

Staphylococcus Aureus Contamination in a Pediatric Dental Clinic

Thais de Cássia Negrini* / Cristiane Duque ** / Ana Carolina Mascarenhas de Oliveira*** / Josimeri Hebling **** / Luis C Spolidorio***** / Denise MP Spolidorio*****

Staphylococcus aureus strains can be disseminated during dental treatment and occasionally lead to contamination and infection of patients and dentists. The **objective** of this study was to determine the frequency and compare the number of *S.aureus* colonies isolated from the nose, hands and tongue of students and patients, as well as from the clinical environment, before and after dental treatment. *Staphylococcus* species were isolated from the tongue, nose and hands of 30 students and 30 patients and from the environment of a Pediatric Dentistry Clinic. The samples were incubated in SMA plates at 37°C for 48 hours. **Results:** The colonies that showed the presence of mannitol fermentation were collected as identification for *Staphylococcus aureus*, using CHROMagar and the coagulase test. The highest amount of *S.aureus* was found in the nose and tongue of children. In relation to dental students, more contamination was observed on gloved hands, followed by the tongue and hands without gloves, before clinical attendance. At the end of dental treatment, *S. aureus* colonies isolated from the gloved hands of students decreased significantly. Considering the clinical environment, the most contaminated areas were the auxiliary table and the storeroom, which was located at the center of the clinic. **Conclusion:** The dental clinic can be considered an environment for *S. aureus* cross-transmission. Preventative measures should be used to avoid the dissemination of pathogenic microorganisms.

Keywords: *Staphylococcus aureus*, Pediatric Dentistry, cross-contamination
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* Thais de Cássia Negrini, DDS, MS, Doctorate Student, Department of Clinical Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo State (UNESP), Araraquara, São Paulo, Brazil.

** Cristiane Duque, PhD, Assistant Professor, Department of Pediatric Dentistry, Federal Fluminense University (UFF), Nova Friburgo, Rio de Janeiro.

*** Ana Carolina Mascarenhas Oliveira, Doctorate student. Department of Endodontics, Piracicaba School of Dentistry, State University of Campinas (Unicamp), Piracicaba, São Paulo, Brazil.

**** Josimeri Hebling, PhD, Associated Professor. Department of Pediatric Dentistry, Araraquara School of Dentistry, University of São Paulo State (UNESP), Araraquara, São Paulo, Brazil.

***** Luis C Spolidorio, PhD, Associated Professor, Department of Physiology and Pathology, Araraquara School of Dentistry, University of São Paulo State (UNESP), Araraquara, São Paulo, Brazil.

***** Denise M P Spolidorio, PhD, Associated Professor, Department of Physiology and Pathology, Araraquara School of Dentistry, University of São Paulo State (UNESP), Araraquara, São Paulo, Brazil.

Send all correspondence to: Cristiane Duque, Federal Fluminense University (UFF), Department of Pediatric Dentistry, Nova Friburgo, Rio de Janeiro. Rua Doutor Silvio Henrique Braune, 22, Centro, CEP.28625-650, Nova Friburgo, Rio de Janeiro, Brasil.

E-mail:cristianeduque@yahoo.com.br

INTRODUCTION

Staphylococcus aureus is a common pathogen found in the human mucosa and skin. This microorganism is considerably instable, has wide dissemination and can be transmitted through the air or person to person, causing cross-contamination.³⁰ In addition to skin infections, *S. aureus* can cause septicemia, pneumonia, osteomyelitis, an abscess or other diseases.⁹ There is a strong relation between the presence of *S. aureus* and the occurrence of serious infections, such as infective endocarditis.²⁵ Antibiotic resistant *S. aureus* strains are considered a public health problem, especially in large hospitals. Loureiro, et al.²⁰ (2000) observed a high frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from the anterior nares of newborns and health care workers of two units in a hospital. Dental practitioners treat a wide range of patients, so it is likely that they will come into contact with people colonized or infected with drug-resistant microorganisms.⁷ High resistance rates against antibiotics used for prophylaxis in dentistry have been detected for pathogens associated with bacterial endocarditis, such as *S. aureus*.^{11,19,24}

Certain aspects of dental practice may contribute to the transmission of microorganisms.² The skin, environment and instruments can be contaminated with saliva, blood or organic debris during routine dental treatment.²⁹ Several

investigators have observed an increase in the amount of microorganisms during clinical procedures in the dental environment, suggesting contamination from aerosols, especially when the high-speed handpieces or ultrasonic scalers are used.^{4,23} Among the species identified in microbiological studies, streptococci from the *viridans* group and *Staphylococcus spp* are the most prevalent microorganisms found on the surfaces of dental equipment.^{4, 12, 23} Kurita et al.¹⁸ (2006) detected MRSA on surfaces of the dental operator, including the air-water syringe and reclining chair. In this same study, nosocomial infection or colonization of MRSA occurred in eight out of 140 patients who had no evidence of MRSA upon admission at a clinic. Antibigrams of 30 antibiotics revealed that the isolates from the eight patients were of the same strain as those from the surfaces of the dental operator, suggesting transmission of *S. aureus* between patients and the dentist via the clinical environment.

