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ORIGINAL PAPER

Detection of capsular genotypes of methicillin-resistant *Staphylococcus aureus* and clonal distribution of the *cap5* and *cap8* genes in clinical isolates

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Abstract
Up until now, the capsular polysaccharides of *Staphylococcus aureus* have been classified into 11 types, of which only 2 types 5 and 8; (encoded by the genes *cap5* and *cap8,* respectively) are present in 80–90% of clinically significant strains. The aim of the present study was to detect the capsular genotypes of methicillin-resistant *S. aureus* (MRSA) clinical isolates and determined their clonal distribution. A total of 262 MRSA clinical isolates from different hospitals in Mexico were analyzed by PCR to determine the genetic characteristics of their capsule expression. Pulsed-field gel electrophoresis and multilocus sequence typing were used to characterize the isolates. The analysis of the capsular genotypes among MRSA isolates showed that 245 isolates (93.5%) contained the *cap5* gene, and that the remaining 17 (6.5%) encoded the *cap8* gene. The MRSA isolates were grouped into four clonal groups. The identification of the capsular genotypes of clinical isolates of MRSA is important information because potential vaccine formulations against *S. aureus* involve capsular polysaccharides.

Keywords MRSA · Capsular genotypes · Clinical isolates · *cp5* · *cp8*

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of both hospital and community-associated infections worldwide (Kateete et al. 2019; Khalid et al. 2018; Harada et al. 2018). The ability of MRSA to cause a variety of infections is due to the expression of multiple virulence factors, including adhesins, cytotoxins, superantigens and exoenzymes, as well as capsular polysaccharides, all of which contribute to the pathogenesis of staphylococcal infections (Haddad et al. 2018). The capsular polysaccharide (CP) is a bacterial cell wall component that protects bacteria from phagocytosis and enhances microbial virulence. The CP prevents the activity of neutrophils against bacteria, preventing chemotaxis and phagocytosis and promoting cell adhesion to prosthetic devices. Up until now, the CP of *S. aureus* have been classified into 11 types, of which only 2 types 5 and 8; (encoded by the genes *cap5* and *cap8,* respectively) are present in 80–90% of clinically significant strains (Keinhörster et al. 2019; Melles et al. 2008; Rozemeijer et al. 2015). Serotypes 5 and 8 have similar trisaccharide repeating units comprised of *N*-acetyl mannosaminuronic acid, *N*-acetyl L-fucosamine, and *N*-acetyl D-fucosamine (Visansirikul et al. 2020). Although the majority of *S. aureus* isolates are type 5 or 8, the remaining 10–20% of the clinical strains, which are nontypeable, are denominated as antigen 336 (Ag336). The 336 antigens also name polysaccharide 336 (PS336) was purified from a strain deposited at ATCC under number ATCC 55,804 and used to serotype S. *aureus* isolates that do not produce capsule (Verdier et al. 2007).

Methicillin resistance is conferred to *S. aureus* strains by the presence of a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*), which carries the *mecA* gene. At least 13 types of SCC*mec* elements have been identified**.** Different MRSA clones have been observed among epidemic MRSA isolates. Perhaps successful lineages of epidemic MRSA clones have an adaptive advantage

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due to their antibiotic resistance and virulence (Lakhundi and Zhang 2018; Challagundla et al. 2018). One strategy for the prevention of staphylococcal infections is the formulation of vaccines, in which CP play an important role. Specific antibodies against CP5 and CP8 have been shown to be protective against *S. aureus* infections (Park et al. 2014; Inouea et al. 2018). The aim of the present study was to detect the capsular genotypes of MRSA clinical isolates and determined their clonal distribution.

Material and methods

Bacterial isolates

Isolates identified as MRSA collected during a 14-year (1999–2012) period were analyzed in this study. These strains were recovered from seven different hospitals in Mexico; three of them located in Mexico City (H1, H2, and H3), two in the north of the country (H4, H5), one in the south (H6), and the other in the west (H7). All strains were recovered from single patients and of different sources: abscess, bronchial aspirate, blood, wound infections, cerebrospinal fluid, pleural fluid, soft tissue, catheter, sputum, and others sources.

