ORIGINAL ARTICLE

TYPIFICATION OF THE STAPHYLOCOCCAL CHROMOSOME CASSETTE OF METHICILLIN-RESISTANT Staphylococcus aureus IN THE STATE OF ARAGUA, VENEZUELA

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ABSTRACT

Objective: Typify the SCCmec cassette in methicillin-resistant strains of Staphylococcus aureus in clinical isolates from health centers in the State of Aragua-Venezuela and compare the presence of SCCmec genotypes among the state health centers and according to the type of infection. Materials and methods: 81 MRSA strains from four health centers of the Aragua-Venezuela State were studied. Methicillin resistance was performed with the Kirby-Bauer method with oxacillin (1 µg) and cefoxitin (30 µg) disks. The mecA gene and SCCmec were analyzed by the multiple PCR technique. Results: Only 55 isolates (67.9%) amplified the mecA gene, and 24 strains (43.6%) amplified SCCmec. SCCmec type I was the most frequent, followed by SCCmec IV and SCCmec III, representing 62.5%, 25% and 12.5%, respectively. SCCmec I was predominant in health center A (80%), while in B and C 60% and 100% respectively were SCCmec IV. At health center D, 50% turned out to be SCCmec I and 50% SCCmec IVd. A relationship was found between the SCCmec and the health center with statistical significance. SCCmec I predominated in skin and soft tissue and respiratory infections with 63.2% and 50%, respectively. There was no association between genotype and type of infection with a p value greater than 0.05. Conclusions: The prevalence of SCCmec I and IV will allow establishing new measures in the use of antibiotics and epidemiological control.

Keywords: Staphylococcus aureus; SCCmec; mecA gene; Molecular Epidemiology; Staphylococcus aureus methicillin resistant (source: MeSH NLM).

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a global public health problem, causing serious infections in hospitals and the community. In 2018, the World Health Organization (WHO) estimated that patients with MRSA infections are 64% more likely to die than patients with non-resistant infections [1]. In addition, by 2017 the WHO included MRSA in the list of the twelve most dangerous pathogens to human health because of its resistance to antibiotics [2]. Resistance to methicillin is caused by the fact that the bacteria synthesizes a penicillin-binding protein known as PBP2a, which has a low affinity for methicillin and the rest of the beta-lactam antibiotics, preventing the entry of this type of antibiotic into the bacterial cell to exert its antimicrobial effect. PBP2a is encoded by the mecA gene, which is found within a mobile chromosomal element, called the staphylococcal chromosome cassette (SCCmec). The mecA gene is distributed in both S. aureus and other methicillin-resistant coagulase-negative staphylococcus species [3,4]. The SCCmec can measure between 21 and 67 Kb, and has a set of genes such as the ccr (ccrAB and ccrC) that encode recombines, in addition to the mec complex that contains the mecA gene, its regulatory genes (mecI, mecR), the acquired genetic determinants, which are produced as a result of the integration of plasmids and transposons, and finally, the sequence of the J region [5]. It is important to know the constitution of the SCCmec because according to recombination events between the ccr and mecA genes, a variety of SCCmec are generated, and these, allow the classification of MRSA according to the SCCmec it possesses. Initially, five types...
of SCCmec (I-V) and a number of variants or subtypes \(^{(5,6)}\) were described; however, new types were recently published as SCCmec VI-XI \(^{(7)}\).

Furthermore, types of SCCmec differ from each other because of their resistance determinants. Therefore, SCCmec I, IV, V, VI and VII code for resistance to beta-lactam antibiotics only, whereas SCCmec II, III and VIII have additional genes for resistance to multiple classes of antibiotics other than beta-lactam antibiotics \(^{(5,8)}\).

On the other hand, MRSA strains can be contracted from the hospital environment (MRSAH) or in the community (MRSEC). MRSAC is characterized by sensitivity to multiple antibiotics and is usually resistant to beta-lactam antibiotics only. It can cause skin and soft tissue infections, including severe cases of necrotizing pneumonia, necrotizing fasciitis, septic thrombophlebitis, and sepsis \(^{(9,10)}\). MRSAH is resistant to several groups of antibiotics, in addition to beta-lactams, and is associated with patients with risk factors such as high antibiotic consumption, prolonged hospital stays, invasive procedures (intravenous catheters, urinary catheters, tracheotomy), bedsores, severe illness, and contact with MRSA colonized patients \(^{(9,10)}\). In addition, MRSAC carries SCCmec IV and V \(^{(5)}\), while MRSAH strains have SCCmec I, II or III \(^{(11)}\).

