Type IV SCC*mec* Found in Decade Old Brazilian MRSA Isolates

Cristina Reinert[1], John Anthony McCulloch[1], Shinya Watanabe[2], Teruyo Ito[2], Keiichi Hiramatsu[2] and Elsa Masae Mamizuka[1]
[1]Department of Clinical Analysis, Pharmaceuthical Medical School, University of São Paulo; São Paulo, SP, Brazil; [2]Department of Bacteriology, Juntendo University; Tokyo, Japan

Methicillin-resistant *Staphylococcus aureus* (MRSA) commonly causes infection in hospitalized patients. Since its appearance in the 1960s, the SCC*mec* has evolved throughout the years into 5 different types (I-V), each bearing a different set of genes. Infection with MRSA SCC*mec* types I, II or III is almost exclusively restricted to hospitalised patients. However, recently, community acquired MRSA (CA-MRSA) infections have been reported with increasing frequency, usually caused by a type IV SCC*mec* MRSA in nosocomial settings. We studied the prevalence of SCC*mec* types in 50 nosocomial strains collected from 1995 to 1999. The SCC*mec* complex type and presence of Panton-Valentine leukocidin (PVL) were determined by PCR. Strains had been previously typed by PFGE and were now typed by MLST. We found that 3 of the isolates studied bore a type IVc SCC*mec* all having different PFGE and MLST profiles (ST3, ST5 and ST88). All strains bearing a type III SCC*mec* belonged to MLST ST239 (Brazilian/Iberian clone). Only the strain which presented the ST5 profile bore the *pvl* gene. The type IVc SCC*mec* strains presented relatively lower levels of resistance to oxacillin in comparison to the type III SCC*mec* strains. The pattern of dissemination of the type IV SCC*mec* remains to be elucidated. The finding of strains carrying a type IV SCC*mec* in the present study among strains isolated at least 7 years ago indicates that clones bearing a type IV SCC*mec* have been present in Brazil for quite some time, and must have gone by undetected.

Key-Words: Nosocomial infections, type IV SCC*mec*, *Staphylococcus aureus*, methicillin-resistant, Brazil.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has established itself as one of the most important nosocomial pathogens in that it is expressively prevalent in the hospital environment and poses a challenge to antibiotic chemotherapy due to its multi-resistant profile. MRSA commonly causes infection in hospitalized patients who possess risk factors for the acquisition of this pathogen [1]. Methicillin resistance in *S. aureus* is conferred by the *mecA* gene, which is itself carried in a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) [2]. Since its appearance in the 1960s, the SCC*mec* has evolved throughout the years into 5 different types (I-V), each bearing a different set of genes. Until recently, infection with MRSA was an event almost exclusively restricted to hospitalised patients, with most nosocomial MRSA strains bearing one of three types of SCC*mec*, either type I, II or III [3]. However, MRSA community infections have been reported with increasing frequency [4-6]. These infections, which are clinically similar to the ones caused by methicillin susceptible *S. aureus* (MSSA), usually affect individuals who do not bear the risk factors for the acquisition of MRSA [7]. Strains causing these infections are designated community acquired MRSA or CA-MRSA. These strains are usually more susceptible to antibiotics of different classes than their nosocomial counterparts and are represented by a larger number of different clones [7-9].

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Address for correspondence: Dr. Cristina Reinert. Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Avenida Professor Lineu Prestes, 580 B17, 05508-900, São Paulo, SP, Brazil. Phone: (+5511) 3091-3663. Fax: (+5511) 3813-2197. E-mail: reinert@usp.br.

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The CA-MRSA strains possess a SCC*mec* type designated type IV, which is physically smaller than the other SCC*mec* types and does not carry antimicrobial resistance genes other than the *mecA* gene [10]. They are usually more virulent than nosocomial MRSA, which, combined with their resistance to beta-lactams turns them into a menace, for infection can be fatal as has been previously reported [4].

An endemic MRSA clone has been shown to be widely disseminated throughout Brazilian hospitals. This clone is known as the Brazilian Endemic Clone (BEC) and has also been found to be disseminated elsewhere in South America, as well as in Europe [11,12]. This well characterised clone has been described as ST239 when typed by Multilocus Sequence Typing (MLST) [13], and carries a type III SCC*mec* [14]. Previous studies [15-17] describing the prevalence and extension of geographic dissemination of this single MRSA clone in Brazil showed it to represent close to 80% of MRSA isolates. This clone was reported as being the most frequent among MRSA isolates recovered from several hospitals from several cities located throughout Brazil's enormous territory and separated by a distance of several thousand kilometres. Souza et al. [11] also reported the predominance (97%) and persistence of this clone among isolates in Brazil. Using Pulsed Field Gel Electrophoresis (PFGE), the BEC has been broken down into different subtypes all related to one another [11], which could be explained by its maintenance in Brazil at least since 1990 [17].