Several sources of bacterial transmission have been investigated and a higher frequency of *S. aureus* has been verified in the mouth and nose of healthy adult individuals.³² Miyake, et al.²² (1991) reported a high incidence of *S. aureus* isolated from the tongue in children. An association between the presence of *S. aureus* and oral mucosa alterations, such as angular cheilitis, erythema, swelling and burning, has been demonstrated in a retrospective study of clinical laboratory data.²⁸ Consequently, preventive measures should be instituted to reduce bacterial contamination from the patient/dentist and to avoid dissemination to the clinical environment. Disposable gloves, face masks and eye glasses have come into widespread use among dental staff to prevent cross-contamination. In addition, hand hygiene procedures have also been used by dentists to reduce/eliminate transmission by skin contact.²⁹ Although infection control has become an essential part of the curricula at dental schools,^{8,27} routine preventive measures have not been applied effectively.¹² The aim of the current study was to determine the frequency and compare the number of *S. aureus* colonies isolated from the nose, hands and tongue of students and patients and from the environment of a pediatric dentistry clinic, before and after dental treatment.

MATERIAL AND METHODS

Staphylococcus species were isolated from 30 undergraduate students, 30 patients aged from 6 to 10 years old and the environment (air) of a Pediatric Dentistry Clinic at the Araraquara Dental School, University of São Paulo State, São Paulo, Brazil. The Ethics Committee of the same institution approved this study and an informed consent was obtained from each subject. The collections were performed by one investigator, using protective equipment (gloves, masks, and goggles), before and after dental treatment. Environmental sampling was carried out prior to clinical activities (between 8:00 and 9:00 a.m.), referred to as “before treatment”, and 2 hours after the clinical appointment began (11:00 a.m), referred to as “after treatment”. For the patient and student sampling, the first collection was performed “before” any dental treatment (between 8:30–9:00

a.m.) and “after” the clinical procedures for the patient had ended (about 11:00 a.m.). The cleaning procedures in the clinic were performed at the middle of the day, before another clinic started (1:00–2:00 p.m.) and at end of the day (6:00–7:00p.m.).

Individual samples were collected with sterile cotton swabs from the nasal mucosa, dorsum of the tongue and bare hands (palm) of students and patients. The samples were stored in 1.0 ml of 0.9% NaCl sterilized solution. For the dental students, samples were also obtained from gloved hands at both periods. Disposable operating gloves, masks and goggles were used by students in the attendances. Additionally, students used rubber dams in their patients during restorative procedures, as a method of reducing microbial contamination. Afterwards, samples collected from students and patients were centrifuged for 1 minute and 100 µl of the resulting solution was inoculated onto plates containing a SMA selective medium (Salt Mannitol Phenol-red Agar, Merk, Darmstadt, Germany) and incubated at 37°C for 48 hours. For the environment sampling, 3 open SMA plates were distributed on each side of the clinic, about 30 minutes before the appointment began, totaling 6 plates (Figure 1). After beginning the appointment, 14 new SMA plates were placed in the clinic, as shown in Figure 2. One of the plates was placed in the center of clinic, where the materials were distributed (ST-storeroom), and one on the auxiliary table (AT), near the area where a student, randomly chosen, was using the high-speed dental handpiece. The collections were performed once a day for 30 consecutive working days. For each day, a different student and patient were randomly selected. Afterwards, all plates were incubated at 37°C for 48 hours.

After growing the cultures, colonies were quantified using a stereoscopic microscope (Stemi DV4, Zeiss, Thornwood, NJ, USA) and the results expressed as CFU, which were counted up to 300 colonies per plate. Indicative colonies of mannitol fermentation by pathogenic *staphylococci* (presence of yellow halo around the colonies) were selected. When a complete change of the culture medium color (red to yellow) was observed, all colonies were collected from the plate for identification as *Staphylococcus aureus*, using a chromogenic medium CHROMagar *S. aureus* and coagulase test-gold standard. Purple color colonies present in the CHROMagar *S. aureus* were considered indicative of *S. aureus*. These colonies were collected for the coagulase test-gold standard (Coagu-Plasma, Laborclin Laboratory Products LTDA.). The coagulase-positive colonies were confirmed as *S. aureus*. For the plates where the counts were more than 300 CFU, fifty colonies were collected and submitted for biochemical testing to identify *S. aureus*.