The molecular typification of SCCmec is performed by polymerase chain reaction (PCR), using the multiple PCR technique, which allows different types of SCCmec to be determined simultaneously, which is very useful in epidemiological studies \(^{(12,13)}\). Acuña et al \(^{(14)}\) applied the multiple PCR technique to typify SCCmec in 21 MRSA strains isolated in the bacteriological laboratory of a hospital in Cumaná, state of Sucre, where they found SCCmec I and IV in outpatients and in adult emergency patients. The presence of SCCmec IV genotypes indicated that the bacteria isolated came from the community and were spreading to hospital services, producing nosocomial infections. In the state of Zulia, González et al \(^{(15)}\) characterized the SCCmec of 54 MRSA strains by multiple PCR and showed that 54% had SCCmec IV; 40%, SCCmec I; while 4% and 2%, SCCmec IA and SCCmec IIIB, respectively.

In the state of Aragua, no previous studies have determined the type of SCCmec circulating in the health centers of the region. This is the reason why the objective of the present investigation was to typify SCCmec in MRSA strains isolated from health centers in Aragua.

**MATERIALS AND METHODS**

**Study design**

Cross-sectional descriptive study conducted between January and August 2015 in patients who attended four health centers in the state of Aragua, Venezuela, called A, B, C and D in this research. Health center A is a private hospital with 72 beds, outpatient consultation and hospitalization. Health center B is a public care facility for patients with diabetic foot complications, which receives an average of 70 people per day and has no hospitalization. Health center C is a public, preventive care facility for adult and pediatric patients with a capacity for 2000 people, while health center D is a public facility and the largest in the region, with a capacity for 400,000 people and 551 hospitalization beds.

**Staphylococcus strains** were isolated from samples of skin, soft tissue, catheters, auricular, ocular, and respiratory secretions, as well as other infections. It was identified whether the samples were from inpatients or outpatients.

These samples were inoculated into blood agar plates and incubated at 35±2 °C in aerobic conditions for 16-18 hours. The standard procedure described in the literature was used for bacterial identification \(^{(16)}\). Finally, of a total of 404 staphylococcus-positive cultures, S. aureus was isolated in 324. The strains were preserved at -20 °C in glycerol until the time of the study.

**Antimicrobial susceptibility test**

Following the guidelines of the Institute for Standardization of Clinical Laboratories for the identification of MRSA, the agar disk diffusion or Kirby-Bauer method was applied \(^{(17)}\). The cefoxitin disc of 30 μg (BD) and the oxacillin disc of 1 μg (BD) were used. The control strain used was S. aureus ATCC 25923.

**DNA extraction**

It was performed on a MRSA pure culture on blood agar after 18-24 hours of incubation. A suspension was prepared in an Eppendorf tube by taking 1 to 5 colonies of the mi-
croorganism and placing them in 50 µL of sterile distilled water and then boiled at 99 °C for 10 min. Finally, it was centrifuged at 30,000 g for 1 min and the supernatant was transferred to a new Eppendorf tube. The concentrated DNA was preserved at -20 °C until the time of testing (13).

**Detection of the mecA gene and SCCmec genotypes of MRSA strains**

The multiple PCR test was performed to identify the SCCmec cassette type and the conditions for amplification according to the methodology previously described by Zhang et al(13). This methodology consisted in using 9 pairs of primers, including the specific primers for SCCmec I, II, III, IVa, IVb, IVc, IVd and V types and subtypes and the primers for the mecA gene. Eight different loci were amplified based on the sequences presented in Table 1. The following conditions were used for the PCR reaction: for the Master Mix, 50 mM KCl, 20 mM Tris-HCl (pH 8.4); 2.5 mM MgCl2, 0.2 mM of each deoxynucleotide triphosphate (dATP, dUTP, dGTP and dCTP). The primers concentrations are shown in Table 1. Additionally, a Go Taq Flexi DNA Polymerase unit (Promega Corp., USA) was used.

For controlling quality of the molecular typification tests, the *S. aureus* ATCC 25923 (methicillin-sensitive) strain was used as a negative control and the *S. aureus* ATCC 43300 (methicillin-resistant) strain was used as a positive control for the mecA gene. The amplification product was subject to electrophoretic migration in 2% agarose gels at 100 v for 30 min. A 100 bp molecular size marker (New England Biolabs, Inc) was used. Finally, the length of the amplicon was compared with the molecular size values recorded in Table 1 for the mecA gene and the SCCmec types and subtypes.

