Chapter 08

Characterization of *Staphylococcus aureus* Isolates that colonize Medical Students in Hospital of the City of Cali, Colombia

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Abstract

Introduction: Nasal carriage of methicillin-resistant Staphylococcus aureus (MRSA) represents a risk for the spread of bacteria. This study characterized the S. aureus isolated from medical students, who were in their clinical rotation at a hospital in the city of Cali.

Materials and methods: 216 students participated in the study and 63 isolates of S. aureus were evaluated for susceptibility and PCR amplification of agr and mecA genes. The origin of MRSA isolates was established by analyzing of agr polymorphisms.

Results: A total of 29.2% of students were colonized by S. aureus and nasal carriage rate was 23.6%, and 14.3% MRSA. Three agr groups (agr I, II, and III) were identified, the agr I group was the most common, with 35% prevalence, this group is of community origin.

Conclusion: A significant presence of asymptomatic carriers of S. aureus was demonstrated in medical students from beginners in their clinical practice, an increase of asymptomatic carriers of S aureus of community origin in more students stay in the hospital.

Keywords

Staphylococcus Aureus; Medical Students; Nasal Carriage; CA-MRSA; HA-MRSA
Introduction

*Staphylococcus aureus* resistant to methicillin (MRSA) is among the bacteria that are currently the cause of global concern [1,2]. It is responsible for a broad range of community-associated MRSA infections, especially in soft tissue of young individuals without risk factors [3-5] and at hospital, specifically in intensive care units (ICU) [1,6]. The most serious cases of MRSA resistance are those mediated by the *mecA* gene, these strains can simultaneously acquire pathogenicity genes that make them develop more aggressive infections [1].

Asymptomatic carriage of *S. aureus* in healthy individuals has been shown to have a high prevalence, especially in children, young adults, and healthcare workers [7-10]. Medical students represent an important portion of the healthcare personnel, and they in frequent contact with the general community or healthcare environment may spread the bacteria to other community members or to susceptible patients, respectively [10-16].

Epidemiological studies conducted in Latin American hospitals report a prevalence of *S. aureus* colonization from 20 to 60% in students from the health area [10-13], with an important presence of MRSA strains in higher percentage than in Europe [14-16].

Epidemiological knowledge of the state of MRSA strains circulating in our environment helps to establish control measures to prevent the transmission of the pathogen through adequate use of bio-safety barriers by the healthcare staff, including medical students. In this sense, two of the aspects that must be considered are: if the *S. aureus* carried by the healthcare staff is of intra-hospital origin or from the community, and how it is disseminated in hospital wards.

Molecular approaches, like analysis of the polymorphism in *agr* genes have permitted determining the variability and origin of isolates generating epidemic outbreaks. According to this gene’s variability pattern, to date, four variants have been defined, denominated as groups I to IV [17]. Isolates belonging to group III are related with
infections associated to the community, while group II is detected predominantly in strains isolated at intra-hospital level [18,19].

The objective of this study was to establish the genetic diversity of isolates of *S. aureus* and detect the presence of the mecA gene in strains isolated from asymptomatic medical students who were in their clinical rotation phase in a hospital in the city of Cali.

**Materials and Methods**

**Study Population**

During the study, 481 medical students were in their pre-clinical and clinical rotation cycle; 216 students signed the informed consent and complied with the inclusion and exclusion criteria, which included not having received antibiotic treatment within the last three months and not presenting respiratory or skin diseases. A total of 119 participants were women and 97 were men who were in hospital practices from October to December 2010 at the San Juan de Dios hospital in the city of Cali. A cross-sectional descriptive study was carried out and it was evaluated and approved by the Ethics and Bioethics Committee of the Faculty of Health at the University.

**Culture Conditions**

Samples were taken with sterile cotton swabs through smears of the nasal mucosa and of the skin in each student. The samples were processed immediately in the microbiology laboratory. The isolates were managed by codes, thus, respecting student confidentiality.

