

Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* with Emergence of Epidemic Clones of Sequence Type (ST) 22 and ST 772 in Mumbai, India[∇]

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A total of 412 methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated between October 2006 and June 2009, representing a mixed hospital- and community-associated patient population from Mumbai, India, were evaluated. MRSA was characterized by multiplex PCR amplification of the Pantone-Valentine leukocidin (PVL) gene and the *mecA* gene, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and multilocus sequence typing (MLST). PCR results were compared with patient risk factors (CDC guidelines) and antimicrobial susceptibility profiles. A total of 395 MRSA strains were *mecA* positive, and 224 were PVL gene positive. A total of 97 *mecA*-positive strains were SCC*mec* III (25%), 136 were SCC*mec* IV (34%), and 162 were SCC*mec* V (41%). All SCC*mec* III strains were multidrug resistant, and all patients had risk factors. Of the SCC*mec* IV and V strains, 73% were multidrug susceptible and 72% of the associated patients had no risk factors. The multidrug susceptibility and absence of patient risk factors in 72% of cases with SCC*mec* IV and SCC*mec* V MRSA demonstrate the presence of community-associated MRSA (CA-MRSA) in Mumbai. Twenty-one percent of these patients had risk factors, signifying CA-MRSA infiltration into hospitals. MLST showed clonal expansion of multidrug-susceptible sequence type (ST) 22 (SCC*mec* IV) and ST 772 (SCC*mec* V), both of which feature in Asian studies and may be slowly replacing the multidrug-resistant ST 239 (SCC*mec* III) in hospitals. The PVL gene-positive methicillin-sensitive *S. aureus* (MSSA) strains were ST 30 and were postulated to be related to the penicillin-resistant *S. aureus* phage type 80/81, notorious for its virulence in the 1950s.

Staphylococcus aureus causes disease ranging from mild skin infection to sepsis, pneumonia, and toxic shock syndrome (32). In the preantibiotic era, mortality from *S. aureus* disease was high and introduction of penicillin had a dramatic impact, which was short-lived due to the emergence of penicillinase-producing *S. aureus* (2, 4, 20). Methicillin was introduced in 1959, and methicillin-resistant *Staphylococcus aureus* (MRSA) strains rapidly emerged and became a major problem in hospitals in the 1960s. These nosocomial MRSA strains were multidrug resistant (MDR), many being susceptible only to glycopeptides (1, 2, 4, 32). The past 10 years have seen reports of reduced susceptibility (vancomycin-intermediate *S. aureus* [VISA]) and resistance (vancomycin-resistant *S. aureus* [VRSA]) to vancomycin (29).

The genome of methicillin-resistant staphylococci contains a 21- to 67-kb heterologous mobile genetic element, termed staphylococcal cassette chromosome *mec* (SCC*mec*), harboring the *mecA* gene and other resistance determinants. Methicillin resistance is mediated by production of an altered penicillin-binding protein, PBP-2a, encoded by the *mecA* gene (1, 2, 5, 9, 15). The tendency of most strains to be heterogeneously resistant to methicillin, oxacillin, and the cephalosporins accounts for many problems that clinical microbiology lab-

oratories encounter with MRSA detection by phenotypic susceptibility methods (15). SCC*mec* characterization relies on variations of the *mec* gene complex, cassette chromosome recombinase (*ccr*) complex, and junkyard regions. Five SCC*mec* types (I to V) have been confirmed in *S. aureus* (13, 14, 21, 26). SCC*mec* VI and VII types have also been discovered, but they are rarer (27).

MRSA strains were previously known for being associated with nosocomial infections (HA-MRSA). However, MRSA strains now cause community-acquired infections (CA-MRSA), especially skin and soft tissue infections (SSTI) and necrotizing pneumonia (2). Although diversity in genomes and antibiotics exists, most CA-MRSA strains carry Pantone-Valentine leukocidin (PVL) genes and possess a small mobile SCC*mec* type IV, V, or VI, which is more easily transferable than the larger SCC*mec* types I, II, and III in HA-MRSA (1, 2). PVL toxin is a bicomponent leukocidin encoded by the *lukS-PV* and *lukF-PV* genes, which reside on a prophage. PVL toxin causes leukocyte destruction, tissue necrosis, and increased disease severity (1, 2, 18). CA-MRSA poses a significant threat to public health. HA-MRSA and CA-MRSA differ on the basis of SCC*mec* types and antimicrobial susceptibilities. Patients with HA-MRSA infections have risk factors like history of exposure to an intensive care unit (ICU), hospital stay, antimicrobial exposure, surgery, and invasive procedures. CA-MRSA infections, on the other hand, are indicated among people living in crowded areas, sharing personal items and gym equipment, as well as intravenous drug users, sports participants, and homosexuals (5, 31).

