than females (7.5±1.1 years vs. 8.9±3.5 years). All children had at least one staphylococcal species in their nasal mucosa. One half of them harboured high levels of *S. aureus* and *Staphylococcus epidermidis*, while occurrence of *Staphylococcus warneri* and *Staphylococcus hominis* was less frequent and at lower levels (Table 1). Salivary *S. aureus*, *S. epidermidis*, *S. warneri* detection was relatively frequent. More specifically, 43% children were positive for *S. aureus*, 6% at high levels. The *S. aureus* nasal and salivary carriage rates were as high as 43%-62% and 33%-53%, respectively. Thirty-four children (35.1%) harboured *S. aureus* in saliva and nasal mucosa, seventeen (17.5%) only in nasal mucosa and eight (8.2%) only in saliva (data not in Table).

*S. aureus* detection in saliva was not affected by children’s gender, but was associated with age

| TABLE 1 - Frequency distributions of children according to staphylococcal florae detected in the nasal mucosa and in saliva. |
| :----------------- | :----------------- | :----------------- | :----------------- |
|                  | *S. aureus*       | *S. epidermidis*  | *S. warneri*      | *S. hominis*      |
| Nasal mucosa     |                  |                  |                  |                  |
| Level, undetected| 47.4% (n=46)      | 44.3% (n=43)     | 95.9% (n=93)     | 91.8% (n=89)     |
| Level, low       | 0                 | 0                 | 0                 | 0                 |
| Level, medium    | 2.1% (n=2)        | 0                 | 3.1% (n=3)       | 3.1% (n=3)       |
| Level, high      | 50.5% (n=49)      | 55.7% (n=54)     | 1.0% (n=1)       | 5.2% (n=5)       |
| Overall detected (carriage rate) | 52.6% (n=51) | 55.7% (n=54) | 4.1% (n=4) | 8.2% (n=8) |
| 95% confidence interval | 42.7%-62.5% |                  |                  |                  |
| Saliva           |                  |                  |                  |                  |
| Level, undetected| 56.7% (n=55)      | 57.7% (n=56)     | 88.7% (n=86)     | 0                 |
| Level, low (100-999 CFU/mL) | 13.4% (n=13) | 16.5% (n=16) | 11.3% (n=11) | 0                 |
| Level, medium (1,000-9,999 CFU/mL) | 23.7% (n=23) | 15.5% (n=15) | 0                 | 0                 |
| Level, high (≥10,000 CFU/mL) | 6.2% (n=6) | 10.3% (n=10) | 0                 | 0                 |
| Overall detected (carriage rate) | 43.3% (n=42) | 42.3% (n=41) | 11.3% (n=11) | 0                 |
| 95% confidence interval | 33.4%-53.2% |                  |                  |                  |
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(OR 4.5; 95CI, 1.1-18.2) and, most importantly, S. aureus detection in nasal mucosa (OR, 17.5; 95CI, 5.2-58.4) (Table 2). These OR estimates were reliable enough as demonstrated by the high values of pseudo-R² and log-likelihood test.

Staphylococcal identification based on biochemical tests is not as efficient as molecular typing. Nevertheless, studies on several clinical and environmental staphylococcal isolates reported that the VITEK® 2 Gram Positive card system identified S. aureus strains correctly in almost 100% of cases, with few misidentifications of S. hominis as S. warneri and non-identifications of S. epidermidis (Spanu et al., 2003; Delmas et al., 2008). Therefore, the present results regarding S. aureus were probably reliable, while the other results were less consistent.

Unstimulated saliva was preferred to alternative sampling methods, such as oral mucosal swab or oral rinse, because microorganisms which come from saliva are frequently spread in the environment during dental therapy, while microorganisms from other respiratory tract secretions are not (Messano et al., 2013a). Therefore, saliva collection is the most specific available method able to distinguish between salivary carriers, who are potential sources of environmental contamination and infection, and mucosal carriers. The use of samples of saliva instead of dental plaque samples was justified by the fact that microbiology of dental plaque is highly site dependent (Simón-Soro et al., 2013) and consistent dental plaque sampling techniques require that multiple sites are collected. On the other hand, the majority of microorganisms detectable in saliva come from dental plaque, as previously demonstrated for cariogenic bacteria, such as mutans streptococci and lactobacilli (Mundorff et al., 1990; Petti & Pezzi, 1996; Petti & Tarsitani, 1998; Petti & Hausen, 2006) and, more recently, for staphylococci (Ohara-Nemoto et al., 2008), thus corroborating the idea that unstimulated saliva is a reliable surrogate of dental plaque.

