Nasal carriage of resistant *Staphylococcus aureus* in a medical student community

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ABSTRACT

*Staphylococcus aureus* can cause a variety of infections due to its high transmissibility, high pathogenic potential and resistance to multiple drugs, factors that contribute to the relevance of infections in healthcare services. The aim of this study was to document phenotypic and genotypic resistance factors of *Staphylococcus aureus* strains, isolated from nasal mucosa of medical students. A nasal swab was collected from the nares (nostrils) of 222 medical students. After collection, the samples were submitted to isolation and identification procedures. From 204 valid samples, 20.6% (42 samples) were positive for *S. aureus*. For the assessment of phenotypic resistance by disk-diffusion technique, from 42 samples, 95.2% showed resistance to erythromycin, 42.8% to clindamycin, 16.6% to cephalotin and 9.5% to oxacillin. The D test showed that 26.2% of samples were resistant to macrolides, lincosamides and streptogramin B. A PCR assay allowed for the evaluation of a genotypic resistance profile, in which 16.6% of the samples were positive for the *mecA* gene, 35.7% positive for the *ermC* gene or *ermA* gene and 28.5% were positive for both genes. These results demonstrate that medical students can enter the healthcare service previously colonized by multidrug resistant strains and become potential spreaders in the hospital environment.

Key words: multidrug resistance, colonization, MLSB resistance, MRSA.

INTRODUCTION

*Staphylococcus aureus* can cause a large variety of infections, most of which are acquired in the hospital environment. The high transmissibility, high pathogenic potential and possible resistance to multiple antibiotics may contribute to the relevance of staphylococcal infections in hospitals and healthcare assistant services. The majority of the human population is colonized by *S. aureus* asymptptomatically (Moreira et al. 2013, Ribeiro et al. 2014).

Data for hospital acquired infections are higher in university hospitals (Nogueira et al. 2009). Nasal and perineum mucosa are the main niches for this bacteria. Colonized and infected individuals are capable of transferring *S. aureus* by...
direct or indirect contact. This phenomenon, called cross infection, is more efficient in hospitals, which can lead to increased risk to patients, especially those undergoing invasive procedures, and an increased use of antibiotics (Muto et al. 2003, Colli et al. 2009, Silva et al. 2012). The importance of *S. aureus* as a pathogen is attributable to the combination of virulence mediated by toxins, its invasive characteristics and profile of antibiotic resistance (Le Loir et al. 2003).

The dissemination of isolates resistant to antibiotics, most used in clinic practice, is a limiting factor for staphylococcal infection treatment (Fitzgerald et al. 2001). An important event during staphylococcal therapy was the appearance of Methicillin-Resistant *Staphylococcus aureus* (MRSA) as a predominant Gram-positive pathogen in nosocomial infections. According to estimates, more than 50% of staphylococcal infections are acquired in healthcare service establishments (Finch 2006, Moreira et al. 2013, Nikfar et al. 2015). MRSA strains are resistant to all beta-lactams (penicillin, cephalosporin, carbapenems and monobactams) due to the expression of low affinity receptors. Beta-lactams clamp to bacterial enzymes that are present in the cell wall, such as Penicillin-Binding Protein (PBP), stopping the normal function of the enzyme. MRSA is capable of synthesizing variants of PBP2, such as PBP2a, which maintains its physiological function, but with low affinity to beta-lactams (Ratti and Sousa 2009, McCulloch et al. 2015). The *mecA* gene, PBP2 encoder, with regulator genes, is located in a mobile genetic element, the staphylococcal chromosomic cassette *mec* (*SSCmec*) (Enrigth et al. 2002). A large variety of types of *SSCmec* are already described, differing from each other according to the number of genes present in their genetic architecture. Some of those types are carriers of determinant genes to multiple antibiotics other than beta-lactams, for instance macrolides, lincosamins, streptogramins, aminoglycosides and tetracycline, therefore when a bacterial cell acquires *SSCmec* it expresses a multidrug resistance phenotype (McCulloch et al. 2015).