DNA extraction

The strains were grown for 18 h at 37 °C on tryptic soy agar plates (Difco, Oxoid, Hampshire, United Kingdom). One colony of each strain was grown in tryptic soy broth for 17 h at 37 °C. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Corporation, Madison WI, USA) following the manufacturer's instructions.

Detection of capsular genotypes

Genomic DNA was used for PCR amplification with primers *cap*5-1 (5′-GGTTTGCTGAAAAACCAGTC-3′) and *cap*5-2 (5′-CCTCATATGCTCCTACATTT-3′), as well as the primers *cap*8-1 (5′-GCGCTACAAACATTAAGC AT-3′) and c*ap*8-2 (5′-TTCTTAGCCTGCTGGCATC-3′). The amplification was carried out in an Eppendorf thermocycler (Eppendorf, Scientific Inc. Hauppauge, NY) under the following conditions: an initial 4-min denaturation step at 94 °C, followed by 25 cycles of 20 s of denaturation at 94 °C, 20 s of annealing at 50 °C, and 50 s of extension at 72 °C, with a final extension step at 72 °C for 10 min (Sau et al. 1997). PCR products were analyzed by electrophoresis on ethidium bromide-stained 1.5% agarose gels (Sigma-Aldrich. Lyon, France). The sizes of the amplicons were 185 bp for capsular type 5 and 170 bp for capsular type 8. The control strains ATCC49521 (*cap5)* and ATCC 49,525 (*cap8)* were included in each assay.

Pulsed-field gel electrophoresis (PFGE)

Whole-genomic DNA was prepared as described previously (Chung et al. 2000). After digestion with *SmaI* endonuclease, DNA was separated in a CHEF-DRII apparatus (Bio-Rad, Birmingham, United Kingdom). The control strains EMRSA16, HPV107 (Iberian), BK2464 (New York-Japan) and USA300, were included in the PFGE gels for comparison. These strains were kindly provided by Prof. Herminia de Lencastre from the Molecular Genetic Laboratory at the Instituto de Tecnologia Quımica e Biologica da Universidade Nova de Lisboa. We used the criteria of Tenover et al. to compare different clones (Tenover et al. 1995).

SCCmec typing and multilocus sequence typing (MLST)

Strains representatives of each clone, throughout the study period, were characterized by SCC*mec* typing and MLST. The multiplex PCR for *mec* element type was performed according to Oliveira, this included eight loci (A through H) and an internal positive control (*mecA* gene) (Oliveira and Lencastre 2002). The MLST was performed according to Enright, the sequences of internal fragments of seven housekeeping genes were amplified by PCR: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*) (Enright et al. 2000). The assignment of alleles and sequence types (ST) was performed using the MLST data base (http://www.mlst.net) and eBURST analysis was performed.

Results

A total of 262 MRSA clinical isolates from different hospitals in Mexico were analyzed by PCR to determine the genetic and epidemiological characteristics of their capsule expression, specifically in genotypes 5 and 8, to which most human *S. aureus* isolates belong to. These isolates were obtained from various body sites associated with *S. aureus* infections. The most common sources of isolates were blood (63/24%), wound infections (52/19.8%), soft tissue (38/14.5%) and bronchial aspirate (30/11.5%); the remaining (79/30.2%) were obtained from ten different sources.

The analysis of the capsular genotypes among MRSA isolates showed that 245 isolates (93.5%) contained the gene for CP type 5 (*cap5*), and that the remaining 17 (6.5%) encoded the gene for CP type 8 (*cap8*). The

distribution of *cap8 gene* decreased across time; MRSA strains type 8 were detected in isolates from 1999 to 2002, (16/17) in two hospitals (H1 and H3), while only one strain (173-H2) isolated in 2010 showed the *cap8* gene, these three hospitals are located in Mexico City. MRSA strains containing the *cap5* gene were present in isolates from all the years under consideration (1999–2012) and in the seven participating hospitals distributed in the center, north, south and west of the country.

