Prevalence and antimicrobial susceptibility of *Staphylococcus aureus* methicilin resistant isolated from medical students

Prevalencia y susceptibilidad antimicrobiana de cepas de *Staphylococcus aureus* meticilino resistentes aisladas de estudiantes de Medicina

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ABSTRACT

Objective: *To establish the prevalence and identify the level of resistance to methicillin, vancomycin and alternative antibiotics in Staphylococcus aureus isolates from medical students in clinical training.*

Materials and methods: *A cross-sectional observational design with non-random sampling was used in medical students during clinical training in a tertiary healthcare facility. Samples were taken from nasal and hands swabs and cultured on blood agar. For beta-hemolytic gram-positive cocci, catalase and coagulase tests were performed and then cultured on mannitol salt agar. Suscep-*

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tibility to cefoxitin, oxacillin, linezolid, clindamycin and trimethoprim sulfamethoxazole was assessed by using the Kirby-Bauer technique, and for vancomycin, an E-test was performed (*Biomerieux®*).

***Results*:** 72 *strains of S. aureus were isolated from* 82 *medical students*. 72.2 % *were identified as methicillin-sensitive* (*MSSA*) *and* 27.8 % *as methicillin-resistant* (*MRSA*). F*our MRSA strains* (20 %) *showed vancomycin intermediate* (*VISA* 4-8 *μg/mL*) *profile*, 65 % *of MRSA isolates was resistant to clindamycin*, 40 % *to linezolid and* 45 % *to trimethoprim sulfamethoxazole*.

C*onclusions*: *MSSA, MRSA and VISA strains are present in nostrils and hands of our medical students, with MRSA showing high resistance levels to clindamycin, TMP-SMX and linezolid, and MSSA levels up to* 45 %. T*hese findings reiterate the need to accomplish good hands hygiene in order to minimize the spread of S. aureus in community and healthcare facilities*.

KEY WORDS

Staphylococcus aureus
MRSA
Microbial drug resistance

RESUMEN

Objetivo: establecer la prevalencia e identificar el perfil de resistencia a meticilina, vancomicina y antibióticos alternativos en aislamientos de *Staphylococcus aureus* provenientes de estudiantes de Medicina en rotaciones hospitalarias.

Materiales y métodos: estudio observacional transversal no aleatorizado en estudiantes de medicina en entrenamiento clínico en un hospital de tercer nivel de complejidad. Las muestras fueron tomadas de hisopados nasales y de manos y cultivadas en agar sangre. A los cocos gram positivos se les realizó pruebas de catalasa, coagulasa y siembra en agar salado manitol. La susceptibilidad a cefoxitina, oxacilina, linezolida, clindamicina y trimetoprim sulfametoxazol se efectuó mediante la técnica de Kirby-Bauer y para la evaluación de la vancomicina el método de E-test (Biomerieux®).

Resultados: 72 cepas de *S. aureus* fueron aisladas de manos y cavidad nasal de 82 estudiantes de medicina, 72,2 % fueron identificadas como meticilino sensibles (SAMS) y 27,8 % como meticilino resistentes (SAMR). Cuatro cepas (20 %) de SAMR mostraron ser vancomicina intermedio (SAVI 4-8 mg / mL), 65 % de los SAMR aislados fueron resistentes a la clindamicina, 40 % al linezolid y 45 % al trimetoprim sulfametoxazol.

Conclusiones: en la cavidad nasal y las manos de estudiantes de medicina están presentes cepas de SAMS, SAMR y SAVI con alto nivel de resistencia para clindamicina, TMP-SMX y linezolid en los SAMR y hasta el 45 % para los SAMS. Estos resultados reiteran la necesidad de realizar una buena higiene de manos para reducir al mínimo la circulación de *S. aureus* en la comunidad y en los servicios de atención de la salud.

