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Research Article

Hands as a Potential Vector for Transmission of Multidrug-Resistant *Staphylococcus aureus* Polyclonal Strains in Dental Clinical Environments

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Abstract

Background: *Staphylococcus aureus* has been considered one of the main opportunistic pathogens for humans, constituting a relevant problem, considering its possible resistance to the main antibiotics used in medical practice. The aim of this study was to detect the presence of oxacillin-resistant *S. aureus* (ORSA) in the hands of undergraduate dentistry students, throughout clinical procedures (before and after dental procedures and hand hygiene). **Methods:** Hand samples were collected at different times (pre-asepsis; immediately after asepsis; and glove and hand surfaces immediately after dental procedures) and seeded in culture media (PCA and MSA). *S. aureus* species were characterized by microbiological identification methods, antimicrobial susceptibility test and genotyping by isoenzymatic markers. **Results:** A total of 14 students (73.7%) had *S. aureus* in both hands. Microbial resistance was observed in 87.1% of *S. aureus* isolates: AMP (54.5%), AMX (26.5%), OXA (12.9%), TET (7.6%), CFE (7.6%), LEX (7.6%), FOX (7.6%), CLI (15.2%) and ERY (44.7%). A total of 132 isolates of *S. aureus* with variable profiles of antimicrobial resistance were identified, 26 of which were characterized as ORSA. Monoclonal and polyclonal patterns were observed in bacteria population. Analyzes of genetic relationship revealed that two or more dentistry students share *S. aureus* isolates through their hands and gloves during their academic clinical activities. **Conclusion:** The spread of multidrug-resistant *S. aureus* mono and polyclonal strains requires a reevaluation of personal hygiene and environment (effective biosafety practices) as a means of preventing the spread and cross-contamination between patients, professionals and academics, and dental clinical environments.

Keywords: Antimicrobial resistance; Dental clinic; MLEE typing; Polyclonality; *Staphylococcus aureus*

Introduction

Staphylococcus aureus are microorganisms especially important with regard to opportunistic infections, it is the most common agent in pyogenic infections and abscesses [1]. In immunocompromised individuals or who have suffered trauma and burns, this microorganism can cause more serious infections such as osteomyelitis and bacteremia, usually associated with metastatic abscesses, which in turn can cause endocarditis. Since the 1940s, there has been a great increase in the incidence of infections associated with hospital environment, caused by resistant strains of *S. aureus,* some showing resistance to more than 20 antimicrobial compounds, including antiseptics and disinfectants [2].

When contracting the potentially infective agent, the organism can behave in two ways: presenting a clinically diagnosable disease, or as an asymptomatic carrier, devoid of symptoms, despite being colonized. The carrier of *S. aureus* becomes a major problem, when in specific environments, such as hospitals, maternity units, outpatient clinics and dental offices [3], as staphylococcal diseases acquire characteristics of professional disease, being quite common clinical-dental infections [4]. The transmission routes are from person to person (cross-infection), by indirect contact (by air) or by direct contact, with this transfer depending on the presence of a source (patients or carriers) [5].

In general, the large number of studies aimed at this purpose seeks to detect carriers of that microorganism in nasal cavities and on the skin [6]. Hands have also been considered an important source of samples of *S. aureus* and one of main means of transmission of that bacteria in hospital environment, contributing significantly to the increase of sources and reservoirs of resistant samples [7].

The study of epidemiological role of hands in transmission of infections among professionals, engaged in dental activities, has shown potential importance of those as source of possible infections within the dental office as well as the possible relationship between samples isolated from different anatomical areas of a same individual, mainly between nasal cavity and hands, suggesting that most *staphylococci* in hands are of nasal origin [8].

Hand hygiene is a primary practice used to reduce the risk and spread of infection. Inadequate hygiene leaves microorganisms in hands that can be transferred to another patient. Hand hygiene must be performed before and after direct contact with patients, before and after the use of gloves, or after contact with inanimate objects in the patient's immediate surroundings [9].

The detection of *S. aureus* in dental care environments is necessary, since the results of present studies provide the academic environment, dental class and other related areas, a significant contribution to future studies and in development of safe techniques in management of patients. The aim of this study was to detect the presence of oxacillin-resistant *Staphylococcus aureus* (ORSA) in the hands of undergraduate dentistry students, throughout clinical procedures (before and after dental procedures and hand hygiene). Species of *S. aureus* were genotyped by isoenzymatic markers and characterized in terms of antibacterial sensitivity profile.

Methods

Sampling

Samples of surfaces of hands and gloves were collected from 19 dentistry students, using swabs (Cral plast, Cotia, São Paulo) previously moistened with PBS solution (100 mM NaCl, 100 mM NaH_2PO_4, pH 7.2), and kept in 2 type Eppendorf tubes containing 1 ml of sterile PBS solution. Samples were obtained at three different times: 1) Pre-asepsis hand surfaces; 2) Hand surfaces immediately after asepsis; and 3) Glove and hand surfaces immediately after dental procedures. At the end of each collection, the samples were identified with date, time and place, then stored in Styrofoam boxes and transported to the microbiology laboratory. Polyvinylpyrrolidone-iodine soap (PVPI) is commonly used in clinics for hand asepsis. However, no training was done especially for research.

Bacterium

Aliquots of 100 µl (1:10 dilution) of the specimens collected were inoculated in PCA culture medium (Plate Count Agar, DifcoTM, São Paulo, Brazil) and in MSA selective culture medium (Mannitol Salt Agar, DifcoTM, São Paulo, Brazil), and incubated aerobically at 35 ºC for 24-48h, in order to determine the total microbial content and identify preliminarily pathogenic *Staphylococcus* or *S. aureus,* respectively. Mannitol positive colonies indicative of *S. aureus* (presence of yellow halo around the colonies) were grown in BHI culture medium (Brain Heart Infusion Broth, DifcoTM, São Paulo, Brazil.) at 37 °C for 24h. *S. aureus* species were characterized by microbiological identification methods (Gram staining, growth in CHROMagar *Staphylococcus aureus* chromogenic medium, biochemical tests such as catalase, coagulase [Coagu-Plasma, Laborclin Produtos para Laboratórios Ltda.], Factor A test agglutination [Staphy test, Probac do Brasil Produtos Bacteriológicos Ltda., Marnes La Coquette, France], mannitol fermentation test, DNAse test and Voges-Proskauer test) and antimicrobial susceptibility test (by the disk-diffusion method) [10, 11] and confirmatory screening for oxacillin resistance [12]. Ampicillin 10 µg (AMP), amoxicillin 10 µg (AMX), oxacillin 1 µg (OXA), tetracycline 30 µg (TET), vancomycin 30 µg (VAN), cephalexin 30 µg (LEX), cephalothin 30 µg (CEF), cefoxitin 30

µg (FOX), 2 µg clindamycin (CLI) and 15 µg erythromycin (ERY) (Control and Diagnostic Products Center - CECON, São Paulo, Brazil) were used for antimicrobial susceptibility testing. The reference strain *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC 25923) was used to ensure the reproducibility and accuracy of disk diffusion susceptibility test.

MLEE Typing

MLEE method (multilocus enzyme electrophoresis) or isoenzymatic typing was used to examine the genetic relationship between two groups of organisms. Bacterial cultures, enzymatic extraction and electrophoresis and specific enzymatic staining were performed as previously described [13]. The enzymatic activities that will be analyzed included: alcohol dehydrogenase (EC 1.1.1.1), sorbitol dehydrogenase (EC 1.1.1.14), mannitol-1-phosphate dehydrogenase (EC 1.1.1.17), malate dehydrogenase (EC 1.1.1.37), glucose dehydrogenase (EC 1.1.1.47), D-galactose dehydrogenase (EC 1.1.1.48), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), catalase (EC 1.11.1.6) and α- and β-esterase (EC 3.1.1.1). *S. aureus* ATCC 25923 enzymes were included in each gel to ensure reproducible results.

The interpretation of patterns was carried out following the general rules commonly accepted in deduction of allelic composition for haploid organisms. The bands on gels were numbered in decreasing order of mobility, and the corresponding alleles were numbered using the same nomenclature. The lack of demonstrable activity for an enzyme was classified as a null allele at corresponding gene locus. Each unique combination of alleles on the examined enzyme locus has resulted in a subtype or electrophoretic (ET) type. The percentage of polymorphic locus, the average number of alleles per locus and the average number of alleles per polymorphic locus were also determined [13].

Data analysis methodology

Nei's statistical methods were used to estimate the genetic distance between *S. aureus* isolates and / or strains (ETs):

$$d_{ij} = -\ln\left[\frac{\sum_k \left|x_k x_{jk}\right|}{\sqrt{\sum_k x_k^2 x_{jk}^2}}\right],$$

where *I* is the normalized gene identity between two populations (ranging from 0 to infinity), a measure of genetic distance based on gene identity (frequency of alleles for all loci, including monomorphic loci) between populations. That genetic distance measures the allelic differences accumulated by locus, which can also be estimated from the amino acid sequences of proteins and even for distantly related species. As a consequence, if sufficient data is available, the genetic distances between any pair of organisms can be measured in terms of d_{ij}. In addition, this measure can be applied to any part of the organism, regardless of the ploidy level or mating scheme. Its interpretation, in terms of enzymatic loci, implies that, on average, zero to an infinite number of allelic substitutions are detected (by electrophoresis) in every 100 loci of a common ancestral strain [14].

