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Research Article

Characterization of Methicillin-Sensitive *Staphylococcus Aureus* Obtained from Invasive Infections and Nasal Carriers of Health Personnel and Medical Students in Cali, Colombia

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Abstract

Introduction: Methicillin-sensitive *Staphylococcus aureus* (MSSA) is among the bacteria that are common sources of intrahospital infections and in the community. The objective of this study was to characterize strains of MSSA obtained from patients with invasive infections, and asymptomatic carriers of health personnel and medical students.

Materials and Method: A descriptive cross-sectional study was performed with MSSA isolates obtained from invasive infections and colonizing health personnel and medical students. The association between the variables was made by analysis by Chi-square test with values of $p < 0.05$, using the statistical package SPSS version 22.0.

Results: The MSSA was detected in 62.2% of cases, 22.8% in clinical samples, 49.8% in students and 27.4% in health personnel. Five profiles or antibiotypes were established, profile 1 with sensitivity to all antibiotics being the most prevalent (31.2%). However, it was found that 16% of the isolates presented a profile of multiresistance to antibiotics. Some isolates presented the *Pvl* and *hgl* genes (5.5%) and were grouped into agrI (20.7%), agr II (14.3%) and agr III (65%).

Conclusion: The results of this study show the important presence of MSSA in invasive infections and in nasal carriers of health personnel and students, several of these isolates have multiresistance to antibiotics and pathogenic genes related to aggressive infections at intra and extra hospital level. Some of these MSSA isolates belong to the agrI group and possess the *pvl* and *hgl* genes, distinctive characteristics of Community-associated Methicillin-resistant *S. aureus* (CA-MRSA).

Keywords: *Methicillin-Sensitive Staphylococcus Aureus*; Epidemiology; Molecular Characteristics; Health Personnel; Medical Students; Clinic Isolates

Introduction

Staphylococcus aureus is one of the microorganisms that is isolated most frequently in hospital and community infections [1]. One of the most important aspects for its study is its high morbidity; it is a causative agent of a wide variety of diseases ranging from simple infections without complications such as folliculitis, cellulitis, and infections of operative site to severe infections such as endocarditis, septicemia, meningitis, pneumonias and bacteremia [1,2]. The bacterium has an extraordinary repertoire of virulence factors that allows it to survive extreme conditions within the human host, within the general aspects of the staphylococcal pathogenesis we have the susceptible strains and those resistant to methicillin, which contain factors or genetic background that can increase their virulence or may allow them to cause clinical

syndromes [3]. Among the pathogenic strains of *S. aureus* stand out those that transport the Panton-Valentine leukocidin (PVL), a virulence factor that is strongly associated with skin infections and severe necrosis pneumonia [4].

This bacterium is usually found colonizing humans and animals [5-7], with the asymptomatic nasopharyngeal carrier being the most frequent source of *S. aureus* and one of the reservoirs for bacterial infection [8-11]. Among asymptomatic carriers, health personnel are reported as important sources of colonization and spread of these, since they are in frequent contact with the sick and healthy community [12-15]. In addition, epidemiological studies conducted in Latin America, establish a prevalence of colonization of *S. aureus* from 20 to 60% in students of the health area [15-17].

In Colombia, there are reports of nosocomial pathogens resistant to methicillin [18-20], but the molecular epidemiological information regarding methicillin-sensitive *S. aureus* (MSSA) is scarce. Some studies have detected the presence of strains of MSSA carrying virulence genes such as PVL, indicating that they are potential reservoirs of severe infections in the community and in hospitals [21,22].

The objective of this study was to characterize strains of MSSA obtained from patients with invasive infections, health personnel and medical students in order to establish reservoirs of MSSA carriers of virulence genes such as *pvl* and *hgl*.

Materials and Method

The study was carried out with a total of 381 *S aureus* isolates, 138 isolates were obtained from patients who were in the Intensive Care Unit (ICU) of the University Hospital San Juan de Dios in the city of Cali with invasive infection by *S. aureus* between 2014 and 2015. Invasive *S. aureus* disease is defined as the isolation of bacteria from a normally sterile site in a patient with clinical signs and symptoms consistent with infection by the bacteria. Fifty-six isolates were obtained from blood, 53 from pus samples from deep wounds, 27 from sputum and the other two samples were obtained from urine.

Two hundred forty three isolates of *S aureus* were from asymptomatic carriers who underwent nasal swabs: 78 isolates were obtained from health personnel (63 isolates were obtained from medical students who rotate in hospital wards and 15 from health workers) and 165 from students medicine without contact with the hospital. The asymptomatic carriers included in the study signed the informed consent and those who did not have respiratory diseases and had not received antibiotic therapy in the last three months were selected.

