

Dissemination of methicillin-resistant *Staphylococcus aureus* SCCmec type IV and SCCmec type V epidemic clones in a tertiary hospital: challenge to infection control

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SUMMARY

Two-hundred MRSA strains from inpatients with healthcare-associated (HA) and 100 MRSA strains from outpatients with community-associated (CA) skin and soft tissue infections (SSTIs) were tested for antimicrobial susceptibility, staphylococcal cassette chromosome mec (SCCmec) typing, Pantone–Valentine leucocidin (PVL) toxin, *seh* and *arcA* genes. Based on SCCmec typing, HA-MRSA isolates were further divided into HA-SCCmec I/II/III MRSA and HA-SCCmec IV/V MRSA, and CA-MRSA isolates into CA-SCCmec I/II/III MRSA and CA-SCCmec IV/V MRSA. SCCmec types were further characterized by pulsed-field gel electrophoresis, *spa* typing and multi-locus sequence typing. Seventy-five (37.5%) HA-MRSA isolates and 83/100 CA-MRSA isolates were SCCmec IV/V genotype. HA-SCCmec IV/V MRSA was associated with malignancy ($P=0.03$) and bone fractures ($P=0.02$) compared to CA-SCCmec IV/V MRSA. HA-SCCmec IV/V MRSA was associated with PVL gene carriage compared to HA-SCCmec I/II/III MRSA ($P<0.001$). ST22-MRSA-IV (EMRSA-15), ST772-MRSA-V, and ST36-MRSA-IV and ST239:EMRSA-I:III were the major clones identified. Our study documents the emergence of SCCmec IV and SCCmec V MRSA clones in an Indian hospital.

Key words: Methicillin-resistant *Staphylococcus aureus*, MLST, Pantone–Valentine leukocidin genes, PFGE, *spa* typing, SCCmec.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is widely recognized as a major cause of nosocomial infection worldwide and the risk factors for infection with these pathogens in hospital populations are well established [1]. Although, traditionally considered to

be a nosocomial pathogen, it is evident that the epidemiology of MRSA infections is rapidly changing.

During the 1990s, various reports of community-associated MRSA (CA-MRSA) infections in healthy individuals appeared in the literature, caused by novel strains which were genetically distinct from traditional healthcare-associated MRSA (HA-MRSA) [2]. CA-MRSA were traditionally regarded as MRSA strains causing infection in previously healthy young patients without prior healthcare contact, generally susceptible to non- β -lactam antibiotics, often carrying Pantone–Valentine leukocidin (PVL) encoding genes,

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and of staphylococcal cassette chromosome *mec* (*SCCmec*) types IV or V [3]. By contrast HA-MRSA predominantly caused infections in patients exposed to healthcare settings, exhibited resistance to most non- β -lactam antibiotics and harboured *SCCmec* types I, II and III [4].

Consequently, as the microbiology and epidemiology of CA-MRSA have evolved, these traditional definitions have broken down, arguing in favour of genotypic classification of strains. As a result there is a growing consensus to define MRSA strains by combinations of genotyping methods such as multi-locus sequence typing (MLST), *spa* gene type and/or pulsed-field gel electrophoresis (PFGE) with *SCCmec* analysis to infer their likely epidemiological origin [5].

Numerous lineages of CA-MRSA have since emerged on every continent, several of which have spread internationally [6, 7]. Currently, more than 20 distinct genetic lineages have been identified worldwide [8], five of which are globally distributed, including ST1-IV (WA-1, USA400), ST8-IV (USA300), ST30-IV (South West Pacific clone), sequence type (ST) 59-V (Taiwan clone), and ST80-IV (European clone) [6, 7, 9]. Among the latter, ST8-IV and ST30-IV may be considered pandemic, as they have been isolated repeatedly from every continent [7, 10].

CA-MRSA strains are increasingly implicated in nosocomial infections. Outbreaks of HA infections caused by CA-MRSA strains have been reported from Australia and the USA suggesting that such strains are spreading in healthcare settings and are replacing traditional HA-MRSA strains in some places [11, 12]. Various lineages typically associated with HA-MRSA, such as ST22-IV (EMRSA-15), are also increasingly identified in CA-MRSA infections [9, 10]. In recent years, ST772-V (Bengal Bay clone) has emerged as a virulent and unusually resistant CA-MRSA strain in Bangladesh and India [13–15], and its spread to the UK and Europe has been documented [10, 16].

