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Original article

Screening of antibiotic-resistant staphylococci in the nasal cavity of patients and healthy individuals

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ABSTRACT

The normal microbiota play critical roles in the general health of an individual and the functions of the microbiota colonized the nasal cavity in maintaining the health of the respiratory tract are well known. The nasal cavity is one of the potential bio-sources of the pathogenic opportunistic bacteria that have the ability to resist standard antibiotics. My aim was an evaluation of the prevalence of antibiotic-resistant staphylococci in the nasal cavity of healthy individuals and compared them with the strains isolated from patients. The work was designed as prospective, descriptive study in Medical University Hospital (MUH) and Botany and Microbiology Department, King Saud University (KSU), Riyadh, respectively. Strain isolation, purification, and preservation were performed according to standard protocols and the identification of pure bacterial cultures was carried out using a fully automatic system (VITEK 2 system). The isolates identified as *Staphylococcus* spp. were subjected to investigation. In patients, 34 out of 6668 isolates were *Staphylococcus* spp. obtained from the nasal cavity, while 32 out of 320 isolates from the nasal cavity of healthy individuals were *Staphylococcus* spp. The results confirmed that all the isolates were resistant to ampicillin and benzylpenicillin, but showed susceptibility to vancomycin, fusidic acid, gentamicin, linezolid, rifampicin, teicoplanin, tetracycline, and trimethoprim/sulfamethoxazole. A significant association ($P < 0.05$) was observed between all the isolates resistant to ampicillin and clindamycin in patients and healthy individuals. The antibiotic-resistant *staphylococci* are prevalent in the nasal cavity among healthy individuals and patients, and a statistically significant association exists between sources of bacterial isolates and antibiotic resistance.

1. Introduction

Several microorganisms, including bacteria, archaea, fungi, and viruses, have been detected and isolated from healthy human tissues and biofluids. "Microbiota" is a scientific term that refers to any non-pathogenic microbes that have the ability to survive and colonize some human parts such as nose, mouth, and skin. The human nasal cavity is a section of the respiratory system and all the parts of the respiratory system receive the inhaled air through the nasal cavity (Bassis et al., 2014; Ramakrishnan et al., 2016; Bomar et al., 2018). Evidence indicates that the normal microorganisms of the nasal cavity maintain the health of the respiratory tract and functions of the defense system (Kumar and Chordia, 2017).

Rasmussen et al. reported that the nasal cavity of a healthy adult is colonized by several opportunistic bacteria such as *Corynebacterium* spp., *Aureobacterium* spp., *Rhodococcus* spp., and *Staphylococcus* spp. Numerous species of fungi have also been isolated from the healthy nasal cavity (Rasmussen et al., 2000). For instance, Sellart-Altisent et al. reported that *Alternaria* spp., *Penicillium* spp., *Aspergillus* spp., and *Cladosporium* may colonize the nasal cavity of healthy humans (Sellart-Altisent et al., 2007). There are several invasive and allergic fungi have been diagnosed in nasal cavity (Robson et al., 1989; deShazo, 1997).

Lina et al. confirmed that the microbiota have the ability to colonize the healthy human nasal cavity and live under constant competition conditions (Lina et al., 2003). Commensal microbes could prevent the colonization of the human nasal cavity by pathogenic bacteria. For instance, *Staphylococcus epidermidis* strains known to produce serine protease Esp2,3, have the ability to block biofilm formation by pathogenic *S. aureus* (Iwase et al., 2010).

Abbreviation: MRSA, methicillin-resistant *Staphylococcus aureus*.

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S. aureus is one of the most pathogenic bacteria associated with human and animal diseases. The growing number of infections caused by *S. aureus* resistant to antibiotics has become a complex health problem. The pathogenesis and epidemiology of *S. aureus* infection are significantly associated with *S. aureus* that colonized the normal nasal carriage determined as a hazard factor for hospital- and community-acquired bacterial infections (Kluytmans et al., 1997; Cole et al., 2001). Kluytmans et al. summarized the bio-factors that control *S. aureus* nasal carriage (Kluytmans et al., 1997). These factors included bacterial adherence compounds, the upper respiratory system infections, nasal abnormalities, histocompatibility antigen types, microbiota of nasal cavity, host age, genetic and immunity factors, repeated needle injections, hormonal situation in women, and hospitalization.

