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ORIGINAL ARTICLE

NASAL COLONIZATION BY GRAM-NEGATIVE BACTERIA IN DENTAL SURGEONS: INTERFACES BETWEEN PREVENTION AND CONTROL MEASURES

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ABSTRACT

The work conditions of dental surgeons (DS), associated with low compliance to precautionary standards, often lead to the colonization and dissemination of infectious agents. To assess the epidemiological and microbiological aspects of nasal colonization by Gram-negative bacteria in DS while teaching (dentistry teachers). The data were collected by application of a questionnaire and a nasal swab. The biochemical identification, the susceptibility profile and the detection of β-lactamases were carried out in *Vitek 2 compact*®. 41 (77.3%) DS participated in the study, nine of them (22.0%) presented nasal colonization by *Enterobacteriaceae*, the participants were predominantly men (27/65.9%), over 50 years of age (24/58.5%). All of them confirmed using procedure gloves and a surgical mask while attending patients, with frequent (95.5%) hand washing although a statistical difference was found regarding personal habits (p=0.03). *Enterobacter aerogenes* (60.0%) was the most prevalent species, followed by *Citrobacter koseri* (20.0%). The intrinsic production of AmpC β-lactamase by *E. aerogenes* species, which is multiresistant to antimicrobials, was present in the nasal cavity of 14.6% of the DS. There were high levels of nasal colonization by *Enterobacteriaceae* in teaching DS (22.0%), 14.6% had been colonized by multiresistant microorganisms and the results were associated with inadequate personal habits.

KEY WORDS: Nasal cavity; Gram-Negative bacteria; occupational risks; dentistry.

INTRODUCTION

Experts and public health entities have focused on health environments because these harbor and promote the dissemination of microorganisms that cause Healthcare-Associated Infections (HAI). Consequently, health workers become potential reservoirs for the crossed transmission of infectious agents, including multiresistant pathogens that are frequently involved in the HAI epidemiological chain (Brasil, 2010; Brasil, 2013).

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In this context, the Gram-negative bacteria from the *Enterobacteriaceae* family (such as *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Proteus* spp.) and the group of non-fermenting Gram-negative bacilli (NFGNB) (such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) are foremost, emerging as a severe public health problem over recent decades (WHO, 2017). These microorganisms do not only develop resistance mechanisms to one or more classes of antimicrobials but also produce new mechanisms that restrict therapeutic options and increase mortality rates (Ahmed-Bentley et al., 2013; Dereli et al., 2013; Ruppé et al., 2015).

Different studies investigate the colonization profile of health workers (HW) regarding pathogenic microorganisms. To the detriment of other professional categories, such as physicians and nurses (Prado-Palos et al., 2011; Costa DM et al., 2014; Leão-Vasconcelos et al., 2015; Lima et al., 2015), two studies have investigated the carrier status among dental workers (Clark, 1974; Horiba et al., 1995).

In the context of dental care, professionals and patients may be exposed to biological materials. From the labor point of view, the work conditions of dental surgeons (DS) favor continuous exposure to a range of microorganisms in blood, oral cavities and respiratory tracts of patients. This condition is enhanced by the use of rotation and ultrasound equipment, enabling the contact of these particles with the worker's respiratory tract (Sousa & Fortuna, 2011; Mutters et al., 2014; Umar et al., 2015; CDC, 2016).

The intense production of droplets and aerosols used in the dental care environment, associated with low compliance to standard precautions, especially the use of masks, contribute to the nasal colonization of these workers by microorganisms that are atypical, virulent and resistant to antimicrobials, such as Gram-negative bacteria, which are not part of a healthy adult's nasal microbiota (Siegel et al., 2007).

This subject is frequently studied in the medical field. However, it is seldom focused on within dental teams, therefore, the relevance of studying infection prevention and control regarding the colonization of health workers by epidemiologically relevant microorganisms. Hence, the purpose of this study was to assess the epidemiological and microbiological aspects of nasal colonization by Gram-negative bacteria of DS engaged in teaching (dentistry professors). These professionals develop clinical practices during their academic activities which favor exposure and colonization by potentially pathogenic microorganisms.

MATERIAL AND METHODS

Data were collected from July to October 2014 for an epidemiological, cross-sectional study. The research project and its respective Free and Informed Consent Term were endorsed by the Research Ethics Committee at *Universidade Federal de Goiás* (protocol 422.360). All DS engaged in teaching at a public Higher Education Institution (HEI) in the city of Goiania (Goias/ Brazil) were invited to take part in the study, while those with suspected upper respiratory tract infection who had taken antimicrobials in the 30 days before the data collection, whether continuously or not, were excluded.