MRSA is a widespread problem in Indian hospitals, where strains have been well characterized [17]. Previous molecular typing of nosocomial MRSA strains recovered from our hospital revealed a similar scenario to that documented for several Indian hospitals where the multi-resistant Brazilian and Hungarian epidemic clone (ST239-MRSA-III) was dominant [17–20]. Recently, MRSA strains carrying *SCCmec* types IV and V were identified in Indian hospitals in and around Bengaluru and Mumbai where

ST772-MRSA-V along with ST22-MRSA-IV are increasingly prevalent and appear to have progressively displaced the previously predominant nosocomial ST239-MRSA-III clone [13–15]. However, these studies did not determine the differences in clinical, demographic and microbiological characteristics of *SCCmec* type IV/V MRSA strains isolated from patients with and without healthcare exposure. The aim of the present study was to determine whether CA-MRSA *SCCmec* type IV and type V strains have emerged in our tertiary-care hospital, located in New Delhi, the capital city of India and to analyse the clinical characteristics and clonal diversity of *SCCmec* IV and *SCCmec* V MRSA strains from patients with HA skin and soft tissue infections (SSTIs). In addition, we investigated the effects of hospital exposure on *SCCmec* IV/V MRSA isolates by comparing them with *SCCmec* IV/V MRSA genotypes isolated from outpatients not exposed to the healthcare setting.

METHODS

This descriptive study was conducted from July 2009 to December 2011 at the All India Institute of Medical Sciences, a 2500-bed teaching hospital providing tertiary care, located in the city of New Delhi, India. The study was approved by the Ethics Committee of the All India Institute of Medical Sciences, New Delhi, India (No. A-25).

Epidemiological definitions

A HA-MRSA SSTI case was defined as one that met one or more of the following criteria: the organism was isolated >48 h after admission to the hospital, the patient had been hospitalized or undergone surgery in the year prior to the MRSA-positive culture results; or an in-dwelling device or percutaneous catheter was present at the time the SSTI specimen for culture was obtained [21]. A case of SSTI infection was classified as CA-MRSA if MRSA was identified in the outpatient setting or <48 h after hospital admission in an individual with no medical history of MRSA infection or colonization, admission to a healthcare facility, dialysis, surgery or insertion of in-dwelling devices in the past year [5].

Patients

To find patients with HA-MRSA SSTIs, we identified all skin and soft tissue cultures obtained >48 h after

hospitalization that grew as MRSA. A total of 237 patients including both adults and children were eligible for enrolment, but 28 were unavailable to study personnel because of discharge before contacting them; another nine patients refused to give their consent. Thus, a total of 200 patients with HA-MRSA SSTIs were enrolled in the study. To find patients with CA-MRSA SSTIs, we identified all skin and soft tissue cultures obtained from outpatients that grew as MRSA and had no HA risk factor in the past year prior to soft tissue culture. A total of 112 patients were eligible for enrolment; however, seven were unavailable to study personnel because of delay in contacting them and another five patients refused to give their consent. Thus, a total of 100 patients with CA-MRSA SSTIs were enrolled. For analysis, we examined only data from the first positive culture. The medical records of each patient were reviewed for patient demographics and clinical information.

Bacterial strains

A total of 300 MRSA strains (200 HA-MRSA, 100 CA-MRSA) were available for the study. All staphylococci were identified by standard biochemical tests. Susceptibility to oxacillin was determined by the cefoxitin (30 µg) disc diffusion test as recommended by Clinical and Laboratory Standards Institute (CLSI) [22]. Strains were confirmed as MRSA by detection of the *femB* gene and *mecA* gene using multiplex PCR [23].

Susceptibility testing

Susceptibility testing was performed on all MRSA isolates using the disc diffusion method to 15 antimicrobial agents: amikacin (30 µg), gentamicin (10 µg), netilmicin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), clindamycin (2 µg), co-trimoxazole (1.25/23.75 µg), erythromycin (15 µg), fusidic acid (10 µg), linezolid (30 µg), mupirocin (200 µg), rifampicin (5 µg), teicoplanin (30 µg) and tetracycline (30 µg). Antimicrobial susceptibility was interpreted according to CLSI criteria. A vancomycin (6 µg/ml) agar screen was used to detect intermediate resistance to vancomycin [22]. Multi-drug resistance (MDR) was defined as resistance to ≥ 3 non- β -lactam antibiotic classes [24].

Molecular typing

All MRSA isolates were subjected to SCCmec typing and detection of PVL genes. SCCmec elements (I–V)

and PVL genes *lukF*–*lukS* were identified as described previously [25, 26]. Based on SCCmec types, the HA-MRSA isolates were further divided into HA-SCCmec I/II/III MRSA and HA-SCCmec IV/V MRSA. Similarly, CA-MRSA isolates were grouped into CA-SCCmec I/II/III MRSA and CA-SCCmec IV/V MRSA. Multiplex PCR detection of the *seh* gene (as a marker for CA-MRSA of clonal lineage ST1/USA400 and *arcA* gene as part of the ACME (arginine catabolic mobile element) cluster for ST8/t008/USA300 were performed on all SCCmec IV isolates as described previously [27]. Controls for the assay used were previously characterized reference isolates 05-01290t127/ST1/*she lukPV* and 06-01172t008/ST8/*arcA lukPV* (kindly provided by B. Strommenger, Robert Koch Institute, Germany).