To isolate species of the Staphylococcus genus, the samples were seeded in *Mannitol salt phenol-red agar* (Oxoid Ltd., Hampshire, United Kingdom) and incubated for 24 to 48 hours at 37 °C. Identification of *S. aureus* was accomplished through fermentation of mannitol in selective agar (medium yellow coloration), the positive reaction of coagulase test, and microscope observation of Gram positive cocci in clusters from a direct extended Gram stain. The *S. aureus* was differentiated from coagulase-negative staphylococci (CoNS) by using the DNase test.
Antibiotic Susceptibility Tests

Antimicrobial susceptibility testing was conducted on paired samples using the agar disk diffusion method. The isolates were classified as susceptible, intermediate susceptibility, or resistant according to the recommendations by the Clinical and Laboratory Standards Institute (CLSI) [20].

To conduct this test, a standardized amount of \( S. \text{aureus} \) was inoculated (standard 0.5 by Mc Farland) in Mueller-Hinton agar medium (Scharlau Chemie S.A.) and then the sensi-disks were placed: oxacillin (OXA, 1 µg), cefoxitin (FOX, 30 µg), cephalxin (LEX, 30 µg), gentamicin (GEN, 10 µg), ciprofloxacin (CIP, 5 µg), erythromycin (ERY, 15 µg), clindamycin (CLI, 2 µg), trimethoprim/sulfamethoxazole (SXT 1.25/23.75 µg), tetracycline (TET, 30 µg), chloramphenicol (CHL, 30 µg), vancomycin (VAN, 30 µg), imipenem (IPM, 10 µg), and penicillin (PEN, 10U) (Oxoid). To verify the action of the sensi-disks, during susceptibility analysis the ATCC 25923 strain of \( S. \text{aureus} \) was used as control.

Determination of MRSA isolates was performed according to the results obtained in the evaluation of oxacillin and cefoxitin, and by detecting the \( \text{mec} \)A gene [21,22].

Molecular Analyses of Isolates

The DNA of the reference strains and of the bacterial isolates was extracted by using the modified protocol by Cheng et al., 2006 [23], based on bacterial lysis using 25% sucrose, 10 mg/ml of lysozyme and 1 mg/ml of Proteinase K, at 56 °C.

To establish resistance to methicillin, the \( \text{mec} \)A gene was amplified, following the protocol reported by Lee [22] and a 1334 pb band of the \( \text{mec} \)A gene was amplified by using primers: MR1, 5’ (478)-GTG GAA TTG GCC AAT ACA GG-(497) 3’ and MR2, 5’ (1816)-TGA GTT CTG CAG TAC CGG AT-(1797) 3’. To determine the four \( \text{agr} \) groups, fragments of 440 bp, 572 bp, 406 bp, and 588 pb, respectively, were amplified independently using
a set of primers, made up of the universal sense primer, with the *agr* gene sequence: 5’- ATG CAC ATG GTG CAC ATG C-´-3’, and one of the specific primers for each group in anti-sense: *agr*I 5’- GTC ACA AGT ACT ATA AGC TGC GAT-3’, *agr*II 5’-GTA TTA CTA ATT GAA AAG TGC CAT AGC-3, *agr*III 5’-CTG TTG AAA AAG TCA ACT AAA AGC TC-3’, and *agr*IV 5’-CGA TAA TGC CGT AAT ACC CG-3´ [18].

The PCR reactions were conducted in 50-μl volume of a reaction mixture composed of MgCl$_2$ 25 mM, 200 μM of the four dNTPs, 0.5 U of Taq DNA polymerase (Invitrogen®), 10 pmol of each primer and 1 μl of DNA solution in a thermocycler GeneAmp PCR system 2400®. In this study *S. aureus* ATCC 25923 strain was used for the positive control.

**Statistical Analysis**

The unit of analysis was the bacterial isolate obtained from the nasal swab from which the microbiological and molecular characteristics were registered and were related with the sociodemographic characteristics of the carriers, like gender and academic semester. The microbiological variables were the presence of *S. aureus* and the degree of susceptibility or resistance to each antibiotic evaluated, categorized into different degrees, thus: resistance (1), intermediate susceptibility (2), and susceptibility (3), according to the standards for each antibiotic established for *S. aureus*. The molecular variables were presence of *mec*A genes and the variants of the *agr* gene. A database was constructed with the variables of interest by using the Excel™ program.

