Microbiology Research International
Vol. 3(1), pp. 14-19, March 2015
ISSN: 2354-2128
Full Length Research Paper

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from medical students of a Brazilian educational institute

Liliane Braga Medeiros[1], Carolina Yumi Gushiken[1], Bruna Pereira Correia[3], Luciana Machado Guaberto[2], Daniela Vanessa Moris[1], Valeria Cataneli Pereira[1] and Marcus Vinicius Pimenta Rodrigues[1*]

[1]Laboratory of Microbiology and Immunology, Presidente Prudente School of Biomedical Sciences, Universidade do Oeste Paulista – UNOESTE, Brazil.
[2]Molecular Genetics Laboratory, Universidade do Oeste Paulista – UNOESTE, Brazil.
[3]Laboratory of Microbiology and Immunology, Presidente Prudente School of Medicina, Universidade do Oeste Paulista – UNOESTE, Brazil.

Accepted 12 February, 2015

ABSTRACT

Staphylococcus aureus is a bacterium that can cause a variety of infections. The great transmissibility, its high pathogenic potential and the possibility of resistance to multiple antimicrobial agents, are relevant items that contribute to the staphylococcal infections. The present work aims to document the spread of isolates and to identify the phenotypic and genotypic factors of resistance evaluate the resistance of these strains of *S. aureus* isolated from nasal mucosa of medical students of an Educational Institution in the State of São Paulo, Brazil. Samples were collected from nasal fossae of fresh students of medicine using sterile swabs were submitted for the identification of *S. aureus.* Phenotypic resistance profile was performed with the technique of disk diffusion method and the genotipical detection of methicillin resistance was performed by the PCR technique to evaluating the presence of *mec*A gene. 222 nasal swab samples of fresh students of medicine were collected, 43 (21.1%) were colonized by *S. aureus*. Out of 43 colonizing strains of *S. aureus*, 86% were resistant to erythromycin, 18.6% to clindamycin and 18.6% to cefoxitin. It was observed that 11 samples showed the D test positive and 8 were MRSA positive. These results demonstrate that the fresh students are already colonized with *S. aureus*, and the monitoring of these reservoirs is important to control the dissemination of these isolates in the hospital environment.

Keywords: Evaluation, *Staphylococcus aureus*, resistance, colonization, medical students.

*Corresponding author. E-mail: marcusvinicius@unoeste.br. Fax: 55 18 3229 1000.

INTRODUCTION

The *Staphylococcus aureus* can cause a variety of infections, many of them acquired in the hospital environment (Casey et al., 2007). Part of the human population is the bearer of this bacteria and the majority does not show any symptoms of infection. This transport pattern, in which one observes bacterial reproduction without interaction immunological or clinical disease, gives the name of "colonization" (Blaser and Falkow, 2009). Both the individual colonized and those who have infection can transmit the *S. aureus* through direct or indirect contact. This phenomenon, known as "crossed transmission", presents dynamic increased within the hospital environment and this fact has a significant contribution to gravity inherent in individuals hospitalized, invasive procedures performed in the hospital, the use of antimicrobials and the occurrence called "understaffing" (that is, a low number of professionals providing assistance to a large number of patients) (Muto et al., 2003).

The great transmissibility, its high pathogenic potential

and the possibility of resistance to multiple antimicrobial agents, are relevant items that contribute to the relevance of staphylococcal infections in hospitals and other health services. An important milestone in staphylococcal therapy was the emergence of *S. aureus* resistant to Methicillin (Methicillin-Resistant *Staphylococcus aureus*, MRSA), that emerged in recent decades as the predominant Gram-positive pathogens in hospital infections. It is estimated that it is involved in over 50% of staphylococcal infections acquired in health care settings (Finch, 2006; Barrett, 2005; Derenski, 2005). MRSA is resistant to all betalactams (penicillins, cephalosporins, monobactams and carbapenems), for expressing a receptor of low affinity for these antibiotics. The gene encoding the protein PBP2a, the *mecA* gene, along with its regulatory gene is located in a mobile genetic element, Staphylococcal Cassette Chromosome *mec* (SCC*mec*) (Enrigth et al., 2002). Some of these types of carriers of determining SCC*mec* are antibacterial multiple genes in addition to the beta-lactams, macrolides, lincosamides, streptogramins, tetracycline, ingsaminoglycosides and thus acquires a bacterial cell when these SCC*mec* at once acquires a phenotype of multiple-resistance (McCulloch, 2006; Ito et al., 2003). The treatment of MRSA infections becomes complicated due to the limited number of secure therapeutic options. The therapeutic choice should consider the sensitivity to antibiotics of each isolated strain (Hanssen and Sollid 2006; Reinert, 2004).