PFGE was performed using *SmaI*-digested DNA as described previously [28] with selected MRSA strains of HA-SCCmec III, HA-SCCmec IV/V and CA-SCCmec IV/V having distinct resistance profiles. Comparison and grouping of the PFGE patterns were performed with InfoQuest™ FP Software v. 5.4 (Bio-Rad Laboratories, USA). PFGE patterns were compared on an unweighted pair-group method with averages (UPGMA) dendrogram based on Dice coefficients, where optimization and band position tolerance were set at 1.0% and 2.3%, respectively [29]. A similarity coefficient of 80% was selected to define the patterns [30].

MLST and *spa* typing were performed on selected representative isolates of major PFGE patterns in different SCCmec types as described previously [31, 32]. Isolates were assigned a ST according to the MLST website (<http://www.mlst.net>) and sequencing scape (Seqscape software, Applied Biosystems, USA).

Spa typing, based on DNA sequencing of the protein A gene variable region was performed, and isolates assigned as described on the Ridom website (<http://spa.ridom.de/>).

Statistical analysis

Each study variable was compared between SCCmec types. Quantitative variables were summarized as mean \pm s.d. and qualitative variables as proportions (%). Odds ratios (ORs) were calculated from logistic regression analysis for SCCmec type IV and type V, along with the 95% confidence intervals (CIs). The ORs for drug resistance were calculated separately for HA-SCCmec I/II/II vs. HA-SCCmec IV/V; and CA-SCCmec IV/V vs. HA-SCCmec IV/V. The

proportion of strains resistant to different drugs in different major clones of CA-MRSA was compared using Fisher's exact test. Multivariable logistic regression analysis was performed to identify the variables independently associated with HA-SCCmec IV/V vs. HA-SCCmec I/II/III, and CA-SCCmec IV/V. All tests were two-tailed and a P value ≤ 0.05 was considered to be statistically significant. Stata v. 12.1 (StataCorp., USA) was used for all analyses.

RESULTS

SCCmec type, PVL genes and characteristics of patients with HA-MRSA SSTIs

The majority of the 200 HA-MRSA patient isolates investigated carried SCCmec III ($n=116$, 58%), followed by SCCmec V ($n=46$, 23%) and SCCmec IV ($n=29$, 14.5%). Very few isolates carried SCCmec II ($n=5$, 2.5%) and SCCmec I ($n=4$, 2%). Thus, of this patient cohort classified epidemiologically as having HA-MRSA SSTIs, 125 (62.5%) were infected with HA-SCCmec I/II/III MRSA and 75 (37.5%) with MRSA of HA-SCCmec IV/V lineages. Forty (20%) isolates carried the genes for PVL, which was strongly associated with SCCmec IV and V MRSA strains (86.2% and 26.1%, respectively, carried PVL genes; $P < 0.001$).

The clinical and epidemiological data of the two groups of inpatients carrying HA-SCCmec IV/V (75) or HA-SCCmec I/II/III (125) were compared to assess associations between carriage of these types and particular risk factors (Table 1). Multivariable analysis confirmed that isolation of HA-SCCmec IV/V MRSA was associated with PVL gene carriage (OR 40.81, 95% CI 11.38–146.32, $P < 0.001$) and HA-SCCmec I/II/III MRSA was associated with burn wounds (OR 0.09, 95% CI 0.008–1.00, $P = 0.050$).

SCCmec type, PVL genes and characteristics of patients with CA-MRSA SSTIs

The most common SCCmec types among 100 isolates from this patient cohort were types V ($n=45$), IV ($n=38$) and III ($n=17$). Interestingly, 65.8% of SCCmec IV isolates carried the PVL gene compared to only 26.7% of type V ($P < 0.001$). The 83 isolates of types IV and V were designated as CA-SCCmec IV/V MRSA and subjected to further analysis. The demographic and clinical characteristics of these patients were compared with the 75 patients with HA-SCCmec IV/V MRSA SSTIs (Table 2). The mean

age of all patients in both cohort groups was 27.95 ± 20.28 years and 104 (65.8%) were male. Multivariable regression analysis identified malignancy (OR 10.57, 95% CI 1.23–90.49, $P = 0.03$) and bone fractures (OR 12.33, 95% CI 1.47–103.34, $P = 0.02$) as independent risk factors significantly associated with HA-SCCmec IV/V MRSA SSTIs.

Antimicrobial resistance

The distribution of resistance to antimicrobials of the MRSA isolates grouped by healthcare exposure status and SCCmec type are presented in Table 3. Compared with HA-SCCmec I/II/III MRSA isolates, a greater proportion of HA-SCCmec IV/V isolates were susceptible to all the antibiotics tested. However, compared to CA-SCCmec IV/V MRSA isolates, a higher proportion of HA-SCCmec IV/V isolates were antibiotic resistant. Ninety-five (76.0%) HA-SCCmec I/II/III isolates and 32 (42.7%) HA-SCCmec IV/V isolates were MDR ($P < 0.001$) whereas only five (6.0%) of CA-SCCmec IV/V MRSA isolates were MDR ($P < 0.001$).