The genotyping results demonstrated that there is an association between capsular serotypes and the results obtained by PFGE, SCC*mec* and MLST; thus, MRSA isolates were grouped into four clonal groups: ST5-MRSA-II-New York-Japan, ST8-MRSA-IV-USA300, ST247-MRSA-I-Iberian and ST36-MRSA-II-EMRSA-16 clones. Two hundred and thirty-five of the 262 strains belonged to the ST5-MRSA-II-New York-Japan clone, and these isolates carried the *cap5* gen; two clonal groups (ST8-MRSA-IV-USA300 and ST247-MRSA-I-Iberian) and the strains with atypical patterns also carried the *cap5* gene, while the *cap8* gene was only associated with the ST36-MRSA-II -EMRSA-16 clone (Fig. 1). All strains were analyzed by PFGE. The PFGE patterns of representative strains of the clones detected in this study and the profiles of control strains are shown in Fig. 2. Graphical presentation of multilocus sequence typing data by ST5 (majority clone) and ST8 (community clone) was performed for eBURST Fig. 3.

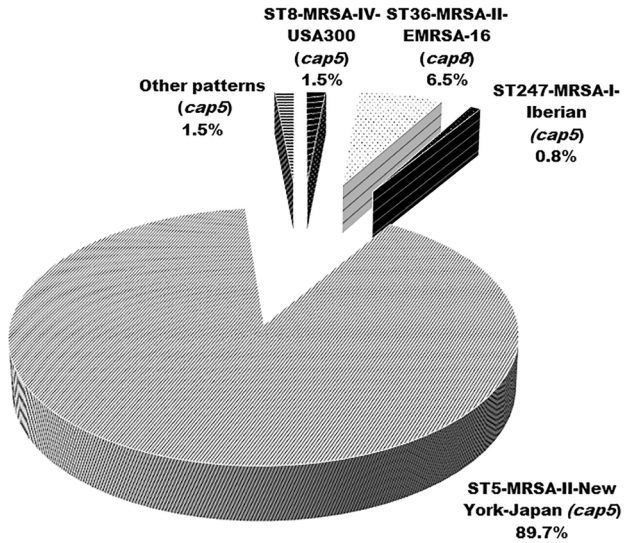

Fig. 1 Distribution of capsule genotypes (*cap5* and *cap8*) among MRSA clinical isolates

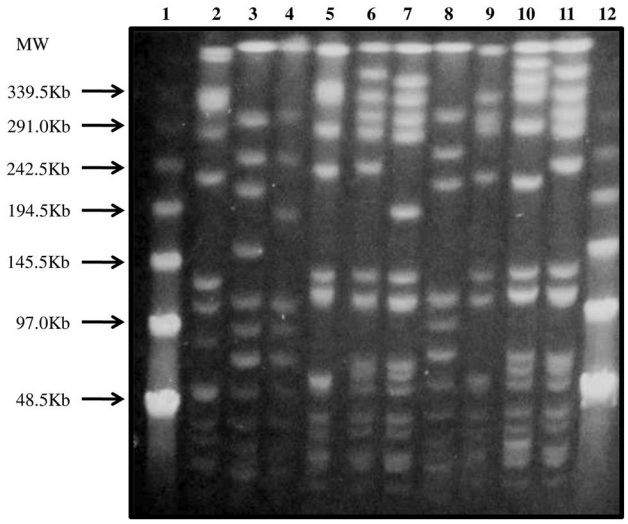

Fig. 2 Examples of pulsed field gel electrophoresis profiles obtained for MRSA clones of clinical isolates and international clones. Lanes 1 and 12 lambda ladder used a molecular size (MW) markers, 2–4 representatives clinical isolates of clones EMRSA-16, lines 5–7 New York-Japan, Iberian and USA300 clones, respectively. Lanes 8–11 control strains EMRSA16, BK2464, HPV107 and USA300

Discussion

This study was carried to characterize the genotypes of capsular polysaccharide expression in MRSA isolates from diverse clinical sources in seven hospitals in Mexico. The results showed that the majority of MRSA isolates (93.5%) contained the *cap5* gene, while a lower percentage contained the *cap8* gene. These results are in agreement with previous studies that reported a predominance of the serotype 5 among MRSA isolates (Verdier et al. 2007; Fournier et al. 1987; Mohamed et al. 2019). The distribution of CP has been analyzed in others countries around the world and the percentages of distribution vary at each study. In a study performed in 2016, in Kuwait 42 (3.1%) *S. aureus* strains were reported to be CP8 (Udo et al. 2020). Liu in 2018 reported that Eighty-one strains (56.64%) were Cap5, 36 strains (25.17%) were identified as Cap8 type, and the remaining 26 strains (18.18%) were non-typeable (Liu et al. 2018). Vadier in 2007, in France reported, 195 (42%) *S. aureus* isolates as CP5, 45% CP8 and 13% were reported as other types (Vadier et al. 2007). In another study carried out in Argentina in 2009, of the total 118 *S. aureus* isolates collected from patients with osteomyelitis, 76 isolates (64%) were capsular type 5 and 42 (36%) were type 8 (21%) (Lattar et al. 2009); similar results were observed in others studies (Fournier et al. 1987; Na'was et al. 1998). Pardo, analyzed 213 *S. aureus* strains that cause skin and soft-tissue infections obtained between 2004 and 2005 in Uruguay, and found that 85 isolates expressed CP8, only 1 expressed CP5 and 4 strains were nontypeable (Pardo