PALABRAS CLAVES

Staphylococcus aureus
SAMR
Farmacorresistencia microbiana

INTRODUCTION

In human body has been recognized over the time a variety of microorganisms mainly bacteria, some of them are commensals and others can cause serious diseases or death. The discovery of drugs that prevent growth or bacterial di-

vision has had a deep impact on reducing the incidence of infectious diseases, mainly bacterial diseases. Nevertheless, over the past century, it has been recognized the resistance to these drugs; between 20 to 40 % of the population served in hospitals had an antibiotic-resistant bacteria strain (1-3).

Staphylococcus aureus has been recognized as one of the microorganisms with most impact even in patients with community acquired infections. S. *aureus* has a remarkable pathogenicity and the ability to adapt to different conditions and avoid the effect of antimicrobial treatment, thus generating huge costs and a high rate of mortality (4,5).

S. aureus has been demonstrated in health care workers; they carry it in their hands and also could be present in medical devices. *S. aureus* causes about 30 deaths per day associated with risk factors such as chronic diseases, immunosuppressive drug regimens or immunosuppressive diseases, where a critical factor in morbidity and mortality is the use of inappropriate antibiotics. Methicillin-resistant, vancomycin-intermediate and a few vancomycin-resistant strains have been isolated from patients treated with glycopeptides and in patients with suspected or confirmed methicillin-resistant *Staphylococcus aureus* (*MRSA*) (6-8).

Many antibiotics such as clindamycin, trimethoprim sulfamethoxazole TMP-SMX, daptomycin and linezolid for MRSA have been tested with similar therapeutic effect to vancomycin, minimal side effects and good recovery for patients (9-13).

The intravenous vancomycin is the core treatment because other drugs such as fluoroquinolones and third-generation cephalosporins are ineffective (14). However, since 1984 it has been recognized the resistance to vancomycin; such strains are designated as vancomycin-resistant *Staphylococcus aureus* (VRSA), trait assigned by the *British Society for Antimicrobial Chemotherapy* to those strains with vancomycin minimum inhibitory concentration (MIC) greater than 8 μg/mL (1,15).

According to the *Clinical Laboratory Standards Institute* (CLSI, formerly NCCLS), *S. aureus* isolates for which vancomycin MIC are 4-8 μg/mL are classified as vancomycin-intermediate (VISA), and isolates for which vancomycin MIC´s are greater than 8 μg/mL are classified as vancomycin-resistant. (16). VISA have been isolated from patients with persistent bacteremia, endocarditis, central nervous system infection, septic arthritis and other infections, and some multiresistant strains (tetracyclines, macrolides, aminoglycosides and teicoplanin), including a VRSA isolated from a nephrostomy tube, exhibit a MIC profile greater than 16 μg/mL (17-23).

Several studies have found some strains of community acquired methicillin-resistant *S. aureus* (CA-MRSA) (24, 25). In Colombia, Sosa *et al*. and Villalobos *et al*. have showed the presence of CA-MRSA by using conventional and molecular typing in children and adults (26,27). Similarly, *S. aureus* is an important nosocomial pathogen with resistance to oxacillin and other antibiotics (hospital-acquired-MRSA, HA-MRSA) (28).

Asymptomatic individuals will always be an important reservoir of *S. aureus* strains; they dwell on skin and can be easily transmitted by fomites or direct contact with others (29).

The objective of this research is to establish the prevalence and identify the profile of resistance to methicillin, vancomycin and alternative antibiotics in *Staphylococcus aureus* isolates from medical students in clinical training.

MATERIALS AND METHODS

A cross-sectional observational design with non-random sampling was used in 82 medical students enrolled (VII to XII semester) with at least two-week clinical training in their main clinical facility in Bogota, Colombia, without antibiotic prescription or respiratory symptoms.

After signing the informed consent previously approved by the ethics committee, samples were taken from nasal and hands swabs (2,3,30,31) and they were processed by gram stain and cultured on blood agar (25). For beta hemolytic gram-positive cocci catalase test was performed; if it was positive, then a coagulase test was performed and then it was cultured on mannitol salt agar (32).