Obtaining bacterial isolates

Bacterial isolates were obtained by culturing the samples on the phenol red mannitol saline agar (Oxoid Ltd., Hampshire, United Kingdom) and incubated for 24 to 48 hours at 37°C. The colonies identified as probable *S. aureus* were confirmed by observing the presence of Gram positive cocci in clusters, from a direct smear with Gram stain and with the positive tests of coagulase and DNAse.

Antibiotic sensitivity tests

The antimicrobial sensitivity test was performed on paired samples using the disk agar diffusion method. The isolates were classified as sensitive, intermediate sensitivity or resistant according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [23].

To carry out this test, a standardized quantity (standard McFarland 0.5) of *S. aureus* was inoculated on a Mueller-Hinton agar medium (Scharlau Chemie SA) and then the sensitiscs were placed: oxacillin (OXA, 1 µg), cefoxitin (FOX, 30 µg), cephalexin (CEF, 30 µg), gentamicin (GEN, 10 µg), ciprofloxacin (CIP, 5 µg), erythromycin (ERI, 15 µg), clindamycin (CLI, 2 µg), trimethoprim / sulfamethoxazole (SXT 1.25 / 23.75 µg), tetracycline (TCY, 30 µg), chloramphenicol (CHL, 30 µg), vancomycin (VAN, 30 µg), imipenem (IMP, 10 µg) and ampicillin (PEN, 10U) (Oxoid).

Molecular analysis of isolations

The DNA of the reference strains and of the bacterial isolates was extracted using the commercial kit (MO BIO Laboratories Inc).

To establish the presence of pathogenicity determinants, the *pvl* and *hgl* genes were amplified according to the protocol described by Lina G., *et al*. [24] The amplification was developed with a denaturation temperature of 94°C for 5 min followed by 30 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 min and a final temperature extension to 72°C for 10 min. The primers luk-PV-1, 50-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-30 were used for the amplification of the *pvl* gene; and luk-PV-2, 50-GCA TCA AST GTA TTG GAT AGC AAA AGC-30. The gene sequence $_{\gamma}$-hemolysin -1, 50-GCC AAT CCG TTA TATA GAA AAT GC-30 and hlg-2, 50-CCA TAG ACG TAG CAA CGG AT-30.

The agr groups were established among the MSSA isolates by independent amplification of the 440 bp, 572 bp, 406 bp and 588 bp fragments, using the set of primers Pan-agr and agrI, agrII, agrIII and agrIV, respectively [25].

PCR reactions were performed in a volume of 50 µl of a reaction mixture composed of 2.0 mM MgCl 2, 0.4 mM dNTP's, 1 U of Taq DNA polymerase (Invitrogen®), 0.5 µM of each primer and 1 µl of

DNA solution in a Gene Amp PCR System 2400 thermal cycler (Perkin-Elmer Instruments®, Norwalk, Conn). The strain ATCC 25923 of *S. aureus* was used as a positive control.

The PCR products were visualized by electrophoresis in 2% agarose gel (100V, 45 min). The band sizes were verified by running a molecular weight marker of 100 bp (Bioline, UK) in parallel and visualized using a UV transilluminator after staining with Syber Green.

Statistical analysis

The unit of analysis was the bacterial isolate with the molecular characteristics and they were related to the sociodemographic characteristics of the study population. The molecular variables were presence or absence of the *pvl* and *hlg* genes and a database was constructed with the variables of interest, using the ExcelTM program.

The type of *S. aureus* clone was determined as a percentage and an analysis of the association between the different variables such as the genetic variant, presence of virulence genes, taking into account the population from which each isolate was obtained.

The significance in the differences in the frequency of the variables between the established groups was determined by statistical analysis, using the Chi-square test. Statistical significance will be assigned for values of $p < 0.05$, considering a confidence level of 95% (alpha) and an error (beta) of 5%. The statistical analyzes were performed using the statistical package (SPSS version 22.0, SPSS, Inc., Chicago, IL, USA). This study was conducted taking into account the technical, scientific and ethical standards established in decree 008430 of 1993 of the Ministry of Health and Social Protection of the Republic of Colombia and submitted to the Ethics and Bioethics Committee of the Faculty.

Results

Phenotypic characterization of the MSSA isolates

According to the results of the antibiotic susceptibility test it was found that 62.2% of the isolates were MSSA, 49.8% was found in the nasal tracing of the students, 27.4% in the personnel of health and 22.8% in clinical samples. The samples of deep wounds and blood were the sites from which the highest number of these isolates of MSSA was obtained, with 10.1% and 8.4% prevalence, respectively. The students presented a risk of 5,347 times more chance of being colonized by MSSA (95% CI: 3,411- 8,381).