A tree with two-dimensional classifications, called dendrogram, was generated using the SAHN clustering method (Sequential, Agglomerative, Hierarchic, Nonoverlapping Clustering Methods) and the UPGMA algorithm (unweighted pair group method using arithmetic mean), based on their respective matrices d_{ij}. Since MLEE provides all levels of relationship, which must be resolved by DNA fingerprints (i.e., identifying the same strain in independent isolates, identifying microevolutionary changes in a strain, identifying groups of moderately related isolates and identifying completely isolates unrelated), a threshold (value: $\overline{d_{ij}}$) was established in the dendrograms to identify groups of identical and highly related isolates ($0 \le d_{ij} < \overline{d_{ij}}$) and *taxon* (plural *taxa*; $d_{ij} \ge \overline{d_{ij}}$). Pearson's product-moment correlation coefficient (ranging from –1 to +1),

$$r_{jk} = \frac{\sum_{i=1}^{n}\left(X_j - \overline{X_j}\right)\left(X_k - \overline{X_k}\right)}{\sqrt{\sum_{i=1}^{n}\left(X_j - \overline{X_j}\right)^2 \sum_{i=1}^{n}\left(X_k - \overline{X_k}\right)^2}},$$

[where X_{ij} represents the value of the character state of the character *i* in OTU (Operational Taxonomy Unit) *j*, X_j is the average of all state values for OTU *j* and *n* is the number of characters sampled], was used as a measure of the agreement between the genetic distance values implied by the UPGMA dendrograms and those of the original d_{ij} genetic distance matrices. Those agreements have been interpreted as follows: $0.9 \le r_{jk}$, very good fit; $0.8 \le r_{jk} < 0.9$, good fit; $0.7 \le r_{jk} < 0.8$, poor fit; $r_{jk} < 0.7$, very poor fit. All of those analyzes were obtained using the NTSYS-pc 2.1 program.

The discriminatory power of MLEE method was established by the numerical index of discrimination (*D*) – Simpson's diversity index –, according to the probability that two unrelated isolates sampled from the test population will be classified in different types [electrophoretic types (ETs), subtypes or strains] [13].

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Results

Microbiological samples, from the hands of 19 dentistry students in academic clinical activities, were investigated for the presence of total bacteria (aerobic growth over PCA), fermenting bacteria — *Staphylococcus* sp. — and non-fermenting mannitol (aerobic growth over MSA), and *S. aureus*, in three different moments: 1) Pre-asepsis hand surfaces (A); 2) Hand surfaces immediately after asepsis; and 3) Glove surfaces (C) and hands (D) immediately after dental procedures (duration of procedures equivalent to ±2h30min) (Supplementary table 1).

Supplementary table 1: Colony forming unit (CFU/ml) of total bacteria (grown on PCA), mannitol^{-} and mannitol^{+} bacteria (grown on SMA), and *S. aureus* (microbiological characterization) from hands of the 19 dentistry students before (A) and after (B) asepsis, and of gloves (C) and hands (D) after dental procedures.

Code of dentistry student (gender)	Dental procedures (Time)	Code of hands and gloves	Total bacteria (CFU/ml)	Mannitol $^{-}$ (CFU/ml)	Mannitol $^{+}$ (CFU/ml)	*S. aureus* (CFU/ml)*	MLEE and antimicrobial susceptibility**
7 (♀)	Absence of procedure	A-RH-7	620	70	–	–	–
		A-LH-7	70	–	30	30	3
		∑	690	70	30	30	3
		B-RH-7	10	–	10	10	1
		B-LH-7	–	–	–	–	–
		∑	70	–	10	10	1
3 (♀)	Dental cleaning and restoration (1h20min)	A-RH-3	80	40	50	50	5
		A-LH-3	550	–	230	80	8
		∑	630	40	280	130	13
		B-RH-3	70	30	20	20	2
		B-LH-3	110	–	110	80	8
		∑	180	30	130	100	10
		D-RH-3	10	–	–	–	–
		D-LH-3	–	–	–	–	–
		∑	10	–	–	–	–
5 (♀)	Endodontic procedures and dental restoration (1h10min)	A-RH-5	140	10	30	30	3
		A-LH-5	390	30	90	80	8
		∑	530	40	120	110	11
		B-RH-5	–	–	–	–	–
		B-LH-5	20	–	–	–	–
		∑	20	–	–	–	–
		D-RH-5	30	20	–	–	–
		D-LH-5	–	10	–	–	–
		∑	30	30	–	–	–

8 (♀)	Dental	A-RH-8	880	300	–	–	–
	restoration:	A-LH-8	4,200	460	20	2	2
	definitive	∑	5,080	760	20	2	2
	cementation of	B-RH-8	60	10	30	3	3
	the dental crown	B-LH-8	110	30	–	–	–
	(1h)	∑	170	40	30	3	3
		D-RH-8	–	–	–	–	–
		D-LH-8	10	–	–	–	–
		∑	10	–	–	–	–
9 (♀)	Dental cleaning,	A-RH-9	10,840	20	40	10	1
	prophylaxis and	A-LH-9	99,280	680	20	20	2
	procedures of	∑	110,120	700	60	30	3
	dental prosthesis	B-RH-9	100	20	10	10	1
	(1h10min)	B-LH-9	120	10	10	10	1
		∑	220	30	20	20	2
		D-RH-9	–	–	–	–	–
		D-LH-9	10	–	–	–	–
		∑	10	–	–	–	–
10 (♂)	Dental	A-RH-10	30	30	20	20	2
	restoration:	A-LH-10	–	–	–	–	–
	temporary	∑	30	30	20	20	2
	cementation of	B-RH-10	40	–	–	–	–
	dental crown	B-LH-10	–	–	–	–	–
	(1h25min)	∑	40	–	–	–	–
		D-RH-10	10	–	–	–	–
		D-LH-10	–	–	–	–	–
		∑	10	–	–	–	–
4 (♀)	Dental	A-RH-4	1,550	10	30	30	3
	restoration:	A-LH-4	860	50	170	80	8
	temporary	∑	2,410	60	200	110	11
	cementation of	B-RH-4	710	–	–	–	–
	dental crown	B-LH-4	10	–	–	–	–
	(1h20min)	∑	720	–	–	–	–

		C-RH-4	80	20	–	–	–
		C-LH-4	170	10	–	–	–
		∑	250	30	–	–	–
		D-RH-4	560	10	20	20	2
		D-LH-4	980	–	–	–	–
		∑	1,540	10	20	20	2
6 (♀)	Dental	A-RH-6	3,430	1,010	–	–	–
	restoration and	A-LH-6	560	480	–	–	–
	polishing	∑	3,990	1,490	–	–	–
	(1h)	B-RH-6	190	210	–	–	–
		B-LH-6	880	700	–	–	–
		∑	1,070	910	–	–	–
		C-RH-6	80	40	–	–	–
		C-LH-6	310	70	–	–	–
		∑	390	110	–	–	–
		D-RH-6	1,210	–	–	–	–
		D-LH-6	20	250	–	–	–
		∑	1,230	250	–	–	–
11 (♀)	Restoration of	A-RH-11	360	80	10	10	1
	dental resin	A-LH-11	2,900	20	120	80	8
	temporary;	∑	3,260	100	130	90	9
	cementation of	B-RH-11	30	–	–	–	–
	dental crown	B-LH-11	690	–	–	–	–
	(1h25min)	∑	720	–	–	–	–
		C-RH-11	10	–	–	–	–
		C-LH-11	–	–	–	–	–
		∑	10	–	–	–	–
		D-RH-11	–	–	–	–	–
		D-LH-11	–	–	–	–	–
		∑	–	–	–	–	–
12 (♀)	Restoration of	A-RH-12	10	10	10	10	1
	dental resin	A-LH-12	40	20	10	10	1
	temporary	∑	50	30	20	20	2

	(1h15min)	B-RH-12	–	–	–	–	–
		B-LH-12	10	10	10	10	1
		∑	10	10	10	10	1
		C-RH-12	–	–	–	–	–
		C-LH-12	–	–	–	–	–
		∑	–	–	–	–	–
		D-RH-12	–	–	–	–	–
		D-LH-12	10	–	–	–	–
		∑	10	–	–	–	–
17 (♀)	Dental cleaning	A-RH-17	–	20	–	–	–
	and polishing of	A-LH-17	70	70	–	–	–
	restorations	∑	70	90	–	–	–
	(55min)	B-RH-17	10	–	–	–	–
		B-LH-17	20	–	–	–	–
		∑	30	–	–	–	–
		C-RH-17	–	–	–	–	–
		C-LH-17	30	–	–	–	–
		∑	30	–	–	–	–
		D-RH-17	–	–	–	–	–
		D-LH-17	–	–	–	–	–
		∑	–	–	–	–	–
18 (♂)	Restoration of	A-RH-18	–	10	–	–	–
	dental resin	A-LH-18	10	20	10	10	1
	temporary;	∑	10	30	10	10	1
	cementation of	B-RH-18	–	–	–	–	–
	dental crown	B-LH-18	–	10	–	–	–
	(1h20min)	∑	–	10	–	–	–
		C-RH-18	40	30	–	–	–
		C-LH-18	–	30	–	–	–
		∑	40	60	–	–	–
		D-RH-18	–	10	–	–	–
		D-LH-18	10	–	–	–	–
		∑	10	10	–	–	–
19 (♂)	Channel sealing,	A-RH-19	1,450	680	350	80	8