The presence of *S. aureus* was determined as percentage of time of permanence and gender. In the group colonized by *S. aureus*, an association analysis was performed among the different variables like resistance phenotype to different antibiotics, presence of *mec*A gene, and the *agr* genetic variant, bearing in mind the academic semester and the number of hours per day of practice: from three hours for third-year students, five hours for fourth-year students, 8 hours for...
fifth-year students, and 12 hours for sixth-year students. Prevalence of the different agr gene variants was determined in MRSA and MSSA phenotypes.

The findings were statistically analyzed using descriptive statistics, Chi-square test ($\chi^2$) and p-value ($p < 0.05$, statistical significant). The risk factor analysis of MRSA colonization was performed using SPSS statistical package (version 20.0, SPSS, Inc., Chicago, IL, USA).

Results

A total of 29.2% (63) of the students who were in the different services at the hospital were colonized by S. aureus. Table 1 shows the isolates according to the students’ academic year; it can be noted that fourth-year students had the highest number of isolates (45%) ($p<0.05$). The frequency of nasal carriage of S. aureus was significantly higher than on skin (20.4% vs. 8.9%; OR=2.870; $p<0.05$) and fifth-year students presented the highest frequency of S. aureus in the nose than on the skin (18.2 vs. 1.8%; OR=5.294; $p=0.094$) (Table 1).

Table 1: Frequency of isolation of S. aureus from medical students.

<table>
<thead>
<tr>
<th>Academic degree</th>
<th>Students Total</th>
<th>Students colonized</th>
<th>OR</th>
<th>CI 95%</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>n (%)</td>
<td>S. aureus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nose (%)</td>
<td>Skin (%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>14.4</td>
<td>9 (14.3)</td>
<td>17 (55)</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>32.9</td>
<td>31 (49.2)</td>
<td>18 (40.9)</td>
<td>13 (68.4)</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>25.5</td>
<td>17 (37.5)</td>
<td>10 (21.3)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>28.2</td>
<td>12 (19)</td>
<td>9 (20.5)</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>100</td>
<td>63 (29.2)</td>
<td>44 (20.4)</td>
<td>19 (8.9)</td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence intervals; statistical significance was based on .

Antibiotic Susceptibility Analysis

Isolates with resistance to β-lactam antibiotics were distributed in a high number of students from all the academic grades, with a variation between 90 and 22% (Table 2). Isolates resistant to antibiotics inhibiting protein synthesis were registered between 18% and 90%, and to antibiotics inhibiting nucleic acid synthesis registered between 11% and 44%.
Table 2: Antibiotic susceptibility patterns of S. aureus isolates.

<table>
<thead>
<tr>
<th>RT (hrs)</th>
<th>OXA (n%)</th>
<th>PEN (n%)</th>
<th>FOX (n%)</th>
<th>LEX (n%)</th>
<th>IPM (n%)</th>
<th>VAN (n%)</th>
<th>CIP (n%)</th>
<th>SXT (n%)</th>
<th>TCY (n%)</th>
<th>GEN (n%)</th>
<th>CLI (n%)</th>
<th>ERY (n%)</th>
<th>CHL (n%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>X</td>
<td>14.8</td>
<td>3</td>
<td>0(0)</td>
<td>1(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>1(100)</td>
<td>0(0)</td>
<td>1(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>1(100)</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>30.9</td>
<td>5</td>
<td>2(63)</td>
<td>17(87)</td>
<td>4(12.9)</td>
<td>29(95.5)</td>
<td>5(10.1)</td>
<td>11(35.5)</td>
<td>12(38.7)</td>
<td>10(29.4)</td>
<td>9(28.6)</td>
<td>7(23.3)</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>23.5</td>
<td>8</td>
<td>2(38.2)</td>
<td>11(100)</td>
<td>3(27.3)</td>
<td>10(90.9)</td>
<td>2(18.2)</td>
<td>10(90.9)</td>
<td>2(18.2)</td>
<td>6(54.5)</td>
<td>7(63.3)</td>
<td>8(72.7)</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>26.2</td>
<td>12</td>
<td>1(16.7)</td>
<td>11(91.7)</td>
<td>2(16.7)</td>
<td>10(90.9)</td>
<td>3(25.0)</td>
<td>4(33.3)</td>
<td>6(50.0)</td>
<td>7(63.3)</td>
<td>5(45.5)</td>
<td>9(75.0)</td>
</tr>
<tr>
<td>Total</td>
<td>236</td>
<td>100</td>
<td>5</td>
<td>5(21.2)</td>
<td>58(92.1)</td>
<td>9(14.3)</td>
<td>57(90.5)</td>
<td>14(22.2)</td>
<td>30(47.6)</td>
<td>19(30.2)</td>
<td>20(41.3)</td>
<td>34(54)</td>
<td>38(28.6)</td>
</tr>
</tbody>
</table>