To evaluate the methicilin pattern of *S. aureus* we used cefoxitin and oxacillin discs, and susceptibility of linezolid, clindamycin, trimethoprim sulfamethoxazole (TMP-SMX) as therapeutic alternative antibiotics was assessed using the Kirby-Bauer technique (9-11,14); vancomycin resistance was tested using E-test (Biomerieux®) (33). Results were reported according to the 2011 CLSI guidelines (16,34). Incubation for all tests was performed at 35 °C under 5 % CO_2 during 24 hours. In some cases, serotyping was performed with commercial antiserum (Staphytect plus® oxoid) (figure 1).

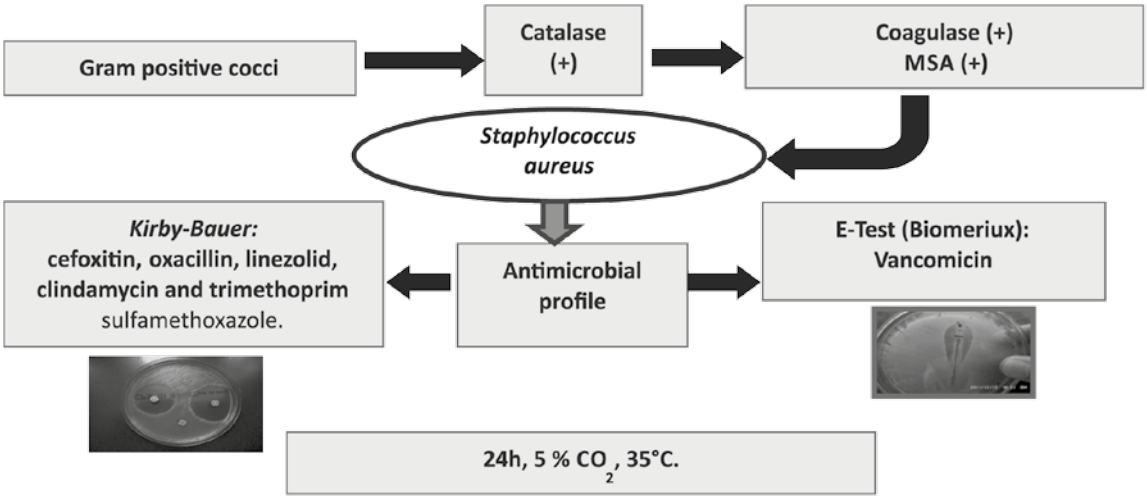

Figure 1. Biotyping and susceptibility test algorithm for S. *aureus*. MSA (manitol salt agar) (16)

RESULTS

164 samples from students were obtained (figure 2), 72 strains of *S. aureus* were isolated, 47 from women (65.3 %) and 25 from men (34.7 %). 72.2 % (52 isolates) were identified as methicillin-sensitive and 27.8 % (20 isolates) as MRSA, 16 from nasal swabs and 4 from hands.

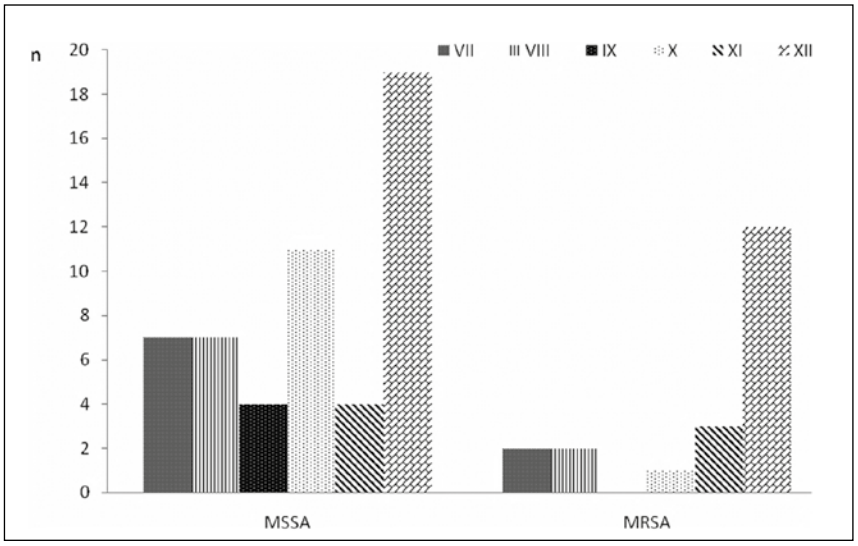

Figure 2. Distribution of strain types of S. *aureus* between academic semesters. MSSA (methicillin sensitive), MRSA (methicillin resistant).

