Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* in an Iranian referral paediatric hospital

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ABSTRACT

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals has been changed in recent years due to the arrival of community-associated MRSA (CA-MRSA) strains into healthcare settings. The aim of this study is to investigate the distribution of staphylococcal cassette chromosome *mec* (SCC*mec*) type V as well as SCC*mec* IV subtypes, which have been associated with community-acquired infection among healthcare-associated MRSA (HA-MRSA) isolates. Antimicrobial susceptibility, SCC*mec* type, *spa* type and the presence of Panton–Valentine leukocidin (*PVL*) genes were determined for all HA-MRSA isolates in an Iranian referral hospital. In this study of 48 HA-MRSA isolates, 13 (27%), three (6.2%), five (10.4%) and one (2%) belonged to SCC*mec* subtypes IVa, IVb, IVc and IVd, respectively. Only two isolates (4.2%) belonged to SCC*mec* types V. Notably, one isolate was found to harbour concurrent SCC*mec* subtypes IVb and IVd. MRSA containing SCC*mec* subtype IVb, IVc and IVd as well as type V isolates were all susceptible to chloramphenicol, clindamycin and rifampicin, while the sensitivity to these antibiotics was lower among MRSA containing SCC*mec* subtype IVa. The most frequently observed *spa* ttype was t037, accounting for 88% (22/25). Three other *spa* type was t002, t1816 and t4478. Large reservoirs of MRSA containing type IV subtypes and type V now exist in patients in this Iranian hospital. Therefore, effective infection control management in order to control the spread of CA-MRSA is highly recommended.

KEY WORDS: Community-acquired infections. Methicillin-resistant Staphylococcus aureus. SCCmec types.

Introduction

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals has changed in recent years due to the emergence of community-associated MRSA (CA-MRSA) strains in healthcare settings.[1] Methicillin-resistant *S. aureus* is characterised by the expression of an altered penicillin binding protein (PBP2a), which is encoded by *mecA* and its regulatory genes. These genes are located in a mobile genetic element termed the staphylococcal cassette chromosome *mec* (SCC*mec*).[2,3] According to molecular analysis, five different allotypes, including SCC*mec* types I–V, based on the combination of the *mec* gene complex and the *ccr* gene complex, have been reported.[2]

Community-associated MRSA strains have been distinguished from healthcare-associated MRSA (HA-MRSA) by molecular analysis. The HA-MRSA strains carry a relatively large SCC*mec* belonging to type I, II, or III, and they are often resistant to many classes of non-β-lactam antimicrobials. In contrast, CA-MRSA isolates carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V. These smaller elements also carry the *mecA* gene and are presumably more mobile. Furthermore, CA-MRSA are usually resistant to fewer non-β-lactam classes of antimicrobials and commonly carry the Panton-Valentine leukocidin (*PVL*) gene, while HA-MRSA strains seldom carry this gene.[4,5]

In addition to the main SCC*mec* types, further classification of type IV SCC*mec* into IVa, IVb, IVc, IVd, IVg and IVh, based on nucleotide differences in the three non-essential junkyard (J) regions, has been reported.[6,7] These regions are located between the *ccr* genes and the downstream chromosomal region.[8]

Occurrence of HA-MRSA is common in Iran[9–11] but reports of CA-MRSA are rare.[12] As the SCC*mec* types IV and V have been associated with community-acquired infection, detection and discrimination of SCC*mec* type V and type IV into subtypes may play an important role in the understanding of the epidemiology and ultimate prevention and control of currently emerging community MRSA clonal outbreaks.[13]

The aim of this study is to investigate the distribution of SCC*mec* type V as well as SCC*mec* IV subtypes among HA-MRSA isolates in an Iranian referral hospital.

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Materials and methods

Bacterial isolates

This study was conducted at the Children's Medical Center, an Iranian referral hospital during November 2010 and October 2011. In an 11-month period, 133 *S. aureus* clinical isolates were collected from hospitalised patients.