RT: residence time of medical students in hospital. P < 0.05.

Note. OXA, oxacillin; PEN, penicillin; FOX, cefoxitin; LEX, cephalxin; IPM, imipenem; VAN, vancomycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; GEN, gentamicin; CLI, clindamycin; ERY, erythromycin; CHL, chloramphenicol.

**Molecular Analysis of Isolates**

Amplification of the mecA gene was detected in the five isolates presenting simultaneous resistance to oxacillin-cefoxitin, and in four isolates resistant only to cefoxitin (14.3%). The multi-drug resistance phenotype was the distinctive characteristic in these MRSA isolates with simultaneous resistance to β-lactam, macrolide, aminoglycoside, and quinolone antibiotics (Table 3).

Table 3: Frequency of MRSA and MSSA strains according of antibiotic susceptibility patterns.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>PEN</th>
<th>FOX</th>
<th>IPM</th>
<th>VAN</th>
<th>CLI</th>
<th>ERI</th>
<th>CHL</th>
<th>GEN</th>
<th>TET</th>
<th>SXT</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 9)</td>
<td>100 (9)</td>
<td>100 (9)</td>
<td>778 (7)</td>
<td>778 (7)</td>
<td>88.9 (8)</td>
<td>100 (9)</td>
<td>778 (7)</td>
<td>778 (7)</td>
<td>100 (9)</td>
<td>66.6 (6)</td>
<td>66.6 (6)</td>
</tr>
<tr>
<td>MSSA</td>
<td>90.7 (49)</td>
<td>88.9 (48)</td>
<td>13 (7)</td>
<td>0 (0)</td>
<td>51.9 (28)</td>
<td>76 (41)</td>
<td>64.8 (35)</td>
<td>20.4 (11)</td>
<td>46.3 (25)</td>
<td>37 (20)</td>
<td>24.1 (13)</td>
</tr>
<tr>
<td>(n = 54)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

MRSA: methicillin-resistant S. aureus; MSSA: methicillin-sensitive S. aureus.

A total of 54 isolates were positive to agr gene amplification with a difference in prevalence of specific agr groups between MRSA and methicillin sensitive S. aureus (MSSA) isolates. Thirty-five bacterial isolates (55.6%) were classified in the agr group I, 31 (49.2%) strains were MSSA and 4 (6.3%) strains were MRSA, the differences were statistically significant (P < 0.05).

Five MRSA isolates (9.3%) were identified in the agr group II; the mecA gene was detected in all isolates and the agr group III was found only in 14 MSSA isolates (22.2%) (Table 4).
The majority of S. aureus strains isolated from students belonged to agr group I followed by agr group III (41 strains), and finally agr group II (9 strains) was distributed among the isolates of fourth-, fifth-, and sixth-year students. The agr groups I to III occurred in the major percentages (25.9% and 18.5%, respectively) in fourth-year students (data not shown).

**Discussion**

The present study evidenced the presence of S. aureus with 29.2% of prevalence in medical students in clinical rotation, and nasal carriage was a risk factor. The tendency is similar in Latin American countries with percentages between 50 and 20% [10-13]. In Europe, a prevalence of nasal carriers of S. aureus is reported at 25% [14-16]. In Asia, prevalence of colonization by S. aureus in Chinese, Malaysian, and Indian medical students is registered in 31% of the cases [24].