**Statistical analysis**

The provenance data of MRSA strains was collected in a Windows XP Excel 2007 database. Statistical analysis of the data was performed with EpiInfo 3.5.1. Descriptive analyses were performed using frequencies and percentages. The Chi-square test with a significance level of p < 0.05 was used to identify differences regarding some characteristics in the strains origin.

**Ethical aspects**

This study was evaluated and approved by the Bioethics Committee of the Teaching and Research Department of the Servicio Autónomo Hospital Central de Maracay. Additionally, patients who participated in the study signed an informed consent form.

**RESULTS**

During the study period a total of 404 staphylococcus-positive cultures from the four health centers in the state of Aragua were analyzed, 80 strains (19.8%) were coagulase-negative staphylococci (CSN) and 324 (80.2%) were *S. aureus*, of the strains analyzed 81 (25%) were MRSA.

Detection of the mecA gene showed that from the 81 MRSA isolates, 55 (67.9%) amplified the mecA gene and 26

<table>
<thead>
<tr>
<th>Primers</th>
<th>5’-3’ sequence</th>
<th>Concentration (µM)</th>
<th>Amplified size</th>
<th>Type SCCmec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I-F Type I-R</td>
<td>GCTTTAAAGAGTGTCGTTACAGGGTGTTCTCCTATAGTATGACGTTCC</td>
<td>0.048</td>
<td>613 pb</td>
<td>SCCmec I</td>
</tr>
<tr>
<td>Type II-F Type II-R</td>
<td>CGTGGAGATGTGAGCGGCGGAATCAATGGTTATGGAACC</td>
<td>0.032</td>
<td>398 pb</td>
<td>SCCmec II</td>
</tr>
<tr>
<td>Type III-F Type III-R</td>
<td>CCATATGTGTACGATGCGCCTTAGTTGCTGTAACAGATCG</td>
<td>0.042</td>
<td>803 pb</td>
<td>SCCmec III</td>
</tr>
<tr>
<td>Type IVa-F Type IVa-R</td>
<td>GCTTATTCGAAGAAAAACCGCTACTCTCTCTGAAAAGGCTG</td>
<td>0.104</td>
<td>776 pb</td>
<td>SCCmec IVa</td>
</tr>
<tr>
<td>Type IVb-F Type IVb-R</td>
<td>TCTGGAATTCTCAGCTGGAAACACATATCGTCTCCCTC</td>
<td>0.092</td>
<td>493 pb</td>
<td>SCCmec IVb</td>
</tr>
<tr>
<td>Type IVc-F Type IVc-R</td>
<td>ACAATATTTGATTATCTGCGAGCCTTGTAGGATTTGCTG</td>
<td>0.078</td>
<td>200 pb</td>
<td>SCCmec IVc</td>
</tr>
<tr>
<td>Type IVd-F Type IVd-R</td>
<td>CTCAAAATACGGAGCCCAATACATGCTCCAGTAATTGCTAAAG</td>
<td>0.28</td>
<td>881 pb</td>
<td>SCCmec IVd</td>
</tr>
<tr>
<td>Type V-F Type V-R</td>
<td>GAACATTGTGTACTTAATGAGCGGCTGAAAGTTTGACCTTGGACACC</td>
<td>0.06</td>
<td>325 pb</td>
<td>SCCmec V</td>
</tr>
<tr>
<td>MecA147-F MecA147-R</td>
<td>GTG AAG ATA TAC CAA GTG ATTATG CGC TAT AGA TGT AAA GGA T</td>
<td>0.046</td>
<td>147 pb</td>
<td>mecA</td>
</tr>
</tbody>
</table>

Source: Zhang et al(13)
(32.1%) did not, with a confidence interval of 56.6% to 77.8% and a 95% confidence level. Among the 55 isolates that tested positive for the mecA gene, only 24 (43.6%) amplified some type of SCCmec, while in 31 isolates (56.4%) no amplification was obtained with confidence intervals between 30.3%-57.7% and 42.3%-68.7%, respectively (Figure 1A, B and C).