Material and Methods

Bacterial Isolates

The bacterial strains used in this study were selected from a collection obtained in a previous study [18] lasting four years (1995 to 1999), in which approximately 300 MRSA strains were collected in a multi-centre investigation involving 27 hospitals in several Brazilian cities. The strains were isolated

from different human clinical samples, from colonization or infection without duplication of single patient, and were characterized by PFGE using *Sma*I, taking into consideration the criteria proposed by Tenover [19]. Approximately 80% of the isolates belonging to this collection had presented the same profile by PFGE (considered to be the BEC), whereas the remainder presented another 9 different profiles.

For the present study, 50 strains were selected from this collection in order to represent the 10 different PFGE clones found by Oliveira et al. [18] These 9 remaining clones presented a more susceptible profile to antibiotics when compared to BEC. Isolates were tested for resistance to oxacillin and vancomycin using the agar dilution method according to the Clinical and Laboratory Standard Institute (CLSI, formerly NCCLS) guidelines [20].

Determination of SCC*mec Complex*

The SCC*mec* complex type was determined by polymerase chain reaction (PCR). The primers used for the classification of type IV SCC*mec* into IVa and IVb subtypes were most kindly provided by Dr. T. Ito [10]. For determining SCC*mec* subtype IVc, primers IVcF (5'-tctattcaatcgttctcgtattt-3') and IVcR (5'-tcgttgtcatttaattctgaact-3'), also provided by Dr. Ito, were used as described previously [10].

MLST

MLST was performed as described previously [21] in order to determine the lineage of some strains.

Determination of the PVL Gene

Presence of the *pvl* gene, which codes for PVL (suggested as a marker for type IV SCC*mec*) [22], was determined by using specific primers as previously described [23]. Strain MR108 was used as a positive control for this reaction.

Results

The types of SCC*mec* found for each strain as well as MLST strain type are described in Table 1. The majority of strains presented a type III SCC*mec*. Three isolates bore a type IVc SCC*mec*, all having different PFGE profiles. The three type IVc SCC*mec* strains had been isolated from clinical specimens obtained from a navel abscess, from a surgical wound secretion and from pleural fluid. As expected, isolates carrying a type IVc SCC*mec* showed resistance to a lower number of antimicrobial agents belonging to different classes, compared to the other type III SCC*mec* strains and seemed not to be genetically related.

All strains bearing a type III SCC*mec* were typed by MLST as belonging to ST239, which is the profile of the BEC. The strains bearing a type IVc SCC*mec*, all presented profiles different to that of the BEC. These strains were characterised as ST3, ST5 and ST88. Of these strains, only the strain which presented the ST5 profile bore the *pvl* gene.

Discussion

The type IVc SCC*mec* ST3 and ST5 strains presented relatively lower levels of resistance to oxacillin (MICs of 8 and 64 mg/mL, respectively) in comparison to the type III SCC*mec* strains, which presented MICs >512 μg/mL.

MLST grouped several different PFGE profiles (A, B, E, H, I, K and M) into a single strain type (ST239), which indicates that MLST's discriminatory power may be lower than that of PFGE. ST239 could thus be broken down into 7 different lineages, which would be invaluable in regional or institutional epidemiological studies. However, MLST unambiguously relates these 7 PFGE profiles with other globally present strains. It thus seems that PFGE is a good technique to analyse a small number of isolates obtained during outbreaks occurring in hospitals or communities in a relatively short period of time (1 to 3 months), because its greater discriminatory power would ensure the detection of lateral transmission even between strains belonging to ST239 determined by MLST [24]. On the other hand MLST is an excellent tool for investigating the clonal evolution of MRSA. The low number of strains used in this study probably does not allow the assessment of the merits of comparisons of these two methods.