The number of *S. aureus* colonies was used to statistically compare the sites of collection from students and patients such as nasal mucosa, dorsum of the tongue and hands, before and after the dental treatment. In addition, the number of colonies isolated from different sites of the clinic environment was also compared. The Kruskal-Wallis test,

complemented by non-parametric multiple-comparison tests (Mann-Whitney), was applied for this purpose. Wilcoxon tests were used to compare the level of contamination of each site of collection before and after dental treatment. All of the statistical tests were considered at a 5% level of significance. The statistical software used was SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 5,547 colonies were isolated and identified as *S. aureus* from the environment and student/patient samples. From these colonies, 4,122 (74.31%) were obtained from the nose, tongue and hands of patients. Of the dental students, 799 (14.4%) colonies were identified as *S. aureus*. This microorganism was not detected in the nose of the students. A total of 51 (0.91%) *S. aureus* colonies were isolated from the clinical environment before it had opened for patients. After the clinical appointment, the amount of *S. aureus* increased (575 colonies –10.3%) (Table 1).

When the number of colonies was compared among the sites of collection for patients, the highest amount of *S. aureus* was found in the nose, followed by the tongue and the hands both before and after the clinical appointment. There was no statistical difference between the frequency of colonies identified as *S. aureus* from the patients' nose for both clinical periods ($p > 0.05$), while a significant reduction in the UFC counts of *S. aureus* for tongue after the clinical appointment was observed ($p < 0.05$) (Table 2).

In relation to dental students, no significant changes in the number of *S. aureus* colonies was observed for bare hands, gloved hands, tongue and nose, when the before and after appointment periods were compared ($p > 0.05$) (Table 3). However, comparing the sites within the same time period, hands with gloves were the most colonized sites before treatment, followed by the tongue and hands without gloves. Nevertheless, there was a strong reduction in the number of colonies from the hands with gloves, while the tongue was the most contaminated site after the clinical appointment ($p > 0.05$) (Table 3).

Considering the clinical environment, the number of detected *S. aureus* colonies from the dental clinic significantly increased after the appointment ($p < 0.05$). The storeroom and auxiliary table were the most contaminated regions of the dental clinic; however, a statistical difference was not observed between them ($p > 0.05$) (Table 4).

DISCUSSION

The dissemination of *Staphylococcus aureus* has been considered an important public health problem. Resistant strains of this microorganism can cause severe infections, mainly in children and hospitalized patients.²⁵ Epidemiologic studies are necessary to identify the principal sources of dissemination of *S. aureus* in clinical environments, such as hospitals or dental offices. In the present study, a high amount of *S. aureus* was isolated from the tongues of children, at both evaluation periods. Miyake *et al.*²² (1991) found similar results in children aged from zero to five years old, indicat-

ing that they may be a source of this pathogen. Additionally, microbial colonization may easily occur in the developing oral ecosystem of children because of their inadequate immune response.¹⁷ Although the oral cavity represents an important region of *S. aureus* dissemination, the highest amount of this pathogen was found in the nose of children. However, *S. aureus* was not detected in the nose of the dental students, which might have been colonized by another species of *Staphylococcus*, such as *S. epidermidis*. Several studies have demonstrated considerable variation on the *S. aureus* frequency isolated from the nose of individuals, both in hospitals and dental offices.^{1,13,20,32}

A high frequency of *S. aureus* colonies was detected from the operator's hands, mainly with gloves, before the dental appointment. The contamination of the gloves may be occurring either from the transfer of microorganisms from the students' hands or by contact with contaminated surfaces. Additionally, operating gloves are not previously sterilized by the manufacturers, becoming another source of dissemination for this microorganism. Best and Kennedy⁵ (1992) demonstrated that contamination from the glove surfaces was reduced after washing with antibacterial agents. The routine washing of gloved hands is recommended prior to a clinical appointment.

After clinical procedures, there was a strong decrease in the presence of *S. aureus* on the students' gloves. This finding suggests the possibility of dissemination of these pathogens to the patients or the clinical environment. Pereira *et al.*²⁶ (1999) demonstrated a considerable frequency of *S. aureus* on the hands of dental students and they believe that there is a transfer of these bacteria to dental equipment after attendance at the clinical appointment. On the other hand, Autio *et al.*² (1980) evaluated the transmission of eight potentially pathogenic organisms, including *S. aureus*, among dental hygiene students and adult patients. They observed that the transfer of this pathogen occurred from patient to operator, because microorganisms were not detected on the dental hygiene student's fingers, likely because the students are required to wash their hands whenever they leave the operating area.