Clindamycin is an alternative drug to treat infections caused by *S. aureus* in case of penicillin intolerance or a methicillin resistant strain, because it stops production of toxins and virulence factors by inhibiting protein synthesis. However, the acquired resistance has limited the use of this drug to staphylococci therapy. There are two mechanisms that result in the resistance to clindamycin, the efflux of macrolides, controlled by the *mrsA* gene; and modification of the binding site at the ribosome, controlled by the *erm* gene (Erythromycin Ribosomal Methylase). The ribosomal methylation confers cross-resistance to macrolides, lincosamins and streptogramin B, called the MLSB phenotype of *Staphylococcus* spp. The presence of genes *ermA* or *ermC* results in this cross-resistance phenotype, leading to the modification of the binding site due to the methylation of the residue A2058, located on the V domain of 23s rRNA (Daurel et al. 2008, Amorim et al. 2009, Moosavian et al. 2014, Saderi et al. 2011, Abbas et al. 2015).

The high frequency of infections by methicillin resistant *Staphylococcus aureus* (MRSA) has shown continued growth in hospital institutions worldwide. Studies of geographic dissemination of multidrug resistant epidemic clones of *S. aureus* in Brazil showed that isolates tested presented the same phenotype resistant to methicillin, and most of them (>70%) were resistant to at least nine antibiotics. For this reason, it is important to take informed actions in prevention, reduction and treatment of infections by MRSA to reduce this transmission of multidrug resistant strains (Vivoni et al. 2006, Ratti and Sousa 2009). Understanding the epidemiology of *Staphylococcus aureus* has important implications for control methods. Because of this, the objective of the present study is to document the dissemination of nasal mucosa isolates from medical students and to identify the phenotypic and genotypic resistance factors.
MATERIALS AND METHODS

The study included students who started medical school in 2012 (first year) and excluded students admitted in different periods as well as those presenting any signs of infection on their upper airways and/or who used antibiotics up to one month before the date of collection. The research was approved by the Committee of Ethics in Research of the University of Western São Paulo - UNOESTE (Plataforma Brasil: 14790013.8.0000.5515). As a control of all reactions, *S. aureus* ATCC 25923 were used. *S. aureus* ATCC 33591, *S. aureus* ATCC 29213 and *S. aureus* ATCC 19095 were used as reference and quality control of susceptibility tests. All samples were identified and tested for susceptibility to erythromycin (15 µg), clindamycin (2 µg), oxacillin (1 µg) and cephoxitin (30 µg), and were genotyped.

Samples were collected in September 2012. Samples were collected from nasal mucosa of participants with a sterile swab, moistened with saline previously sterilized, with gentle circular movement three times. The collected material was placed into sterile dry tubes and immediately sent to the Laboratory of Microbiology. Samples were striated in Mannitol Salt Agar (selective medium for *Staphylococcus*), incubated at 37°C for 24 hours and submitted to identification procedures. Gram stains were performed to evaluate the morphology and specific coloration as well as the purity of isolates. After that, colonies were submitted to catalase and coagulase tests.

Additional acidification tests of Maltose, Trehalose, mannitol and Polymyxin B were assayed for differentiation of other coagulase positive *Staphylococcus* species.

Susceptibility testing was performed on all isolates using the disk diffusion method, and interpretation of the test was done in agreement with the Laboratory Standards Institute protocol (CLSI 2012). The inoculum was prepared from cultures in Brain Heart Infusion (BHI) broth incubated for 4-6 hours before adjusting turbidity to 0.5 on the McFarland scale. Once the inoculum density was adjusted, the bacteria were spread with a sterile swab on the surface of Mueller Hinton Agar and subsequently the disks impregnated with the antibiotics, oxacillin, cephoxitin, clindamycin and erythromycin were placed on the agar surface. The D test was performed to evaluate the profile of clindamycin induced resistance, using disks impregnated with erythromycin and clindamycin at a distance of 20 mm from one another and then incubated at 35°C for 24 hours. Subsequently, antibiotic activity was evaluated by measuring the inhibition halo formed and interpreted based on the CLSI (CLSI 2012).

Determination of the genotypic resistance profile consisted of amplification of genes meca, ermA and ermC by Polymerase Chain Reaction (PCR) (Coutinho et al. 2010), and analysis by electrophoresis running in a 1.7% agarose gel in 1x TBE buffer (Tris 90mM, Boric acid 90mM, EDTA 2mM pH 8.0) stained with ethidium bromide 0.5 µg/ml. Gels were compared to a 100bp Ladder (Amersham Pharmacia Biosciences Inc.) and photographed under ultraviolet light.