Among these isolates, 66.9% were resistant to penicillin, and 2.5% to cefazolin. Among the non-β-lactam antibiotics, the greatest number of resistant isolates occurred in erythromycin (30.5%), clindamycin (22%) and trimethoprim / sulfamethoxazole (17.3%).

According to the profile of sensitivity and resistance found among the MSSA isolates, 5 profiles or antibiotypes were established, profile 1 with sensitivity to all the antibiotics evaluated being the most prevalent (31.2%), followed by profile 2 with resistance to penicillin or gentamicin (26.2%) and profile 5 (16%) in isolates with resistance to more than four antibiotics (Table 1).

Ant	Isolates N (%)	MSSA n (%)	Sample Site						Sensitive profile	Resistance profile
			T N (%)	Bl N (%)	Sp N (%)	Nt N (%)	Ct N (%)	Ur N (%)		
1	76 (19,9)	74 (31,2)	-	-	1 (0,4)	73 (30,8)	0	0	AMP, OXA, CEF, FOX, SXT, ERI, TCY, CLI. IMP, VAN, GEN, CIP	-
2	69 (18,1)	62 (26,2)	12 (5,1)	11 (4,6)	2 (0.8)	37 (15,6)	0	0	FOX, OXA, VAN, CEF, SXT, ERI, TCY, CLI, CIP, IMP	PEN ó GEN
3	82 (21,5)	36 (15,2)	10 (4,2)	9 (3,8)	2 (0,8)	13 (5,5)	2 (0,8)	0	FOX, OXA, VAN, CEF, SXT, TCY, CLI, CIP, IMP	PEN y ERI/ GEN
4	35 (9,2)	27 (11,4)	1 (0,4)	0	2 (1,6)	24 (10,1)	-	0	FOX, OXA, VAN, CEF, SXT, TCY, GEN, IMP	PEN, ERI, CLI/ CIP
5	119 (31,2)	38 (16)	1 (0,4)	0		36 (15,2)		1 (0,4)	FOX, OXA, TCY, CEF, IMP, VAN, , SXT	PEN, ERI, GEN, CIP/ CLI
Total	381	237 (62,2)	24 (10,1)	20 (8,4)	7 (3)	183 (77,2)	2 (0,8)	1 (0,4)		

Table 1: Distribution of MSSA isolates between antibiotypes and clinical samples and asymptomatic carriers of health personnel and medical students. n = 381

Nt= Nasal trace (health personnel and students): Ant= Antibiotype

Clinical samples: Bl=Blood; Wd= wound; Ur= Urine; Sp=Sputum; Ct= Catheter tips

oxacillin (OXA, 1 µg) , cefoxitin (FOX, 30 µg), cephalexin (CEF, 30 µg), gentamicin (GEN, 10 µg), ciprofloxacin (CIP, 5 µg), erythromycin (ERI, 15 µg), clindamycin (CLI, 2 µg), trimethoprim / sulfamethoxazole (SXT 1.25 / 23.75 µg), tetracycline (TCY, 30 µg), chloramphenicol (CHL, 30 µg), vancomycin (VAN, 30 µg), imipenem (IMP, 10 µg) and ampicillin (PEN, 10U).

Molecular characteristics of the MSSA isolates

Table 2 shows the molecular characteristics of the MSSA isolates, in this study the presence of the agrI (20.7%), agr II (14.3%) and agr III (65%) groups was determined in a significant manner ($p < 0.05$). The agr IV was not found. The risk of being present among the MSSA isolates was 1,671; 3,370 and 2,609, respectively.

The genes coding for the PVL and HGL toxins were detected significantly each in 5.5% of the MSSA isolates

	MSSA n=237 n (%)	MRSA N=144 n (%)	P-value
agrI	49 (20.7)	3 (2.1)	0.003
agrII	27 (14.3)	10 (6.9)	0.000
agrIII	154 (65)	36 (25)	0.000
pvl	13 (5.5)	33 (22.9)	0.000
hgl	13 (5.5)	25 (17.4)	0.000
Medical students	118 (49.8)	47 (32.6)	0.001
Health personnel	65 (27.4)	13(9)	0.000
Clinical samples	54 (22.8)	84 (58.3)	0.000

Table 2: Distribution of the MSSA and MRSA isolates between the detected genes, clinical samples and asymptomatic carriers of health personnel and medical students.

MRSA: Methicillin-resistant *Staphylococcus aureus*;

MSSA: Methicillin-sensitive *Staphylococcus aureus*

Discussion

In this study, MSSA was isolated in 62.2%, the ability of MSSA to cause various diseases in humans such as endocarditis, osteomyelitis, pneumonia, bacteremia and toxic shock syndrome (TSS), indicates that the pathogenesis of this bacterium is highly complex 2-4. Although, methicillin-resistant *S. aureus* (MRSA) is reported as the cause of the most complicated and difficult to treat infections, approximately half of the hospital infections caused by *S. aureus* are caused by MSSA isolates, which suggests that the latter, despite not having *SCCmec*, they must have genetic advantages that favor their dissemination [26,27]. In this study, a frequency of MSSA of 22.8% was found in deep infections such as abscesses and bacteremia's. The origin of this bacterium can be from the nasal colonization of the patients, as it has been demonstrated in many cases, the nasal colonization of the patient preceded the infection [10,11].