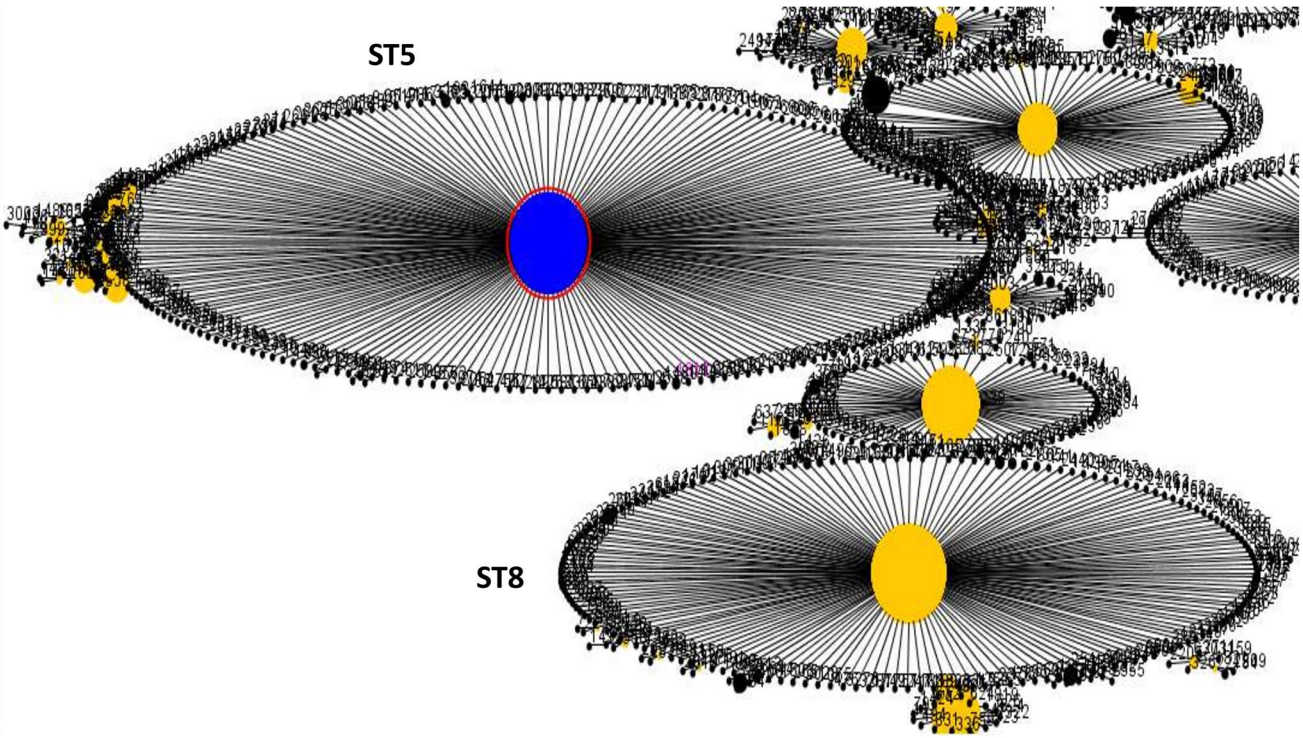

Fig. 3 Analysis of eBURST of the ST5 and ST8 clonal complex of MRSA. The relatedness between isolates in the MRSA MLST database that shared alleles at seven loci with the allelic profile of ST5 and ST8 is displayed as a dendrogram

et al. 2009). However, these results differ from our findings that showed the predominance of CP5. In another study, E. Sutter, studied 91 MRSA isolates during 2004–2005 and reported that type 5 and type 8 capsular genotypes were detected in only 58% of the strains, while the remaining 42% were either *cap5* or *cap8*, but did not express a capsule in vitro and reacted strongly with type 336PS antisera. The author suggested that the lack of capsule expression is common among clinical isolates of the most prevalent CA-MRSA clone, USA300 (Sutter et al. 2011). In contrast, our results showed that the strains analyzed descendants of the USA300 clone expressed the capsular genotype *cap5*.