In the current study, the dissemination of *S. aureus* to the clinical environment increased at the end of dental procedures. The most contaminated sites were the auxiliary table and storeroom. This result could be explained by the intense circulation of people in the clinic and the use of high-speed dental handpieces. Additionally, invasive dental procedures might result in a higher level of contamination when compared to any other clinical procedure in a dental clinic.³ Bernardo, *et al.*⁴ (2005) found a variable prevalence of *S. aureus* in different areas of a dental clinic and suggested the role of aerosol in the clinic contamination. The aerial spread of microorganisms has been demonstrated after buccal prophylaxis and radiographic examinations.² Cochran *et al.*⁶ evaluated the rubber dam as an infection control barrier during standard restorative procedures. Microbial collection was performed during preparation and placement of amalgam and composite resin restorations with and without the

rubber dam, and during handpiece and air-water syringe spraying with and without the rubber dam. The results showed a significant reduction in microorganisms with the use of the rubber dam -70% to 88% and 95% to 99%, respectively; and 90% to 98% when all data were combined. These results indicate that a rubber dam provides an excellent barrier to the potential spread of infectious disease in the dental office, when associated with the use of gloves, a mask and protective eyewear.

In the present study, it is speculated that much of the *S. aureus* contamination detected in the clinical environment came from other sources, such as direct contact, skin exfoliation or improper handling of plates. However, to avoid cross-contamination, all the plates and cotton swabs were manipulated by one researcher, who was wearing a mask and gloves as preventive measures, during all of the sampling procedures.

It was concluded that the dental clinic could be considered an environment for *S. aureus* cross-transmission. High levels of methicillin-resistant staphylococci have been detected on the dental chair and floors of a dental office,¹⁴ suggesting that preventive measures should be indicated to avoid the dissemination of these microorganisms. Dentists should use masks, special protective glasses and perform anti-sepsis procedures on their hands, before and after appointments.³⁰ Suzuki *et al*²⁹ (1997) compared the ability of four skin disinfectants to prevent horizontal transmission of resistant *S. aureus*, demonstrating that ethanol was highly effective in preventing microbial contamination. Some equipment routinely used in the dental office can not be sterilized, such as the nitrous oxide inhalation nose piece. A sim-

ple cleaning method, involving alkaline glutaraldehyde, provides adequate disinfection of this rubber piece and avoids cross-infection.³¹

The dentist should conduct rigorous anamneses to investigate the medical condition of the patients and to avoid the dissemination of resistant strains in the environment.⁴ To reduce the amount of microorganisms in the patients' mouth, chlorhexidine rinses could be indicated.¹⁶ Gautier *et al*¹⁰ (2000), testing different monthrinses in volunteers in a dental clinic, demonstrated that chlorhexidine and triclosan have inhibitory effects against several microorganisms, including *S. aureus*. The operatory area should be cleaned regularly with disinfectant solutions, such as 70 percent ethanol, to eliminate the precipitate particles and pathogenic microorganisms.²⁹ Additionally, disposable plastic covers could be used by the dentist during treatment to control the surfaces at risk for contamination and by decontaminating and sterilizing equipment.¹⁵ Recently, infection control guidelines and published research pertinent to dental infection control principles and practices were revised by the Centers for Disease Control and Prevention (CDC) and this information must be applied by the dentist as a matter of routine in the dental office.^{15,21}

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Table 1. Total of *S. aureus* colonies isolated from patients, dental students and clinical environment, before and after clinical attendance.

Condition	Patients			Dental Students				Clinical environment			
	N*	T	H	N	T	H	Hg	C	S	AT	
Initial	1500	621	45	0	136	81	417	43	8	-	
Final	1500	425	31	0	122	24	19	404	86	85	
Total	3000	1046	76	0	258	105	436	447	94	85	

* N –nose, T – tongue, H – hands, Hg – hands with gloves, C – clinic, S – storeroom, AT – auxiliary table.

Table 2. Median and range of *S. aureus* colonies (CFU) isolated from patients, before and after clinical attendance.