The treatment of MRSA infections becomes complicated due to the limited number of secure therapeutic options. The therapeutic choice should consider the sensitivity to antibiotics of each isolate (Hanssen and Sollid 2006; Reinert, 2004). However, the prevalence in Brazil in the isolation of MRSA strains ranges from 40 to 80%, and those isolated SCC*mec* Type III is the most prevalent in nosocomial infections, often exhibiting resistance to aminoglycosides, chloramphenicol, lincosamides, macrolides, quinolones, sulphamethoxazole, tetracyclines, and trimethoprim with only the large rifampicin susceptibility to glycopeptides and therefore the demand for glycopeptídeonos cases these infections is high (Gardella et al., 2005; Sader et al.,1993).

The spread of antibiotic resistance in hospital settings, where the pressure is greater represented by antibiotic therapy, determines selective for the proliferation of resistant strains (Muto et al., 2003; Fitzgerald and Musser, 2001) advantages. The spread of resistant strains ("cross-transmission") from patient to patient by health professionals, devices and equipment have a defined role in the dissemination of resistance (Chaves et al., 2005).

MRSA infections are associated with considerable morbidity and mortality, and are more expensive to manage compared to other infections. This considerable increase in expenses related to the management of these infections are due to prolonged hospitalization, increased care in isolation, and additional health care and financial burden on secondary care. The major impact of MRSA on morbidity, mortality and costs, without been widely documented in the United States and in European countries, that have a high prevalence of MRSA nosocomial (Finch, 2006; Barrett, 2005).

The proportion of patients colonized with MRSA in a Intensive Care Unit (ICU) is the greatest risk factor for acquisition of MRSA in hospitalized young patients there (Merrer et al., 2000). Patients colonized can contaminate the environment with their strains thereby facilitating cross transmission. This fact underscores the importance of caution and isolation of patients colonized or infected with MRSA measures, complemented by effective protocols of hygiene, cleanliness and maintenance of hospital environments (Hardy et al., 2006). This study also aims to identify the prevalence of molecular factors of resistance to macrolides and beta-lactams in *S. aureus* strains isolated from nasal mucosa of first-year students of medicine from a private institution of higher learning in the state of São Paulo.

MATERIALS AND METHODS

Sample

This study was approved by the ethics committee and research CCPq Protocol: 1565 protocol platform Brazil: 14790013.8.0000.5515. The study included students who entered medical school at the University of Oeste Paulista - UNOESTE in Presidente Prudente - SP, Brazil, in 2012. We excluded those admitted in different period, which showed no sign of infection of the upper airways and had used antibiotics within one month before the date of collection.

Disk diffusion test

Using sterile swabs and Brain Heart Infusion (BHI), which is a derivative of nutrients through the brain and heart, peptone and dextrose that it become an non-selective medium for the isolation and cultivation of most anaerobic bacteria such as *Staphylococcus*, *Streptococcus* and other fastidious microorganisms, agar tubes identified, samples of nasal fossa of the medical students were collected. The BHI remained in the oven at 37°C for 24 h. The samples were placed on plates containing Mannitol Salt agar and incubated at 37°C for 24 h. Gram stain to evaluate the morphology and purity was performed. After confirming these characteristics, evidence of catalase and tube coagulase, as recommended by Koneman et al. (1997) were performed. The antimicrobial susceptibility test was performed for all isolates positive for *S. aureus* using the technique of disk diffusion method from impregnated discs as recommended by CLSI-Clinical Laboratory Standards Institute (2011) criteria. The drugs used were: Oxacillin (1 µg), Cefoxitin (30 µg), Vancomycin (30 µg), Clindamycin (2 µg) and Erythromycin (15 µg).

Polymerase Chain Reaction (PCR) for the detection of *mecA* gene

To determine the genotype for the PCR amplification of the *mecA* gene by PCR technique, the PCR was performed in microcentrifuge

Table 1. Oligonucleotides for detection of the *mec*A gene.

Function	Name	Sequence 5'a nucleotide 3'	Amplified product
Primer	*mecA*1	AAA ATC GAT GGT AAA GGT TGG	533 bp
Primer	*mecA*2	AGT TCT GCA GTA CCG GAT TTG	533 bp

Source: Murakami et al. (1991).

