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Methicillin-resistant *Staphylococcus* spp. in the nasal cavity of dental surgeon's professors

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The aim of the study is to investigate the methicillin-resistant *Staphylococcus* spp. nasal colonization among dental surgeon professors. Dental surgeon professors of a Higher Education Institution (HEI) responded to a questionnaire covering sociodemographic, employment and behavioral data, and were subjected to clinical specimen collection by nasal swab. Identification and susceptibility testing of bacteria were performed by automated method (Vitek 2 compact™). Susceptibility to mupirocin was tested by disk-diffusion method. The detection of *mec*A and *lukS*-F genes was performed by PCR. The genetic similarity among the isolates was determined by Pulsed Field Gel Electrophoresis. Four (9.7%) dental surgeon professors were colonized by methicillin-resistant *Staphylococcus* spp. and claim have provided care to patients without wearing surgical masks (1/4) and/or gloves (4/4), and had the habit of keeping surgical masks on the chin (1/4). Two *S. aureus* and one *S. epidermidis* isolates were *mec*A gene positives. MLS_B complex (inducible), mupirocin and sulfamethoxazole/trimethoprim resistance were also detected. The *lukS-F* gene was not detected in any *S. aureus* and no genetic similarity was found among the isolates. Dental surgeon professors were found to be colonized with methicillin-resistant *Staphylococcus* spp. and declared noncompliance to infection control practices, posing risk of infection to themselves, patients, students and their families.

Key words: Occupational dentistry, antimicrobials/antimicrobial resistance, dental education, infection control, bacteria, infectious disease(s).

INTRODUCTION

The dental surgeon (DS) provides treatment to patients with various medical problems by peculiar procedures including the continuous use of instruments that generate droplets and aerosols, which enhances his exposure to a

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wide variety of microorganisms, including pathogenic bacteria, favoring the colonization (Centers for Disease Control and Prevention 2016; Harrel and Molinari, 2004; Centers for Disease Control and Prevention 2013). Additional factors that make healthcare workers (HCW) vulnerable to colonization are the non-adherence to biosecurity measures (Centers for Disease Control and Prevention 2016; Siegel et al., 2007; Centers for Disease Control and Prevention, 2003). Additionally, unlike hospital settings, the DS works in clinics where, sometimes, the clinical care is conducted in the same area where the reprocessing of dental reusable devices/ instruments is performed, due to the absence of a specific area for this purpose (Alvarenga et al., 2010).

Colonization status poses risks to the HCW since, in an episode of imbalance of the microbiota and immune system, an endogenous infection may be developed (Kim et al., 2018; Zervou et al., 2014; Albrich and Harbarth 2008). In addition, it poses risks to the patient, once the colonized HCW becomes a reservoir and a potential source of bacteria in the epidemiological chain of Healthcare-Associated Infections (HAI) (Ugolotti et al., 2018; Kim et al., 2018; Zervou et al., 2014; Costa et al., 2014; Albrich and Harbarth 2008). However, studies on the colonization of dental HCW with multidrug-resistant bacteria are scarce (Khairalla et al., 2017), particularly, in clinical practice in higher educational institutions, which reflects the reality of clinical care treatments in outpatient dental clinics.

Among the more relevant multidrug-resistant bacteria in the context of HAI, methicillin-resistant *Staphylococcus aureus* (MRSA) stands out. This infectious agent is associated with high morbi-mortality rates worldwide (Grundmann et al., 2006) and belongs to the ESKAPE group (*Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa* and *Enterobacter* spp.), composed of bacteria that are often multidrug-resistant (Rice, 2008). Coagulase-negative *Staphylococcus,* especially, methicillin-resistant *Staphylococcus epidermidis* (MRSE), previously reported as contaminant, also represent important pathogens in the context of the HAI (Soumya et al., 2017; Becker et al., 2014). Thus, the aim of this paper was to investigate the nasal colonization of DS professors with methicillin-resistant *Staphylococcus* spp. These HCW were chosen because they practice in various medical specialties and dedicate themselves to the academic teaching and guidance for students in dental clinical practice.

