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ARTÍCULO ORIGINAL

Methicillin-resistant *Staphylococcus aureus* causes both community-associated and health care-associated infections in children at the Hospital Universitario de Santander

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a frequent cause of infection in the pediatric population. Initially, MRSA was restricted to hospitals; however, outbreaks in the community among people without health care-related risk factors have been reported worldwide. Currently, MRSA is a frequent cause of both hospital and community-associated infections.

Objective: To describe the relationships between the molecular characteristics of MRSA isolates (staphylococcal cassette chromosome mec (SCCmec) type and Panton-Valentine leukocidin (PVL) carriage) and the characteristics of infection (the origin and localization of infection) in pediatric patients at the Hospital Universitario de Santander in Bucaramanga, Colombia.

Materials and methods: A total of 43 MRSA isolates were obtained from hospitalized pediatric patients. SCCmec typing (I-V), SCCmec IV subtyping and PVL carriage were determined and related to the clinical characteristics.

Results: Among the MRSA isolates studied, SCCmec IVc was present in 77%, followed by 16% for SCCmec I and 2% for SCCmec IVa. Two isolates were not typeable (NT). PVL genes were carried by 88% of the MRSA isolates, including the SCCmec IVc/IVa and SCCmec I isolates. SCCmec IV caused both community-acquired infection (CAI) (47%) and nosocomial infection (HAI) (53%). SCCmec IV, PVL-positive MRSA was associated with both CAI (47%) and HAI (53%) and caused mostly SSTI and osteoarticular infection.

Conclusions: These findings suggest that the presence of community-associated MRSA (CA-MRSA) (SCCmec IV and PVL positive) causes both health care-associated infection (HCAI) and nosocomial infection (HAI) in pediatric patients in Colombia.

Key words: Methicillin-resistant *Staphylococcus aureus*, patients, child.

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***Staphylococcus aureus* resistente a meticilina causante de infecciones comunitarias y de infecciones asociadas a la atención en salud en pacientes pediátricos del Hospital Universitario de Santander**

Introducción. *Staphylococcus aureus* resistente a la meticilina (SARM) es un agente frecuente de infección en la población pediátrica. Aunque inicialmente las cepas de SARM estaban restringidas a los hospitales, se han reportado a nivel mundial brotes de infección por SARM en individuos sin factores de riesgo y, actualmente, SARM es una causa frecuente de infecciones hospitalarias y comunitarias.

Objetivo. Describir la relación entre las características moleculares de aislamientos de SARM (casete cromosómico estafilocócico mec SCCmec y leucocidina Panton-Valentine) y el origen de la infección y su presentación clínica en pacientes pediátricos del Hospital Universitario de Santander en Bucaramanga, Colombia.

Materiales y métodos. Se incluyeron 43 aislamientos de SARM obtenidos de niños hospitalizados. La clasificación del SCCmec (I-V) y la subclasificación del SCCmec-IV se realizaron en todos los aislamientos. Además, los genes de la leucocidina Panton-Valentine se detectaron mediante amplificación por PCR. Las características moleculares fueron asociadas con las características clínicas de cada paciente.

Resultados. Entre los 43 SARM tipificados, el SCCmec-IVc fue el más frecuente con 77 %, seguido por el

Author contributions:

Clara Isabel González and Luis Miguel Sosa: Experiment design, reagents, materials and analysis tools.

Mayra Alejandra Machuca: Performance of experiments.

All authors contributed equally to the data analysis and the drafting of the article.

SCCmec-I con 16 % y el SCCmec-IVa con 2 %. Tres aislamientos no pudieron ser tipificados. Los genes de la leucocidina Pantone Valentine se detectaron en 88 % de los SARM en aislamientos portadores del SCCmec-IVc/IVa y el SCCmec-I. Los SARM SCCmec-IV positivos para la leucocidina Pantone-Valentine se asociaron con infecciones adquiridas en la comunidad (47 %) y en el hospital (53 %) con compromiso de piel y tejidos blandos, y en los casos más graves, con compromiso osteoarticular.

Conclusiones. Estos resultados sugieren la presencia de cepas SARM-CO (SCCmec-IV positiva para PVL) causantes de infecciones adquiridas en la comunidad y en el medio hospitalario en pacientes pediátricos en Colombia.

Palabras clave: *Staphylococcus aureus* resistente a meticilina, pacientes, niño.