The isolates were examined by traditional culture method for *S. aureus* strains and further confirmation was obtained by standard biochemical tests including mannitol salt agar fermentation, catalase, coagulase and DNase.[14]

Methicillin-susceptible *S. aureus* (MSSA) strains were differentiated from MRSA using Mueller-Hinton agar containing 2 mg/mL oxacillin with 4% NaCl. Isolates growing on the plates were considered to be MRSA, while isolates that did not grow on this medium were considered to be MSSA (Clinical and Laboratory Standards Institute [CLSI] guidelines).

Cases were considered healthcare–associated if the clinical culture from which MRSA was isolated was obtained 48 h after admission to the hospital with a history of hospitalisation or residence in a long-term healthcare facility within six months prior to the culture.[15] A CA-MRSA infection was defined according to the Centers for Disease Control and Prevention (CDC) definition: any MRSA infection diagnosed for an out-patient or within 48 h of hospitalisation if the patient lacks the following healthcare-associated MRSA risk factors such as haemodialysis, surgery, residence in a long-term care facility or hospitalisation during the previous year, the presence of an indwelling catheter at the time of culture, or previous isolation of MRSA.[4]

DNA isolation

Extraction of DNA from MRSA strains was performed using lysostaphin digestion.[16] The pellet of a 1 mL overnight culture was resuspended with 350-µL lysis buffer (Tris-HCl 0.01 mol/L, EDTA 0.01 mol/L), to which 10-µL lysostaphin were added. The sample was incubated at 37°C overnight. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1 by volume) was added, and nucleic acid was precipitated by ethanol using a standard protocol.

Detection of **mecA** *gene*

All presumptive MRSA isolates were confirmed by amplifying a *mecA* fragment using the following primers: forward, 5′-ACTGCTATCCACCCTCAAAC-3′; reverse, 5′- CTGGTGAAGTTGTAATCTGG-3′ using a previously reported method.[9] *S. aureus* strain ACTC33591 was used as a positive control.

Detection of **PVL** *gene*

Primers used were 5′-ATCATTAGGTAAAATGTCTGCA CATGATCCA- 3′; and 5′-GCATCAACTGTATTGGATAG CCAAAAGC-3′.[17] *S. aureus* strain ACTC49775 was used as a positive control for detection of *PVL* genes.

*Determination of SCC***mec** *type*

A multiplex polymerase chain reaction (PCR) assay with specific primers for SCC*mec* subtypes IVa, IVb, IVc, IVd, and types V[13] was performed on all MRSA isolates. The PCR mixture contained 50 mmol/L KCl, 20 mmol/L Tris-HCl (pH 8.4), 2.5 mmol/L $MgCl_2$, 0.2 mmol/L each deoxynucleoside triphosphate (dATP, dUTP, dGTP and dCTP), various concentrations of the respective primers, one unit TaqDNA polymerase and 10–1000 ng template DNA.[13]

Amplification was performed in a thermal cycler (Applied Biosystems, Foster City, CA) beginning with an initial denaturation step at 94°C for 5 min, 30 cycles at 94°C for 60 sec, 55°C for 60 sec (annealing), 72°C for 30 sec, 72°C for 10 min (final extension) followed by a hold at 4°C. All PCR products were loaded on 2% (w/v) agarose gel with ethidium bromide and analysed by gel electrophoresis.

Antimicrobial susceptibility test

A standardised Kirby-Bauer disc-diffusion method was performed on Mueller-Hinton with the following antibiotics: vancomycin, clindamycin, rifampicin, amikacin, co-amoxiclav, penicillin, chloramphenicol, trimethoprim/sulphamethoxazole, cefazolin and cephalothin.[9]

spa *typing*

To determine the *spa* type of MRSA containing SCC*mec* subtype IVb, IVc and IVd as well as type V,

Table 1. Antimicrobial susceptibility of MRSA isolates containing SCC*mec* types IV and V.