	temporary	A-LH-19	1,280	20	560	80	8
	restoration	∑	2,730	700	910	160	16
	(1h10min)	B-RH-19	70	10	–	–	–
		B-LH-19	1,670	40	120	80	8
		∑	1,740	50	120	80	8
		C-RH-19	490	10	30	30	3
		C-LH-19	200	20	30	30	3
		∑	690	30	60	60	6
		D-RH-19	60	10	10	10	1
		D-LH-19	10	20	40	40	4
		∑	70	30	50	50	5
20 (♀)	Instrumentation,	A-RH-20	1,200	–	1,160	80	8
	canal filling and	A-LH-20	10	20	–	–	–
	temporary dental	∑	1,210	20	1,160	80	8
	restoration	B-RH-20	–	–	–	–	–
	(3h05min)	B-LH-20	10	20	–	–	–
		∑	10	20	–	–	–
		C-RH-20	30	10	–	–	–
		C-LH-20	–	–	10	10	1
		∑	30	10	10	10	1
		D-RH-20	–	–	10	10	1
		D-LH-20	–	10	–	–	–
		∑	–	10	10	10	1
21 (♀)	Ultrasonic	A-RH-21	1,620	–	–	–	–
	scraping of	A-LH-21	30	–	–	–	–
	dental biofilms,	∑	1,650	–	–	–	–
	dental cleaning	B-RH-21	20	–	–	–	–
	and prophylaxis	B-LH-21	20	–	–	–	–
	(1h05min)	∑	40	–	–	–	–
		C-RH-21	290	10	–	–	–
		C-LH-21	70	–	–	–	–
		∑	360	10	–	–	–
		D-RH-21	700	40	–	–	–

		D-LH-21	–	–	–	–	–
		∑	700	40	–	–	–

♀ and ♂ means gender female and mate, respectively. MD and ME means right hand and left hand, respectively. * Identification of *S. aureus* by microbiological methods: Gram staining, growth on chromogenic medium CHROMagar *Staphylococcus aureus*®, biochemical tests such as catalase test, coagulase test, clumping factor A test, mannitol fermentation test, DNAse test and Voges-Proskauer test. ** Number of isolates used for characterization by MLEE and antimicrobial susceptibility.

Analyzing the 19 students included in the present study, all (100%) showed contamination on the surfaces of hands pre-asepsis by total bacteria, 18 (94.7%) showed contamination by non-fermenting mannitol bacteria, 14 (73.7%) showed contamination by *Staphylococcus* sp. and 14 (73.7%) showed contamination by *S. aureus*. The results of contamination of the surfaces of hands, immediately after asepsis, revealed that 17 (89.5%) students still had total bacteria, 9 (47.4%) had non-mannitol fermenting bacteria, and 6 (31.6%) had both *Staphylococcus* sp. and *S. aureus*. Soon after dental clinical procedures, samples of surfaces of gloves and hands were evaluated in 13 and 18 of the 19 dental students, respectively. A total of 11 (84.6%) students showed contamination by total bacteria on the surface of their gloves, 9 (69.2%) students showed contamination by non-fermenting mannitol bacteria, and 3 (23.1%) students showed contamination by *Staphylococcus* sp. and *S. aureus*. Once those gloves were discarded, 13 (72.2%) students showed contamination by total bacteria on the surface of their hands, 9 (50.0%) students showed contamination by non-fermenting mannitol bacteria, and 3 (16.7%) students showed contamination by *Staphylococcus* sp. and *S. aureus.*

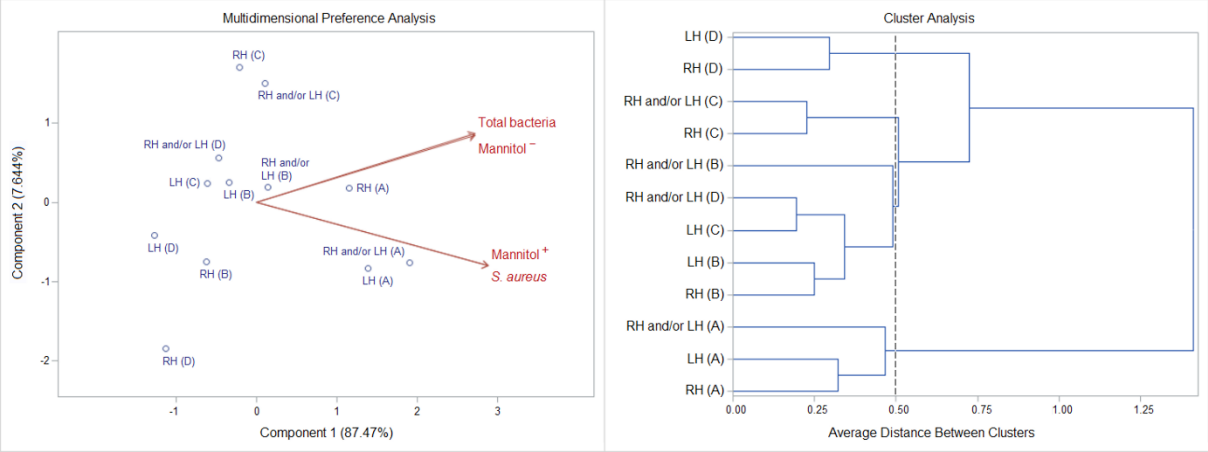
Data on the frequency of microbial contamination of the hands of dental students were submitted to multivariate statistical using the SAS® version 9.2 [principal component analysis (PCA) and interactive biplot construction; cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5)], which revealed similarity profiles as follows (Table 1 and Fig. 1):

Table 1: The incidence of total bacteria, *Staphylococcus* sp. and *S. aureus* coming from hands and gloves of the dentistry students. The data obtained in the frequency were submitted to multivariate statistical (SAS® version 9.2), using principal component analysis (PCA) and interactive biplot construction, and using cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5): sampling (A, B, C and D) versus total bacteria (PCA), mannitol$^-$ bacteria (MSA), mannitol$^+$ bacteria (MSA) and *S. aureus.*

Sampling	Surface	Number and frequency of dentistry students							
		Total bacteria		Mannitol$^-$		Mannitol$^+$		*S. aureus*	
		n	%	*n*	%	*n*	%	*n*	%
A. Pre-aseptic hand Surfaces	Right hand	17	89.5	17	89.5	10	52.6	10	52.6
	Left hand	18	94.7	15	78.9	12	63.2	12	63.2
	Right and/or left	19	100.0	18	94.7	14	73.7	14	73.7
B. Hand surfaces immediately after asepsis	Right hand	13	68.4	6	31.6	4	21.1	4	21.1
	Left hand	15	78.9	8	42.1	4	21.1	4	21.1
	Right and/or left	17	89.5	9	47.4	6	31.6	6	31.6
C. Glove surfaces immediately after dental procedures	Right hand	10	76.9	9	69.2	2	15.4	2	15.4
	Left hand	8	61.5	7	53.8	2	15.4	2	15.4
	Right and/or left	11	84.6	9	69.2	3	23.1	3	23.1
D. Hand surfaces immediately after dental procedures	Right hand	8	44.4	5	27.8	3	16.7	3	16.7
	Left hand	9	50.0	6	33.3	1	5.6	1	5.6
	Right and/or left	13	72.2	9	50.0	3	16.7	3	16.7

PCA culture medium (Plate Count Agar): analysis of total bacteria. MSA selective culture medium (Mannitol Salt Agar): analysis of mannitol$^-$ and mannitol$^+$ microorganisms (preliminarily pathogenic *Staphylococci* and *S. aureus*, respectively).
S. aureus: species were characterized by Gram staining, growth in CHROMagar *Staphylococcus aureus* chromogenic medium, catalase, coagulase, Factor A test agglutination, mannitol fermentation test, DNAse test and Voges-Proskauer test.

Figure 1: Incidence profile of dentistry students displaying bacterial contamination on surfaces of the hands and gloves from different sampling moments: A. Pre-aseptic hand surfaces; B. Hand surfaces immediately after asepsis; C. Glove surfaces immediately after dental procedures; and D. Hand surfaces immediately after dental procedures. The data obtained in the frequency were submitted to multivariate statistical (SAS® version 9.2), using principal component analysis (PCA) and interactive biplot construction, and using cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5): sampling (A, B, C and D) versus total bacteria (PCA), mannitol^{-} bacteria (MSA), mannitol^{+} bacteria (MSA) and *S. aureus*.



(i) Sampling A (pre-aseptic hand surfaces): right hand $^{RH\ (A)}$, left hand $^{LH\ (A)}$, and right and/or left hand $^{RH\ and/or\ LH\ (A)}$ were characterized by high values of total bacteria, mannitol^{-} and mannitol^{+} bacteria, and *S. aureus*;

(ii) Sampling C (glove surfaces immediately after dental procedures): right hand $^{RH\ (C)}$, and right and/or left hand $^{RH\ and/or\ LH\ (C)}$ were characterized by high values of total bacteria and mannitol^{-}, but < sampling A;

(iii) Sampling D (hand surfaces immediately after dental procedures): right hand $^{RH\ (D)}$ and left hand $^{LH\ (D)}$ were characterized by low values of total bacteria, mannitol^{-} and mannitol^{+} bacteria, and *S. aureus*;

(iv) Sampling B (hand surfaces immediately after asepsis), and partial samplings C and D: right hand $^{RH\ (B)}$, left hand $^{LH\ (B)}$, right and/or left hand $^{RH\ and/or\ LH\ (B)}$, left hand $^{LH\ (C)}$ and right and/or left hand $^{RH\ and/or\ LH\ (D)}$ were characterized by intermediate values of those observed between sampling A (RH, LH, and RH and/or LH), C (RH, and RH and/or LH) and D (RH and LH).