From the 24 MRSA strains that amplified SCCmec, the most predominant cassette among the isolates was found to be the SCCmec type I, followed by SCCmec IV (subtypes IVb and IVd) and SCCmec III represented by 62.5%, 25% and 12.5%, respectively. SCCmec II and SCCmec V were not found (Table 2).

The largest number of MRSA strains with some type of SCCmec amplification was found in health center A (15 strains), where SCCmec I was found to be predominant (80%), followed by SCCmec III (20%). In the case of health center B, a total of 5 strains amplified SCCmec, where it was found that three of them (60%) turned out to be SCCmec IV (subtypes IVb and IVd) and 40%, SCCmec I. In health center C, two MRSA strains were obtained that amplified SCCmec IV, one amplified subtype IVb and the other subtype IVd. In health center D, only two MRSA isolates amplified SCCmec, one of which was SCCmec I and the other SCCmec IVd. A correlation was found between the genotype isolated and the health center (p = 0.032) (Table 2).

Of the 24 strains analyzed, 19 were isolated in skin and soft tissues, 4 in secretions from respiratory infections and 1 in blood culture. Of the 19 isolates from skin and soft tissues, SCCmec I was the most predominant (63.2%), followed by SCCmec IV (26.3%), where 15.8% were subtype IVb, and 10.5% subtype IVd. Four strains were obtained in respiratory samples that amplified SCCmec, from which two (50%) carried SCCmec I, while one was SCCmec III and one was SCCmec IVd. No relationship was found between genotype and type of infection (p = 0.870) (Table 2).

**DISCUSSION**

In this study, 25% of MRSA data was similar to the data obtained by Dorante et al (18), who found that out of 117 S. aureus isolates in a hospital in the state of Aragua, 24.7% were MRSA. Likewise, Chavez et al (19) reported that, in a hospital in Medellín (Colombia), out of 35 isolates, 28.6% were MRSA. However, Guillen et al (20) found in Paraguay that, from 77 strains, 18.7% were MRSA, slightly lower than the percentage reported in this study.

On the other hand, detection of MRSA using mecA gene identification by PCR, showed discrepancies with the results obtained with the use of oxacillin and cefoxitin discs because from the 81 MRSA isolates, only 55 amplified the mecA gene. Other studies have reported similar results, such as Acuña et al (14), who observed that from 21 MRSA strains, only 19 amplified the mecA gene. In contrast, the research by Chavez et al (19) and Guillen et al (20) reported that all of the MRSA strains they studied possessed the mecA gene. In this study, the strains that did not amplify the mecA gene had their identification and their phenotypic methicillin resistance profiles confirmed.

According to the results obtained, it is possible that resistance is related to some mechanism other than the expression of PBP2a. One of them may be the hyperproduction of β-lactamases by S. aureus strains, known as BORSA (Borderline oxacillin-resistant S. aureus) (21). In addition, it would be possible to imply that the lack of amplification of the mecA gene may be due to the fact that the strain carries the mecC gene, which is not detectable by conventional methods, and is responsible for 2% of MRSA infections in humans. The mecC gene is 70% homologous to the mecA gene and synthesizes a transpeptidase that is 60% homologous with PBP2a (22).
Table 2. Genotype frequency of the staphylococcal chromosome cassette and its distribution according to health centers and sample type.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Type of staphylococcal chromosome cassette (SCCmec)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IVb</td>
<td>IVd</td>
<td>V</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Number of isolates</td>
<td>15 (62,5)</td>
<td>0 (0)</td>
<td>3 (12,5)</td>
<td>3 (12,5)</td>
<td>3 (12,5)</td>
<td>0 (0)</td>
<td>24 (100)</td>
<td></td>
</tr>
<tr>
<td>Health centers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>12 (80)</td>
<td>0 (0)</td>
<td>3 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>15 (100)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2 (40)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (40)</td>
<td>1 (20)</td>
<td>0 (0)</td>
<td>5 (100)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td></td>
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<tr>
<td>D</td>
<td>1 (50)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td></td>
</tr>
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<td>Sample type</td>
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<td></td>
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</tr>
<tr>
<td>Hemoculture</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>12 (63)</td>
<td>0 (0)</td>
<td>2 (10)</td>
<td>3 (16)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>19 (100)</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>2 (50)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td>4 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Regarding the typification of SCCmec, 24 strains amplified some type of SCCmec, with SCCmec I being the most predominant, followed by SCCmec IV (subtypes IVb and IVd) and SCCmec III in a smaller proportion. Distribution of SCCmec genotypes was different in health centers A, B, C and D, health center A had the highest number of strains found, with a clear predominance of SCCmec I and, in less frequency, type III, which confirms its in-hospital origin and demonstrates that those isolates carrying type III, must possess resistance to a wide variety of antibiotics other than β-lactams (5,8). Center B occupied the second position according to the total number of isolates, with predominance of SCCmec type IV (subtypes IVb and IVd), followed by type I. The presence of SCCmec IV in health center B, an outpatient center for diabetic foot complications, seems to indicate its origin in the community (5,8). However, in the same center, the finding of SCCmec I is related to MRSA-H strains (5,8), which could predict the possible dissemination of strains acquired in the hospital environment at this health center.