MATERIALS AND METHODS

This study was conducted in a Higher Education Institution (HEI) in the Central-West region of Brazil. The institution has a total of 106 dental offices organized in polyclinics, providing an average of 4.500 consultations per month in several dental specialties. All DS involved in academic teaching and guidance activities of the institution were invited to participate. The faculty team was composed of 53 DS, and 43 of them were in clinical practice. The inclusion criteria were: To be a dental surgeon, to be an employee of the HEI, and to have a role in providing guidance in academic clinical activities during the period of the samples collection. The exclusion criteria were: Suspicion of upper respiratory tract infection at the time of the samples collection, and who were using or had used any antimicrobial in the last 30 days prior to the samples collection. The project was approved by the Ethics and Research Committee (protocol number 509.774) and the Informed Consent was read and signed by the participants.

Data and sample collection

The eligible DS who agreed to participate in the study responded to a questionnaire related to socio-demographic, employment and behavioral aspects. Nasal specimens were obtained by sterile swab moisturized with sterile saline (0.9%) (Askarian et al., 2009; Scarnato et al., 2003), and were stored in tubes containing *Stuart* transport medium (Copan®, Brescia, Italy). The tubes were transported to the laboratory of bacteriology at room temperature and processed within 12 h.

The nasal swab was immersed in Brain Heart Infusion (BHI) broth and mixed on vortex for 1 min and incubated for 18/24 h at 35°C. Following incubation, the broth culture was inoculated onto mannitol salt agar and tryptic soy agar (TSA) supplemented with 4.0% NaCl and 6 µg/mL of oxacillin (primary culture), followed by incubation at 35°C for up to 72 h. The colonies suggestive of *Staphylococcus* sp. were initially identified by their macroscopic and microscopic characteristics, by Gram stain, and streaked onto mannitol salt agar and incubated at 35°C for 24 h to isolate pure cultures. Colonies were subcultured onto nutrient agar and incubated for 24 h at 35°C, to perform the test of catalase production and storage into microtubes containing tryptic soy broth with 20% of glycerol, at -20°C.

The biochemical identification (Vitek™ 2 GP card) and evaluation of antimicrobial susceptibility, the detection of methicillin resistance and the induced resistance to the Macrolide-lincosamide-streptogramin B group (MLS_B) (Vitek™ 2 - AST-GP-P585) were performed by automated method using the Vitek 2 Compact™ system, according to the manufacturer instructions for use. Susceptibility to mupirocin (20 µg) was analyzed by disk-diffusion method (Clinical and Laboratory Standards Institute, 2015) and the interpretation of the test was done following the recommendations of the British Society for Antimicrobial Chemotherapy (British Society for Antimicrobial Chemotherapy, 2015). The standard strain (ATCC 25923) was used as a quality control.

mecA and *lukS-F* genes detection

All *Staphylococcus* sp. identified by the Vitek 2 Compact™ system were submitted to genomic DNA extraction (Aires de Sousa et al., 2007) and subjected to PCR for detection of *mec*A gene (Murakami et al., 1991), which is responsible for the alternative pathway for the synthesis of a modified PBP (PBP2a or PBP2'), using the primers: F 5'-TCCAGATTACAACTTCACCAGG-3' and R 5'-CCACTTCATATCTTGTAACG-3'. Cycle condition: 4 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 53°C, 1 min at 72°C, and an additional extension of 4 min at 72°C. The detection of *lukS*-F gene, which encodes the Panton-Valentine leukocidin (PVL), was performed in all *S. aureus* identified, using the primers: PVL1: 5' - ATCATTAGGTAAAATGTCTGGACATGATCCA- 3' and PVL2: 5'-GCATCAASTGTATTGGATAGCAAAAGC – 3' (Lina et al., 1999), under the following cycle condition: 5 min at 94°C, 25 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C and an additional extension of 7 min at 72°C.

Table 1. Sociodemographic, employment and behavioral characteristics of dental surgeon professors (N = 41).

Variable	N	%
Age (years)		
<50	17	41.5
≥50	24	58.5
Experience (years)		
01 - 15	12	29.3
16 - 30	18	43.9
31 - 45	11	26.8
Clinical practice area*		
Esthetic/prosthetic dentistry	18	43.9
Periodontics/implantology/buccomaxilofacial surgery	11	26.8
Endodontics	07	17.0
Pediatric dentistry	08	19.5
Clinical activity (working hours per week)		
01 - 10	18	43.9
11 - 20	12	29.3
21 - 40	07	17.1
< 40	04	9.8
Currently working in hospital settings		
Yes	05	12.2
No	36	87.8
Ever worked in hospital settings		
Yes	12	29.3
No	29	70.7

* Possibility of more than one alternative.