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Staphylococcus aureus is responsible for infections ranging from skin and soft tissue infections to severe diseases, such as endocarditis, bacteremia, necrotizing pneumonia and osteomyelitis (1). Methicillin resistance is encoded by the *mecA* gene, which is carried in the staphylococcal cassette chromosome *mec* (SCCmec) (2,3). Based on the class of the *mec* complex, *ccr* type and composition of the J regions, eight types of SCCmec, called I-XI, have been described (4,5).

For many years, methicillin-resistant *S. aureus* (MRSA) was restricted to hospitals, causing infections associated with the health care setting (HAI) (6). However, since the early 2000s, outbreaks of community-associated MRSA (CA-MRSA) have been reported worldwide in diverse community populations (7,8). Recently, several reports described the spread of CA-MRSA in the hospital setting, which is beginning to replace typical hospital-associated MRSA (HA-MRSA), especially in the United States and Taiwan, where CA-MRSA prevalence is very high (9). CA-MRSA has been reported to be causing infections in Latin American countries, such as Brazil (10), Uruguay (11), Colombia (12) and Argentina (13). In Colombia, CA-MRSA isolates have recently been reported to be causing HAI in hospitals in Bogotá and Bucaramanga (12,14).

Phenotypic and molecular differences between HA- and CA-MRSA have been described, as follows: i) HA-MRSA strains carry SCCmec types I-III (15), whereas CA-MRSA strains carry SCCmec IV and V (16,17); ii) HA-MRSA tends to be multiresistant, whereas CA-MRSA tends to be susceptible to narrow-spectrum non-beta-lactams, such as clindamycin,

trimethoprim-sulfamethoxazole and tetracyclines (18); and iii) a high percentage of CA-MRSA strains carry the genes *lukS-PV* and *lukF-PV*, encoding Pantone-Valentine leukocidin (PVL) (18), an important virulence factor that has been associated with skin abscesses and necrotizing pneumonia (19-21). The PVL genes are present in CA-MRSA strains with a frequency >75% and are largely absent from HA-MRSA strains (7,22-23). In the present study, we characterized the relationships between the molecular features (SCCmec type and PVL carriage) and the characteristics (the origin and localization of infection) of infection in pediatric patients at the Hospital Universitario de Santander in Bucaramanga, Colombia.

Materials and methods

Clinical isolates

MRSA isolates were obtained from isolated cases of local or systemic infections in a pediatric population (age range: 0-13 years old), who were hospitalized in a third-level university hospital. The isolates were collected in the Clinical Laboratory of the Hospital Universitario de Santander in Bucaramanga, Colombia, during the period from March 2007 to March 2009. The antibiotic susceptibilities of the *S. aureus* isolates were assessed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (24). The antibiotics tested included erythromycin, clindamycin, ciprofloxacin, gentamicin, tetracycline, oxacillin, rifampicin, vancomycin and trimethoprim-sulfamethoxazole. Clinical and epidemiological information was obtained from the medical records of each patient. Each MRSA infection was classified as a community-associated infection (CAI), nosocomial infection (HAI) or health care-associated infection (HCAI), according to the following definitions:

- CAI: Clinical condition and isolation of MRSA within 48 hours of hospitalization in the absence of risk factors.

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- HAI: No obvious clinical disease at admission, isolation of MRSA after 72 hours of hospitalization or presence of risk factors (use of invasive devices, surgery, dialysis and previous hospitalization in the last three months).

- HCAI: Clinical condition and isolation of MRSA at admission or within 48 hours of hospitalization and presence of risk factors (25).

Detection of *nuc* and *mecA* genes

All isolates were confirmed as MRSA by PCR amplification of the *nuc* and *mecA* genes according to a previously described protocol (26,27).

SCCmec typing and PVL gene detection

The SCCmec type (I-V) was determined based on combinations of the *mec* complex and *ccr* type using independent PCRs. SCCmec IV subtypes were identified using a multiplex PCR under reported conditions (28). Amplification of the PVL genes (*lukS/F* PV) was performed as previously reported (20). The COL, N315, MW2, E-MRSA-16 and RN4220/pG01 strains were included as reference strains and were supplied by the Network on Antimicrobial Resistance in *S. aureus* (NARSA).