Herein, we evaluated the predominance of *Staphylococcus* strains resistance to antibiotics isolated from the nasal cavity of patients and healthy individuals at the Medical University Hospital (MUH) and Department Botany and Microbiology, King Saud University (KSU), Riyadh, respectively.

2. Material and methods

2.1. Design of the experiment

In this study, the work was carried out in (MUH) and Botany and Microbiology Department, KSU, Riyadh from 1/1/2016 to 1/1/2017. Written informed consent and ethical approvals were obtained in conformity to the directions of the Ethics Committee (17/0449/IRB, Institutional Review Board of College of Medicine, KSU, Saudi Arabia). The data obtained from the Medical Microbiology Department in MUH were compared with those acquired from the healthy individuals in Botany and Microbiology Department to evaluate the association between the two sources of *Staphylococcus* strains. The study was a completely randomized design and the clinical samples and healthy individuals were randomly selected.

2.2. Isolation, purification, and preservation of strains

The microbial strains were cultivated from the nasal cavity of healthy individuals (N = 50) on blood agar (base blood medium [Sigma-Aldrich, USA] contained defibrinated sheep blood (5%) [Watin-Biolife, Saudi Arabia, Riyadh]) using wet sterile cotton swabs. The incubation of the plates were done at 37 °C for 24 h and purification was carried out by triple streaking method from the single colonies grown on the surface of blood agar using new blood agar medium. The purity of the bacterial cultures was determined using cultural and microscopic characteristics; all cultures with same characteristics were considered as a single or pure culture. The preservation of the pure bacterial cultures was carried out in sterile glycerol solution (30%) at −80 °C.

2.3. Identification of microbial isolates

The identification of all isolates were performed using a fully automated enclosed system (VITEK 2, Biomeriux, USA). The manufacturers' guidelines were followed using AST-GN69, AST-XN06, or AST-GN69 cards. Strain identification was performed using a single colony of the bacterial isolates after cultivation on blood agar, followed by MacConkey Agar (Oxoid, UK). Only the isolates identified as *Staphylococcus* spp. were used in the subsequent tests.

2.4. Antibacterial susceptibility testing

VITEK 2 system was used to perform the antibacterial susceptibility tests. The antibacterial susceptibility tests using AST-GP71 card was carried out in fully automated system in Vitek 2 instrument according to the manufacturer's guidelines. The susceptibility test was performed using pure cultures obtained from single colonies and cultivated on blood agar at 35 °C for 19 h.

2.5. Statistical analysis

Statistically significant association between the isolates from patients and healthy individuals was analyzed using Pearson's chi-square test. The percentage of bacterial isolates resistance to antibiotics, relative risk, and odds ratio were calculated using statistical software of SPSS (IBM SPSS Statistics 25).

3. Results

The evaluation of the predominance of *Staphylococcus* species resistance standard antibacterial agents isolated from healthy individuals and patients was done, and the association between the clinical isolates and the isolated obtained from healthy individuals was investigated. Furthermore, the presence of potential antibiotic-resistant *Staphylococcus* in the isolates from healthy individuals was analyzed.

3.1. Clinical bacterial isolates from nasal cavity

The data obtained from 6668 clinical bacterial isolates were analyzed and summarized in Table 1. The results revealed the isolation of 0.7% of pathogenic bacteria from the nasal cavity of patients. *Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Proteus mirabilis, Acinetobacter* spp., *S. epidermidis, Enterococcus faecalis, E. faecium, Streptococcus* group A, group B, and *S. pneumonia* were absent in the nasal cavity samples from the patients, but *Klebsiella pneumonia, S. aureus* resistance to methicillin (MRSA), *P. aeruginosa*, and *S. aureus* were isolated. Among the clinical isolates, 12.4%, 1.5% and 0.0% of the isolates were MRSA, *S. aureus* and *S. epidermidis* respectively. The results showed that 65.9% of the isolates from the nasal cavities of patients were MRSA and 67.5% were *Staphylococcus* spp.