		SCC*mec* IV subtypes					
	All HA-MRSA	IVa	IVb	IVc	IVd	IVb+IVd	SCC*mec* type V
	n (%)	*n* (%)	*n*	*n*	*n*	*n*	*n*
Cephalotin	41 (85)	12 (92)	3	5	0	1	0
Cefazolin	41 (85)	12 (92)	3	5	0	0	0
Trimethoprim sulfamethoxazole	43 (90)	12 (92)	3	5	0	1	2
Chloramphenicol	9 (19)	5 (39)	0	0	0	0	0
Penicillin	48 (100)	13 (100)	3	5	1	1	2
Amikacin	45 (94)	13 (100)	3	5	1	1	0
Co-amoxiclav	47 (98)	13 (100)	3	5	0	1	2
Rifampicin	2 (4)	1 (8)	0	0	0	0	0
Clindamycin	12 (25)	7 (54)	0	0	0	0	0
Vancomycin	0 (0)	0 (0)	0	0	0	0	0
Total	48	13	3	5	1	1	2

Table 2. Distribution of different *spa* types among CA-MRSA isolates.

	spa types					
SCC*mec* type	t037	t4478	t002	t1816	*PVL*	Total
IVa	13	0	0	0	0	13
IVb	3	0	0	0	0	3
IVc	4	1	0	0	0	5
IVd	0	0	1	0	0	1
IVb+IVd	0	0	0	1	0	1
V	2	0	0	0	0	2

the polymorphic X-region of the *S. aureus* protein A (*spa*) gene was sequence-typed.[18]

Results

Forty-eight (36%) of the 133 *S. aureus* clinical isolates were HA-MRSA. HA-MRSA was isolated from 25 (52%) male and 23 (48%) female patients (age range: 10 days to 10 years). The majority of these isolates were obtained from patients age under one year (30/48, 62.5%).

Among the 48 MRSA isolates, 13 (27%), three (6.2%), five (10.4%) and one (2%) belonged to SCC*mec* subtypes IVa, IVb, and IVc and IVd, respectively. Only two isolates (4.2%) belonged to SCC*mec* type V. Notably, one isolate was found to harbour concurrent SCC*mec* subtypes IVb and IVd (Table 1).

The majority of the MRSA isolates containing SCC*mec* types IV and V were obtained from skin and soft tissue infections (10/25). Other isolates were derived from blood (n=9), trachea (n=3), eye (n=2) and urine (n=1).

Methicillin resistance was confirmed by PCR in all HA-MRSA isolates and all had the *mecA* gene, while no isolates were found to harbour the *PVL* gene. All MRSA isolates were susceptible to vancomycin but resistant to penicillin. Most isolates were resistant to cephalotin and cefazolin. MRSA containing SCC*mec* subtype IVb, IVc and IVd as well as type V were all susceptible to chloramphenicol, clindamycin and rifampicin, while the sensitivity to these antibiotics was lower among MRSA containing SCC*mec* subtype IVa (Table 1).

All 25 MRSA containing SCC*mec* type IV or type V isolates were *spa*-typeable and three *spa* types were detected (Table 2); the most frequently observed *spa* type was t037, accounting for 88% (22/25) of all isolates tested. Three MRSA were associated with three other spa types (t002, t1816 and t4478) (Table 2).

Discussion

It is a matter of debate as to whether SCC*mec* has been acquired horizontally from other staphylococcal species in the non-hospital environment[19,20] or derived from existing hospital-acquired strains (HA-MRSA) transmitted to the community.[21,22] The type IV SCC*mec* is found in *S. aureus* isolates with several different genetic backgrounds, is smaller than the other SCC*mec* types, and generally does not contain any additional resistance genes, which may facilitate its mobility.