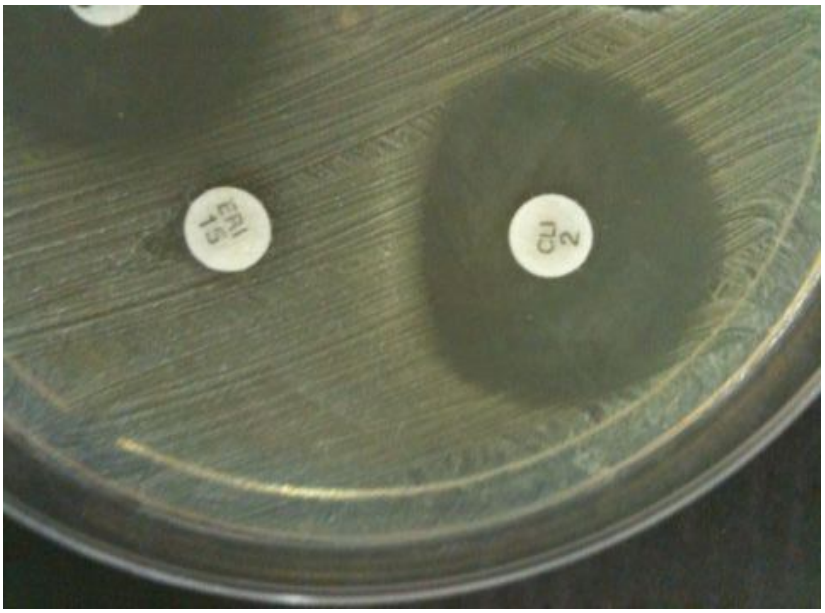

Figure 1. Test D positive. You can see the halo flattening forming the letter "D" due to the induction of by erythromycin resistance.

tubes in 0.5 ml total volume of 25 µl containing 1 µM of each primer (Table 1), 2 U of Taq polymerase, and 100 mM of dNTP 0.75 mM MgCl2 and 150 ng of nucleic acid. Incubation was carried out in an appropriate thermocycler using the parameters described by Murakami et al. (1991) consisting of 40 cycles of denaturation at 94°C for 30 s, annealing of primers at 55°C for thirty seconds and extension at 72°C for one minute. After completion of the 40 cycles, the tubes were incubated at 72°C for 5 min before cooling to 4°C. International reference strains with positive controls (*S. aureus* ATCC 33591) and negative (*S. aureus* ATCC 29213) were used in all reactions carried out. The amplification efficiency was evaluated by electrophoresis on a 2% agarose gel in 1x TBE buffer (90 mm Tris, 90mm Boric acid, 2 mM EDTA pH 8.0) stained with ethidium bromide 0.5 µg/ml. The amplified products were compared with a 100bp marker (Amersham Pharmacia Biosciences Inc.) and then visualized and photographed under ultraviolet light.

RESULTS

A total of 222 samples were collected, from these 18 samples (8.1%) were excluded because students used antimicrobials. Among the 204 samples studied, 86 were collected from men (42.2%) and 118 women (57.8%). These samples were subjected to the identification process and 43 samples (21.1%) were positive for *S. aureus*.

In assessing the strength of the 43 *S. aureus* isolates, 4 samples (9.3%) were resistant to oxacillin, 8 samples (18.6%) were resistant each to cefoxitin and clindamycin, while 40 samples (93.0%) were resistant to erythromycin. 11 samples (25.6%) were found to be positive for the D test (Figure 1). All the isolates that were resistant to clindamycin were also resistant to erythromycin. All isolates were susceptible to vancomycin (Figure 2). By PCR, it was possible to confirm that all eight samples (18.6%) resistant to Cefoxitin (positive for MRSA) showed the expression of *mec*A gene.