Sites	Condition	
	Before attendance	After attendance
Nose	500 (500-500)* Aa**	500 (500-500) Aa
Tongue	125 (57.5-352.5) A,b	55 (0-162.5) B,b
Hands	10 (0-12.5) A,c	0 (0-10) A,c

* Values are medians (lower/upper quartiles)

**Medians followed by the same uppercase letters in the rows (Wilcoxon, p>0.05) and lowercase letters in the columns (Mann-Whitney, p>0.05) do not differ statistically.

Table 3. Median and range of *S. aureus* colonies (CFU) isolated from dental students, before and after clinical attendance.

Sites	Condition	
	Before attendance	After attendance
Nose	0 (0-0)* Aa**	0 (0-0) Aa
Tongue	5 (0-50) A,b	10 (0-50) A,b
Hands	0 (0-20) A,b	0 (0-10) A,c
Hands with gloves	0 (0-12.5) A,b	0 (0-10) A,c

* Values are medians (lower/upper quartiles)

**Medians followed by the same uppercase letters in the rows (Wilcoxon, p>0.05) and lowercase letters in the columns (Mann-Whitney, p>0.05) do not differ statistically.

Table 4. Median and range of *S. aureus* colonies (CFU) isolated from clinical environment, before and after clinical attendance.

Sites	Condition	
	Before attendance	After attendance
Clinic	1.7 (1.7-3.7)* A	7.5 (5.6-12.7) B,a**
Storeroom	-	25 (10-40) b
Auxiliary table	-	25 (20-40) b

* Values are medians (lower/upper quartiles)

**Medians followed by the same uppercase letters in the rows (Wilcoxon, p>0.05) and lowercase letters in the columns (Mann-Whitney, p>0.05) do not differ statistically.

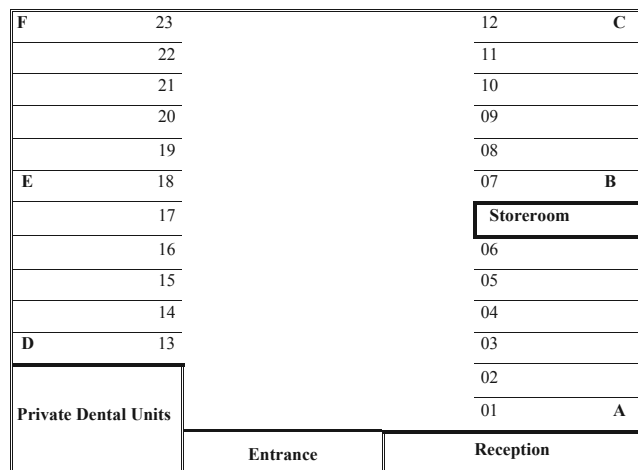


Figure 1. Schematic design of the Pediatric Dentistry clinic. The numbers represent the location of dental units. The letters represent the open MAS plates placed 30 minutes before beginning clinical work (n=6).

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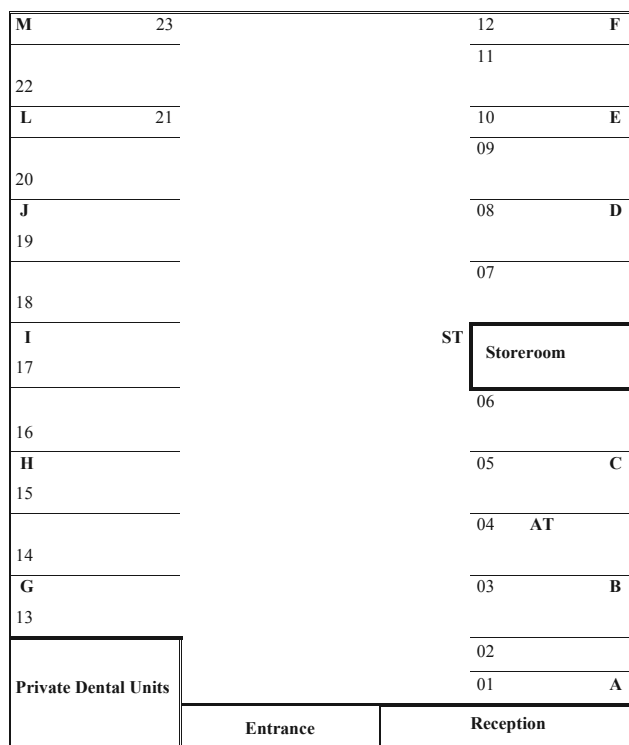


Figure 2. Schematic design of the clinic. Open plates - A to M - clinic, ST - storeroom, AT - auxiliary table. Each number represents the location of a dental unit. The letters represent the open MSA plates placed at the start of the clinic appointment and retrieved two hours later (n=14).

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