Ethics statement

The research and informed consent protocols were approved by the Ethics Committee at the Universidad Industrial de Santander in accordance with the ethical standards of the 1964 Declaration of Helsinki (Acta No. 15 27/08/2007). Each child's parents or guardians provided written informed consent for the review of medical information, and all of the information was confidential.

Results

In the period between March 2007 and March 2009, there were 126 *S. aureus* infections in pediatric patients, of which 42% were caused by MRSA. In total, 8% of the children were aged <1 month; 40%, from 1-24 months; 35%, from 2-10 years old, and 17%, >10 years old.

In this study, 43 non-duplicated MRSA isolates were included. Most of the MRSA isolates were susceptible to the majority of the antibiotics tested; only one isolate presented a multiresistant phenotype (resistant to four antibiotics). In total, 44% (19/43) of the MRSA isolates exhibited resistance to tetracycline; 42% (18/43), to erythromycin; 30% (13/43), to ciprofloxacin; 26% (11/43), to clindamycin; 19% (8/43), to gentamicin, and 5% (2/43), to TMP-SMX. All strains were resistant to oxacillin and susceptible to vancomycin and rifampicin.

SCCmec typing and PVL gene detection

The type SCCmec IVc was the most frequent in MRSA clinical isolates (77%, 33 isolates). Additionally, 2% (1 isolate) was SCCmec type IVa, and 16% (7 isolates) were type I. Two isolates were not typeable (NT) following the search for the five SCCmec types. The *lukS/F* PV genes were detected in 88% (38/43) of the MRSA isolates and were present in all SCCmec types identified. The *lukS/F* PV genes were detected in 100% (1/1) and 91% (30/33) of the SCCmec type IVa and SCCmec type IVc isolates, respectively. Most of the SCCmec type I isolates carried the *lukF/S* PV genes (86%, 6 isolates).

Clinical analysis

Among the infections, 46% were CAIs, 19% were HCAIs, and 35% were HAIs (table 1).

The most frequent clinical manifestation was skin infection, affecting 53% (23/43) of pediatric patients, followed by soft tissue infection in 28% (12/43) and osteoarticular infection in 12% (5/43). More severe infections were present at low frequencies, such as bacteremia in 5% (2/43) and complicated pneumonia in 2% (1/43) (table 2). Classification was performed according to the definitions described before (29).

Expression of the molecular markers SCCmec and PVL and the origin of MRSA infection are included in table 1. MRSA carrying SCCmec types IVc and

Table 1. Molecular characteristics of MRSA isolates from community, nosocomial and health care infections

Molecular marker		CAI (n=20) n (%)	HCAI (n=8) n (%)	HAI (n=15) n (%)	Total (n=43) n (%)
SCC <i>mec</i>	Type I	4 (20)	0	3 (20)	7 (16)
	Type IVc	16 (80)	6 (75)	11 (73)	33 (78)
	Type IVa	0	1 (13)	0	1 (2)
	NT	0	1 (13)	1 (7)	2 (5)
PVL	LPV (+)	19 (95)	7 (88)	12 (80)	38 (88)
	LPV (-)	1 (5)	1 (12)	3 (20)	5 (12)

Table 2. Molecular characteristics of MRSA isolates and infection type

Molecular marker		Isolate localization / Infection type				
		SI (n=23) n (%)	STI (n=12) n (%)	OAI (n=5) n (%)	B (n=2) n (%)	Pn (n=1) n (%)
SCC <i>mec</i>	Type I	4 (17)	1 (8)	1 (20)	1 (50)	0
	Type IVc	16 (70)	11 (92)	4 (80)	1 (50)	1 (100)
	Type IVa	1 (4)	0	0	0	0
	NT	2 (9)	0	0	0	0
PVL	LPV (+)	20 (87)	10 (83)	5 (100)	2 (100)	1 (100)
	LPV (-)	3 (13)	2 (17)	0	0	0

SI: skin infection; STI: soft tissue infection; OAI: osteo-articular infection; B: bacteremia; Pn: pneumonia

IVa and PVL positivity caused CAI, HCAI and HAI at high frequencies. In total, 80% of CAIs, 88% of HCAIs and 74% of HAIs were caused by isolates with SCCmec type IV, and the isolates carrying SCCmec type I caused 20% of CAIs and 20% of HAIs. SCCmec type IVc and type I isolates that were obtained from all localizations were similarly PVL-positive isolates, but in a large percentage, these isolates were related to skin and soft tissue infections (table 2).