Pulsed Field Gel Electrophoresis

The chromosomal DNA macrorestriction profile of the isolates was determined by Pulsed Field Gel Electrophoresis (PFGE), after bacterial chromosome digestion with *Sma*I (Chung et al., 2000). The PFGE was performed with 1% agarose gel in Tris-Borate-EDTA 0.5X buffer solution using the CHEF DRII system (Bio-Rad Laboratories). Images were captured with the Molecular Imager Gel Doc XR (Bio-Rad™) and analyzed by BioNumerics program (version 5.0; Applied Maths, Ghent, Belgium). The construction of the dendrogram was established by using the similarity coefficient of Dice (Dice, 1945), based on the position and presence of the bands and the phylogenetic analysis algorithm UPGMA (Unweighted Pair-Groups Method), using unweighted average clustering (Sneath and Sokal, 1975). The tolerance and optimization parameters were set to 0.7 and 1.0%. Each cluster of isolates will be defined as a grouping of profiles (n ≥ 2), presenting a similarity coefficient above 80% (Carriço et al., 2005).

RESULTS

Forty-one (77.3%) of the 53 DS professors actively involved in teaching participated in this study. Table 1 presents the socio-demographic and employment characteristics of participants. Of the 41 DS professors, 31.7% (13/41) were colonized in the nasal cavity with *Staphylococcus* spp. and 9.7% (4/41) were colonized with methicillin-resistant *Staphylococcus*. Table 2 presents the socio-demographics, employment and behavioral risk characteristics of the four DS professors colonized with methicillin-resistant *Staphylococcus*, who are identified as A, B, C and D. Cases of upper respiratory tract infections (tonsillitis), before sample collection, and use of antimicrobials (clavulanic acid and amoxicillin combined with clavulanic acid), not within the 30 days prior sample collection, were confirmed by 2/4 DS professors.

Three MRSA were isolated, denominated MRSA 1 (from DS professor A), MRSA 2 (from DS professor B), MRSA 3 (from DS professor C) and 1 MRSE (from DS professor D). Isolates MRSA 1 and MRSA 3 were susceptible to cefoxitin screen test (disk-diffusion), but *mec*A gene positive (Figure 1), thus considered MRSA. MRSA 2 was resistant to oxacillin and to cefoxitin screen test, although *mec*A gene negative. Inducible resistance to MLS_B complex was observed in two (50.0%) of the isolates (MRSA 1 and MRSE) (Table 3).

MRSA 1 was mupirocin-resistant and MRSE was trimethoprim/sulfamethoxazole-resistant. MRSE also presented intermediate resistance to quinolones, ciprofloxacin and norfloxacin (Table 4). All methicillin-resistant *Staphylococcus* spp. were susceptible to

Table 2. Sociodemographic, employment and behavioral characteristics of dental surgeon professors colonized with methicillin-resistant *Staphylococcus* spp. (N = 4).

Socio-demographic and employment characteristics	Dental surgeons*			
	A	B	C	D
Age (years)	≥50	≥50	<50	≥50
Experience (years)	25	37	14	31
Clinical practice area*	Esthetic/ prosthetic dentistry	Esthetic/ prosthetic dentistry	Pediatric dentistry	Endodontics
Clinical activity (working hours per week)	11 and 20	1 and 10	1 and 10	11 and 20
Behavioral risk characteristics				
Hand hygiene not performed upon changing torn gloves	X	X	X	
Gloves not used during some patient care			X	X
Dental care has been provided to patients without using gloves	X	X	X	X
Dental care has been provided to patients without using surgical masks		X		
Surgical cloth mask has been used by dentist	X	X		X
Habit of wearing masks on the chin	X			
Not changing wet masks			X	

*Dental surgeons colonized with methicillin-resistant *Staphylococcus* spp. identified by the letters A, B, C and D.