Prevalence of *S. aureus* was estimated by point in a 95% confident interval. Statistical Kappa were calculated to evaluate the concordance between PCR and disk-diffusion results (Vieira and Garret 2005). Frequency of resistance genes showed by strains were compared between individuals gender by Chi-square test with correction for continuity of Yates, or exact Fisher, depending on data frequency. All comparisons were performed on Bioestat 5.3 software, with 5% significance level (p<0.05) (Ayres et al. 2007).

RESULTS

The total of 222 samples were collected from nasal mucosa of medical students and 8.1% (18 samples)
excluded from those who were being treated with antibiotics. From 204 valid samples, 86 (42.2%) were collected from male participants and 118 (57.8%) from female. After identification, 42 samples (20.6%; IC95% = 15.0% to 26.1%) were positive for *Staphylococcus aureus* (SA). Resistance to erythromycin was observed in 40 samples (95.2% of SA) and 3 showed intermediate resistance; 18 samples (42.8% of SA) were resistant to clindamycin and 2 presented intermediate resistance; 7 samples (16.6%) were resistant to cephoxtin and; 4 (9.5%) were resistant to oxacillin, and 1 showed intermediate resistance (Table I). D test showed 11 samples (26.2%) resistant to MLSB (Figure 1).

Genotype analysis demonstrated 07 samples (16.6%) resistant to cephoxtin, presenting expression of gene meca, characterized MRSA strains. Resistance to macrolides was observed in 15 samples (35.7%) expressing ermA gene. 15 showed gene ermC and 12 (28.5%) both genes (Table II). In addition, 1 strain (8.3%) was positive for all 3 genes tested (meca, ermA and ermC), signifying a potential multidrug resistant strain (Fig. 2). Furthermore, 2 strains (4.7%) showed non-expressed ermA, as presented by resistance to erythromycin.

### Table I

<table>
<thead>
<tr>
<th>Profile</th>
<th>Erythromycin (n=42)</th>
<th>Clindamycin (n=42)</th>
<th>Cephoxitin (n=42)</th>
<th>Oxacilin (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensible</td>
<td>4.76 (n=2)</td>
<td>52.38 (n=22)</td>
<td>83.33 (n=35)</td>
<td>88.10 (n=37)</td>
</tr>
<tr>
<td>Resistant</td>
<td>95.24 (n=40)</td>
<td>47.62 (n=20)</td>
<td>16.67 (n=7)</td>
<td>11.90 (n=5)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test.

### Table II

<table>
<thead>
<tr>
<th>Profile</th>
<th>meca (n=42)</th>
<th>ermA (n=42)</th>
<th>ermC (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>16.67 (n=7)</td>
<td>64.29 (n=27)</td>
<td>64.29 (n=27)</td>
</tr>
<tr>
<td>Negative</td>
<td>83.33 (n=35)</td>
<td>35.71 (n=15)</td>
<td>35.71 (n=15)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*chi-square test.

**Figure 1** - D-test positive. Halo formed as letter “D” form due to erythromycin-induced resistance.

**Figure 2** - Electrophoresis gel signifying amplified meca (533 bp), ermA (421 bp) and ermC (572 bp) genes from the same strain. L: ladder (100 bp). C-: negative control (*S. aureus* ATCC 29213).
Results obtained from Kappa estimates for concordance between detection of resistance are summarized at Tables III, IV and V. In relation to the prevalence of *S. aureus* resistance gene carrier strains, there were no difference observed in males and females (p<0.05).

**DISCUSSION**

Resistance to multiple antibiotics frequently observed in *Staphylococcus aureus* strains due to defense mechanisms against drugs has lead to limiting therapeutic choices and a prolonged treatment period of infections (Lima et al. 2015). In the present study, 20.6% of samples were positive for *S. aureus*. This prevalence corroborate to the data of Catão et al. (2012), that reported prevalence of 20.0% positive samples for *S. aureus* collected from a hospital team of public healthcare employees. Atique et al. (2012) identified 33.3% samples positive for *S. aureus* isolated from the nasal mucosa of students, while Silva et al. (2012) discussed that colonization can vary from 20 to 30% in samples isolated from healthcare workers. Results presented by Pereira and Cunha (2009) showed that 30 of 104 positive samples for *Staphylococcus* collected from nursing students were *S. aureus*. Predominant rates of MRSA strains can vary mainly due to the size and type of healthcare establishment (Pereira and Cunha 2009).