CA-MRSA cases are being reported all over the world, yet

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there have not been many studies regarding the actual incidence of CA-MRSA infections in India (5, 8). This study comprises MRSA strains isolated in the laboratory, including simultaneous documentation of clinical histories, risk factors, and antimicrobial susceptibility patterns. PCR amplification of PVL genes, *SCCmec* typing, and multilocus sequence typing (MLST) enabled characterization of MRSA (4, 6, 16, 18, 23, 24, 32). This gave insight into *SCCmec* types isolated in the laboratory and the frequency of PVL⁺ MRSA and CA-MRSA.

MATERIALS AND METHODS

Bacterial reference strains and clinical isolates. PVL⁺ *S. aureus* and *SCCmec* reference strains were kindly provided by Jerome Etienne, INSERM, Lyon, France, and Dilip Mathai and Anand Manoharan, CMC, Vellore, India. The local MRSA strains were collected in the microbiology laboratory. The hospital, being a tertiary care center, also receives numerous requests for culture from other hospitals, nursing homes, and general practitioners from all over Mumbai, India. Thus, the MRSA in this study represents a mixed patient population from hospitals and communities in the city.

Isolation and confirmation of MRSA by phenotypic methods. MRSA strains were isolated from clinical samples (nasal and axillary screening swabs, blood, pus, soft tissue, wound swabs, respiratory samples, and body fluids) from October 2006 to June 2009. Staphylococci were identified morphologically and biochemically by standard laboratory procedures. *S. aureus* was identified by tube coagulase and latex agglutination test for coagulase and protein A (Bio-Rad), along with mannitol fermentation on mannitol salt agar (Himedia). Methicillin resistance was confirmed using a cefoxitin disk on Mueller-Hinton agar (Oxoid) and observation of growth on oxacillin screen agar (Himedia) according to CLSI guidelines. Antimicrobial susceptibility testing was performed by disk diffusion on Mueller-Hinton agar (Oxoid) according to CLSI guidelines.

Documentation of patient risk factors and antimicrobial susceptibility patterns. A standard form was filled out for each patient whose sample grew MRSA, with details like the name, age, sex, phone number, and hospital admission specifics. Clinical diagnosis along with risk factors (Centers for Disease Control and Prevention [CDC] guidelines) for acquiring MRSA (previous ICU or hospital stay, antimicrobial exposure, surgery or invasive procedures, etc.) was documented, along with antimicrobial susceptibility patterns (by disk diffusion according to CLSI guidelines) (5).

DNA extraction. Rapid DNA extraction was performed from bacterial colonies as previously described (32).

Molecular characterization by PCR amplification and DNA sequencing. Five PCR assays carried out using an MJ Research PTC-200 thermocycler were used for characterization of the MRSA. The first four were multiplex PCRs, and the last was a multilocus sequence typing (MLST) assay, comprised of PCRs for seven housekeeping genes in *S. aureus* along with DNA sequencing methods.

MRSA-PVL PCR-1. The PCR mixture contained four sets of primers: PVL (toxin gene), *mecA* (methicillin resistance), *nuc* (specific for *S. aureus*), and SS756 (IC) (*Staphylococcus* species) (18, 32). PCR was performed in a 25- μ l reaction volume with 2.5 μ l DNA, 1 \times *Taq* buffer, 100 μ mol deoxynucleoside triphosphate (dNTP) mixture (Fermentas), 5 mM MgCl₂, 12.5 pmol SS756 (IC), 7.5 pmol each PVL, *mecA*, and *nuc* primers (MWG Biotech, India), and 1 unit Hotstar *Taq* DNA polymerase (Qiagen). Amplification was performed with denaturation at 95°C for 15 min, followed by 10 cycles of 95°C for 45 s, 67°C for 45 s, and 72°C for 1.5 minutes; 25 cycles of 95°C for 45 s, 57°C for 45 s, and 72°C for 1.5 minutes; and extension at 72°C for 10 min.