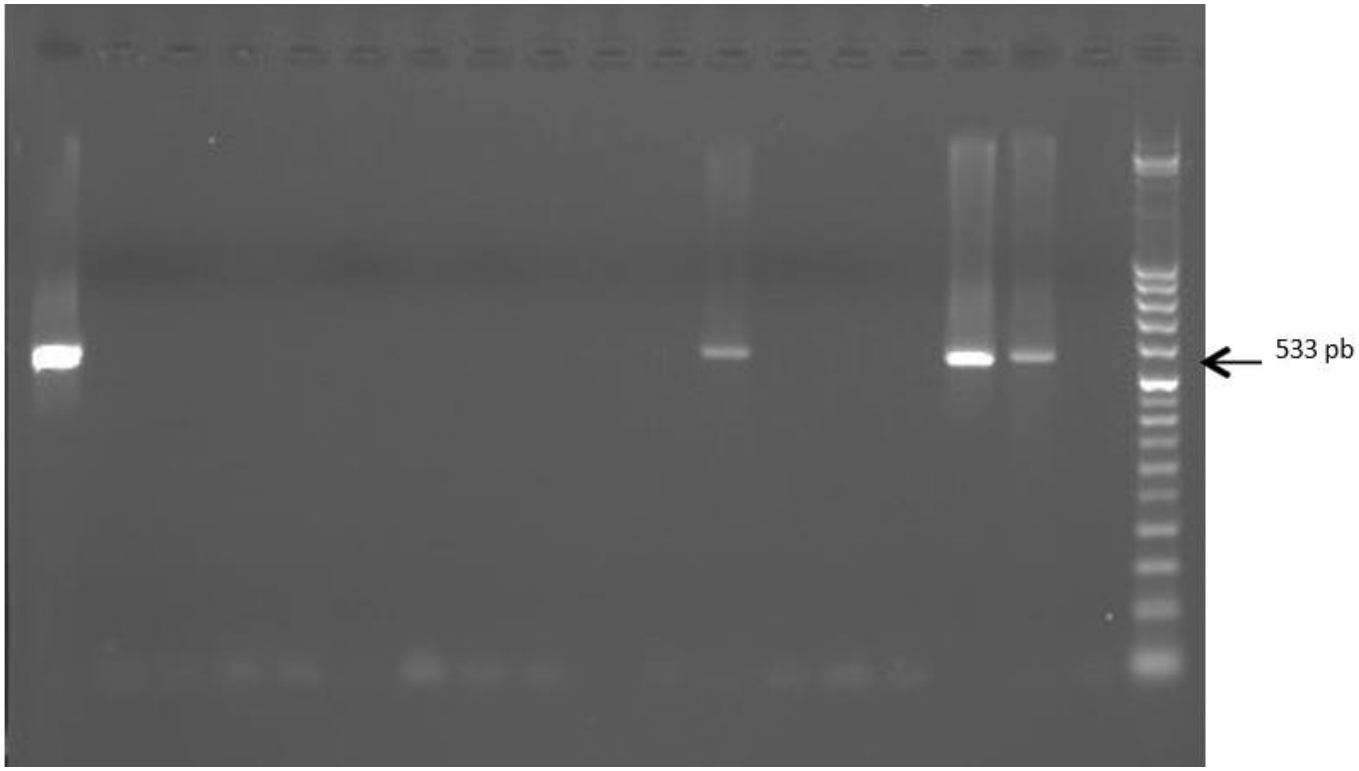

Figure 1. Electrophoresis for detection of *mec*A gene in *Staphylococcus* spp. isolated from the nasal cavity of dental surgeons. Columns 1 to 16: Strains of *Staphylococcus* spp.; column 17: Positive control (USA 300); column 18: Negative control; column 19: 50 bp molecular weight marker. Column 1: Methicillin-resistant *Staphylococcus aureus* (MRSA) 1; Column 2: MRSA 2; Column 12: MRSA 3; Column 16: Methicillin-resistant *Staphylococcus epidermidis* (MRSE).

Table 3. Phenotypic and genotypic characterization of resistance to methicillin and phenotypic resistance to MLS_B complex of methicillin-resistant *Staphylococcus* spp. (N = 4) isolated from the nasal cavity of dental surgeon professors.