Although some studies report increased frequency of nasal carriers as medical students have greater exposure to the hospital environment during clinical rotations [25,26], our results did not show significant differences among the nasal carriers and the time of permanence in the hospital environment. However, a significant number of isolates resistant to imipenem was determined in third-year students, and a higher number of isolates with resistance to aminoglycosides, erythromycins, and quinolones in students with greater time of permanence in the hospital (Table 2).
Our study determined a high frequency of *S. aureus* isolates resistant to penicillin, cephalexin, imipenem, erythromycin, chloramphenicol, clindamycin, tetracycline, gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin, giving way to the presence of multi-drug resistant isolates. These isolates can evolve in response to selective pressure exerted on microflora residing in patients, which are then passed on to students, or can be directly generated in the microflora of students.

Similar results were obtained in studies conducted by Lee [22], all isolates with resistant to cefoxitin presented the *mecA* gene. Datta [21] demonstrated that cefoxitin induces the expression of the PBP2a protein encoded in the *mecA* gene, the cefoxitin disk diffusion test had the best diagnostic performance among the phenotypic methods for detection of MRSA.

One of the factors that permit the presence of MRSA strains in asymptomatic carriers is due to antibiotic intake that strengthens overgrowth of organisms on the skin and on mucosa surface [2,6]. It has been demonstrated that use of penicillin is associated with acquisition of MRSA, likewise, use of fluoroquinolones, macrolides, and cephalosporins increase the risk of having these strains [27].

Although in Colombia it is reported that the majority of MRSA strains are not of nosocomial origin [2], an increasing number of reports indicate an epidemiological change with the prevalence of strains of community origin [5,28].

This study determined that strains of hospital origin (AH-MRSA) presented resistance to β-lactam antibiotics and to multiple antibiotics (Table 3) and, according to the *agr* gene analysis these isolates were located in the *agr* group II, a group compatible with hospital origin [17,18].

The presence of AH-MRSA strains in asymptomatic carriers are a potential risk of disseminating in the community, additionally, these strains are resistant to multi-drug with few therapeutic alternatives.
However, we also found isolates with characteristics of community origin (AC-MRSA) in four MRSA isolates grouped in the *agr* I. Nasal carriage of MRSA, become an important reservoir and source of pathogen propagation among the personnel, the community, and the patient.

As observed in Table 3, MSSA isolates also presented characteristics of resistance multi-drug. Studies conducted in Spain reveal that MSSA isolates susceptible also show resistance to erythromycin (13.5%), clindamycin (11.5%), and ciprofloxacin (1.9%) [16].

In addition, 49.2% and 22.2% of MSSA isolates were grouped in *agr* I and III, respectively, according to this approach is associated molecular Community origin.

Several studies have found that the MRSA and MSSA circulating in Colombia have genetic similarities to clone USA300 [29,30]. The spread between asymptomatic carriers of MSSA influence the presence of MRSA in the community. This clone has as distinctive feature the presence of the marker ST-8 leucocidin Panton Valentine (PVL) and Hemoliisna (HDL), and belongs to *Agr* I group with a high power of dissemination. The spread would be favored perhaps by origin from MSSA isolates that are colonizing the asymptomatic carriers [30].

Although, some of the risk factors for colonization by *S. aureus* are age, gender, hospitalizations, use of antibiotics, and some diseases and habits [3,12]. Among the population analyzed, these variables were not evaluated, which became a limitation of the study. The results of this study, however, evidence the important presence of asymptomatic carriers *S. aureus* in medical students from the time they begin their clinical practice. Additionally, increased MRSA colonization was found in students as they spent more time in the hospital, as it’s seen in fifth year students showing the highest number of carriers.
Acknowledgments

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References


Top 10 Contributions on Microbiology


13. Prates KA, Torres AM, Garcia LB, Ogatta SF, Cardoso CL, et al. Nasal carriage of methicillin-resistant *Staphylococcus*


20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-
third Informational Supplement M100-S23. CLSI, Wayne, PA, USA, 2013.