3.2. Staphylococcus isolates from healthy nasal cavity

Fig. 1 shows that approximately one-third of the bacterial isolates from the nasal cavity of healthy individuals were *S. aureus* and the other bacterial isolates were not *S. aureus* strains. Furthermore, 33.3% of the bacterial isolates were *S. epidermidis*, while *S. capitis* and *S. hominis* subsp. *hominis* represented 6.7% and 26.7% of the bacterial isolates, respectively.

3.3. Antibiotic susceptibility testing of Staphylococcus spp.

The results shown in Table 2 demonstrate that all *Staphylococcus* spp. isolated and identified from the patients were vancomycin, linezolid, and teicoplanin-susceptible strains whereas those isolated from the healthy individuals showed susceptibility to vancomycin, cefoxitin, gentamicin, linezolid, moxifloxacin, rifampicin, teicoplanin, and trimethoprim/sulfamethoxazole. More than 90% of the bacterial isolates from patients were resistant to ampicillin, oxacillin, amoxicillin/clavulanic acid, cefoxitin, cefaclor, and benzylpenicillin, while over 90% of the isolates from healthy individuals were resistant to only two antibiotics (ampicillin and benzylpenicillin). Statistical analysis indicated a significant association ($P < 0.05$) between ampicillin- and clindamycin-resistant strains isolated from patients and healthy individuals.

Table 1
Pathogenic bacterial isolates from the nasal cavity of patients.

Clinical bacterial isolates		Total isolates from all clinical samples (N)	Clinical isolates from nasal cavity (N)	%
Staphylococcus spp.	Methicillin-Resistant *Staphylococcus aureus* (MRSA)	251.0	31.0	12.4
	Staphylococcus aureus	339.0	5.0	1.5
	Staphylococcus epidermidis	231.0	0.0	0.0
Non-Staphylococcus spp.	*Klebsiella pneumoniae*	704.0	9.0	1.3
	Pseudomonas aeruginosa	804.0	2.0	0.2
	Escherichia coli	1594.0	0.0	0.0
	Proteus mirabilis	144.0	0.0	0.0
	Enterobacter cloacae	225.0	0.0	0.0
	Pseudomonas aeruginosa	1425.0	0.0	0.0
	Acinetobacter spp.	276.0	0.0	0.0
	Enterococcus faecalis	249.0	0.0	0.0
	Enterococcus faecium	109.0	0.0	0.0
	Streptococcus group A	85.0	0.0	0.0
	Streptococcus group B	186.0	0.0	0.0
	Streptococcus pneumonia	46.0	0.0	0.0
	Total	6668.0	47.0	0.7

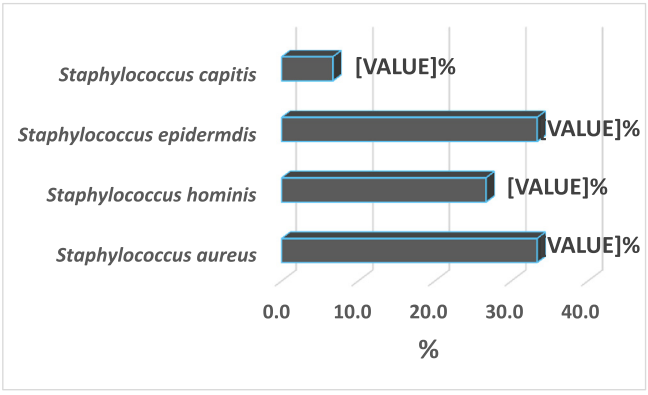

Fig. 1. Percentage of *Staphylococcus* species isolated from healthy individuals (N = 32).