SCCmec-typing PCR-2. The PCR mixture contained nine sets of primers: CIF, ccrC, RIF, *mecV*, DCS, *ccrB*, KDP, *mecIII*, and *mecR1* (targeting constituents of *SCCmec*), as well as *mecA-149* (IC) (methicillin resistance) (24). PCR was performed in a 25- μ l reaction volume with 2.5 μ l DNA, 1 \times *Taq* buffer, 100 μ mol dNTP mixture (Fermentas), 4 mM MgCl₂, 12.5 pmol each CIF, DCS, *ccrB*, KDP, *mecIII*, *mecR1*, and *mecA-149* (IC), 2.5 pmol each RIF and *mecV*, 20 pmol *ccrC* primers (MWG Biotech, India), and 0.75 units Hotstar *Taq* DNA polymerase (Qiagen). Amplification was performed with denaturation at 95°C for 15 min; 30 cycles of 95°C for 25 s, 56°C for 25 s, and 72°C for 1 min; and extension at 72°C for 4 min.

SCCmec IV-subtyping PCR-3. The PCR mixture contained six sets of primers: IVa, IVb, IVc, IVd, IVg, and IVh (*SCCmec* IV subtypes), as well as *ccrB-210* (IC) (*SCCmec* IV strains) (23). PCR was performed in a 25- μ l reaction volume with 2.5 μ l DNA, 1 \times *Taq* buffer, 100 μ mol dNTP mixture (Fermentas), 4 mM MgCl₂, 5 pmol each *ccrB-210*, IVa, and IVb, 10 pmol each IVc and IVd, 20 pmol each

TABLE 1. Frequency of the PVL gene in MRSA among each *SCCmec* type isolated^a

MRSA source	No. of strains	No. of strains of PVL phenotype					
		<i>SCCmec</i> III (n = 97)		<i>SCCmec</i> IV (n = 136)		<i>SCCmec</i> V (n = 162)	
		PVL ⁺	PVL ⁻	PVL ⁺	PVL ⁻	PVL ⁺	PVL ⁻
Pus/tissue	186	24	72	7	60	23	
Wound swabs	66	17	19		18	12	
Blood	51	18	16	3	4	10	
Screening swabs	37	16	6	1	9	5	
Respiratory	28	12	5		7	4	
Fluids	21	6	4	2	3	6	
Miscellaneous	6	4	1			1	
Total	395	97	123	13	101	61	

^a *SCCmec*, staphylococcal cassette chromosome *mec*; PVL, Panton-Valentine leukocidin gene.

IVg and IVh (MWG Biotech, India), and 0.5 units Hotstar *Taq* DNA polymerase (Qiagen). Amplification was performed with denaturation at 95°C for 15 min; 35 cycles of 95°C for 30 s, 48°C for 30 s, and 72°C for 1.5 minutes; and extension at 72°C for 5 min.

ccr-typing PCR-4. The PCR mixture contained primers *ccrAB* α 1-F, *ccrAB* α 2-F, *ccrAB* α 3-F, *ccrAB* β 2-R, *ccrAB* α 4-F, *ccrAB* α 4-R, *ccrCY*-F, and *ccrCY*-R (*ccr* types) (16). PCR was performed in a 25- μ l reaction volume with 2.5 μ l DNA, 1 \times *Taq* buffer, 100 μ mol dNTP mixture (Fermentas), 5 mM MgCl₂, 30 pmol each *ccrAB* α 3-F and *ccrAB* β 2-R, 25 pmol each *ccrAB* α 4-F and *ccrAB* α 4-R, 0.25 pmol *ccrAB* α 1-F, 0.125 pmol *ccrAB* α 2-F, 0.75 pmol each *ccrCY*-F and *ccrCY*-R primers (MWG Biotech, India), and 2 units Hotstar *Taq* DNA polymerase (Qiagen). Amplification was performed with denaturation at 95°C for 15 min, followed by 10 cycles of 95°C for 2 min, 60°C for 1 min, and 72°C for 2 min; 20 cycles of 95°C for 2 min, 50°C for 1 min, and 72°C for 2 min; and extension at 72°C for 2 min.