Clonality of CA-SCCmec IV/V, HA-SCCmec IV/V MRSA and HA-SCCmec III isolates

The *Sma*I macrorestriction fragment profiles of 48 HA-SCCmec IV/V, 41 CA-SCCmec IV/V MRSA and 20 HA-SCCmec III MRSA isolates revealed 19 clones with four major clusters predominating (clone 4, $n=32$, 29.4%; clone 14, $n=22$, 20.2%; clone 6, $n=15$, 13.8%; clone 1, $n=11$, 10.1%) and together representing 80% of all isolates tested (Fig. 1). Each major clone comprised 2–10 subtypes. Except for clone 6, which was exclusive to the HA-SCCmec III MRSA group, the remaining three major clones were found in each patient cohort with SCCmec IV/V MRSA isolates. Clone 4 isolates carried only SCCmec IV and were detected in both CA- (40.6%) and HA- (59.4%) SCCmec IV isolates, often exhibiting PVL carriage (75%). Similarly, clone 14 was highly associated with SCCmec type V (86.4%) and less so with SCCmec type IV (13.2%) isolates; 50% of this clone carried PVL. Clone 1 comprised only SCCmec IV isolates and these often exhibited PVL (81.8%).

Comparison of local with international clones

PFGE was performed with epidemic MRSA strains (EMRSA)-1, -15, -16 from the UK, MW2, WIS, 655 U and Mu-50 (vanomycin-intermediate *S. aureus*) along with representative strains of major clones

Table 1. Characteristics of patients with HA-MRSA, skin and soft tissue infection as per SCCmec type isolated (*n* = 200)

Characteristics	HA-SCCmec I/II/III MRSA (<i>n</i> = 125)	HA-SCCmec IV/V MRSA (<i>n</i> = 75)	OR	95% CI	<i>P</i>
Age (years), mean ± s.d.	34.58 ± 18.84	31.42 ± 22.87	0.99	0.98–1.01	0.29
Sex					
Male	83 (66.4)	52 (69.3)	1.0		
Female	42 (33.6)	23 (30.7)	0.87	0.47–1.62	0.67
Patient location					
Medical ward	56 (44.8)	31 (41.3)	1.0		
Surgical ward	56 (44.8)	37 (49.3)	1.02	0.37–2.85	0.96
ICU	13 (10.4)	7 (9.3)	1.22	0.45–3.36	0.69
Duration of hospital stay (days) before culture	18.5 ± 18.89	15.65 ± 11.96	0.99	0.97–1.01	0.26
0–9	41 (32.8)	27 (36.0)	1.0		
10–19	34 (27.2)	22 (29.3)	0.98	0.48–2.03	0.96
≥20	38 (30.4)	23 (30.7)	0.92	0.45–1.87	0.82
Unknown	12 (9.6)	3 (4.0)	0.38	0.10–1.47	0.16
Panton–Valentine leukocidin	3 (2.4)	37 (49.3)	39.6	11.56–135.7	<0.001
Risk factors associated					
Intravenous catheter in use at time of culture	89 (70.2)	51 (68.0)	0.86	0.46–1.60	0.63
Urinary catheter in use at time of culture	52 (41.6)	28 (37.3)	0.84	0.46–1.51	0.55
Inter-hospital transfer	19 (15.2)	9 (12.0)	0.76	0.32–1.78	0.53
Intra-hospital transfer	38 (30.4)	31 (41.3)	1.6	0.89–2.93	0.12
Previous hospitalization (<6 months)	55 (44.0)	37 (49.3)	1.24	0.70–2.20	0.46
ICU admission before culture	35 (28.0)	25 (33.3)	1.29	0.69–2.39	0.43
Endotracheal intubations	26 (20.8)	9 (12.0)	0.52	0.23–1.18	0.12
Prosthetic devices	3 (2.4)	8 (10.7)	4.86	1.25–18.92	0.023
Underlying diseases/conditions					
Diabetes mellitus	12 (9.6)	9 (12.0)	1.28	0.51–3.21	0.59
Bone fracture	7 (5.6)	8 (10.7)	2.01	0.70–5.80	0.16
Osteomyelitis	9 (7.2)	1 (1.3)	0.17	0.02–1.40	0.1
Malignancies/leukaemia	17 (13.6)	10 (13.3)	0.98	0.42–2.26	0.96
Chronic renal disease	2 (1.6)	5 (6.7)	4.39	0.83–23.34	0.08
HIV infection	0 (0.0)	1 (1.3)	0.0	0.0	0.2
Sepsis	19 (15.2)	10 (13.3)	0.86	0.38–1.96	0.72
Multi-organ failure	6 (4.8)	2 (2.7)	0.54	0.11–2.76	0.46
Respiratory tract infections	7 (5.6)	8 (10.7)	2.01	0.67–5.80	0.2
Non-infectious dermatosis*	19 (15.2)	14 (18.7)	1.28	0.60–2.74	0.52
Infected burns	15 (12.0)	1 (1.3)	0.1	0.01–0.77	0.027
Recent antimicrobial therapy†					
β-lactams	99 (79.2)	56 (74.7)	0.77	0.39–1.52	0.46
Aminoglycosides	56 (44.8)	31 (41.3)	0.87	0.49–1.55	0.63
Metronidazole	36 (28.8)	13 (17.3)	0.52	0.25–1.06	0.07
Quinolones	32 (25.6)	17 (23.7)	0.85	0.43–1.67	0.64
Linezolid	23 (18.4)	14 (18.7)	1.02	0.49–2.13	0.96
Glycopeptides	17 (13.6)	13 (17.3)	1.33	0.61–2.93	0.48
Clindamycin	8 (6.4)	2 (2.7)	0.4	0.08–1.94	0.26
Co-trimoxazole	3 (2.4)	2 (2.7)	1.11	0.18–6.83	0.91
Mupirocin	2 (1.6)	1 (1.3)	0.83	0.07–9.32	0.88
Total number of antibiotics received					
Monotherapy	17 (13.6)	17 (22.7)	1.0		
Dual combination therapy	37 (29.6)	20 (26.7)	1.61	0.73–3.52	0.24
Triple or multiple combination therapy	61 (48.8)	38 (50.7)	0.87	0.44–1.71	0.68
No antibiotic therapy	10 (8.0)	0 (0.0)	—	—	—
Mortality	6 (4.8)	2 (2.7)	0.54	0.11–2.76	0.46