The data were obtained from a structured questionnaire and the collection of a nasal swab. The questionnaire focused on the socio-demographic, occupational and behavioral characteristics of the DS related to their clinical practice, considering risk factors for the colonization of HW.

The swab was moistened in 0.9% sterilized saline solution, inserted in the front portions of the participants' nostrils (approximately 1cm^2) and turned five times to retrieve the microorganisms (Scarnato et al., 2003; Askarian et al., 2009). The swabs were then inoculated in BHI (Brain Heart Infusion) broth, vortexed and incubated at 35°C for up to 48h. Cultures with growth signs were seeded and isolated in *MacConkey* agar. The biochemical identification, analysis of susceptibility profile to the antimicrobials and phenotypic detection of β-lactamase production were performed by an automated method in the *Vitek 2* compact® system. For the identification of *Enterobacteriaceae* and NFGNB the Gram-negative card was used. This card has a negative control space and 47 biochemical tests.

The antimicrobials analyzed by the AST-N 239 card were: amikacin, cefepime, ceftazidime, ceftriaxone, cefuroxime, cefuroxime axetil, ciprofloxacin, colistin, ertapenem, gentamycin, imipenem, meropenem, piperacillin/ tazobactam sodium, tigecycline, ampicillin, ampicillin/ sulbactam and cefoxitin.

The behavioral characteristics of the DS were studied according to three categories, namely, hand washing, personal habits and use of individual protection equipment (IPE), scored between 0 and 24 points, according to the current recommendations in best practices in HAI prevention and control (CDC, 2003; Brasil, 2006; Brasil, 2009; Brasil, 2010; Brasil 2013; CDC, 2016).

The data were analyzed in the Statistical Package for the Social Sciences (SPSS). Initially, a descriptive analysis of the study population was performed. Quantitative variables were presented as mean and standard deviation (SD) and qualitative variables as absolute (n) and relative (%) frequencies. A comparative analysis of the demographic and behavioral variables of colonized and non-colonized individuals was performed using Fisher's Exact Test (qualitative variables) or Student's T-test for independent samples (quantitative variables). Variables with $p < 0.05$ were considered statistically significant.

RESULTS

41 (77.3%) of the 53 DS teaching in the Dentistry course, participated in the research project. Nine of them (22.0%) presented nasal colonization by at least one Gram-negative bacteria. Also, one DS (2.4%) presented concomitant colonization by different species.

In Table 1, the socio-demographic and occupational characteristics of the DS are described, comparing colonized and non-colonized groups. The predominant characteristics were men (n= 27; 65.9%). The mean age was 49.7 years (SD: 10.4 years; range 25-68) and more than 15 years of clinical and/or teaching activity (n= 29; 70.7%).

Table 2 presents the comparison of behavioral variables between colonized and non-colonized individuals. All participants confirmed using procedure gloves and a surgical mask during patient care. Nevertheless, two DS (22.2%) who were colonized stated that they did not change the surgical masks during care, even in cases of moisture and/or visible contamination, with a statistically significant difference in relation to the non-colonized group (p = 0.04). The variable "leaving the mask on the chin" was significantly associated with the presence of nasal colonization (p=0.049).

A statistical difference was found between colonized and non-colonized individuals regarding personal habits, such as long nails, nail biting and putting pens in the mouth (p=0.03) (Table 3).

Concerning upper respiratory tract infections and antimicrobial use, 19 (46.3%) stated frequent infections and had also taken antimicrobials in the previous year, with no statistical difference between colonized and non-colonized groups for the two variables analyzed (p> 0.05). Among the individuals who reported the use of antimicrobials (n = 19), the most frequent drugs were amoxicillin (n=10; 52.6%, azithromycin (n=4; 20.0% and ciprofloxacin (n=3; 15.8%).

Ten Gram-negative bacteria were isolated from the nasal cavity of the DS, all of which from the *Enterobacteriaceae* family. The most frequent species was *Enterobacter aerogenes* (n= 6; 60.0%), followed by *Citrobacter koseri* (n=2; 20.0%), *Escherichia coli* (n=1; 10.0%) and *Klebsiella oxytoca* (n= 1; 10.0%). The multicolonized DS presented concomitant colonization by *Klebsiella oxytoca* and *Citrobacter koseri*. Regarding the study population (n= 41), the prevalence of *Enterobacter aerogenes* was 14.6%, *Citrobacter koseri* 4.9%, *Escherichia coli* 2.4% and *Klebsiella oxytoca* 2.4%.