Microbial density (CFU/ml) has proved to be variable and dependent on each dental student, taking into account their entry into the academic dental clinic, that is, prior to hand hygiene and asepsis. However, the average density of bacterial contamination prior to hand asepsis was shown to be significantly higher for total bacteria (7,216.3 ±24,962.5), followed by non-fermenting mannitol bacteria (330.0 ±417.8 CFU/ml), *Staphylococcus* species (160.0 ±320.3 CFU/ml) and *S. aureus* (45.9 ±51.6 CFU/ml). That average density was shown to be lower on the surfaces of hands immediately after asepsis (total bacteria: 271.6 ±467.5 CFU/ml; non-mannitol fermenting bacteria: 62.1 ±206.5 CFU/ml; *Staphylococcus* sp.: 16.8 ±39.0 CFU/ml; and *S. aureus* 11.7 ±28.3 CFU/ml), and on the surfaces of gloves (total bacteria: 163.1 ±209.6 CFU/ml; non-mannitol fermenting bacteria: 56.9 ±77.3 CFU/ml; *Staphylococcus* sp.: 7.7 ±17.9; e *S. aureus*: 7.7 ±17.9 CFU/ml) and hands (total bacteria: 258.3 ±495.9 CFU/ml; non-mannitol fermenting bacteria: 22.2 ±58.2 CFU/ml; *Staphylococcus* sp.: 4.4 ±12.5 CFU/ml; e *S. aureus*: 4.4 ±12.5 CFU/ml) immediately after dental procedures.

Data on the average density (CFU/ml) of microbial contamination of the hands of dental students were submitted to multivariate statistical using the SAS® version 9.2 [principal component analysis (PCA) and interactive biplot construction; cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5)], which revealed similarity profiles as follows (Table 2 and Fig. 2):

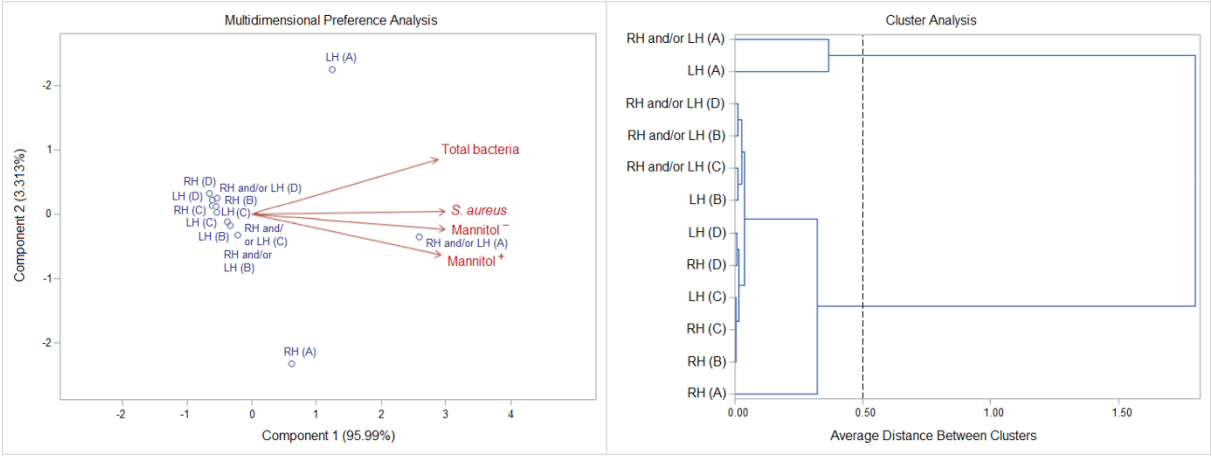
Table 2: Average density (CFU/ml) of bacterial contamination (total bacteria, mannitol$^-$ and mannitol$^+$ bacteria, and *S. aureus*) on surfaces of the hands and gloves from dentistry students obtained in different sampling moments. The data obtained (average values of CFU/ml) were submitted to multivariate statistical (SAS® version 9.2), using principal component analysis (PCA) and interactive biplot construction, and using cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5): sampling (A, B, C and D) versus total bacteria (PCA), mannitol$^-$ bacteria (MSA), mannitol$^+$ bacteria (MSA) and *S. aureus*.

Sampling	Surface	CFU/ml							
		Total bacteria		Mannitol $^-$		Mannitol $^+$		*S. aureus*	
		A	±SD	A	±SD	A	±SD	A	±SD
A. Pre-aseptic hand Surfaces	Right hand	1,284.2	±2,477.3	150.5	±266.9	90.5	±270.7	17.9	±25.9
	Left hand	5,932.1	±22,633.2	179.5	±246.1	69.5	±135.4	28.0	±33.8
	∑	7,216.3	±24,962.5	330.0	±417.8	160.0	±320.3	45.9	±51.6
B. Hand surfaces immediately after asepsis	Right hand	72.6	±161.4	17.4	±48.5	3.7	±8.3	2.3	±5.3
	Left hand	198.9	±430.3	44.7	±159.2	13.2	±36.1	9.5	±25.0
	∑	271.6	±467.5	62.1	±206.5	16.8	±39.0	11.7	±28.3
C. Glove surfaces immediately after dental procedures	Right hand	86.9	±143.5	18.5	±24.8	4.6	±11.3	4.6	±11.3
	Left hand	76.2	±102.1	38.5	±62.7	3.1	±8.5	3.1	±8.5
	∑	163.1	±209.6	56.9	±77.3	7.7	±17.9	7.7	±17.9
D. Hand surfaces immediately after dental procedures	Right hand	143.9	±334.3	5.0	±10.4	2.2	±5.5	2.2	±5.5
	Left hand	114.4	±318.6	17.2	±58.4	2.2	±9.4	2.2	±9.4
	∑	258.3	±495.9	22.2	±58.2	4.4	±12.5	4.4	±12.5

Sampling A and B consisted of 19 dental students and sampling C and D were composed of 13 and 18 students, respectively. CFU/ml correspond to colony forming unit per milliliter. PCA culture medium (Plate Count Agar): analysis of total bacteria.

MSA selective culture medium (Mannitol Salt Agar): analysis of mannitol $^-$ and mannitol $^+$ microorganisms (preliminarily pathogenic *Staphylococci* and *S. aureus*, respectively). *S. aureus*: species were characterized by Gram staining, growth in CHROMagar *Staphylococcus aureus* chromogenic medium, catalase, coagulase, Factor A test agglutination, mannitol fermentation test, DNAse test and Voges-Proskauer test.

Figure 2: Average density (CFU/ml) of bacterial contamination (total bacteria, mannitol^{-} and mannitol^{+} bacteria, and *S. aureus*) on surfaces of the hands and gloves from dentistry students obtained in different sampling moments: A. Pre-aseptic hand surfaces; B. Hand surfaces immediately after asepsis; C. Glove surfaces immediately after dental procedures; and D. Hand surfaces immediately after dental procedures. The data obtained (average values of CFU/ml) were submitted to multivariate statistical (SAS® version 9.2), using principal component analysis (PCA) and interactive biplot construction, and using cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5): sampling (A, B, C and D) versus total bacteria (PCA), mannitol^{-} bacteria (MSA), mannitol^{+} bacteria (MSA) and *S. aureus*.



(i) Sampling A (pre-aseptic hand surfaces): left hand $^{LH\ (A)}$, and right and/or left hand $^{RH\ and/or\ LH\ (A)}$ were characterized by high values of average density (CFU/ml) of total bacteria, mannitol^{-} and mannitol^{+} bacteria, and *S. aureus*. Note: right hand $^{RH\ (A)}$ was only characterized by high value of average density (CFU/ml) of mannitol^{+} bacteria.

(ii) Sampling B (hand surfaces immediately after asepsis), C (glove surfaces immediately after dental procedures), D (hand surfaces immediately after dental procedures), and A partial: right hand $^{RH\ (ABCD)}$, left hand $^{LH\ (BCD)}$, and right and/or left hand $^{RH\ and/or\ LH\ (BCD)}$ were characterized by low values of average density (CFU/ml) of total bacteria, mannitol^{-} and mannitol^{+} bacteria, and *S. aureus*.

Among the 132 isolates of *S. aureus* identified in the samples (A, B, C and D), 72 (54.5%), 35 (26.5%), 17 (12.9%), 10 (7.6%), 10 (7.6%), 10 (7.6%), 20 (15.2%) and 59 (44.7%) isolates were resistant to AMP, AMX, OXA, TET, LEX, CEF, FOX, CLI and ERY, respectively. All isolates were sensitive to VAN.