Markers for methicillin resistance	MRSA 1	MRSA 2	MRSA 3	MRSE
Oxacillin	S	R	S	R
Minimum inhibitory concentration for oxacillin (mcg/mL)	≤0.25	≤4	0.5	≤4
Cefoxitin (screening test)	-	+	-	+
mecA gene	+	-	+	+
Markers for inducible resistance to MLS_B complex				
Clindamycin	R	I	S	R
Inducible Clindamycin resistance test	+	-	-	+
Phenotypic resistance to MLS_B complex	MLS_B (i)	-	-	MLS_B (i)
Erythromycin	R	R	S	R

S= susceptible; I=intermediate; R=resistant; (+) = positive; (-) = negative; Inducible resistance to Macrolide, Lincosamide and Streptogramin B - MLS_B (i).

Table 4. Susceptibility profile of methicillin-resistant *Staphylococcus* spp. (N = 4) isolated from the nasal cavity of dental surgeon professors.

Antimicrobials	MRSA 1	MRSA 2	MRSA 3	MRSE
Mupirocin	R	I	S	S
Benzylpenicillin	R	R	R	R
Ciprofloxacin	S	S	S	I
Norfloxacin	S	S	S	I
Trimethoprim/Sulfamethoxazole	S	S	S	R

*S= susceptible; R=resistant; I=intermediate; P =positive; N =negative.

moxifloxacin, vancomycin, teicoplanin, gentamicin, tigecycline, linezolid, rifampicin and fusidic acid. All (100%) isolates were *luk-F* gene negative. There was no genetic similarity among the MRSA isolates.

DISCUSSION

Biohazard exposure is widely addressed in guidelines for HCW and there has been a wide discussion about blood borne pathogens (Kuhar et al., 2013; Schillie et al., 2013), however little discussion about multidrug-resistant bacteria has taken place (Centers for Disease Control and Prevention, 2016; Centers for Disease Control and Prevention, 2003). Most studies about multidrug-resistant bacteria colonization in HCW address those who work in hospital settings (Albrich and Harbarth, 2008). Thus, it highlights the importance of analyzing the nasal colonization with methicillin-resistant *Staphylococcus* spp. among DS professors (9.7% - 4/41) working in clinical practice orientation in HEI. These professionals, as well as those who work in hospitals, provide direct patient care and are exposed to biohazards and are at risk of acquiring HAI. A similar prevalence of nasal colonization with MRSA was reported among DS from a university in Egypt (9.7% - 3/31) (Khairalla et al., 2017).

The four professionals colonized with methicillin-resistant *Staphylococcus* spp. worked in specialty clinics in endodontics, pediatric dentistry or esthetic/prosthetic dentistry, areas where the use of rotational instruments is frequent. It is well known that medical devices/ instruments that generate droplets and aerosols increase the dispersion of particles in the air containing water, saliva, pathogenic microorganisms and even blood, factors which contribute to the colonization of HCW (Harrel and Molinari, 2004; Centers for Disease Control and Prevention, 2003). Additionally, colonized DS professors presented risk behaviors such as low compliance with standard and transmission-based precautions. Improper use and/or no use of gloves were reported (Table 2), which exposes the hands of these HCW to contamination with infectious agents and may be transferred to the nasal cavity. Removing gloves to facilitate the dental procedure was reported by about 50% of dentists participating in a study in Poland (Garus-Pakowska et al., 2017). The colonization of the gloves, in turn, leads to contamination of hands with direct contact. Colonization of nasal cavity and hands among DS with MRSA was also confirmed in dental clinics at a university in Egypt (Khairalla et al., 2017).

Improper use and/or no use of surgical masks were also reported by DS professors colonized with methicillin-

resistant *Staphylococcus* spp. (Table 2). In Poland, 6.5% of dentists reported never use protective equipment, including procedure masks, which is the main protective barrier against nasal cavity colonization (Garus-Pakowska et al., 2017). A study that assessed the contamination of different areas of DSs' faces during dental procedures identified the presence of spatters throughout the face being more concentrated around the nose, probably due to close proximity of the HCW to the oral cavity to obtain a better view of the area (Nejatidanesh et al., 2013).