Resistance to oxacillin was observed in 11.9% of samples positive for *S. aureus*, which differs

**TABLE III**

<table>
<thead>
<tr>
<th>Comparison (gene x antibiotic)</th>
<th>Kappa</th>
<th>Concordant results</th>
<th>p</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA x oxacillin</td>
<td>0.2364</td>
<td>80.95%</td>
<td>0.0487*</td>
<td>Weak</td>
</tr>
<tr>
<td>mecA x cephoxitin</td>
<td>0.8286</td>
<td>95.24%</td>
<td>&lt;0.0001*</td>
<td>Excellent</td>
</tr>
<tr>
<td>ermA x erythromycin</td>
<td>-0.0917</td>
<td>59.52%</td>
<td>0.1400</td>
<td>No concordance</td>
</tr>
<tr>
<td>ermC x erythromycin</td>
<td>0.1651</td>
<td>69.05%</td>
<td>0.0259*</td>
<td>Weak</td>
</tr>
</tbody>
</table>

p<0.05.

**TABLE IV**

<table>
<thead>
<tr>
<th>Comparison (D test x resistance test)</th>
<th>Kappa</th>
<th>Concordant results</th>
<th>p</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>D test x erythromycin</td>
<td>0.0349</td>
<td>30.95%</td>
<td>0.1940</td>
<td>No concordance</td>
</tr>
<tr>
<td>D test x ermA</td>
<td>-0.0060</td>
<td>42.86%</td>
<td>0.4791</td>
<td>No concordance</td>
</tr>
<tr>
<td>D test x ermC</td>
<td>0.1617</td>
<td>52.38%</td>
<td>0.0789</td>
<td>No concordance</td>
</tr>
</tbody>
</table>

p<0.05.

**TABLE V**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Kappa</th>
<th>Concordant results</th>
<th>p</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA x ermA</td>
<td>-0.2000</td>
<td>28.57%</td>
<td>0.0154*</td>
<td>Weak</td>
</tr>
<tr>
<td>mecA x ermC</td>
<td>0.1200</td>
<td>47.62%</td>
<td>0.0975</td>
<td>No concordance</td>
</tr>
<tr>
<td>ermA x ermC</td>
<td>-0.5556</td>
<td>28.57%</td>
<td>0.0002*</td>
<td>Weak</td>
</tr>
</tbody>
</table>

* p<0.05.
from those described by Kobayashi et al. (2009), who evaluated antimicrobial resistance in clinical isolates of *Staphylococcus aureus* in a public hospital in Goiânia. They found that 68.5% of samples showed a resistance profile to oxacillin, a difference which can be attributed to the fact that the samples from Kobayashi et al. (2009) study were from a hospital where MRSA was endemic, giving a higher rate of MRSA isolation. Faria et al. (2011) isolated *S. aureus* obtained from nasal vestibules of nursing students and showed that 9.8% of samples were oxacillin resistant, data that corroborate the findings of the present study. Faria et al. (2011) also found 87.5% of samples resistant to erythromycin, data that corroborate our study, where 95.2% of samples were observed to have the same resistance. Nogueira et al. (2009), while assessing hospital infection notifications, found 66.6% of samples resistant to erythromycin, a level that differs from the findings of this study. These results show that elevated levels of *S. aureus* resistance to erythromycin emphasize the importance of research regarding these bacteria and resistance to this antibiotic. Silva et al. (2010) evaluated the prevalence of *S. aureus* colonizing healthcare professionals in a reference hospital in Recife, Brazil, and identified 17.2% of isolates resistant to cephoxitin, supporting the prevalence observed in this study. Differently, in Pernambuco, Brazil, Silva et al. (2012) reported that 2.4% of samples isolated from nurses in a hospital environment were resistant to cephoxitin. In the present study, concordance between results found for resistance to cephoxitin by gene amplification and disk-diffusion tests showed an excellent rate (95.24%), signifying that the amplification by PCR of the *mecA* gene indicates a correlation between this gene and resistance to cephoxitin. Some studies have already shown the efficacy of cephoxitin to be a marker for detecting resistance to methicillin by the *mecA* gene (Colli et al. 2009).