MRSA-MLST PCR-5. Each PCR mixture contained a set of primers (*arc*, *aro*, *glp*, *gmk*, *pta*, *tpi*, and *yqi*) (6). PCRs were performed in a 25- μ l reaction volume with 2.5 μ l DNA, 1 \times *Taq* buffer, 100 μ mol dNTP mixture (Fermentas), 4 mM MgCl₂, 12.5 pmol primers (MWG Biotech, India), and 0.5 unit Hotstar *Taq* DNA polymerase (Qiagen). Amplification was performed with denaturation at 95°C for 15 min, followed by 30 cycles of 95°C for 1 min, 51°C for 1 min, and 72°C for 1 min and extension at 72°C for 5 min.

Electrophoresis of PCR amplicons. The amplicons from PCR-1, -2, -3, and -5 were separated on 4% agarose gels in 1 \times TAE buffer (Sigma) using a 50-bp reference ladder (Fermentas), and those of PCR-4 were separated on 2% agarose gels in 1 \times TAE buffer (Sigma) using a 100-bp reference ladder (Fermentas). Gels were documented under a UV transilluminator. Amplicons of PCR-5 were purified using a gel extraction spin column PCR cleanup kit (Chromobio Biotech) and sequenced by the ABI 3130 genetic analyzer (6). All PCRs were validated using appropriate positive and negative controls and by DNA sequencing.

RESULTS

Strain collection. A total of 412 MRSA strains were cultured from October 2006 to June 2009. Of these, 61% were from the IPD and 39% from the OPD. Of the IPD samples, 98 came from the ICU and 154 came from the wards. Isolates were from pus/tissue (*n* = 186), wound swabs (*n* = 66), blood (*n* = 51), screening swabs (*n* = 37), respiratory tract (*n* = 28), fluids (*n* = 21), and miscellaneous sources (*n* = 6) (Table 1).

Multiplex PCR results. A total of 412 cultured MRSA strains were subjected to PCR-1 for *nuc*, *mecA*, and PVL genes. Of these, 395 proved to be MRSA genotypically (presence of *mecA* and *nuc*) and 224 were PVL gene positive. The 395 MRSA strains were subjected to PCRs 2, 3, and 4. In PCR-2, 97 strains were *SCCmec* III, 136 were *SCCmec* IV, and 162 were *SCCmec* V. In PCR-3, 135 *SCCmec* IV strains were

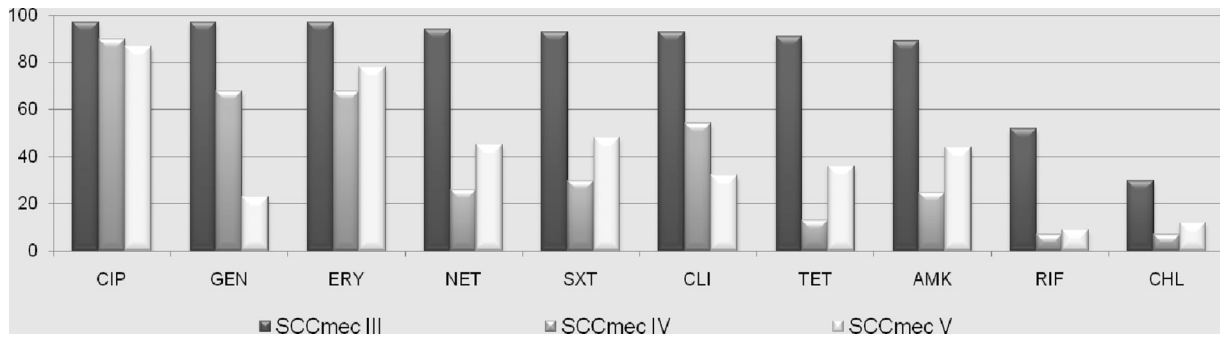


FIG. 1. Antimicrobial resistance patterns of MRSA from the laboratory. CIP, ciprofloxacin; GEN, gentamicin; ERY, erythromycin; SXT, trimethoprim-sulfamethoxazole; NET, netilmicin; AMK, amikacin; TET, tetracycline; CLI, clindamycin; RIF, rifampin; CHL, chloramphenicol (resistance data represented as percentages among SCCmec types III, IV, and V).