3.4. Antibiotic susceptibility testing of S. aureus

Fig. 2 shows that all *S. aureus* isolates obtained from the nasal cavity of patients and healthy individuals were resistant to ampicillin and benzylpenicillin but showed susceptibility to vancomycin, fusidic acid, gentamicin, linezolid, rifampicin, teicoplanin, tetracycline, and trimethoprim/sulfamethoxazole. No significant association ($P < 0.05$) was observed between *S. aureus* strians obtained from the nasal cavity of patients and healthy individuals based on susceptibility test for azithromycin, cefoxitin, cefaclor, clindamycin, erythromycin, and moxifloxacin.

3.5. Minimum variance criterion

The minimum variance criterion of bacterial isolates was evaluated with Ward's method in SPSS. Fig. 3 shows that the bacterial isolates from the nasal cavity of healthy individuals could be

Table 2
Antibiotic susceptibility testing of *Staphylococcus* spp. isolated from the nasal cavity of patients and healthy individuals.

	Patients				Healthy individuals				$\chi(1)^{*}$	p
	S		R		S		R			
Antibiotics	N	%	N	%	N	%	N	%		
Vancomycin**	34	100	0	0	32	100	0	0		
Amoxicillin/Clavulanic acid	3	8.8	31	91.2	22	68.8	10	31.2	25.1	0.00
Ampicillin*	1	2.9	33	97.1	2	6.3	30	93.8	0.416	0.519
Azithromycin	20	58.8	14	41.2	26	81.3	6	18.8	3.92	0.048
Cefoxitin	2	5.9	32	94.1	32	100	0	0	58.46	0.00
Cefaclor	3	8.8	31	91.2	24	75	8	25	29.86	0.00
Ciprofloxacin	20	58.8	14	41.2	30	93.8	0	0	17.95	0.00
Clindamycin*	21	61.8	12	35.3	22	68.8	10	31.3	1.146	0.564
Erythromycin	19	55.9	14	41.2	26	81.3	6	18.8	5.23	0.073
Fusidic acid	18	52.9	2	5.9	18	56.3	14	43.8	22.96	0.00
Gentamicin	27	79.4	6	17.6	32	100	0	0	7.37	0.025
Imipenem	4	11.8	30	88.2	24	75	8	25	26.98	0.00
Levofloxacin	21	61.8	13	38.2	30	93.8	0	0	16.54	0.00
linezolid**	34	100	0	0	32	100	0	0		
Moxifloxacin	19	55.9	15	44.1	32	100	0	0	18.27	0.00
Oxacillin	2	5.9	32	94.1	24	75	8	25	32.98	0.00
Benzylpenicillin	0	0	34	100	2	6.3	30	93.8	2.191	0.139
Rifampicin	33	97.1	0	0	32	100	0	0	0.95	0.328
Teicoplanin**	34	100	0	0	32	100	0	0		
Tetracycline	26	76.5	6	17.6	30	93.8	2	6.3	4.22	0.121
Trimethoprim/sulfamethoxazole	29	85.3	5	14.7	32	100	0	0	5.09	0.024

R = resistance, S = susceptible, (100 − [R% + S%] = intermediate %).
* Statistically significant association between source of bacterial isolates and antibiotic resistance ($P < 0.05$).
** No statistics were computed because the results were identical among all the isolates.