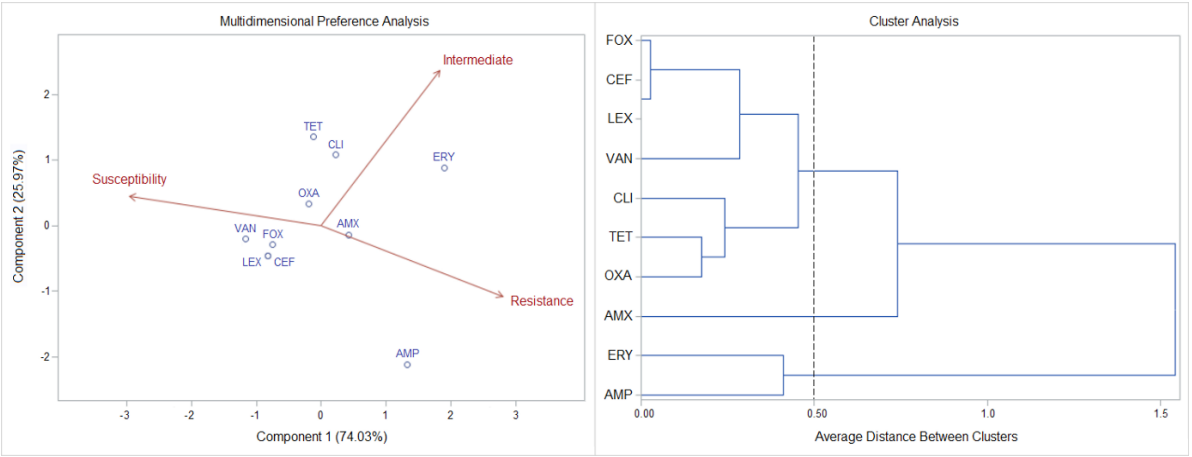
Data on the frequency of antibacterial susceptibility profiles of 132 *S. aureus* isolates obtained from surfaces of the hands and gloves from fourteen dentistry students were submitted to multivariate statistical using the SAS® version 9.2 [principal component analysis (PCA) and interactive biplot construction; cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5)], which revealed similarity profiles as follows (Table 3 and Fig. 3):

Table 3: Antibacterial susceptibility patterns of 132 *S. aureus* isolates obtained from surfaces of the hands and gloves from fourteen dentistry students in different sampling moments. The data obtained in the frequency were submitted to multivariate statistical (SAS® version 9.2), using principal component analysis (PCA) and interactive biplot construction, and using cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5): antimicrobial versus antimicrobial susceptibility profile (resistant, intermediate and susceptible).

Antimicrobial	Antimicrobial susceptibility profile					
	Resistant		Intermediate		Susceptible	
	n	%	*n*	%	*n*	%
AMP	72	54.5	0	0.0	60	45.5

AMX	35	26.5	6	4.5	91	68.9
OXA	17	12.9	6	4.5	109	82.6
TET	10	7.6	11	8.3	111	84.1
VAN	0	0.0	0	0.0	132	100.0
LEX	10	7.6	0	0.0	122	92.4
CEF	10	7.6	0	0.0	122	92.4
FOX	10	7.6	1	0.8	121	91.7
CLI	20	15.2	11	8.3	101	76.5
ERY	59	44.7	16	12.1	57	43.2
∑	243	18.4	51	3.9	1026	77.7
Ampicillin 10µg (AMP), amoxicillin 10µg (AMX), oxacillin 1µg (OXA), tetracycline 30µg (TET), vancomycin 30µg (VAN), cephalexin 30µg (LEX), cephalothin 30µg (CEF), cefoxitin 30µg (FOX), clindamycin 2µg (CLI), and erythromycin 15µg (ERY).						

Figure 3: Antibacterial susceptibility patterns of 132 *S. aureus* isolates obtained from surfaces of the hands and gloves from fourteen dentistry students in different sampling moments: A. Pre-aseptic hand surfaces; B. Hand surfaces immediately after asepsis; C. Glove surfaces immediately after dental procedures; and D. Hand surfaces immediately after dental procedures. The data obtained in the frequency were submitted to multivariate statistical (SAS® version 9.2), using principal component analysis (PCA) and interactive biplot construction, and using cluster analysis (CA) and dendrogram interpretation (threshold ≤ 0.5): antimicrobial versus antimicrobial susceptibility profile (resistant, intermediate and susceptible). Ampicillin 10µg (AMP), amoxicillin 10µg (AMX), oxacillin 1µg (OXA), tetracycline 30µg (TET), vancomycin 30µg (VAN), cephalexin 30µg (LEX), cephalothin 30µg (CEF), cefoxitin 30µg (FOX), clindamycin 2µg (CLI), and erythromycin 15µg (ERY).



(i) AMP and ERY: high frequency of *S. aureus* isolates displaying resistance to ampicillin and erythromycin;

(ii) AMX: intermediate frequency of *S. aureus* isolates displaying resistance to amoxicillin;

(iii) CEF, CLI, FOX, LEX, OXA, TET and VAN: high frequency of *S. aureus* isolates displaying susceptibility to cephalothin, clindamycin, cefoxitin, cephalexin, oxacillin, tetracycline and vancomycin.

A total of 26 isolates of oxacillin-resistant *S. aureus* (ORSA) were identified, 18 isolates resistant to multiple drugs (Supplementary table 2), as interpreted in CLSI documents:

Supplementary table 2: Antibacterial susceptibility patterns of 132 *S. aureus* isolates obtained from surfaces of the hands and gloves from fourteen dentistry students in different sampling moments: A. Pre-aseptic hand surfaces; B. Hand surfaces immediately after asepsis; C. Glove surfaces immediately after dental procedures; and D. Hand surfaces immediately after dental procedures. RH and LH means right hand and left hand, respectively.

Code of dentistry student	Code of hands and gloves	Code of *S. aureus* isolates	Antibacterial susceptibility *									
			AMP	AMX	OXA	TET	VAN	LEX	CEF	FOX	CLI	ERY
3 (♀)	A-RH-3	1	S	S	S	S	S	S	S	S	S	R
	A-RH-3	2	S	S	S	S	S	S	S	S	S	R
	A-RH-3	3	S	S	S	S	S	S	S	S	S	R
	A-RH-3	4	S	S	S	S	S	S	S	S	S	S
	A-RH-3	5	S	S	S	I	S	S	S	S	S	R
	B-RH-3	6	R	R	S	S	S	S	S	S	S	R
	B-RH-3	7	R	R	S	S	S	S	S	S	S	R
	A-LH-3	8	S	S	S	I	S	S	S	S	S	R
	A-LH-3	9	S	S	S	S	S	S	S	S	S	R
	A-LH-3	10	S	S	S	I	S	S	S	S	S	R
	A-LH-3	11**	S	S	S	S	S	S	S	R	S	R
	A-LH-3	12	S	S	S	S	S	S	S	S	S	R
	A-LH-3	13	S	S	S	I	S	S	S	S	S	R
	A-LH-3	14	S	S	S	S	S	S	S	S	S	R
	A-LH-3	15	S	S	S	S	S	S	S	S	S	R
	B-LH-3	16	S	S	S	S	S	S	S	S	S	R
	B-LH-3	17	R	S	S	S	S	S	S	S	S	R
	B-LH-3	18	S	S	S	S	S	S	S	S	S	R
	B-LH-3	19	S	S	S	S	S	S	S	S	S	R
	B-LH-3	20	S	S	S	S	S	S	S	S	S	R
	B-LH-3	21	S	S	S	S	S	S	S	S	S	R
	B-LH-3	22	S	S	S	S	S	S	S	S	S	R
	B-LH-3	23	S	S	S	S	S	S	S	S	S	R

4 (♀)	A-RH-4	24	R	R	S	S	S	S	S	S	S	I
	A-RH-4	25	S	S	S	S	S	S	S	S	S	I
	A-RH-4	26	R	R	S	S	S	S	S	S	S	R
	D-RH-4	27	R	R	S	I	S	S	S	S	S	R
	D-RH-4	28	S	S	S	S	S	S	S	S	S	R
	A-LH-4	29	R	R	S	S	S	S	S	S	S	S
	A-LH-4	30	R	R	S	S	S	S	S	S	S	S
	A-LH-4	31	R	R	S	S	S	S	S	S	S	S
	A-LH-4	32	R	R	S	S	S	S	S	S	S	S
	A-LH-4	33	R	R	S	S	S	S	S	S	S	S
	A-LH-4	34	R	S	S	S	S	S	S	S	S	S
	A-LH-4	35	R	R	S	S	S	S	S	S	S	S
	A-LH-4	36	R	R	S	S	S	S	S	S	S	S