These data are important for vaccine development because even though 80 to 90% of MRSA clinical isolates produce capsular polysaccharides type 5 or 8; a type 5/type 8 capsular vaccine would be inadequate for 10 to 20% of the strains that are not typeable (Ma et al. 2004; O'Brien et al. 2000). Vadier, proposes that adding the 336 antigen to a types 5/8 capsular vaccine should increase its coverage to 100% of all *S. aureus* infection isolates (Vadier et al. 2007). Although in our study not typeable *S. aureus* strains were not detected, this does not mean they cannot be circulating in our community, perhaps for their detection it would be necessary to analyze a bigger sample including *S. aureus* strains of carriers and methicillin sensitive. Because methicillin-sensitive *S. aureus* strains and strains isolated from healthy carriers have greater clonal diversity, and clonality is associated with capsular type (*cap5*, *cap8* and not typeable).

A limited number of MRSA lineages have emerged from the transfer of SCC*mec* into successful methicillin-susceptible *S. aureus* (MSSA) clones. Using MLST typing Enright et al. demonstrated that MRSA clones evolved from five different groups of related genotypes or clonal complexes (CC), each arising from a distinct ancestral genotype (Enright et al. 2003). In this study, the ST36-MRSA-II-EMRSA-16 clone (*cap8*) was only found in isolates from 1999 to 2002 and in a single isolate from 2010. This was because clone EMRSA-16 (*cap8*) was present from 1999 to 2002 and was replaced in late 2002 by New York Japan clone (*cap5*) (Velazquez et al. 2004) and a sporadic isolate of EMRSA-16 was found in 2010. This clone is one of the dominant types of MRSA found in a UK hospital (Moore and Lindsay 2002) and is widely disseminated in Canada (Simor et al. 2002) and Greece (Aires de Sousa et al. 2003). The multiresistant ST5-MRSA-II-New York-Japan clone (*cap5*) was present in 89.7% of the MRSA strains analyzed; this clonal group was observed throughout the all study and is the predominant clone in clinical isolates in Mexico (Velazquez et al. 2013, 2004; Cornejo et al. 2010; Echaniz et al. 2006). A recent study showed the evolution of clonal complex 5 (CC5), that includes the ST5-MRSA-II-New York–Japan clone of Mexican isolates (Challagundla et al. 2018). This clone was initially described in New York and Tokyo, and is now widespread in USA and other parts of the

world (Roberts et al. 1998; Aires de Sousa et al. 2003; Chung et al. 2004; McDougal et al. 2003). Strains belonging to the ST8-MRSA-IV-USA300 clone ($n=4$) and ST247-MRSA-I-Iberian clone ($n=2$), which also carry the *cap5* gene were present in two hospitals (H4 and H6) in 2008 and 2010, respectively. It has been reported that virulence gene profiles are strongly associated with *S. aureus* clonal lineages (Ferry et al. 2006; Thurlow et al. 2012).

Conclusions

This study showed that the prevalence of *cap5* in clinical isolates of MRSA is very high (93.5%). The results obtained showed that the presence of *cap5* and *cap8* is closely associated with the clonal origin of the strains. Our results suggest the potential benefits of using *S. aureus* CP5 in the preparation of vaccines due to the prevalence of encapsulated strains in Mexican hospitals. The identification of the capsular genotypes of clinical isolates of MRSA is important information because capsular polysaccharides are part of potential vaccine formulations against *S. aureus*.

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Declarations

Conflict of interest None declared.

Ethics approval and consent to participate This research was performed according to the principles expressed in the Declaration of Helsinki with approval from the Ethics Committee of the Instituto Nacional de Salud Publica; CI: 647.

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