On the other hand, several studies have shown the cross-transmission of the bacteria, mainly through health personnel [8,10,11]. In this case, the prevalence of MSSA was 27.4% in health personnel. If we take into account that the greatest number of isolates were causing invasive infections (blood) and infections of the skin and soft tissues (abscess), the health personnel can be one of the sources of the microorganism, being colonized in a prolonged way and transmitting it to the patient. The infection will begin when there is a break in the cutaneous-mucosal barrier that precipitates the passage of the microorganism to adjacent tissues and to the bloodstream [1,3].

In some studies, it has been found that infections by this pathogen are more frequent among patients with a low socio-economic level, which would be a factor that may be predominant in this study, because the hospital cares for this type of patients, including patients in street condition.

In this study, we found five sensitivity profiles or antibiotypes among MSSA. Although, the antibiotype 1 with sensitivity to all the antibiotics evaluated was the most prevalent (31.2%), a significant number of isolates presented antibiotypes with resistance patterns to more than three and four antibiotics such as antibiotype 4 (11.4%) and 5 (16%). The resistance patterns of these antibiotypes included antibiotics such as penicillin, erythromycin, clindamycin, ciprofloxacin and gentamicin, antibiotics that are usually prescribed for the treatment of MSSA infections.

It was observed that the clinical samples presented a higher prevalence of antibiotypes 2 and 3 (those that showed resistance to one and two antibiotics, respectively); however, health personnel and students presented nasal colonization with MSSA that presented the 5 antibiotypes, including multi-resistant antibiotypes such as antibiotype 4 (10.1%) and antibiotype 5 (15.2%). These isolates with multiresistance characteristics found in health personnel and students may have evolved in response to selective pressures exerted on the patient-resident micro-flora that then passes to students and health personnel, or may have directly generated in the microflora of these asymptomatic carriers. The presence of these strains in asymptomatic carriers is a potential risk of spreading in the community and causing pathologies. Also, if we take into account that these strains are multi-resistant to antibiotics, we will be left with few therapeutic alternatives for their management.

The molecular analysis of the MSSA isolates, could determine the presence of the gene that codes for the PVL in 5.5% of the cases, the gene that codifies for the HGL toxin was also, detected.

The leukocidal PVL is an exotoxin encoded by the *Luk*S / *Luk*F genes and acts on the outer membrane of polymorphonuclear leukocytes, monocytes and macrophages causing the opening of calcium channels; consequently, they cause the release of calcium and inflammatory mediators, which leads to tissue apoptosis and necrosis [28]. In recent years, the presence of MSSA ST398 has been reported, which is characterized by the presence of type A protein t571, is sensitive to all antibiotics except for macrolides and has PVL [29-31] and has been reported in colonized patients [32,33], and in samples of infected patients [29, 30].

The high prevalence of *S. aureus* positive to PVL has been reported in regions of Indonesia and has been associated with severe infections in the community and in hospitals [22].

Escobar., *et al*. have suggested that the origin of Community-associated MRSA strains (CA-MRSA) in Colombia is due to the emergence of MSSA clones related to the USA300 clone that subsequently acquired SCC *mec* Ivc [21].

The CA-MRSA strains have a lower resistance to non-β-lactam antibiotics, they have a greater power of dissemination, tend to cause a greater proportion of skin and soft tissue infections and are much more aggressive in causing the disease due to the activity ant staphylococcal. Since they are usually producers of a series of exotoxins (particularly, Panton-Valentine leukocidin) and other virulence factors such as the HGL toxin and belong to the agrI group [34].

In this study the MSSA isolates were found to belong to the agrI group in 20.7%. this group is related to strains of community origin. While, groups agrII (14.3%) and agrIII (65%) are more related to infections of hospital origin.

Conclusion

The results of this study show the significant presence of MSSA in invasive infections and nasal carriers of health personnel and students. Several of these isolates presented profiles with multiresistance to antibiotics, especially penicillin, erythromycin, clindamycin, gentamicin and ciprofloxacin.

The presence of the *pvl* and *hgl* genes among MSSA isolates was also evident, being potential reservoirs of aggressive infections at intra and extra-hospital level.

S. aureus strains carrying toxins such as PVL and belonging to the agrI group, are considered a potential source of CA-MRSA.

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Conflict of Interest

The authors have no conflict of interest to declare.

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