OR, Odds ratio; CI, confidence interval; ICU intensive care unit.

Data are no. (%) of patients, unless otherwise specified.

* For example, psoriasis, eczema, pemphigus vulgaris, etc.

† Preceding antibiotics used for the condition for which the patient was hospitalized.

P values <0.05 are highlighted in bold.

Table 2. Association of study characteristics in patients with CA-SCCmec type IV/IV and HA-SCCmec type IV/IV infections

Characteristics	CA-SCCmec IV/IV MRSA (n=83)	HA-SCCmec IV/IV MRSA (n=75)	OR	95% CI	P
Age (years), mean \pm s.d.	24.7 \pm 17.1	31.4 \pm 22.9	—	—	0.04
Sex					
Male	52 (62.7)	52 (69.3)	1.0		
Female	31 (37.4)	23 (30.7)	0.74	0.38–1.44	0.38
Panton–Valentine leukocidin	40 (48.2)	37 (49.3)	1.05	0.56–1.95	0.89
Underlying diseases/conditions					
Non-infectious dermatosis*	1 (1.2)	14 (18.7)	18.82	2.41–147.01	0.005
Bone fracture	1 (1.2)	8 (10.7)	9.79	1.19–80.26	0.034
Osteomyelitis	5 (6.0)	1 (1.3)	0.21	0.024–1.85	0.16
Malignancies/leukaemia	1 (1.2)	10 (13.3)	12.62	1.57–101.10	0.017
Respiratory tract infections	0 (0.0)	8 (10.7)	—	—	0.002
Chronic renal disease	0 (0.0)	5 (6.67)	—	—	0.02
Wound infection	4 (4.8)	3 (4.0)	0.82	0.18–3.80	0.80
Infected ulcer	2 (2.4)	2 (2.7)	1.11	0.15–8.08	0.92
Abscess	11 (13.3)	3 (4.0)	0.27	0.07–1.02	0.05
Cellulites	2 (2.3)	2 (2.7)	1.12	0.15–8.08	0.92
Infected burns	0 (0.0)	1 (1.3)	—	—	0.48

OR, Odds ratio; CI, confidence interval.

Data are no. (%) of patients, unless otherwise specified.

* For example, psoriasis, eczema, pemphigus vulgaris, etc.
P values <0.05 are highlighted in bold.

found here (Fig. 1). The predominant clone 4 showed close similarity (>90%) in DNA pattern to the epidemic UK EMRSA-15 clone. Of note, none of the isolates showed PFGE patterns of the USA400 CA-MRSA lineage. In addition, all HA-SCCmec IV and CA-SCCmec IV MRSA isolates were negative for *arcA* and *seh* genes.

Antimicrobial resistance patterns in different clones

The resistance to various antimicrobials varied from clone to clone and there was no correlation with resistance profiles and specific clones. Nevertheless, two broad groups were evident: one with isolates resistant only to β -lactams or 1–3 other antibiotics (clones 1, 2, 4, 12–17), and those resistant to almost all antibiotics tested (clones 3, 5–11, 18, 19). Isolates of clone 6 showed more resistance than other clones, with ~80% resistance to fluoroquinolones, tetracycline, erythromycin and clindamycin; clone 4 was uniformly susceptible to gentamicin, netilmicin, amikacin and co-trimoxazole.

Spa typing and MLST

MLST and *spa* typing were performed on 38 representative strains of the major clones 1, 4, 6 and 14 ($n=30$)

and sporadic clones ($n=8$). Table 4 shows that the strains were assigned to 14 STs and 17 *spa* types. The 12 SCCmec V strains belonged to four STs and *spa* types with eight of them identified as ST772-SCCmec-V-t657 and the remainder were each unique. The SCCmec IV strains (13) clustered in four STs and the dominant ST22 consisted of diverse *spa* types. The 13 SCCmec III strains were grouped into six STs and *spa* types with 10 strains belonging to ST239 or the closely related ST241, ST1097 and ST1310.