Discussion

HCAI and HAI are generally caused by isolates of MRSA with the molecular characteristics of HA-MRSA strains, such as SCCmec I, the absence of PVL and antimicrobial multiresistance patterns. However, since the emergence of CA-MRSA strains carrying SCCmec IV, the PVL genes and an antimicrobial sensitivity pattern phenotype, CA-MRSA isolation in hospitals has increased significantly in the United States (30,31), in certain European countries, such as France (32) and Italy (33), and in Latin American countries, including Brazil (10), Argentina (14), Uruguay (11) and Colombia (12). In Colombia, for the last few years, the presence and circulation of CA-MRSA isolates have been described in hospitals in different regions, and the prevalence of this strain type in the hospital environment has been estimated to be approximately 39% (12,34). In our study, 74% of HAIs and 88% of HCAIs were caused by isolates with the molecular characteristics of CA-MRSA, which were higher percentages than previously reported (12,34). Therefore, these results confirm that CA-MRSA strains have been introduced into and have become established in hospitals in Colombia. Of the 43 isolates analyzed in our study, only seven carried SCCmec I, similar to what has been reported in certain European countries such as Austria and the United Kingdom, where the frequency of HA-MRSA strains in hospitals has typically decreased (35).

SCCmec type IV has the greatest variability among the SCCmec types (36), and seven subtypes (IVa to IVg) have been identified (28). This subtype identification has been important for understanding the mechanisms of the insertion and acquisition of this mobile genetic element and has also been used to identify new circulating clones (37). The SCCmec subtype IVc was identified for the first time in Japan, and this variant was associated with HAI (2). Although similar results were observed in other countries, such as France (38), in Sweden, this subtype has been associated with CAI (39). Nevertheless, HAI in countries such as the United States and Australia most frequently presents the subtype IVa (27). In our study, 77% of the isolates were classified as SCCmec IVc, and 2% were classified as SCCmec IVa. Both were present in all three types of infection. This result supports the previous reports of studies in our country, which have shown the predominance of the SCCmec IVc subtype among isolates, followed by a smaller proportion for SCCmec IVa (40,41).

As it has been previously described, most CA-MRSA isolates are PVL positive (22). Among the MRSA isolates tested in our study, 88% carried the *lukS/F-PV* genes, and these genes were detected in all SCCmec types identified (SCCmec IVc, IVa and I). In Colombia, there have been several reports of infections caused by PVL-positive MRSA strains carrying SCCmec IVc belonging to clonal complex 8 (CC8) in hospitals (13,41-43). In a recent previous work, we reported the ST8-MRSA-IVc clone as the main strain causing HAI (13). This finding suggests the possibility that the PVL-positive and SCCmec IVc isolates detected in our study could be related to the CC8. Despite this observation, additional molecular markers should be analyzed to confirm the clonal complex to which our isolates belonged. In contrast, HA-MRSA strains (SCCmec type I) usually do not

carry the *lukS/F* PV genes. However, 80% of HA-MRSA isolates carried these genes in our hospital (13). This finding is interesting because several countries report the absence or a low percentage of these genes in HA-MRSA isolates. Thus, in European countries such as Spain, the United Kingdom, France, Italy and Ireland, the PVL genes are absent among HA-MRSA isolates (44-46), and in a previous study in Colombia, these genes were detected in less than 9% of isolates (40). This result could reflect a different epidemiology of HA-MRSA strains in our country, resulting in a high frequency of the PVL genes.

Although the role of PVL in the severity of *S. aureus* infection is controversial (47-49), PVL production in MRSA has been associated with severe skin infections, such as abscesses and necrotizing pneumonia. As in a previous report that we published (50), in the present study, all pediatric patients developed moderate to severe infections and had to be treated at a high-complexity hospital. This finding could be related to the observed high frequency of PVL, which was greater than 80%.

Therefore, this descriptive study confirms the presence of strains with the molecular characteristics of CA-MRSA, such as SCCmec IV and PVL positivity, causing both HCAI and HAI in pediatric patients in Colombia. Finally, due to CA-MRSA emergence and establishment in hospitals, it is necessary to implement control measures and appropriate management of infection to prevent CA-MRSA dissemination in both hospitals and the community environment.

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Conflicts of interest

None to report.

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