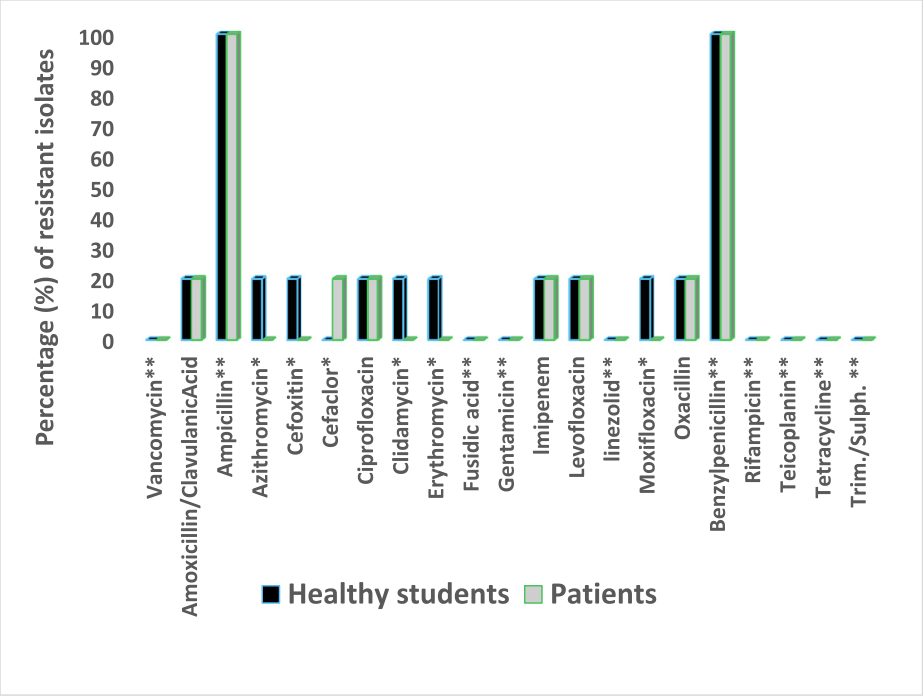

Fig. 2. Prevalence of *Staphylococcus aureus* strains resistant to antibiotics in the nasal cavity of patients and healthy individuals. *No statistically significant association between the isolates from patients and healthy individuals, as analyzed with Pearson's chi-square test ($P < 0.05$). **No statistics were computed because the resistance to antibiotics was constant.