Cases of upper respiratory tract infections were reported by HCW colonized with methicillin-resistant *Staphylococcus* spp. Albrich and Harbarth (2008) showed that the prevalence of HCW colonized with MRSA who had subsequent infections was 5.1%. The most frequent diseases were cutaneous and soft tissue, followed by upper respiratory tract infections. Among dentists, prolonged exposure to procedures in which there is production of aerosols was associated with the presence of symptoms such as persistent or productive cough, nasal congestion, runny nose, sneezes, eye irritation, cutaneous eruptions, pruritus or dry skin (Allsopp et al., 1997). These findings indicate bacterial colonization as an adjuvant in the occurrence of adverse effects in occupational health (Costa et al., 2014; Albrich and Harbarth, 2008).

There was no genetic similarity among the MRSA isolates, implying an absence of clones and transmission among DS professors. However, the colonized status increases the possibility to spread these bacteria from symptomatic professionals with upper tract infections to patients, their family members, community setting as well as the occurrence of outbreaks (Lis et al., 2009; Lu et al., 2008). Furthermore, it is worth highlighting the potential of direct transmission from professor to students, since they are in continuous contact during clinical orientation and practice at HEIs. In addition, the risks those noncompliant DS professors pose on students, with regards to the preventive measures, since the professor is considered to be a role model and can influence students' behavior and skills (Morais et al., 2017; Betancourt et al., 2011).

In this study, a genotypic resistance pattern (presence of *mecA* gene) with a phenotypic methicillin-susceptible profile was detected in two isolates (MRSA 1 and MRSA 3). It can be explained by a phenomenon called heteroresistance, when two subpopulations coexist in a culture, where all cells can carry the genetic information for resistance, however only a small number expresses it, therefore, in the absence of genotypic characterization of isolates, these could be wrongly identified as methicillin-susceptible (Andrade-Figueiredo and Leal-Balbino, 2016). The opposite, isolate with phenotypic methicillin-resistant profile and genotypic susceptibility profile (absence of *mecA* gene) (MRSA 2), was also identified. Two possibilities may explain these findings. Firstly, it is the hyperproduction of β-lactamase, which results in partial hydrolysis of the beta-lactam ring, or the modification of other Penicillin-Binding Proteins, known as Borderline resistance, and the treatment of infections caused by this microorganism could be inefficient even with the use of high doses of oxacillin (Hryniewicz and Garbacz, 2017). Secondly, it is the presence of a *mecA* gene homologue, the $mecA_{LGA251}$ gene, known as *mecC* gene (Ito et al., 2012). Bacteria that carry this gene can colonize and cause disease in humans and in a wide range of other host species and it was able to adapt rapidly in high concentrations of oxacillin *in vitro* (Milheiriço et al., 2017).

MRSE was the microorganism that showed to accumulate the highest number of drug resistance mechanisms, being intermediate to ciprofloxacin and norfloxacin, and resistant to trimethoprim/sulfamethoxazole. Similar results were reported for MRSE isolated from HCW in a cancer hospital centre (Costa et al., 2014), pointing to the need for follow-up cultures of these microorganisms given the multidrug-resistance and the difficulty for the infection treatment (Soumya et al., 2017). Inducible MLS_B complex resistance was observed in MRSE and MRSA 1 isolates and resistance to mupirocin in MRSA 1. Total resistance rate has been shown to be higher in MRSA isolates of dental staffs than in isolates from environmental surfaces in dental service (Khairalla et al., 2017). It should be noted that the topical use of mupirocin is the most widely used treatment option for decolonization and its high rate of resistance has been related to mistakes in how bacterial decolonization is conducted (McConeghy et al., 2009).

In conclusion, DS professors were colonized in the nasal cavity with methicillin-resistant *Staphylococcus* spp. with different resistance mechanisms and reported noncompliance with preventive measures, such as the use of gloves and surgical masks. These findings highlight that DS professors are reservoirs of these infectious agents which pose a threat to their own health and place them as potential disseminators. Educational and strategic activities to increase adherence to standard and transmission-based precautions are required not only for the HCW's own safety but also for the patient, students, other dental staff and community/family contact safety, and to ensuring quality of academic education, since students mirror professors' behavior.

It should be noted that the results of this study were reported individually to the DS professors, with a letter explaining the implications of being colonized and the preventive measures to be taken. In addition, a newsletter containing the results of the research was delivered to the HEI directors in order to clarify the importance of sending the results to the Dental Infection Control Committee of the HEI for implementation of appropriate precautions.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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