The D test can differentiate between Staphylococci that have inducible resistance (*erm* gene) and those that have resistance mediated by efflux pump (*mrsA* gene) (Moosavian et al. 2014). In regard to the induced resistance to MLSₜ, 11 samples (26.2%) showed positive results. Colli et al. (2009) studied *S. aureus* isolated from nasal and lingual mucosa of adult patients, and identified resistance to clindamycin in 22.2% of strains. Controversially, the prevalence of 47.2% of samples resistant to clindamycin observed, Catão et al. (2012) demonstrated susceptibility to clindamycin in 100% of samples isolated from nasal mucosa of healthcare workers. Divergence between prevalence profile might be related to the mechanisms of induced resistance to MLSₜ that is dependent on gene expression (*erm*) as well as environmental factors that induce the resistance (Ungureanu 2010).

Presence of gene *mecA*, which encodes the penicillin-binding proteins (PBPs), was shown to be related to the resistance to methicillin (MRSA) and oxacillin. PBPs are important for cell wall synthesis and are targets of beta lactam antibiotics. MRSA strains are able to express the variant of PBP2, called PBP2a, maintaining its physiological function but, with low affinity to betalactams (Askari et al. 2012, Lima et al. 2015). In this study, strains showed resistance to cephoxitin, evidenced by PCR to evaluate presence of the *mecA* gene, characterizing the MRSA strain. After a similar study to identify samples displaying MRSA profile that infected nasal mucosa in patients in the intensive care unit (ICT), Ropke et al. (2002) found a prevalence of 14% MRSA strains, corroborated by the present report, in which a prevalence of 16.6% was observed in the nasal mucosa from medical students. On the other hand, Mimica and Mendes (2007), in a multi-center study identified that the prevalence of MRSA colonization is variable and dependent on the country, with some rates higher than 80%.

The excessive and inappropriate use of clindamycin has increased the number of *S.
Staphylococcus aureus strains resistant to MLS$_B$, and the rate can vary according to the extent of treatment in each country (Abbas et al. 2015, Otsuka et al. 2007). In the present study, 64.2% of samples were found positive for $ermA$ and 64.2% for $ermC$. Moosavian et al. (2014) investigated the genotypic profile of $S. aureus$ samples in university hospitals of Iran, and found a 41.1% prevalence of $S. aureus$ with $ermA$ and 17.7% with $ermC$.

Prevalence of resistance genes to macrolides in $S. aureus$ reported by Schmitz et al. (2000) showed that 74.2% and 8.6% of samples from 24 European hospitals were positive for $ermA$ and $ermC$ genes, respectively, and 8.6% of samples presented both genes. This differs from 64.2% of samples that presented positive results for $ermA$, and the same percentage for $ermC$, and 28.5% for both genes in the present study. Teodoro et al. (2012) characterized the resistance to MLS$_B$ in MRSA strains analyzing the distribution of $erm$ (A, B and C) and $mrs$ (A) genes in samples from different hospitals, which from a total of 39 MRSA strains, 53.8% presented the $ermA$ gene and 30.8% $ermC$. Moreover, 7.7% of samples were positive for both genes. Present findings corroborate the previous study that 8.3% of samples were positive for $mecA$, $ermA$ and $ermC$ genes characterizing a profile of multidrug resistance.

CONCLUSIONS

Considering the findings, healthcare students, for instance medical students, can be colonized by multidrug resistant $S. aureus$. The monitoring of irrational use of antibiotics by the population is fundamental to prevent the emergence of new multidrug resistant strains. Students and healthcare professionals that attend a nosocomial environment have to be oriented and aware of hygiene protocols, and the use of personal protective equipment (PPE) is essential to prevent and control the dissemination of infectious agents, mainly multidrug resistant strains.

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dada em isolados clínicos de *Staphylococcus aureus* e *Pseu-