DISCUSSION

Our study documents the emergence of MRSA isolates typical of CA genotypes in patients with HA-MRSA SSTIs in an Indian hospital. Remarkably, at our institute a large proportion (37.5%) of isolates classified epidemiologically as HA-MRSA had a CA genotype. These data confirm the reported spread of CA-MRSA SCCmec IV/IV strains in hospital settings in Europe, the USA [33, 34] and India [13, 15]. Given the vulnerable population within the hospital setting, it is unclear how infections with isolates that contain SCCmec IV/IV may differ in symptoms and severity from those caused by the traditional HA-SCCmec I/II/III isolates. The introduction of

Table 3. Antimicrobial resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from patients with skin and soft tissue infections grouped by SCCmec type and healthcare exposure status*

Antimicrobial agents (μg)	HA-SCCmec I/II/III MRSA ($n=125$)	HA-SCCmec IV/V MRSA ($n=75$)	OR	95% CI	P^\dagger	CA-SCCmec IV/V MRSA ($n=83$)	OR‡	95% CI‡	$P^{\dagger\ddagger}$
Amikacin	69 (55.2)	28 (37.3)	0.48	0.27–0.87	0.015	4 (4.8)	11.77	3.88–35.63	<0.001
Gentamicin	62 (49.6)	25 (33.3)	0.51	0.28–0.92	0.026	17 (20.5)	1.94	0.95–3.98	0.07
Netilmicin	23 (18.4)	10 (13.3)	0.68	0.31–1.53	0.35	1 (1.2)	12.62	1.57–101.10	0.017
Chloramphenicol	14 (11.2)	6 (8.0)	0.69	0.25–1.88	0.47	3 (3.6)	2.32	0.56–9.62	0.25
Ciprofloxacin	91 (72.8)	42 (56.0)	0.48	0.26–0.87	0.016	20 (24.1)	4.01	2.03–7.90	<0.001
Levofloxacin	87 (69.6)	40 (53.3)	0.50	0.28–0.90	0.022	20 (24.1)	3.60	1.83–7.09	<0.001
Clindamycin	82 (65.6)	43 (57.3)	0.70	0.39–1.27	0.24	17 (20.5)	5.22	2.58–10.53	<0.001
Co-trimoxazole	88 (70.4)	32 (42.7)	0.31	0.17–0.57	<0.001	42 (50.6)	0.73	0.39–1.36	0.32
Erythromycin	106 (84.8)	53 (70.7)	0.43	0.22–0.87	0.018	33 (39.8)	3.65	1.88–7.09	<0.001
Fusidic acid	25 (20.0)	14 (18.7)	0.92	0.44–1.90	0.82	3 (3.6)	6.12	1.68–22.25	0.006
Mupirocin	7 (5.6)	3 (4.1)	0.71	0.18–2.84	0.63	0 (0.0)	—	—	0.064
Rifampicin	44 (35.2)	16 (21.3)	0.50	0.26–0.97	0.040	3 (3.6)	7.23	2.01–25.96	0.002
Tetracycline	87 (69.6)	34 (45.3)	0.36	0.20–0.66	0.001	5 (6.0)	12.94	4.70–35.59	<0.001

OR, Odds ratio; CI, confidence interval.

* According to the CDC definition [7].

† All P values determined using odds ratios and not derived from t test or χ^2 test. P values <0.05 highlighted in bold.

‡ Analyses of antimicrobial resistance patterns of HA-SCCmec-IV/V and CA-SCCmec-IV/V MRSA isolates. All strains were susceptible to vancomycin (screen agar method), linezolid and teicoplanin.

P values <0.05 are highlighted in bold.

Table 4. *SCCmec*, *PVL* status, *MLST* and *spa* typing for selected strains of the major and sporadic *PFGE* clones

<i>SCCmec</i> (n)	<i>PVL</i> status	<i>MLST</i> profile (no. of isolates)	ST	<i>spa</i> type (no. of isolates)	<i>PFGE</i> clones (no. of isolates)	Epidemic clone
V (12)	1	6, 2, 6, 2, 7, 13, 5 (1)	ST689	t019 (1)	2 (1)	Asia (SLV of ST1)
	8	1, 1, 1, 1, 22, 1, 1 (9)	ST772	t657 (9)	14 (9)	
	1	22, 1, 14, 109, 12, 4, 3 (1)	ST1289	t2526 (1)	16 (1)	
	1	1, 1, 1, 159, 1, 1, 1 (1)	ST2039	t386 (1)	8 (1)	
IV (13)	3	7, 6, 1, 5, 8, 8, 6 (6)	ST22	t005 (1), t891 (2), t1152 (2), t3107 (1)	4 (6)	United Kingdom (EMRSA-15)
	3	2, 2, 2, 2, 3, 3, 2 (3)	ST36	t4410 (3)	1 (3)	Southwest Pacific (USA1100)
	1	7, 6, 1, 5, 8, 5, 6 (2)	ST217	t1328 (2)	4 (2)	
	0	2, 2, 2, 2, 6, 3, 2 (2)	ST30	t021 (2)		
III (13)	1	2, 3, 1, 1, 4, 4, 3 (6)	ST239	t030 (6)	6 (6)	Brazilian (SLV of ST8)
	0	2, 3, 1, 1, 4, 4, 30 (2)	ST241	t037 (2)	6 (1), 9 (1)	
	0	1, 4, 1, 8, 4, 4, 3 (1)	ST72	t347 (1)	8 (1)	
	0	2, 3, 6, 1, 4, 4, 3 (2)	ST1097	t2952 (2)	3 (2)	
	0	2, 3, 1, 1, 4, 4, 30 (1)	ST1310	t4410 (1)	8 (1)	
	0	1, 3, 1, 1, 4, 4, 11. 1)	ST673	t1309 (1)	16 (1)	