5 (♀)	A-RH-5	37	S	S	S	S	S	S	S	S	S	R
	A-RH-5	38	S	S	S	I	S	S	S	S	S	R
	A-RH-5	39	S	S	S	I	S	S	S	S	S	R
	A-LH-5	40	S	R	S	R	S	S	S	S	S	R
	A-LH-5	41**	R	R	S	S	S	R	R	R	R	R
	A-LH-5	42	R	R	S	R	S	S	S	S	S	I
	A-LH-5	43	R	R	S	R	S	S	S	S	S	I
	A-LH-5	44	R	R	S	R	S	S	R	S	S	I
	A-LH-5	45	R	R	S	R	S	S	S	S	S	S
	A-LH-5	46	S	R	S	R	S	S	S	S	I	I
	A-LH-5	47	S	R	S	R	S	S	S	S	S	R
8 (♀)	B-RH-8	48	R	R	S	R	S	R	S	S	S	R
	B-RH-8	49	R	R	S	I	S	S	S	S	S	R
	B-RH-8	50	S	S	S	S	S	S	S	S	S	S
	A-LH-8	51	S	S	S	S	S	S	S	S	S	S
	A-LH-8	52	R	R	S	S	S	S	S	S	S	S
9 (♀)	A-RH-9	53	S	S	S	S	S	S	S	S	S	R
	B-RH-9	54**	R	R	R	I	S	S	S	S	S	R
	A-LH-9	55	R	R	I	S	S	S	S	S	S	R
	A-LH-9	56**	S	S	R	S	S	S	S	S	S	R
	B-LH-9	57	S	S	S	S	S	S	S	S	S	R
10 (♂)	A-RH-10	58	R	R	S	I	S	S	S	S	S	R
	A-RH-10	59	R	R	S	I	S	S	S	S	S	S

11 (♀)	A-RH-11	60**	R	R	R	S	S	S	S	S	S	R
	A-LH-11	61**	S	S	R	S	S	S	S	S	S	S
	A-LH-11	62**	S	S	R	S	S	S	S	S	S	S
	A-LH-11	63**	S	S	R	S	S	S	S	S	S	S
	A-LH-11	64	S	S	S	S	S	S	S	S	S	S
	A-LH-11	65**	S	S	R	S	S	S	S	S	S	S
	A-LH-11	66**	S	S	R	S	S	S	S	S	S	S
	A-LH-11	67**	S	S	R	S	S	S	S	S	S	S
	A-LH-11	68**	S	S	R	S	S	S	S	S	S	S
12 (♀)	A-RH-12	69	S	S	S	S	S	S	S	S	R	R
	A-LH-12	70**	R	R	S	S	S	R	R	R	R	R
	B-RH-12	71	S	S	S	S	S	S	S	S	R	R
15 (♀)	A-RH-15	72	S	S	S	S	S	S	S	S	S	S
	A-RH-15	73	S	S	S	S	S	S	S	S	S	S
	C-RH-15	74	R	S	S	S	S	S	S	S	S	S
	C-RH-15	75	R	S	S	S	S	S	S	S	S	S
	C-RH-15	76	R	S	S	S	S	S	S	S	S	S
	A-LH-15	77	R	S	I	S	S	S	S	S	R	R
	A-LH-15	78	R	S	S	S	S	S	S	S	R	R
	A-LH-15	79	S	S	S	S	S	S	S	S	S	s
	A-LH-15	80	S	S	S	S	S	S	S	S	R	R
16 (♀)	A-LH-16	81**	R	R	I	S	S	R	R	R	R	R
	A-LH-16	82**	R	S	S	S	S	R	R	R	R	R

18 (♂)	A-LH-18	83	S	S	S	S	S	S	S	S	I	I
19 (♂)	A-RH-19	84**	R	S	R	S	S	S	S	S	R	R
	A-RH-19	85	R	S	S	S	S	S	S	S	S	S
	A-RH-19	86	R	R	S	S	S	S	S	S	S	S
	A-RH-19	87	R	S	S	S	S	S	S	S	S	S
	A-RH-19	88**	R	S	R	S	S	S	S	S	R	R
	A-RH-19	89	R	S	S	S	S	S	S	S	S	S
	A-RH-19	90	R	R	I	S	S	S	S	S	R	R
	A-RH-19	91	R	I	S	S	S	S	S	S	S	S
	C-RH-19	92**	R	S	I	R	S	R	R	R	R	R
	C-RH-19	93**	R	R	S	S	S	R	R	R	R	R
	C-RH-19	94**	R	R	R	S	S	S	S	S	S	S
	D-RH-19	95	R	S	S	S	S	S	S	S	R	R
	A-LH-19	96	R	I	S	S	S	S	S	S	S	S
	A-LH-19	97	R	S	S	S	S	S	S	S	S	S
	A-LH-19	98	R	S	S	S	S	S	S	S	S	S
	A-LH-19	99	R	S	S	S	S	S	S	S	S	S
	A-LH-19	100	R	S	S	S	S	S	S	S	R	R
	A-LH-19	101	R	S	S	S	S	S	S	S	S	S
	A-LH-19	102	R	S	S	S	S	S	S	S	S	S
	A-LH-19	103	R	I	S	S	S	S	S	S	S	S
	B-LH-19	104	R	S	S	S	S	S	S	S	S	S
	B-LH-19	105	R	S	S	S	S	S	S	S	S	S
	B-LH-19	106	R	S	S	S	S	S	S	S	S	S
	B-LH-19	107	R	S	S	S	S	S	S	S	S	S
	B-LH-19	108	R	S	S	S	S	S	S	S	S	S
	B-LH-19	109	R	S	S	S	S	S	S	S	S	S
	B-LH-19	110	R	S	S	S	S	S	S	S	S	S
	B-LH-19	111	R	S	S	S	S	S	S	S	S	S
	C-LH-19	112**	R	S	S	S	S	R	R	R	R	R
	C-LH-19	113**	R	S	R	S	S	R	R	R	R	R
	C-LH-19	114**	S	S	S	S	S	R	R	R	R	R
	D-LH-19	115**	S	I	R	S	S	S	S	S	S	S
	D-LH-19	116	S	S	S	S	S	S	S	S	S	S
	D-LH-19	117	R	R	S	S	S	S	S	S	S	S
	D-LH-19	118**	R	S	R	S	S	S	S	I	I	I

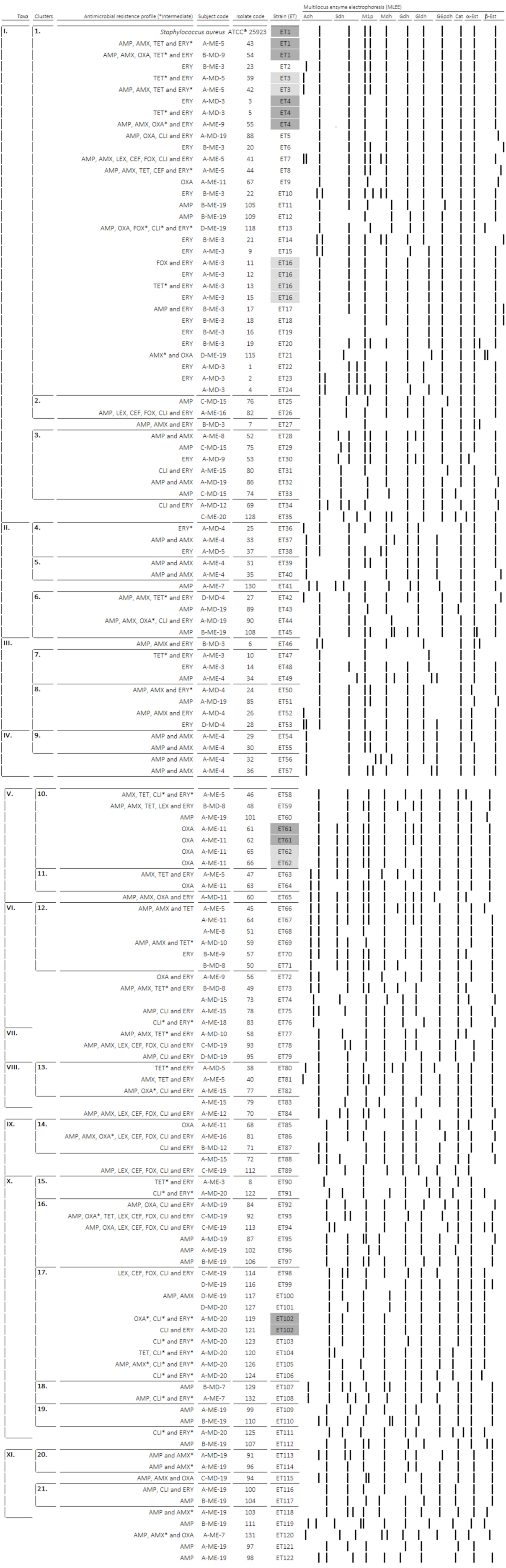
20 (♀)	A-RH-20	119	S	S	I	S	S	S	S	S	I	I
	A-RH-20	120	S	S	S	R	S	S	S	S	I	I
	A-RH-20	121	S	S	S	S	S	S	S	S	R	R
	A-RH-20	122	S	S	S	S	S	S	S	S	I	I
	A-RH-20	123	S	S	S	S	S	S	S	S	I	I
	A-RH-20	124	S	S	S	S	S	S	S	S	I	I
	A-RH-20	125	S	S	S	S	S	S	S	S	I	I
	A-RH-20	126	R	I	S	S	S	S	S	S	I	I
	D-RH-20	127	S	S	S	S	S	S	S	S	S	S
	C-LH-20	128	S	S	S	S	S	S	S	S	S	S
7 (♀)	B-RH-7	129	R	S	S	S	S	S	S	S	S	S
	A-LH-7	130	R	S	S	S	S	S	S	S	S	S
	A-LH-7	131**	R	I	R	S	S	S	S	S	S	S
	A-LH-7	132	R	S	S	S	S	S	S	S	I	I
			AMP	AMX	OXA	TET	VAN	LEX	CEF	FOX	CLI	ERY
Σ (Resistant to antimicrobial – R)			72 (54.5%)	35 (26.5%)	17 (12.9%)	10 (7.6%)	0 (0.0%)	10 (7.6%)	10 (7.6%)	10 (7.6%)	20 (15.2%)	59 (44.7%)
Σ (Intermediate to antimicrobial – I)			0 (0.0%)	6 (4.5%)	6 (4.5%)	11 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	11 (8.3%)	16 (12.1%)
Σ (Susceptible to antimicrobial – S)			60 (45.5%)	91 (68.9%)	109 (82.6%)	111 (84.1%)	132 (100%)	122 (92.4%)	122 (92.4%)	121 (91.7%)	101 (76.5%)	57 (43.2%)

*R, I and S corresponds to resistant, intermediary and sensible, respectively. ** ORSA (oxacillin-resistant *S. aureus*). Ampicillin 10µg (AMP), amoxicillin 10µg (AMX), oxacillin 1µg (OXA), tetracycline 30µg (TET), vancomycin 30µg (VAN), cephalexin 30µg (LEX), cephalothin 30µg (CEF), cefoxitin 30µg (FOX), clindamycin 2µg (CLI), and erythromycin 15µg (ERY).