20 % of MRSA (4 of 20 strains) showed VISA (4-8 μg/mL) profile, all were isolated from nasal swabs from students up to XII semester. Out of 16 MRSA - vancomycin sensible (VSSA < 2μg/mL) strains, 12 (60 %) were identified from nasal swabs and 4 (20 %) from hands. All *Staphylococcus aureus* (52 MSSA and 20 MRSA) were tested for antimicrobial susceptibility to alternative antibiotics. Thirteen (65 %) of MRSA isolates were resistant to clindamycin, 8 (40 %) to linezolid and 9 (45 %) to trimethoprim sulfamethoxazole (figure 3).

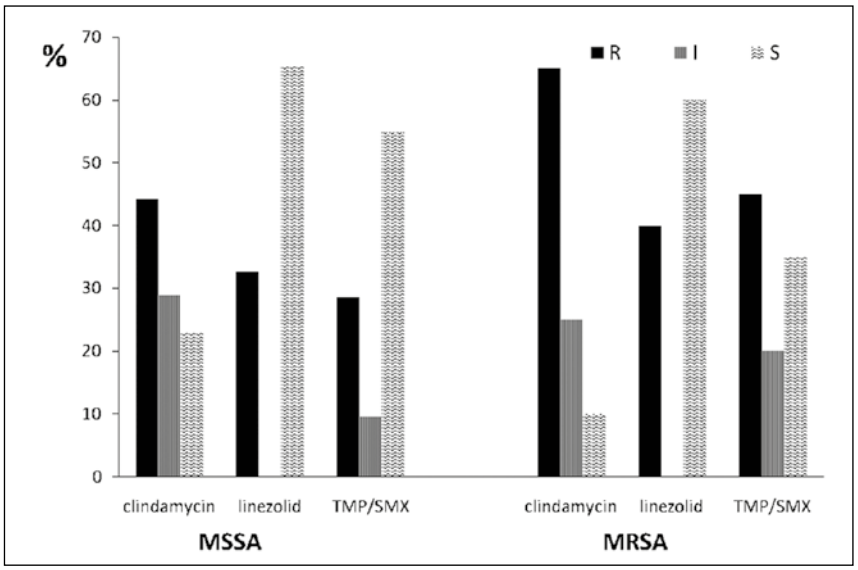

Figure 3. Antimicrobial susceptibility profile of MSSA and MRSA to other antibiotics, TMP/SMX: trimethoprim/sulfamethoxazole

DISCUSSION

We describe the distribution of methicillin-sensitive, methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus* strains identified in nasal swabs and hands from 82 medical students in clinical training in a tertiary healthcare facility with 350 beds and all medical and surgical facilities. Our school of Medicine in Bogota, Colombia has approximately 600 students (250 in basic sciences and 350 in clinical training).

In a previous work performed between 2009-2011, our group surveyed 155 students and obtained a total of 455 gram-positive cocci (49.7 %), 46 gram-positive bacilli (7.4 %) and 41 (6.6 %) gram-negative bacilli from hands, nostrils, oral cavity and stethoscopes. For gram positive cocci, MRSA was most prevalent with 22.7 % isolated from hands (29 %) and nostrils (23 %). Levels of 25 % resistance to ampicillin/sulbactam and 37 % to cephalexin were observed (35), Gandia *et al* found a 30 % of MRSA in medical students in Sinu, Colombia (36). In both cases, the rate of isolated MRSA is similar to that of this report.

Our work found four VISA strains in nasal swabs from students of last semester and it is worth noting the presence of that kind of strain in a clinical setting. On the contrary, one study in healthcare workers in Nicaragua didn't found VISA and showed a low prevalence of MRSA (<11.6 %) yet it found 15 % of multi-resistance mainly to erythromycin and clindamycin (2). In a Cuba research study it highlight the fact that medical students are continuously exposed to environment highly loaded with microorganisms, including all types of *S. aureus* (25).