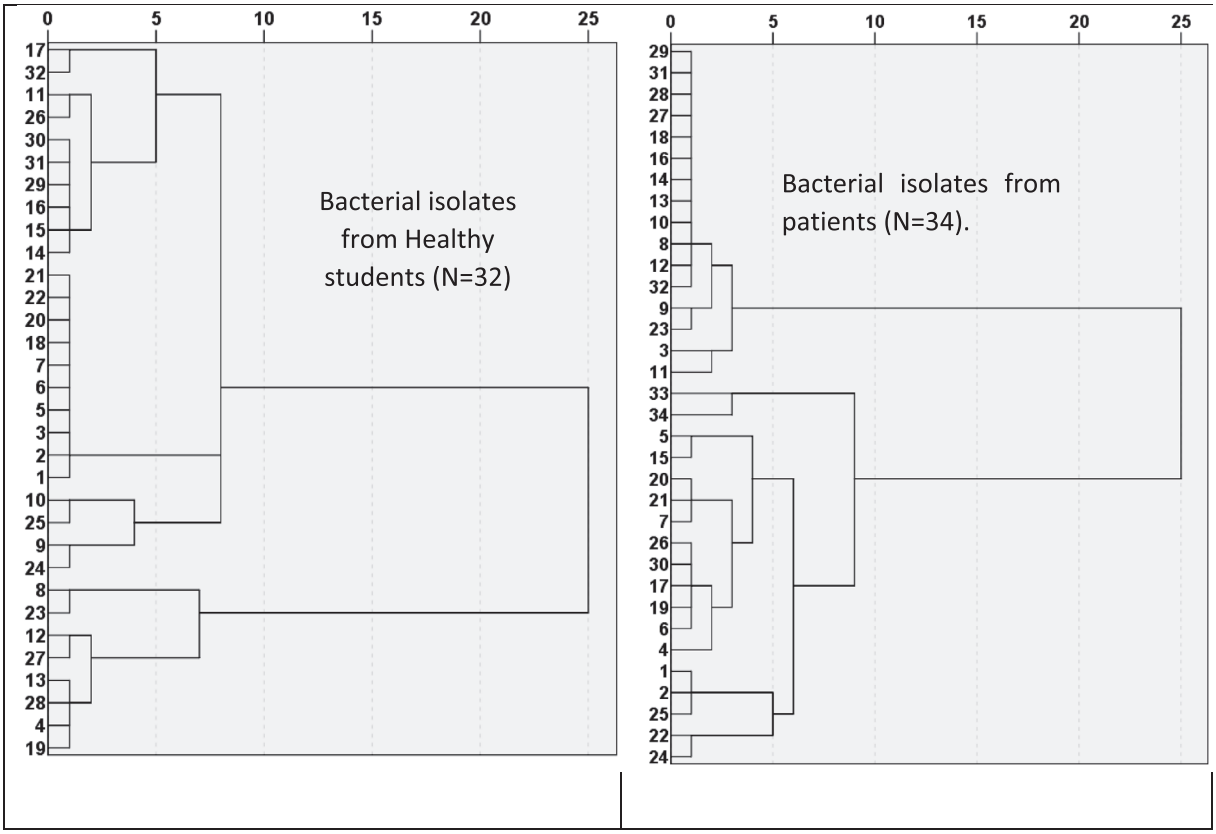

Fig. 3. Dendrogram using Ward linkage (rescaled distance cluster) of *Staphylococcus* isolates from nasal cavity of patients and healthy subjects. Numbers on Y axis indicate the number of bacterial isolates and numbers on X axis indicate the distance or dissimilarity between clusters.

Table 3
Risk estimate of antibiotic-resistant *Staphylococcus* isolates.

Strains*	Cohort (resistance = resistance)			Odds ratio for case (patient/healthy)		
	Value	95% Confidence interval		Value	95% Confidence interval	
		lower	lower		lower	Upper
Azithromycin-resistant strain	2.039	0.882	4.715	2.683	0.871	8.266
Clindamycin -resistant strain	1.129	0.568	2.244	1.2	0.430	3.349
Cefaclor-resistant strain	3.444	1.864	6.366	18.60	5.394	64.141
Erythromycin-resistant strain	2.074	0.905	4.754	2.758	0.907	8.386
Fusidic acid-resistant strain	0.134	0.033	0.546	0.080	0.016	0.394
Imipenem-resistant strain	3.333	1.797	6.182	15.00	4.578	49.150
Oxacillin-resistant strain	3.556	1.930	6.551	24.00	6.464	89.103
Tetracycline-resistant strain	2.833	0.613	13.086	3.200	0.599	17.102

* Antibiotics resisted by all bacterial isolates (from patients and healthy individuals/from patients or healthy individuals) were excluded.

divided into nine groups, wherein 31.25% of the isolates were classified in one group. We found that 0%, 100%, 50%, 40%, 0%, 30%, 0%, 20%, 40%, 20%, 0%, 30%, 0%, 0%, 0%, 30%, 100%, 0%, 0%, 20%, and 0% strains were resistant to vancomycin, amoxicillin/clavulanic acid, azithromycin, ampicillin, cefoxitin, cefaclor, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, imipenem, levofloxacin, linezolid, moxifloxacin, oxacillin, benzylpenicillin, rifampicin, teicoplanin, tetracycline and trimethoprim/sulfame thoxazole, respectively. In patients, the bacterial isolates were divided into nine groups; the largest group represented 33.3% of the isolates, and 0%, 100%, 100%, 0%, 100%, 91.60%, 0%, 0%, 0%, 0%, 0%, 100%, 0%, 0%, 0%, 100%, 100%, 0%, 0%, 0%, and 0% were resistant to vancomycin, amoxicillin/clavulanic acid, azithromycin, ampicillin, cefoxitin, cefaclor, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, imipenem, levofloxacin, linezolid, moxifloxacin, oxacillin, benzylpenicillin, rifampicin, teicoplanin, tetracycline, and trimethoprim/sulfamethoxazole, respectively.

3.6. Risk estimate

Risk estimate of the predominance of antibiotic-resistant strains of *Staphylococcus* isolated from the nasal cavity of healthy individuals and patients is shown in Table 3. The results reported risk factor in patients over healthy individuals for all strains except fusidic acid-resistant strains. In patients, the risk prevalence for oxacillin-resistant strains was 3.5 times higher than that reported in healthy individuals, while the risk prevalence for fusidic acid-resistant strains was less than a fold. Clindamycin-resistant strains showed almost the same risk factor in patients and healthy individuals.