The isolated bacteria were susceptible to several antimicrobials, such as Ampicillin, Ampicillin/Sulbactam and Cefoxitin, exhibiting resistance phenotypes that are considered intrinsic to each species. In this study, the phenotypic production of extended-spectrum β-lactamases (ESBL) and carbapenemases was not observed, only the intrinsic production of AmpC β-lactamase by *E. aerogenes* species, present in the nasal cavity of 14.6% of the professors.

Table 1. Socio-demographic and occupational characteristics of dentistry professors colonized by Gram-negative bacteria.

Socio-demographic and occupational characteristics	Colonized (n=9)	Not colonized (n=32)	Total (N=41)	p**
Age range (years)				
<50	4 (44.4)	13 (40.6)	17 (41.5)	0.84
≥50	5 (55.6)	19 (59.4)	24 (58.5)	
Sex				
Female	2 (22.2)	12 (37.5)	14 (34.1)	0.39
Male	7 (77.8)	20 (62.5)	27 (65.9)	
Experience (years)				
01 – 15	3 (25.0)	9 (75.5)	12 (29.3)	0.92
16 – 30	4 (22.2)	14 (77.8)	18 (43.9)	
31 – 45	2 (18.2)	9 (81.8)	11 (26.8)	
Specialties				
Cosmetic dentistry/Prosthesis	6 (66.7)	12 (37.5)	18 (43.9)	0.12
Periodontology/OMFS*/Dental implants	2 (22.2)	9 (28.1)	11 (26.8)	0.72
Endodontics	0 (0.0)	6 (21.9)	6 (14.6)	0.12
Pediatric dentistry	2 (22.2)	6 (18.8)	8 (19.5)	0.82
Others	3 (22.2)	7 (21.9)	10 (24.4)	0.98
Discipline				
Diagnosis	1 (11.1)	2 (6.3)	3 (7.3)	0.62
Children's clinic	2 (22.2)	7 (21.9)	9 (22.0)	0.98
Surgical clinic	0 (0.0)	2 (6.3)	2 (4.9)	0.44
Primary care clinic	3 (33.3)	11 (34.4)	14 (34.1)	0.95
Clinical training	6 (66.7)	17 (53.1)	23 (56.1)	0.47
Prosthesis	3 (33.3)	6 (18.8)	9 (22.0)	0.35
Clinical activities (hours/week)				
01 – 10	4 (44.5)	14 (43.8)	18 (43.9)	0.71
11 – 20	3 (33.3)	9 (28.1)	12 (29.3)	
21 – 40	2 (22.2)	5 (15.6)	7 (17.1)	
> 40	0 (0.0)	4 (12.5)	4 (9.7)	
Work in hospital environment				
Yes	0 (0.0)	6 (18.8)	6 (14.6)	0.16
No	9 (100.0)	26 (81.2)	36 (85.4)	
Worked in hospital environment				
Yes	2 (22.2)	10 (31.3)	12 (29.3)	0.60
No	7 (77.8)	22 (68.7)	29 (70.7)	

*OMFS – Oral and Maxillofacial surgery; ** Fisher's Exact Test.

Table 2. Behavioral characteristics of dentistry professors colonized by Gram-negative bacteria.