PVL, Panton–Valentine leukocidin; *MLST*, multi-locus sequence typing; *ST*, sequence type; *PFGE*, pulsed-field gel electrophoresis; *SLV*, single locus variant.

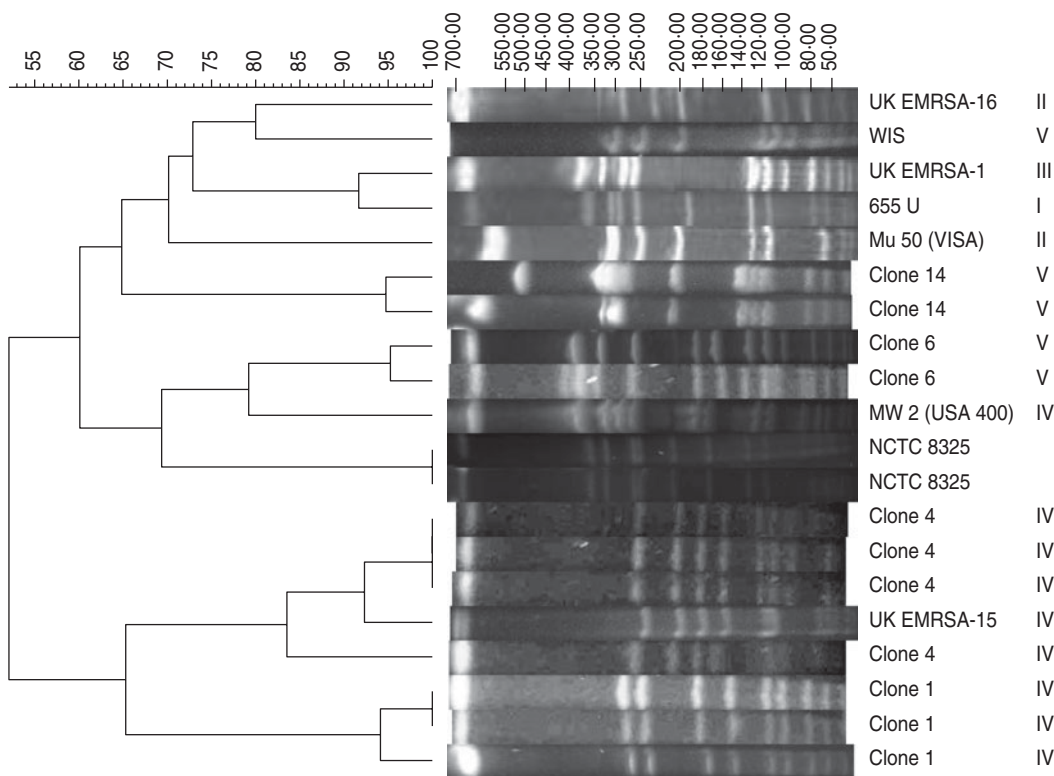


Fig. 1. Dendrogram based on similarities derived from the unweighted pair group method with arithmetic mean (UPGMA) and Dice coefficients, using InfoQuest™ FP software (Bio-Rad). The scale at the top represents similarity. Pulsed-field gel electrophoretic patterns of *Sma*I macrorestriction fragments of *EMRSA*-1, -15, -16, MW2, WIS, 655 U, Mu-50 along with representative strains of the *SCCmec* types IV/V major clones 1, 4, 6 and 14.

SCCmec IV/V strains into our hospital population did not result in a change in the spectrum or severity of illness in this group. Compared to patients with CA-SCCmec IV/V SSTIs, it was observed that HA-SCCmec IV/V SSTIs were significantly associated with malignancy and bone fractures. Patients with these conditions are predisposed to use healthcare facilities, are generally exposed to antibiotics, tend to have interventions performed, and hence present opportunities to contract MRSA in the healthcare facility. Exactly why the SCCmec IV/V strains are successful in hospital settings such as ours remains unknown but mathematical models predict the replacement of traditional HA-MRSA strains by CA-MRSA strains, due to their higher growth rate and greater genetic fitness [35, 36].