In addition, SCCmec type IV was mainly found in health centers C and D. However, the number of MRSA strains associated to some type of SCCmec was low, which could be explained by the low resources from most bacteriology laboratories and this affects detection and identification of MRSA strains. The low number of detected MRSA strains and SCCmec is considered a limitation to interpret the results of this study. Therefore, further research will be necessary to deepen the data about distribution of SCCmec in both health centers, being health center D the largest and most important one in the region.

Results related to the high frequency of SCCmec I are similar to those reported in other regions of Venezuela and in other cities of Latin America. In fact, Acuña et al (14) found in a hospital in Cumaná (Venezuela) the predominance of SCCmec I (14 of 19 MRSA isolates), followed by SCCmec IV (3 of 19 MRSA strains). In Valdivia, Chile, SCCmec I was identified, followed by SCCmec IV (25). The cited investigations also coincide with the predominance of SCCmec I found in health center A. On the other hand, the presence of SCCmec IV in health centers B and C, coincides with that proposed by Romero et al (24) and Castellano et al (25) in hospitals in the state of Zulia, as well as that referred to by Sanchez et al (26) in hospitals in Medellín (Colombia). A study carried out in a hospital in Cali (Colombia) (19) reported that 26.6% of MRSA strains carried SCCmec II, unlike what was observed in this study in the four health centers investigated. This result could be due to differences in the predominance and distribution of SCCmec between hospitals and the geographical area of reference. In fact, the studies published to date in other regions of Venezuela have not identified SCCmec II (14,15,24,25).

Identifying the presence of SCCmec I in health centers in the state of Aragua could improve therapeutic options for the treatment of MRSA infections (5,8). However, the presence of SCCmec IV also gains relevance, because of its exclusive resistance to beta-lactam antibiotics and because it is related to MRSA-C (5,8). MRSA-C isolates with SCCmec IV, and to a lesser extent type V, also carry the genes for Panton-Valentine Leukocidin toxin (PVL) (5), while MRSA strains acquired in the hospital environment, have SCCmec II or III, and in very few cases LPV has been found (5,8). That is why it has been proposed that the identification of PVL in hospital strains carrying SCCmec IV allows to corroborate its origin and to clarify the epidemiological panorama (5,8).

In relation to the infection localization, most of the typed strains came from skin and soft tissues, with predominance of SCCmec I, confirming the hospital origin of the infections and orienting antibiotic therapy, since type I is a carrier of resistance to beta-lactam antibiotics (5,8). Results from this study differ from those found by Romero et al. (24), who found a high percentage of SCCmec IV isolates in skin and soft tissue samples.
From the 55 MRSA isolates that amplified the mecA gene, 56.4% did not amplify the SCCmec, and it is possible to assume that primers were used to detect SCCmec I to V and their subtypes, which was proposed by Zhang et al. (13). This was another limitation for this study. The possible existence of other SCCmec would indicate the presence of other genotypes with new antibiotic resistance determinants.

In conclusion, the identification of MRSA by SCCmec typification showed evidence of the predominance of SCCmec I and III related to MRSA acquired in the hospital environment and SCCmec type IV associated to the community. It was shown that there is a correlation between the isolated genotype and the health center. In skin and soft tissue samples, SCCmec I predominated; however, no correlation was found between SCCmec and the type of infection.

It is recommended to carry out prospective studies regarding the detection of SCCmec, including the main health centers in the region, the use of alternative methods to verify methicillin resistance, and the introduction of new primers to identify the existence of other SCCmec. In addition, it is recommended to include the assessment of hyperproduction of beta-lactamases and the determination of minimum inhibitory concentrations of oxacillin for those MRSA strains that do not amplify the mecA gene.

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**Conflicts of interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

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