- Oxacillin-resistant *S. aureus* with multidrug-resistant phenotype (*n* = 10): isolates 54, 56, 60, 84, 88, 94, 113, 115, 118 and 131.
- Oxacillin-resistant *S. aureus* with non-multi-resistant phenotype (*n* = 7): isolates 61, 62, 63, 65, 66, 67 and 68
- *S. aureus* intermediate to oxacillin and *S. aureus* resistant to cefoxitin with multidrug-resistant phenotype (*n* = 2): isolates 81 and 92
- Cefoxitin-resistant *S. aureus* with multidrug-resistant phenotype (*n* = 6): isolates 41, 70, 82, 93, 112 and 114.
- *S. aureus* resistant to cefoxitin with non-multiresistant phenotype (*n* = 1) isolated 11.

The isoenzymatic patterns of multiresistant *S. aureus* isolates ($n = 132$), including the reference strain ATCC 25923, were reproducible in different gels after three repetitions of each electrophoretic run (that is, reproducibility was excellent for the replicated samples: ≥ 95%). According to the haploid nature of *S. aureus*, those patterns demonstrated the following characteristics (Fig. 4):

Figure 4: Allelic profiles of 122 electrophoretic types (ET; strains) of *S. aureus* isolated from the hands of dentistry students, and its characteristics and distributions in clusters/taxa. MLEE typing: Adh (alcohol dehydrogenase EC 1.1.1.1), Sdh (sorbitol dehydrogenase EC 1.1.1.14), M1p (mannitol-1-phosphate dehydrogenase EC 1.1.1.17), Mdh (malate dehydrogenase EC 1.1.1.37), Gdh (glucose dehydrogenase EC 1.1.1.47), Gldh (D-galactose dehydrogenase EC 1.1.1.48), G6pdh (glucose-6-phosphate dehydrogenase EC 1.1.1.49), Cat (catalase EC 1.11.1.6) and α-Est and β-Est (α- and β-esterase EC 3.1.1.1)



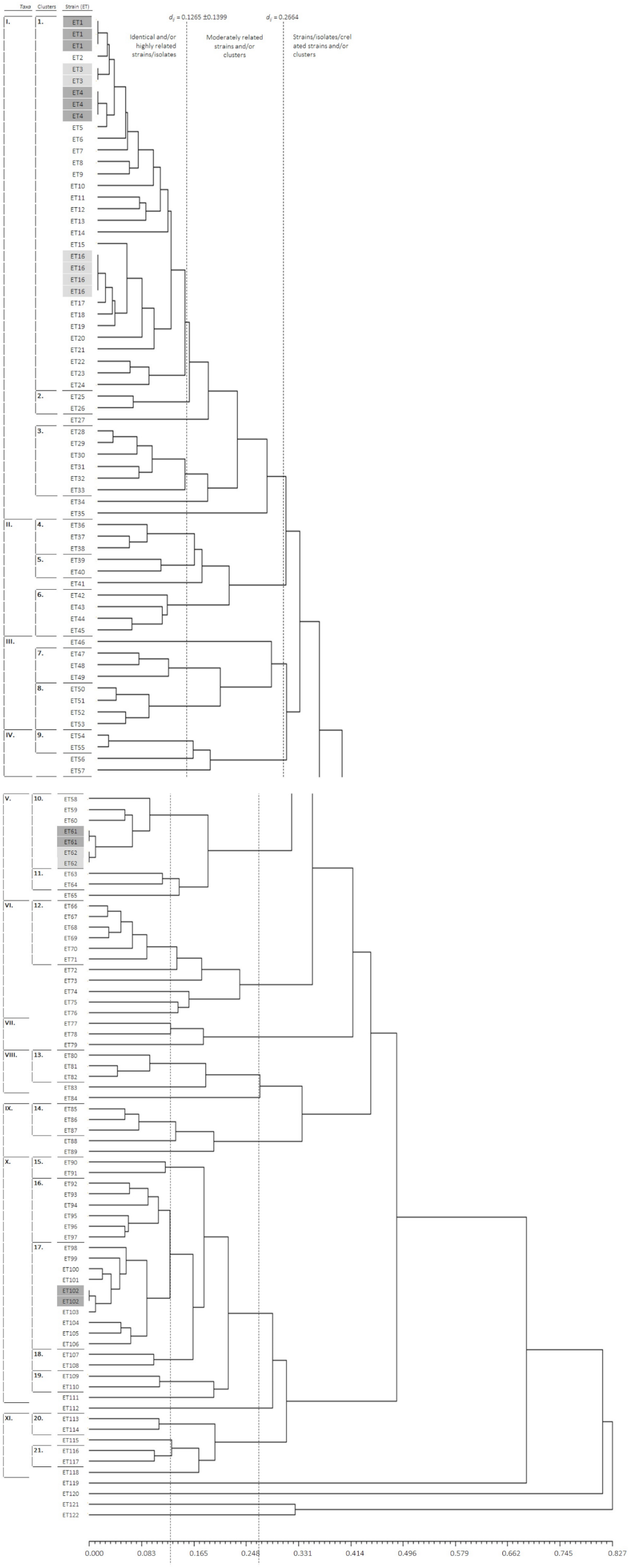
Polymorphic locus ($n = 29$): Adh-1 (allele *a* 6.06%), Adh-2 (alleles *a* 9.85%, *b* 3.03%, *c* 8.33% and *d* 2.27%), Adh-3 (alleles *a* 4.55% and *b* 64.39%), Adh-4 (alleles *a* 3.03%, *b* 2.27%, *c* 16.67% and *d* 11.36%), Sdh-1 (alleles *a* 3.03%, *b* 10.61% and *c* 6.82%), Sdh-2 (alleles *a* 11.36% and *b* 7.58%), Sdh-3 (alleles *a* 65.15% and *b* 3.79%), Sdh-4 (alleles *a* 1.52% and *b* 5.30%), M1p-1 (allele *a* 2.27%), M1p-2 (allele *a* 67.42%), M1p-3 (allele *a* 23.48%), M1p-4 (allele *a* 32.58%, *b* 1.52% and *c* 0.76% – rare allele), Mdh-1 (alleles *a* 7.58% and *b* 2.27%), Mdh-2 (alleles *a* 75.76% and *b* 4.55%), Mdh-3 (allele *a* 9.09%), Mdh-4 (allele *a* 3.79%), Gdh-1 (alleles *a* 3.79%, *b* 0.76% – rare allele and *c* 10.61%), Gdh-2 (alleles *a* 73.48% and *b* 7.58%), Gldh-1 (alleles *a* 6.82%, *b* 18.18% and *c* 2.27%), Gldh-2 (alleles *a* 62.12%, *b* 3.79%, *c* 9.85% and *d* 3.79%), G6pdh (alleles *a* 9.09%, *b* 4.55%, *c* 71.21%, *d* 2.27%, *and* 3.03% and *f* 0.76% – rare allele), Cat (alleles *a* 6.06%, *b* 6.82% and *c* 87.12%), α-Est-1 (allele *a* 1.52%), α-Est-2 (alleles *a* 4.55%, *b* 6.82% and *c* 81.06%), α-Est-3 (alleles *a* 8.33% and *b* 2.27%), β-Est-1 (allele *a* 2.27%), β-Est-2 (alleles *a* 11.36%, *b* 2.27% and *c* 7.58%), β-Est-3 (alleles *a* 62.12%, *b* 9.09% and *c* 2.27%) and β-Est-4 (allele *a* 3.03%).

- Average number of alleles per locus: 2.3928.
- Average number of alleles per polymorphic locus: 2.2857.
- Electrophoretic types (ET): 122 strains (92.4% of total isolates).
- Discriminatory power: 0.998177261, that is, 99.8% probability that two unrelated isolates of *S. aureus* sampled in test population will be classified into different strains.
- Clonality patterns: monoclonal and polyclonal (very frequent).