DISCUSSION

The prevalence of *S. aureus* from this study was 21.1%, which was supported by data described by Catã et al. (2012), who found 20.0% prevalence. Atique et al. (2012) reported the prevalence of 33.3% of *S. aureus* in the nasal mucosa of the students, while Goldmann (1992) in their study showed that the incidence was 20.0 and 40.0% of patients of *S. aureus*. Data presented by Pereira et al. (2009) in assessing the nasal colonization of 109 *Staphylococcus* samples collected from 104 students of undergraduate nursing, showed that 30

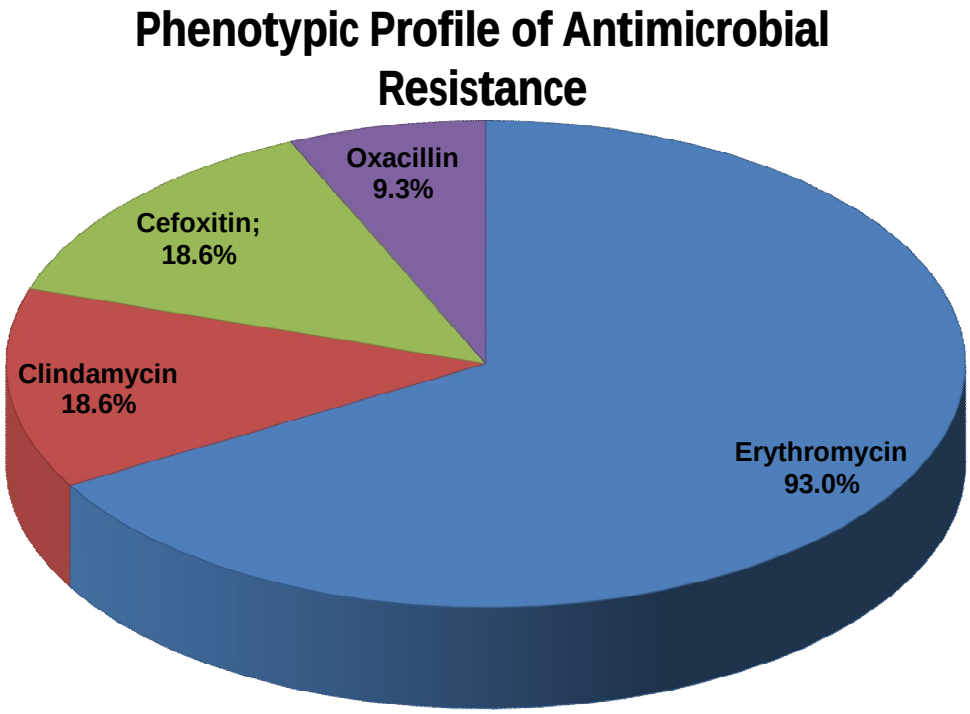

Figure 2. Distribution of the percentage of resistance to antimicrobials in *Staphylococcus aureus* isolates.

(27.5%) samples were positive for *S. aureus*. This data corroborate with those found in this study. Prevalence rates of MRSA may vary, particularly as a function of the size and type of the medical institution (Pereira and Cunha, 2009).

In this study resistance to oxacillin was observed in 9.3% of samples of *S. aureus*, which differ from those described by Ciraj et al. (2009) who analyzed different clinical isolates and found 17.33% reported rates of resistance oxacillin. Also reports described by Kobayashi et al. (2009) in a study on evaluation of antimicrobial resistance in clinical isolates from hospitalized patients, found 68.5% of samples resistant to oxacillin. These authors also reported that resistance to clindamycin occurred in 94.7% of *S. aureus* isolates resistant to erythromycin, similar data were observed in our study where 100% of *S. aureus* resistant to erythromycin also showed resistance to clindamycin.

Campiotto et al. (2010) found 75.0% of erythromycin resistance among the samples taken from the nasal mucosa of health professionals, and in our study 93.0% of samples positive for *S. aureus* were resistant to erythromycin. In another study, Colli et al. (2009) reported having found 66.6% of samples resistant to erythromycin. These results determine high rates of resistance of *S. aureus* to erytromicina and emphasize the importance of research of resistance of these bacteria to this antibiotic.

In this study, 11 samples (25.6%) that had inducible resistance phenotype to Macrolides, Lincosamides and Streptogramins. Colli et al. (2009) in a study to assess the resistance of *S. aureus* isolated from nasal or lingual sites of adult patients showed that 22.2% of the strains exhibited induced resistance to clindamycin. Catão et al. (2012) in his study, where samples were collected from the nasal cavity of an employee health service, demonstrated that the analyzed samples showed 100% of sensitivity phenotype to clindamycin, diverging to those presented in this study, where it was observed that 18.6% of strains were resistant to clindamycin.