Behavioral characteristics	Colonized (n=9)	Not colonized (n=32)	Total (N=41)	p**
HW† performed				
Before putting on gloves	8 (88.9)	32 (100.0)	40 (97.6)	0.22
After removing gloves	9 (100.0)	30 (93.8)	39 (95.1)	0.61
After touching contaminated surfaces	8 (88.9)	20 (62.5)	28 (68.3)	0.13
When changing torn gloves	3 (33.3)	19 (59.4)	22 (53.7)	0.26
When changing procedure gloves for sterile gloves	5 (55.6)	15 (46.9)	20 (48.8)	0.72
Most frequent HW method				
Water and soap	8 (88.9)	28 (87.5)	36 (87.8)	1.00
Water and soap or 70.0% alcohol	1 (11.1)	4 (12.5)	5 (12.2)	
Personal habits				
Long nails	0 (0.0)	3 (9.4)	3 (7.3)	0.47
Nail biting	0 (0.0)	1 (3.1)	1 (2.4)	1.00
Putting pens in the mouth	0 (0.0)	2 (6.3)	2 (4.9)	1.00
Conducts regarding IPE use				
Using gloves in care delivery to all patients	9 (100.0)	32 (100.0)	41 (100.0)	1.00
Always use gloves in patient care	4 (44.4)	18 (56.3)	22 (53.7)	0.71
Use mask in patient care	9 (100.0)	32 (100.0)	41 (100.0)	1.00
Always use a mask for patient care	7 (77.8)	30 (93.8)	37 (90.2)	0.20
Uses tissue mask	0 (0.0)	03 (9.4)	03 (7.3)	1.00
Have used tissue mask	7 (77.8)	20 (62.5)	27 (65.9)	0.69
Mask use				
When moist	6 (66.7)	24 (75.0)	30 (73.2)	0.62
In case of blood and/or saliva contamination	7 (77.8)	29 (90.6)	36 (87.8)	0.30
Never change mask during care	2 (22.2)	0 (0.0)	2 (4.9)	0.04*
Reuse mask in more than one care period	2 (22.2)	2 (6.3)	4 (9.8)	0.20
Reuse mask in more than one period when supervising students	2 (22.2)	3 (9.4)	5 (12.2)	0.30
Habit of leaving the mask on the chin	1 (11.1)	16 (50.0)	17 (41.5%)	0.049*
Habitual nose scratching during mask use	4 (44.4)	14 (43.8)	18 (43.9%)	1.00

*Data for $p<0.05$; **Fisher's Exact Test; †HW – Hand washing; ‡IPE – Individual protection equipment.

Table 3. Mean and standard deviation of total score for behavioral characteristics and hand washing, personal habits and IPE use by dentistry professors.

	Colonized (n=9)	Not colonized (n=32)	p**
Hand washing	4.8 (1.3)	4.8 (1.3)	0.76
Personal habits	4.0 (0.0)	3.8 (0.5)	0.03*
IPE† use	9.7 (1.7)	10.3 (1.7)	0.30
Mask use	8.2 (1.5)	8.8 (1.4)	0.30
Total score	18.4 (2.6)	18.9 (2.3)	0.61

*Data for $p<0.05$; **Student's T-test for independent samples; †IPE – Individual protection equipment.

DISCUSSION

Nasal cavity colonization by MDR microorganisms poses occupational health risks, enabling the development of non-invasive respiratory conditions, such as sinusitis and otitis; invasive conditions like pneumonia, bronchopneumonia and meningitis, mainly in vulnerable situations such as hospitalization, immunosuppression and indiscriminate use of antimicrobials (Safdar et al., 2005; Kusahara et al., 2012; Saadatian-Elahi et al., 2013; Almeida et al., 2014). Workers carrying MDR may disseminate these pathogens in the community, home and care environments (Safdar et al., 2005; Lemon et al., 2010; Kusahara et al., 2012; Lim et al., 2014).

Among the 41 DS investigated, 9 (22.0%) presented nasal colonization by Gram-negative bacteria from the *Enterobacteriaceae* family. Although not found in this study, possibly be due to the small number of participants, NFGNB have been isolated from this location and are related to important nosocomial infections (Azim et al., 2010; Winter-de-Groot et al., 2013). We therefore believe it is important to investigate NFGNB in the nasal cavity of DS, as these pathogens are commonly related to opportunistic respiratory infections (Mao et al., 2015), periodontal conditions (Souto et al., 2014) and contamination of the dental environment (Umar et al., 2015). In addition, they present important virulent factors that facilitate invasion and dissemination in the human organism, with high morbidity and mortality rates and high resistance to antimicrobials (Winn Jr et al., 2012; Nóbrega et al., 2013; Royer et al., 2015; Ruppé et al., 2015).

Enterobacteriaceae is a family of heterogeneous microorganisms, whose natural habitat is the intestinal tract of humans and other animals (Tortora et al., 2012). This family's resistance profile is currently considered a severe worldwide public health issue (Ahmed-Bentley et al., 2013). In a previous study, the microorganisms cited were the most isolated on patients and on the surfaces around patients in the hospital environment (La Fauci et al., 2018). In addition, similarly to NFGNB, *Enterobacteriaceae* have been described as contaminants of the dental environment, and as causes of recurrent gingival and periodontal conditions (Conti et al., 2009; Pereira et al., 2013; Umar et al., 2015).

Enterobacteriaceae do not naturally inhabit the nasal and oropharyngeal cavity of healthy individuals (Winn Jr et al., 2012). The presence of these microorganisms in these cavities increases, in vulnerable situations, the risk of respiratory infections such as sinusitis, pneumonias and bronchopneumonias (Safdar et al., 2005; Lemon et al., 2010; Kusahara et al., 2012).