SCCmec IVc. One strain was SCCmec IVa. Results of PCR-4 correlated with those of PCR-2. A total of 97 strains were ccrAB3, 136 were ccrAB2, and 162 were the ccrC type. Of 412 MRSA strains, 17 proved to be methicillin-resistant coagulase-negative staphylococci (MRCNS) and PVL⁺ methicillin-susceptible *S. aureus* (MSSA) (Table 1).

Antimicrobial susceptibility patterns. Of the SCCmec III strains, 38% were MDR and the rest were susceptible only to chloramphenicol, rifampin, vancomycin, and linezolid. Of the SCCmec IV strains, 83% were susceptible to many antimicrobial classes and the rest were susceptible to three classes, none being MDR. Of the SCCmec V strains, 64% were susceptible to many antimicrobial classes, 24% were susceptible to three classes, and 12% were MDR (Fig. 1).

Patient independent risk factors. All patients with SCCmec III strains had risk factors for MRSA infections. Of the patients with SCCmec IV strains, 24% had risk factors and 76% had no documented risk factors. Of the patients with SCCmec

V strains, 32% had risk factors and 68% had no documented risk factors (Table 2; Fig. 2).

Multilocus sequence typing. MLST was performed for 13 representative SCCmec III MRSA strains and 42 representative SCCmec IV and SCCmec V MRSA strains, with similar antimicrobial susceptibility patterns and PVL positivity. For comparative purposes, MLST was also performed for 11 PVL⁺ MSSA strains. Each PCR amplicon was sequenced and analyzed (4, 5). All 13 SCCmec III MRSA strains were sequence type (ST) 239, with the allelic profile 2-3-1-1-4-4-3. All 29 SCCmec IV MRSA strains and 13 SCCmec V MRSA strains were PVL gene positive. All 29 SCCmec IV strains were ST 22, with the allelic profile 7-6-1-5-8-8-6, and all 13 SCCmec V strains were ST 772, with the allelic profile 1-1-1-1-22-1-1. All 11 PVL⁺ MSSA strains were ST 30, with the allelic profile 2-2-2-2-6-3-2 (Table 3).

DISCUSSION

From the 412 strains culture confirmed as MRSA, 395 were *mecA* positive by PCR. Of these MRSA strains, 64% were found to be PVL gene positive by PCR. The highest number of MRSA strains in this study were isolated from SSTI, and 67% of these strains were PVL⁺. From 2006 to 2009, the isolation of MRSA from pus and tissue samples at the hospital has increased considerably compared to that from other sources. We are aware that a larger study surveying both community and hospitalized patients in a defined geographical area may

TABLE 2. Patient breakdown and risk factors for HA-MRSA among the SCCmec types

Patient status or risk factor	No. of strains per group			Total
	SCCmec III	SCCmec IV	SCCmec V	
Patient location				
Inpatient status	97	54	91	242
Outpatient status		82	71	153
Risk factors for HA-MRSA				
Time from admission to culture >48 h	72	16	10	98
Previous hospitalization	78	23	35	136
Interhospital transfer	23	6	7	36
ICU stay	87	3	8	98
Hospital stay	95	14	23	132
Surgery	63	12	17	92
Catheterization	96	14	23	133
Intubation	84	3	7	94
Other invasive procedures	78	20	37	135
Immunocompromised status, chronic illness	95	25	35	155
Antimicrobial exposure	75	29	32	136
Patients with other risk factors	97	32	52	181
Patients without risk factors		104	110	214

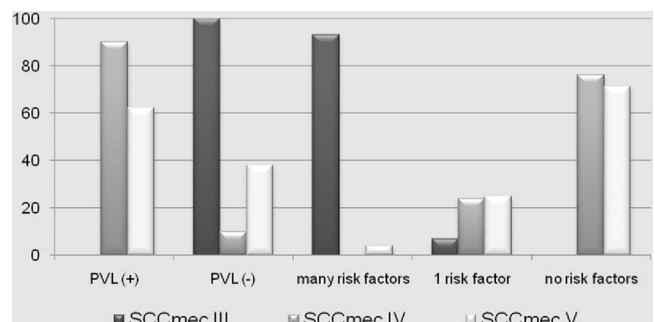


FIG. 2. Characteristics of the MRSA strains in the study: PVL gene positivity and patient risk factors.