All 43 samples were tested for genotypic resistance evaluation by detection of the *mecA* gene by PCR, in which was possible to confirm that all samples that showed phenotypic resistance to Cefoxitin (8 sample) were positive for the *mecA* gene (Figure 3) and were classified as Methicillin-Resistant *Staphylococcus aureus* (MRSA). These data differ from those reported by Pereira and Cunha (2009) who found no sample of MRSA in samples collected from nursing students. Another study aimed at detection of MRSA in nasal mucosa in patients admitted to the intensive care unit (ICU), Rowe et al. (2002) reported a prevalence of 14% of MRSA, a value of approximate prevalence with the result obtained in this study (18.6% for MRSA). However, Mimica and Mendes (2007), and other studies conducted in various parts of the world, reported that the prevalence of MRSA varies and depends on the country, and in some places rates are over 80%.

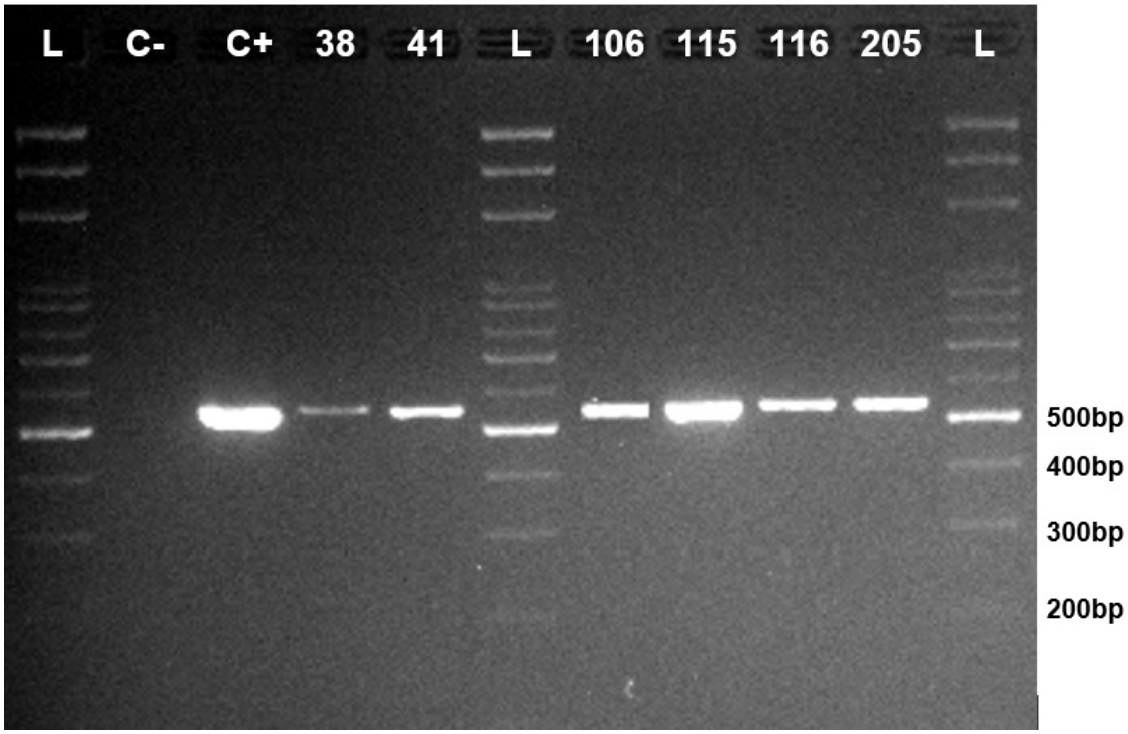

Figure 3. Agarose gel electrophoresis to search the *mecA* (533 bp) gene L: 100 bp ladder, 38; 41; 106; 115; 116 and 205: samples of *S. aureus* isolated from nasal cavities of medical students positive for the *mecA* gene. **C-:** negative control (*S. aureus* ATCC 29213) and **C+:** positive control (*S. aureus* ATCC 33591).

CONCLUSION

Considering the results, it can be concluded that students in courses related to health care services, such as medical students, are colonized by *S. aureus* with multidrug resistance profile. Monitoring the colonization of health professionals and students colonized by bacteria with multiple resistances attending the hospital environment profile is crucial in order to establish measures to prevent and control the spread of these strains in the hospital setting.

ACKNOWLEDGEMENTS

We want to thank the Central Research Coordination (CCPq), Western São Paulo University UNOESTE) for the technical and financial support.

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Citation: Medeiros LB, Gushiken CY, Correia BP, Guaberto LM, Moris DV, Rodrigues MVP, Pereira VC, 2015. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from medical students of A Brazilian education institute. Microbiol Res Int, 3(1): 14-19.