In this study, as expected, HA-SCCmec IV/V isolates were more susceptible to multiple antibiotics compared to HA-SCCmec I/II/III. However, similar to previous reports [11], HA-SCCmec IV/V strains demonstrated higher antimicrobial resistance rates compared to CA-SCCmec IV/V strains. Differences in antimicrobial susceptibility indicate that, in patients with healthcare contact, SCCmec IV/V MRSA strains exhibit characteristics of traditional HA-MRSA strains. Our findings support the hypothesis of Gonzalez *et al.* [37] that as typical CA-MRSA strains proliferate in healthcare settings, where antimicrobial selection pressures are high, they will continue to acquire additional antimicrobial resistance genetic elements, causing them to appear similar to more traditional MRSA isolates, with respect to resistance profiles. Our finding of PVL genes in 49% of HA-SCCmec IV/V isolates is not surprising since SCCmec IV/V strains have been associated largely with SSTIs and PVL production [12]. However, the sporadic (2.6%) PVL-positive SCCmec III HA-MRSA isolates is an issue of potential concern and could result in the emergence of MDR HA-MRSA isolates with increased virulence, despite the fact that the role of PVL as a virulence factor is a matter of much debate.

Molecular typing revealed that 73% of the isolates belonged to four major genetic lineages, with the remaining sporadic isolates showing a high degree of genetic diversity, suggesting the possibility of new strains being imported into the hospital from the expanding community reservoir. Interestingly, the highly infectious CA-MRSA strains USA300 and USA400 were not detected in our hospital or community. Previous characterization of nosocomial

MRSA strains recovered in our hospital from patients with SSTIs identified a major MRSA clone closely related to the ST239:EMRSA-I:III which was MDR [17]. In the present study, selected strains of the major PFGE clone 6 also showed genetic relatedness to the lineage ST239:EMRSA-I:III with *spa*-type t030. ST239 is the major endemic HA-MRSA clone in many Asian countries, although recent studies show that it is being gradually replaced by emerging CA-MRSA clones [13–15] as was also observed in our study.

The ST22-SCCmec IV MRSA (EMRSA-15) strain is a global pandemic HA-MRSA clone and interestingly it was recovered from both inpatients and outpatients in our hospital. This suggests that outpatients may represent an important reservoir for MRSA dissemination within the hospital, when admitted as inpatients and reinforces the observation of Alcoceba *et al.* [38] regarding the movement of EMRSA-15 from the community to the hospital setting.

The SCCmec V MRSA isolates that were genotyped were of *spa* type t657 and ST772, similar to the types reported recently in Mumbai and elsewhere in India [13–15]. The decreasing prevalence of the HA-MRSA strain ST239-MRSA-III in hospital in India since 2006, coupled with an increase in prevalence of ST772-MRSA-V and ST22-MRSA-IV, has led to the suggestion that these strains may be replacing the ST239-MRSA strain in Indian hospitals [13–15]. The emergence of the MDR clone ST772-MRSA-V in our hospital is a worrying development, and is indicative of the need for enhanced surveillance to ensure that these strains do not spread.

Another relevant finding of the present study concerns the presence of the ST36:EMRSA-16:IV (major PFGE clone 1) clones of *spa*-type t4410. Similar to the finding of Söderquist *et al.* [39], it was found to have the same ST as the EMRSA-16 (ST36-MRSA-II) strain, which is one of the predominant EMRSA strains in Europe. Furthermore, the described isolate was PVL positive, as were the majority of CA-MRSA strains, but this differs from EMRSA-16 ST36, which is PVL negative. The PFGE pattern of the isolate differed by >6 bands compared to EMRSA-16. To our knowledge, this is the first report of the ST36:EMRSA-16:IV clone in an Asian hospital.

This study had certain limitations. All of the observations were derived from a single hospital and therefore, may only reflect local trends and, thus, might not

be representative of other settings in India or other countries. Further, only representative SCCmec IV/V strains of the major PFGE clones were genotyped by MLST and *spa* typing. Nevertheless, our data clearly demonstrate that the SCCmec type IV and type V epidemic clones of ST22 (EMRSA-15) and ST772 have entered into our hospital and now cause a substantial proportion of serious HA-MRSA infections.

In summary, the high proportions of HA-MRSA strains carrying SCCmec types IV and V, together with the considerable occurrence of PVL-positive MRSA strains, confirm the extensive infiltration of CA-MRSA genotypes in our hospital. We found that, following healthcare exposure, SCCmec IV/V strains have characteristics similar to those of other HA-MRSA strains isolated from these patients. Moreover, HA-SCCmec IV/V strains exhibited an antimicrobial susceptibility pattern in-between those of CA-SCCmec IV/V and HA-SCCmec I/II/III. This finding raises concerns that CA-SCCmec IV/V and HA-SCCmec IV/V strains may exchange genetic material resulting in an organism uniquely adapted to produce aggressive SSTIs similar to CA-MRSA strains which carry PVL genes as well expressing resistance to multiple antimicrobial agents generally associated with the current SCCmec I/II/III strains. The dissemination of these epidemic CA-MRSA clones in both inpatients and outpatients represents a significant challenge to infection control.

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DECLARATION OF INTEREST

None.

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