TABLE 3. Characterization of HA-MRSA, CA-MRSA, and PVL⁺ MSSA by MLST^a

SA type (n)	SCCmec (n)	PVL status (n)	MLST profile ^b	ST:CC ^c	Epidemic clone
MRSA (55)	III (13)	- (13)	2-3-1-1-4-4-3	239:8	Brazilian (SLV of ST 8)
	IV (29)	+ (28)	7-6-1-5-8-8-6	22:22	United Kingdom (EMRSA-15)
	V (13)	+ (13)	1-1-1-1-22-1-1	772:1	Asia (SLV of ST 1)
MSSA (11)	NA ^d	+ (11)	2-2-2-2-6-3-2	30:30	Southwest Pacific (USA 1100)

^a MLST, multilocus sequence type.
^b MLST profile: arc-aro-glp-gmk-pti-yqi.
^c ST, sequence type; CC, clonal complex.
^d NA, not applicable.

be more comprehensive. However, consecutive samples were included to try and represent the situation at a tertiary care center laboratory which serves as a referral laboratory by default. Molecular characterization using multiplex PCR assays showed that 25% of the MRSA strains were SCCmec III, 34% were SCCmec IV, and 41% were SCCmec V. None of the SCCmec III MRSA strains were PVL⁺, but 90% of SCCmec IV and 62% of SCCmec V strains were PVL⁺. While all SCCmec III strains showed multidrug-resistant phenotypes, all SCCmec IV strains and around 88% of SCCmec V strains were multidrug susceptible (Fig. 1) (10). All patients with SCCmec III strain infections had multiple risk factors for MRSA infections. On the other hand, 76% of patients with SCCmec IV strains and 68% of patients with SCCmec V strains had no documented risk factors (Table 2 and Fig. 2). This particular multidrug susceptibility pattern and absence of risk factors among patients with SCCmec IV and V strains show the emergence of CA-MRSA (Fig. 1 and 2) (5). This is an alarming trend, as such a large portion of patients who had no apparent risk factors seem to have acquired these infections, hence posing a major threat to public health. Even though these “community-acquired” infections were relatively susceptible to multiple antimicrobial classes compared to HA-MRSA, 60% of patients had to be hospitalized or have surgery, as the infections were rather severe, a majority of them being caused by PVL⁺ MRSA (2). No SCCmec III MRSA strain in this study was PVL positive (Table 4).

Thirteen SCCmec III MRSA strains were found to be ST 239, corresponding with the sequence of the Brazilian/Hungarian clone belonging to clonal complex (CC) 8. ST 239 strains are the endemic HA-MRSA clone in many Asian countries, although recent studies show that they are being steadily dis-

placed by emerging CA-MRSA clones (5, 10, 12, 19, 22). ST 239, a single-locus variant (SLV) of USA 300, is thought to have evolved from ST 8 (the USA 300 clone lineage) and ST 30 (the *S. aureus* phage type 80/81, USA 1100 clone lineage) (5, 15, 17, 19, 28, 31). A total of 29 SCCmec IV (28 of subtype c and 1 of subtype a) MRSA strains were ST 22 (CC 22), corresponding with the sequence type of the epidemic United Kingdom EMRSA-15 strain (5, 10, 12, 22). It has now spread to other parts of the world, especially in many Asian countries. All 13 SCCmec V MRSA strains were ST 772 (CC 1), corresponding with the sequence type of other strains from India (New Delhi and Karnataka) collected by another group in a previous study, information about which has been deposited in the MLST database but is unpublished as yet. The 772 sequence type, an SLV of ST 1 (USA 400) (MW2), was first reported in Bangladesh and has also been reported in Malaysia this year (1, 25). A global epidemiological trial showed the occurrence of ST 239, ST 22, and ST 772 *S. aureus* in India (8). In this study, the numbers of SCCmec III MRSA strains isolated in the laboratory have been gradually decreasing from 2006 to 2009, whereas the isolation of SCCmec IV and SCCmec V MRSA has increased further with each year (10). The MLST data, along with the other parameters studied, illustrate clonal expansion of the ST 22 and ST 772 lineages among strains in Mumbai. MLST carried out for 11 PVL⁺ MSSA strains showed that all were ST 30 (CC 30), indicating that they are related to the USA 1100 clone from the Southwest Pacific United States (Table 3) (5, 8, 15, 28). The ST 30 PVL⁺ MSSA strains are postulated to be descendants of the historical penicillin-resistant epidemic *S. aureus* phage type 80/81 (5, 15, 28). The ST 30 clone has been implicated as the predominant clonal type in CA-MRSA isolates (SCCmec IVc) in Singapore (11). Since the present study findings show that the SCCmec IVc cassette is present among the CA-MRSA strains, it is possible that the ST 30/SCCmec IVc MRSA may emerge as an important community-acquired epidemic clone (like USA 1100) in Mumbai in the future, as it has in Singapore (11, 28).