It is possible that some S. aureus positive subjects were misclassified as negative, because the lowest detection limit of salivary staphylococci was 100 CFU/mL. Therefore, salivary carriage rates could be even higher than the rate reported in this study. In addition, these data did not establish whether positive children were permanent or transient carriers. However, from the point of view of S. aureus transmission, low-level carriers are unlikely to spread these microorganisms in the environment, while it does not matter whether carriers are persistent or transient, because they are both potential sources of infection during dental therapy.

The results of the present study suggest that a proportion ranging between one third and one half of healthy children are salivary S. aureus carriers at levels higher than 100 CFU/mL. The majority of these children are also nasal carriers. These data are in agreement with previous surveys on similar samples. Oral carriage rates in 7 to 15-year-olds from the UK was 92% for overall staphylococci and 64% for S. aureus. This study used the oral rinse technique which produces higher carriage rates than unstimulated saliva sampling technique, because staphylococci may also originate from oro-pharyngeal mucosa and not only from saliva (Jackson et al., 2000). Another survey from the UK on 7 to 8-year-olds, which used the oral rinse technique, reported an oral S. aureus carriage rate of 37.1% (Millar et al., 2001). Using the oral rinse technique, in orthodontic patients from Japan (mean age, 17 years), only 5.6% subjects were positive for S. aureus and 11.2% for S. epidermidis (Kitada et al., 2009). In plaque samples from British children aged 4-5 years S. aureus was never detected, while S. epidermidis, S. warneri, S. hominis were found (Ready et al., 2003). S. aureus carriage rate was 46% among

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference group</th>
<th>Risk group</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>6-9 years</td>
<td>10-12 years</td>
<td>4.5*</td>
<td>1.1-18.2</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>0.4</td>
<td>0.1-1.3</td>
</tr>
<tr>
<td>S. aureus in nasal mucosa</td>
<td>Undetected</td>
<td>Detected</td>
<td>17.5*</td>
<td>5.2-58.4</td>
</tr>
</tbody>
</table>

*Statistically significant at 95% level. Pseudo-R², 0.24; Log-likelihood χ² 3df, 31.21, p<0.0001.
young adults from Japan (mean age, 27 years), while S. epidermidis, S. hominis and S. warneri rates were 41%, 12.5% and 11%, respectively. In 37.5% of subjects with detectable S. aureus levels in saliva, these microorganisms were not detected in the nasal mucosa (Ohara-Nemoto et al., 2008). Finally, S. aureus was detected in samples from nose and tongue of 6 to 10-year-old Brazilian children attending a paedodontic clinic (Negrini et al., 2009). These data globally suggest that oral S. aureus carriage rate in healthy children and adolescents is approximately 20-30%. In addition, one third of children harbour salivary levels higher than 1,000 CFU/mL (Table 1), in agreement with previous studies (Ohara-Nemoto et al., 2008).

These data do not allow us to infer a high risk for S. aureus transmission in dental healthcare settings during dental therapy. However, hypotheses can be made with the help of published studies reporting that dental therapy may promote the dissemination of airborne human bacteria (Messano et al., 2013b) in the environment. Indeed, staphylococci, S. aureus and even MRSA were detected during dental therapy with high-speed instruments (Rautemaa et al., 2006; Szymańska & Dutkiewicz, 2008; Negrini et al., 2009; Kimmervle et al., 2012) and oral surgery (El-Maaytah et al., 2007). Collectively, these data suggest that the risk of airborne contamination by S. aureus is probably low in general dental practice. Nevertheless, since S. aureus and, occasionally, MRSA were detected in the dental environment (Petti & Polimeni, 2011; Roberts et al., 2011; Petti & Polimeni, 2012), contamination seems prevalently due to contact with hands.

In conclusion, the present study reported a high S. aureus salivary carriage rate among healthy paedodontic patients, suggesting that it would be advisable to ascertain whether the suggested measures to control for environmental contamination (Kohn et al., 2003) are also effective against S. aureus.

REFERENCES