PVL has consistently been found in CA-MRSA strains, so much so that it has been proposed as an epidemiological marker for CA-MRSA strains [22]. Studies have shown the physiopathological involvement of PVL in skin infections and necrotising pneumonia [25,26]. Out of three type IVc SCC*mec* strains identified in this present study, only one bore the *pvl* gene. This strain had been isolated from pleural fluid. Unfortunately we did not have the opportunity to follow this case. The other type IVc SCC*mec* strains found did not bear the *pvl* gene and had been isolated from a surgical wound secretion and a navel abscess.

The finding of type IV SCC*mec* in nosocomial strains in Brazil should be, like the emergence of VISA [27], of great concern to the medical community. CA-MRSA is emerging as significant community pathogens, especially in previously healthy children who bear no recognizable risk factors. It is predominantly associated with skin and soft tissue infections (especially abscesses and cellulitis). When present, risk factors are generally similar to those for infection with MSSA [28]. Some CA-MRSA strains have entered the hospital environment and caused outbreaks of infection, which has led to difficulty in distinguishing these strains from traditional nosocomial ones. CA-MRSA strains usually bear genes for PVL, which is associated with furunculosis and necrotizing pneumonia, and sometimes possess other virulence genes such as those for toxic shock syndrome or exfoliative toxins [29]. The pattern of dissemination of the type IV SCC*mec* remains to be elucidated. Whether the flow of type IV SCC*mec* strains is greater from the community to the hospital environment or vice versa is still unknown and might be revealed in future studies. What also remains to be determined is whether it might give rise to other SCC*mec* types in the future by

Table 1. Phenotypic and genotypic properties of MRSA strains from different inpatients.

PFGE Type (n)	Antibiotic resistance[a]	Oxacillin MIC (μg/mL)	Allelic profile[b]	ST	SCC*mec* type	*pvl* genes
B (1)	Oxa, Sxt, Ery, Amp, Tob, Tet, Gen, Cip, Cli	256	2-3-1-1-4-4-3	239	III	Absence
D (1)	Oxa, Chl, Ery, Tob	8	1-4-1-4-12-1-10	5	IVc	Presence
E (1)	Oxa, Sxt, Chl, Ery, Amp, Tob, Tet, Gen, Cip, Cli	>512	2-3-1-1-4-4-3	239	III	Absence
H (1)	Oxa, Sxt, Ery, Amp, Tob, Tet, Gen, Cip, Cli	>512	2-3-1-1-4-4-3	239	III	Absence
I (1)	Oxa, Sxt, Ery, Amp, Tob, Tet, Gen, Cli	>512	2-3-1-1-4-4-3	239	III	Absence
J (1)	Oxa, Chl, Ery, Amp, Tob, Gen	>512	22-1-14-23-12-4-31	88	IVc	Presence
K (1)	Oxa, Sxt, Ery, Amp, Tob, Tet, Gen, Cip, Cli	>512	2-3-1-1-4-4-3	239	III	Absence
L (1)	Oxa, Sxt, Ery, Amp, Tet	64	1-1-1-9-1-1-12	3	IVc	Absence
M (1)	Oxa, Sxt, Chl, Ery, Amp, Tob, Tet, Gen, Cip, Cli	256	2-3-1-1-4-4-3	239	III	Absence

[a]Antibiotics used: Oxa=oxacillin; Van=vancomycin; Sxt=sulphamethoxazole-trimethoprim; Chl=cloranphenicol; Ery=erythromycin; Amp=ampicillin; Tob=tobramycin; Tet=tetracycline; Gen=gentamicin; Cip=ciprofloxacin; Cli=clindamycin; [b]Allelic profile assignment (*arcC-aroE-glpF-gmk-pta-tpi-yqiL*).

gaining or losing some of its genes. Indeed, other SCC*mec* types (V) have already been described. Whether these new types evolved from the type IV SCC*mec* or from the other older types (I to III) is still unknown.

Recently, a case of CA-MRSA strains isolated from a community infection in Brazil was reported [30]. The finding of strains carrying a type IV SCC*mec* in the present study among strains isolated at least 7 years ago, and moreover the report of a recent outbreak of type IV SCC*mec* strains at a Brazilian university hospital, reported by Trindade et al. [31], indicates that clones bearing a type IV SCC*mec* have been present in Brazil for quite some time, and must have escaped undetected. These strains are disseminating in hospitals where they may be adapting to vanquish the intense antimicrobial selective pressure present in a nosocomial environment. The detection and monitoring of strains carrying a type IV SCC*mec* in both hospital and community environments is crucial if we are to better understand their epidemiology and ultimately control their dissemination.

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