In India, probably due to overcrowding and poor personal hygiene, CA-MRSA has spread. Although it mainly manifests in severe soft tissue and skin infections requiring surgical drainage, it is now becoming pronounced in bacteremias affecting neonates, especially from lower economic sections, and breast abscesses in lactating mothers, becoming increasingly common in urban areas. In this study, all patients with SCCmec III MRSA had risk factors, but a significant 21% of patients with strains of the SCCmec IV and SCCmec V MRSA also had

TABLE 4. Characteristics of MRSA SCCmec types in the study

SCCmec	PVL status	Drug of resistance (% of resistant strains) ^a	Infection (% with infection) ^b
III	100% PVL ⁻	MDR (100)	BSI (19) RTI (12)
IV, V	75% PVL ⁺	ERY (74) CLI (42) CIP (90) TET (25)	SSTI (66) BA (6)

^a MDR, multiple drug resistance; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; TET, tetracycline.
^b BSI, bloodstream infection; RTI, respiratory tract infection; BA, breast abscess; SSTI, other skin and soft tissue infections.

risk factors. Therefore, it can be concluded that 25% were true HA-MRSA strains and 54% were true CA-MRSA strains (5, 22, 30). Since 21% of strains had the SCCmec IV or V cassette but the patients had risk factors, the spread of these genotypes, earlier associated only with CA-MRSA, into the hospital environment is indicated (5, 30).

SCCmec characterization by multiplex PCR assays, multilocus sequence typing, antimicrobial susceptibility testing, and documentation of patient risk factors enabled successful characterization of strains isolated in the laboratory, confirming the emergence of CA-MRSA in Mumbai, with dominant epidemic clones like ST 22 (EMRSA-15) and ST 772. Since the numbers of SCCmec IV and V MRSA strains isolated in the laboratory have been increasing and the number of SCCmec III MRSA strains has been decreasing every year from 2006 to 2009, it is probable that the ST 22 and ST 772 clones may be replacing the ST 239 clone in the hospital (5, 10, 12, 22). There have been many studies conducted and reviews written in the last few years about CA-MRSA in hospital settings, blurring the fine line that differentiates it from HA-MRSA (5, 22, 30). It can also be concluded that defining CA-MRSA merely by the absence of risk factors for health care exposure in patients would underestimate the real burden of its incidence today, since many CA-MRSA clones have successfully established themselves in hospital settings (5, 30). Since it has been hypothesized that all the major MRSA clones could have evolved from one common ancestor, *S. aureus* phage type 80/81, the ST 30 PVL⁺ MSSA strains in the hospitals or communities today may one day evolve into "successful" MRSA epidemic clones themselves (28). With the recent pandemic swine origin influenza virus A (H1N1), CA-MRSA is adept enough to cause severe pneumonia with increased morbidity and mortality (3, 7). Just as the SCCmec IV ST 22 (EMRSA-15) has spread over Europe and Asia, the SCCmec V ST 772, which currently seems to be native to Asia, may emerge as a powerful global epidemic MRSA strain in the years to come.

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We have no conflict of interest to declare.

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