Seventy two percent of healthcare workers from a university hospital in Bucaramanga, Colombia, were nasal carriers of *S. aureus* with 11.6 % of MRSA; likewise, other microorganisms such as *Enterobacter aerogenes* (6 %), *Proteus mirabilis* (2.3 %), *Haemophilus influenzae*, *Citrobacter koseri* and *Providencia rettgeri* (1.1 %) were isolated.

Particularly regarding *S. aureus*, a high level of resistance to TMP-SXT (50 %) and low resistance to clindamycin (7.7 %) is remarkable (37). In that paper, a multicenter resistance study was performed between 2001 and 2009 in intensive care units, with 11.2 % of *S. aureus*, and 45.6 % of MRSA. Our isolates showed similar level of resistance to TMP-SXT but an important proportion of MRSA were resistant to clindamycin as well.

Continuous screening MRSA-colonized people and the eradication of microorganisms by using an appropriated hand washing technique and nasal hygiene seem to be of the utmost importance. Hand hygiene is a key component in reducing infection; however, despite of hand washing, MRSA was recovered from healthcare workers even after using alcohol, chlorhexidine, or soap and water, which indicates that *S. aureus* is a very persistent bacteria (38), other study shown that a 39.3 % of hands of medical students were colonized by *S. aureus*, and 2,1 % by MRSA, with a poor adherence to hand washing protocol (39).

More than 20 thousand isolates of *Staphylococcus aureus* were collected between 2004 and 2009, 41.2 % were MRSA and 58.8 % MSSA. A total of 4 % MRSA and MSSA isolates exhibited a MIC ≥2 μg/mL to vancomycin. Linezolid, minocycline, tigecycline and vancomycin were the most active agents against *S. aureus*; the susceptibility to vancomycin in MRSA decreased from 100 % in 2004 to 95.77 % in 2009. Similarly, in MSSA isolates, susceptibility to vancomycin decreased from 100 % in 2004 to 91.07 % in 2009. Evidence suggests that although the number of isolates of *S. aureus* with reduced susceptibility to vancomycin has increased significantly from 2004 to 2009, in our work, an important level of resistance of both MRSA (≥40 % to alternative antibiotic used in case of vancomycin failure [linezolid, TMP-SMX and clindamycin]) and MSSA (≥ 28 %) was found (13).

In their review, Matlow *et al*. focus on several recommendations to prevent transmission of antimicrobial-resistant bacteria in the office and clinic, such as the use of alcohol for hands, identification of colonized or infected patients, isolation of some patients (for example: those with draining abscesses or wounds, uncontained diarrhea, fecal incontinence or cystic fibrosis), routine hand-hygiene practices for all patients, contact precautions improvement, general housekeeping and equipment cleaning, disinfecting and sterilizing to avoid transmission, consultation with experts such as public health officers or hospital infection control personnel (4).

Some studies have showed differences between MSSA or MRSA decolonization. Screening is useful in high-risk units to identify the reservoir and initiate contact precautions, this strategy being more effective than decolonization; however, screening and decolonization will be more important to prevent infection risk in MRSA carriers (40).

CONCLUSIONS

Our results indicate that MSSA, MRSA and VISA strains are present in an important proportion in nostrils and hands of our medical students. It highlights the high MRSA resistance level, between 40 %-65 % to clindamycin, TMP-SMX and linezolid, and MSSA resistance, up to 45 %. Patients and health care workers are commonly recognized as a source of pathogenic microrganisms, but medical students had not been recognized as performers in MRSA transmission.

A controversial issue is the effectiveness of routine surveillance cultures in order to detect MRSA colonized patients, healthcare workers and now, medical students, and the role of decolonizing agents for example with mupirocine in the non-outbreak clinical setting (4). These findings reiterate the need to accomplish a good personal hygiene in order to minimize the spread of pervasive, persistent and pathogenic *S. aureus* in community and healthcare facilities. Finally, medical students should be included in hospital infection control programs.

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DISCLOSURE

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