In this study, as in other reports,[13,23,24] subtype IVa was the most frequent subtype (13/23, 56.5%). Although subtype IVa is described and frequently found in CA-MRSA from the USA and Australia, and has then been identified around the world, including Iran,[2,3,23–26] reports of subtype IVb have been scarce since it was first described in the USA.[2,13,23,24]

In this study, the second most frequent SCC*mec* type IV subtype was IVc (5/23, 21.7%). However, Berglund *et al*. reported this subtype as a predominant subtype among MRSA isolates.[2] In the study by Zhang *et al*. 74 (16.34%), five (1.1%) and four (0.88%) isolates belonged to SCC*mec* subtypes IVa, IVb and IVc, respectively, and no SCC*mec* subtype IVd was found.[13] In the study by Berglund *et al*., 45%of MRSA isolates contained type IV SCC*mec* (69% subtype IVc, 24% subtype IVa, 10% subtype IVd), and no SCC*mec* subtype IVb was found.[2] Okuma *et al*.[23] reported that among 32 type IV SCC*mec* isolates from the USA and Australia, 31 were subtype IVa and only one isolate was subtype IVb. In a study conducted in Iran in 2005 among 156 MRSA isolates, 19 (12%) belonged to SCC*mec* type IV (42.1% IVa, 36.8% IVc, 2.1% IVd), but did not include IVb.[24]

Susceptibility to more than two non-lactam antimicrobials and the presence of *PVL* gene has been used as a proxy defining criteria to identify CA-MRSA.[27,28] Although it has been reported that SCC*mec* subtypes IVa and IVb do not carry additional genes for antibiotic resistance,[3,23] the present study showed that more than half of the MRSA isolates containing SCC*mec* subtype IVa were multidrug-resistant. Almost all the isolates of this type were resistant to penicillin, amikacin, cephalotin, cefazolin, trimethoprim/sulfamethoxazole and co-amoxiclav. The multidrug resistance pattern of this subtype might be due to subsequent acquisition of additional genes following exposure to antibiotic selection pressures in hospitals. On the other hand, children might be at higher risk of infection by SCC*mec* type IV-bearing isolates than adults.[4] Although paediatric strains were also more likely to be susceptible to gentamicin, clindamycin, ciprofloxacin and rifampin,[29] in this study these strains were also more likely to be susceptible to vancomycin and chloramphenicol.

SCC*mec* type V is similar to type IV and has been found in CA-MRSA isolates predominantly in Australia[30] and Taiwan[31–33] while strains bearing SCC*mec* type V remain limited largely to Asia, Europe and the USA.[4] In this study only two isolates contained SCC*mec* type V (8%).

Although it has been reported that *PVL* is widely associated with the presence of SCC*mec* IV and sporadically with SCC*mec* V,[34] in the present study all type IV subtype- and type V-bearing MRSA isolates were *PVL*-negative.

To the best of the authors' knowledge, this is the first report of t002, t1816, t4478 *spa* types in Iran. Interestingly, the *spa* type t002 was only detected in MRSA isolates that contained SCC*mec* subtype IVd. In addition, *spa* type t1816 was only detected in an MRSA isolate that contained concurrent SCC*mec* subtype IVb and SCC*mec* subtype IVd. spa type t4478 was seen only in one MRSA isolate, which contained concurrent SCC*mec* subtype IVc. In this study, t037 was a common *spa* type among MRSA containing type IV subtype and type V *PVL*-negative isolates. However, t002 has been reported as a common *spa* type with similar healthcare-associated characteristics,[35] but this *spa* type was only detected in one MRSA isolate carrying SCC*mec* subtype IVd .

The emergence of CA-MRSA strains in the hospital setting has important implications. Large reservoirs of MRSA containing type IV subtypes and type V now exist in patients in the authors' hospital. Therefore, effective infection control management in order to control the spread of CA-MRSA in hospital is highly recommended.[4] In addition, surveillance programmes should be implemented to determine the extent of CA-MRSA dissemination. □

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