The genetic diversity of 132 isolates of *S. aureus* from the hands of dental students (122 strains / electrophoretic types) was analyzed using the Nei distance and a UPGMA dendrogram (Fig. 5). The genetic diversity of total bacterial population ranged from 0 to 0.827; that is, on average, the population of isolates contained 0 to 82.7 allelic substitutions for every 100 loci, starting from a common ancestor. Taking into account the average genetic distance ($d_{ij} = 0.1265 \pm 0.199$) obtained from total population of bacteria, these analyzes also allowed to verify the coexistence (threshold: $0.1265 > d_{ij} \geq 0$ – identical or highly related strains/isolates) or non-coexistence (threshold: $0.2664 > d_{ij} \geq 0.1265$ – moderately related isolates/strains/clusters; $d_{ij} \geq 0.2664$ – distantly related isolates/strains/clusters) from strains/bacterial clusters and to infer possible microbial propagation events among dental students, as well as microevolutionary bacterial changes and better adaptations. Eleven highly polymorphic *taxa* (from I to XI) were identified from the d_{ij} value above 0.2664 (genetically related *taxa* at a distance). Then, isolates were identified / strains or moderately related groups ($0.2664 \geq d_{ij} > 0.1265$) in each of those large *taxa*: 104 isolates distributed in 21 groups ($0.1265 > d_{ij} \geq 0$) and 28 non-grouped isolates ($d_{ij} \geq 0.1265$). Each of those clusters comprises two or more bacterial isolates interpreted as identical or highly related isolates ($0.1265 > d_{ij} \geq 0$). The interpretation of UPGMA dendrogram and, consequently, the qualitative distribution (that is, the epidemiological characteristic of bacterial population) and the quantitative distribution of those isolates / strains and clusters of each *taxon*, are described below:

- *Taxon* X comprised the largest number of clusters ($n = 5$), followed by *taxa* I and II ($n = 3$), I, III and XI ($n = 3$), IV, VI, VIII and IX ($n = 1$) and VII ($n = 0$).
- *Taxon* I comprised the largest number of isolates ($n = 43$, including *S. aureus* ATCC 25923), followed by *taxa* X ($n = 23$), VI ($n = 11$), I and II ($n = 10$) , III ($n = 8$), XI ($n = 6$), IX ($n = 5$), IV and VIII ($n = 4$) and VII ($n = 3$).
- Five isolates may possibly represent even less common *taxa* (ET84, ET112, ET119, ET120, ET121 and ET122).
- Most clusters comprise isolates of *S. aureus* (identical and / or highly related) exhibiting varying profiles of antimicrobial resistance (except clusters 5, 9, 19 and 20).
- Isolates of identical *S. aureus* and/or highly related are found on the surface of hands and gloves (right and/or left) of two or more dental students, before and/or after asepsis (more often), as well as after academic dental procedures (low frequency).

Figure 5: Genetic relationship between 122 strains (132 isolates) of *S. aureus* isolated from hands of dentistry students, including the reference strain *S. aureus* ATCC 25923. UPGMA dendrogram (r_{jk} = 0.80644: good concordance) generated from the genetic distance matrix (Nei, 1972) and the genetic interpretation of the patterns MLEE.



Discussion

Recognizing the potential threat that exists, as the hands are an important source of infection and transmission of *S. aureus* in dental environment [8], provides the identification of how biosafety interferes in prevention of bacterial infections in the dental field. As expected, in samples obtained before the asepsis of dental students' hands, a significant presence was noticed, mainly in the identification of the total microbial content, in addition to the presence, even if in a smaller number of *S. aureus* strains. After asepsis of hands, there was a significant decrease in the total microbial content, but the potentially pathogenic strains of *S. aureus* remained present in hands after asepsis, gloves and hands after procedures.

S. aureus has become a paradigm for hospital infections, due to its high frequency and its pathogenicity, which enables it to produce diseases, both in immunocompromised and healthy individuals, its easy dissemination and resistance to antibiotics. In 2017, in the United States, about 119,247 bloodstream infections, 19,832 deaths were associated with methicillin-resistant *S. aureus* (MRSA), most of those infections were in debilitated people in healthcare settings [15].

Hygiene incorporates behavioral and procedural rules that prevent transmission of bacteria and consequently the possibility of spreading bacteria such as methicillin-resistant *S. aureus* (MRSA). Any infection prevention and control strategy must be supported by changes in attitude and adopted by all. Among the main components of MRSA prevention and control include hand and environment hygiene [16]. According to Loftus et al. (2018), hand hygiene should be performed before and after direct contact with patients, before

and after use of gloves, or after contact with inanimate objects in the vicinity of patient, in order to reduce risk and spread of infection [9].

Emphasizing the importance of hand washing as a preventive action in the control of infection by microorganisms, the West China Stomatology hospital at Sichuan University, in face of the 2019-nCoV pandemic, proposed that especially dental professionals should wash their hands before examining the patient, before dental procedures, after touching patients, after touching surroundings and equipment. More caution should be taken by dental professionals to avoid touching their eyes, mouth and nose [17].

Strains of *S. aureus* can be disseminated during dental treatment and occasionally lead to contamination and infection of patients and dentists. In one study, *Staphylococcus* species were isolated from the tongue, nose and hands of 30 students and 30 patients and from the Pediatric Dentistry Clinic. The highest count of *S. aureus* was found on the patients' nose and tongue. In relation to dentistry students, the greatest contamination was observed in gloved hands, followed by the tongue and hands without gloves before clinical care. At the end of dental treatment, *S. aureus* colonies isolated from gloves decreased significantly. Considering the clinical environment, the most contaminated area was the auxiliary table. Dental clinic can be considered a favorable environment for the cross transmission of *S. aureus*. Therefore, preventive measures must be used to prevent the spread of pathogenic microorganisms [18].

As for the antimicrobial susceptibility test, there is an expressive resistance of some strains of *S. aureus* isolated from hands and gloves of dentistry students, in dental procedures before the antibiotics used in the study, mainly ampicillin (54.5% of the isolates), erythromycin (44.7% of the isolates) and amoxicillin (26.5% of the isolates) consecutively, in contrast vancomycin (100% of the isolates) proved to be totally efficient in the same study. A similar study, involving saliva and nasal secretion samples from 100 nursing professionals, found that 43.0% tested positive for *S. aureus*, of 74 nasal secretion samples with *S. aureus*, 14.9% had resistance to oxacillin; 91.9% to penicillin; 44.6% to erythromycin and 41.9% to clindamycin. Of 12 positive saliva samples, 16.7% were resistant to oxacillin; 100.0% to penicillin; 33.4% to erythromycin and 25.0% to clindamycin [19].

The isoenzymatic electrophoresis profiles of potentially pathogenic *S. aureus* isolates were reproducible in different gels after three repetitions of each electrophoretic run, thus it has been understood that the reproducibility was excellent for the replicated samples (≥ 95%). A genetic relationship was observed between 122 strains of 132 isolates of *S. aureus*, including the reference strain *S. aureus* ATCC 25923, from the hands of dentistry students. Analyzing UPGMA Dendrogram, there was good agreement (r_{jk} = 0.80644) generated from the genetic distance matrix [14] and the genetic interpretation of MLEE patterns.

It is recommended, therefore, to intensify actions of educational process within the scope of concepts about the importance of hand hygiene and asepsis procedures by health professionals, cross-microbial infections and antimicrobial resistance in dental and hospital clinical environments. Neglect in hand hygiene is a major cause of infection in healthcare settings. The present study suggests that *S. aureus* represents a great risk to dental practice, however it is necessary to deepen new specific studies, since for the comparison of results there was an absence of published articles with the presence of multiresistant *S. aureus* in an environment dental care. The spread of antimicrobial resistance calls for a reassessment of personal hygiene and the environment as a means to avoid the problem. The first safety challenge for patient care is the adoption of international infection control guidelines. Hand hygiene must follow a global standard for patient care in all health services ensuring at least a reduction in the possibilities of MRSA transmission.

Most isolates of *S. aureus* are sensitive to almost all antibiotics tested and commonly used to treat infections or antibiotic prophylaxis: vancomycin ^{VAN} (100% of isolates are sensitive); cephalexin ^{LEX}, cephalotin ^{CEF} and cefoxitin ^{FOX} (<93% of isolates are sensitive); oxacillin ^{OXA} and tetracycline ^{TET} (<85% of isolates are sensitive); clindamycin ^{CLI} (<77% of isolates are sensitive); amoxicillin ^{AMX} (<69% of isolates are sensitive); ampicillin ^{AMP} and erythromycin ^{ERY} (<46% of isolates are sensitive). Although at low frequency, multiple drug resistant *S. aureus*, especially *S. aureus* (ORSA), are found in hands and gloves of dental students during their academic clinical activities. MLEE typing method has a high discriminatory power and reproducibility for species of *S. aureus*. Monoclonal and mainly polyclonal patterns occur in the population of *S. aureus*, coming from the surface of hands and gloves of dental students, either under pre-asepsis conditions or immediately after asepsis and post-dental procedures. Analyzes of diversity and genetic relationship show that isolates of *S. aureus*, genetically identical and/or highly related and, therefore, grouped in the same group, exhibit variable phenotypes of resistance to multiple drugs. Those analyzes also reveal that two or more dentistry students share *S. aureus* isolates through their hands and gloves during their academic clinical activities.

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Ethical considerations

This study was initiated after approval by the Research Ethics Committee (CAAE: 25227319.0.0000.5578), according to the current resolution for Research Ethics in Human Beings No. 466/12 of National Health Council (Ministério da Saúde, DF), in addition to prior signature of Free and Informed Consent Form.

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