In this study, six *Enterobacter aerogenes*, two *Citrobacter koseri*, one *Escherichia coli* and one *Klebsiella oxytoca* were isolated. The species identified are similar to those found by other researchers (March et al., 2010; Prado-Palos et al., 2011; Leão-Vasconcelos et al., 2015).

In these species, the phenotypic production of ESBL and carbapenemases were not detected, only the intrinsic production of AmpC β-lactamases by the isolated *Enterobacter aerogenes*. This resistance profile differs from the findings by March et al. (2010) at a long-term care institution, where 27.5% (n=19) of the HW were colonized by multiresistant bacteria (MDR), 14.5% (n=10) ESBL producers and 1.5% (n=1) metallo-β-lactamase (MβL).

In the study by Adler et al. (2014), the rate of *Enterobacteriaceae* producing ESBL was lower, as about 3.5% (n=35 of the workers at a rehabilitation center were colonized. On the other hand, the results are similar to the findings by Leão-Vasconcelos et al. (2015), in which none of the 64 *Enterobacteriaceae* species isolated from HW was characterized as ESBL and carbapenemase producer. Additionally, the phenotypic production of AmpCβ-lactamases was detected in 67.2% (43/64) of the isolated bacteria, supporting the present findings.

According to the criteria proposed by Magiorakos et al. (2012), AmpC producing *Enterobacteriaceae* may be considered an MDR microorganism, because this enzyme promotes the hydrolysis of penicillins, third-generation monobactams and cephalosporins, such as ceftazidime and ceftriaxone, and carbapenems when massively expressed. In addition, it is not degraded by the action of the β-lactamase inhibitors, limiting the therapeutic options (Ruppé et al., 2015; Saadatian-Elahi et al., 2013). Therefore, it can be inferred that 14.6% of the DS, active in teaching, were colonized by MDR microorganisms.

The statistical associations found in this study evidence that the incorrect ($p = 0.049$) or prolonged use of the surgical mask ($p = 0.04$) contributed to the nasal colonization by Gram-negative bacteria in the participating DS, facilitating the contact of pathogenic microorganisms with the workers' nasal mucosa, as well as the relation between their personal habits and the colonization process.

Official publications by the CDC (Centers for Disease Control and Prevention) recommend the use of double-filter, disposable masks that fully cover the mouth and nose, permitting normal breathing without irritating the skin. The masks should be disposed off after each care session or when moistened (CDC, 2003; CDC, 2016).

Wearing the surgical mask incorrectly round the neck, covering the mouth only and even non-use of the mask at the recommended times have been described earlier (Nobre et al., 2001; Barbosa et al., 2009). In addition, there is evidence that after four hours of use the surgical mask gradually loses its bacterial filtration efficiency (BFE). It should be highlighted that, when the surgical masks are used correctly and cover the nose and mouth, their BFE is superior to 95.0% (CDC, 2003; Siegel et al., 2007; CDC, 2016).

Aerosols, droplets and secretions from dental procedures, containing infectious microorganisms and touching can contaminate the external surface of the surgical mask. In addition, breathing makes the mask moist. In case of any humidity, the masks should be replaced (CDC, 2003; Brasil, 2006; CDC, 2016). Therefore, it is clear that inadequate use of contaminated masks during care contributed to the worker's nasal colonization ($p=0.04$).

In regard to behavioral data, a statistical difference was found between the scores of colonized and non-colonized individuals for personal habits. Natural or artificial nails are reservoirs of pathogenic organisms, as well as inanimate objects that may penetrate the worker's nasal and oral mucosa by direct or indirect contact (Hernández-Chavarría et al., 2003; Brasil, 2009).

Studies have identified Gram-positive and Gram-negative bacteria and fungi when scraping HW's nails, highlighting the significant and proportional difference in the number of colonies depending on their length (Hernández-Chavarría et al., 2003; Cassettari et al., 2006). Artificial nails increase the risk of perforation as well as microbial density when compared to natural nails and cause a decrease in hand washing frequency (McNeil et al., 2001).

The small number of participants limited this study, but its findings clearly show the risk for DS who engage in teaching activities, as well as their patients. Due to the nature of their work, part of their academic activities do not take place within the clinical environment. Therefore, the prevalence of nasal colonization among exclusively clinical DS might be even higher. Our findings indicate the need for further studies among DS and other members of the dental care team.

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