4. Discussion

Investigation of antibiotic-resistant bacteria among healthy subjects and patients is an important scientific purpose associated with community health. This information may help predict the prevalence of dangerous pathogenic bacteria, including opportunistic pathogens, among healthy subjects. Furthermore, it may reveal some bio-sources of pathogenic bacteria resistant to the standard antibiotics. *S. aureus* is renowned for its ability to gain resistance to antibacterial agents, and the risks multiply once the bacterial strains acquire resistance to multiple antibiotics (Chambers and DeLeo, 2009). In this study, 12.4% of MRSA were identified from the non-healthy nasal cavity; however, this number is not an indicator that the lower respiratory tract infected by MRSA, as per the findings reported by Sarikonda et al. (2010). Although the nasal cavity of healthy humans is known to be colonized by *S. epidermidis* (Chen et al., 2016), we failed to isolate this bacterium from the nasal cavity of patients but isolated it from healthy individuals (33.3% of the isolates were *S. epidermidis*). More than 60% of the bacterial isolates from the nasal cavity of patients that underwent refractive surgery were *S. epidermidis* (Kitazawa et al., 2016).

Herein, we confirmed that all the *S. aureus* strains obtained from the patients and healthy individuals were susceptible to vancomycin, linezolid, and teicoplanin. Cell wall biosynthesis of *S. aureus* is inhibited by vancomycin, a glycopeptide antimicrobial agent applied for the treatment of MRSA diseases (McGuinness et al., 2017). Vancomycin-resistant *S. aureus* strains were frequently identified in several studies performed in different countries (Hiramatsu et al., 1997; Centers for Disease Control and Prevention (CDC), 2002); however, we failed to report similar observation. Vancomycin remains a viable option for the treatment of bacterial infections resulted from *S. aureus*. The results reported with linezolid and teicoplanin were the same as those observed with vancomycin, wherein the isolation of *Staphylococcus* strains resistant to linezolid and teicoplanin has been previously reported (Tsiodras et al., 2001; Cepeda et al., 2003; Stefani et al., 2010); however, we could not observe these results in the present study. The most important risk indicator found in the present work was the isolation of the strains that have resistance to ampicillin and benzylpenicillin; more than 90% of the bacterial strains were ampicillin- and benzylpenicillin-resistant *Staphylococcus* species in both patients and healthy individuals. Ampicillin may be used to treat microbial diseases caused by *S. aureus* except for the strains resistant to penicillin or methicillin. The results obtained herein suggest that ampicillin may be excluded for the treatment of all infections caused by *Staphylococcus*. We found that the nasal cavity of healthy individuals is not a bio-source for cefoxitin-resistant *Staphylococcus* isolates, which were obtained from more 90% of patients. However, the nasal cavity of healthy individuals served as a potential bio-source for the strains resistant to oxacillin. Methicillin resistance in species of *Staphylococcus* is screened using cefoxitin and oxacillin disc diffusion test (Jain et al., 2008; Broekema et al., 2009). In healthy individuals, the oxacillin test results showed that approximately one-third of the isolates were methicillin-resistant staphylococci, although cefoxitin test results confirmed that all the strains were non-methicillin-resistant staphylococci. Velasco *et al.* reported that cefoxitin test is the best analysis method to screen methicillin resistance in staphylococci (Velasco et al., 2005). Risk estimate of oxacillin-resistant staphylococci indicated that the risk prevalence of these strains is more three times among patients as compared with healthy individuals. Phenotypic resistance to standard antibiotics for the isolates from patients or healthy individuals confirmed that *Staphylococcus* species may be classified into nine groups; this classification may help us detect the groups of antibiotics that may be used to treat the bacterial diseases caused by staphylococci. Thus, the antibiotic-resistant *staphylococci* are prevalent in the nasal cavity among healthy individuals and patients, and a statistically significant

association exists between sources of bacterial isolates and antibiotic resistance. The risk factor of prevalence of approximately all the isolates resistant to antibiotics is higher in patients than in healthy individuals.

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