ORIGINAL ARTICLE

Epidemiology of *Staphylococcus aureus* in Bangalore, India: emergence of the ST217 clone and high rate of resistance to erythromycin and ciprofloxacin in the community

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Abstract

This study aimed to determine the antibiotic susceptibility pattern of *Staphylococcus aureus* (SA) and the circulating clones in Bangalore, India. Susceptibility testing was performed for all cases of SA infections in a tertiary-care hospital. Panton-Valentine leucocidin (*PVL*) encoding genes were detected, and sequence type and *spa* type were determined. Out of the 92 collected strains, 52.2% were methicillin-resistant SA (MRSA), isolated from community-acquired (CA) infections in 60.4% and hospital-acquired (HA) infections in 39.6%. *S. aureus* isolates were also highly resistant to erythromycin (54.3%) and ciprofloxacin (70.6%) in methicillin-susceptible SA (MSSA) and MRSA, as well as in CA and HA infections. MRSA were found to be significantly more resistant to gentamicin ($p < 0.001$), cotrimoxazole ($p < 0.001$) and ciprofloxacin (p 0.001) than MSSA, but no significant difference was observed between CA- and HA-SA. ST217 appeared as a new emerging and prevalent clone, but ST772 remained the predominant clone, all being *PVL*-positive isolates. Our study points out the high prevalence of MRSA, even in the community, and the worrying increase of resistance to ciprofloxacin and erythromycin among CA-MSSA. Emergence of clone ST217 is reported for the first time in India.

Keywords: Antibiotic resistance, community-acquired infection, emergence, India, ST217, ST772, *Staphylococcus aureus*
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important causes of antibiotic-resistant healthcare-associated infections worldwide. MRSA was initially associated with hospital-acquired (HA) infections and since the 2000s with community-acquired (CA) infections [1]. In Europe, the percentages of invasive MRSA extend from less than 5% in Northern European countries to more than 25% in southern and Eastern European countries [2]. In the US military health system and over a period extending from 2005 to 2010, 54% of cases of hospital-onset bacteremia were due to MRSA [3]. The prevalence of CA-MRSA infections varies widely among countries [4]. In the United States, CA-MRSA is one of the most common cause of skin and soft tissue infections (SSTIs) in patients from emergency departments, and the clone USA300 (ST8-SCCmec IV) is largely predominant [5]. In Europe, the prevalence of CA-MRSA is much lower but is increasing, especially in countries where the incidence of HA-MRSA is low, such as Denmark or the Netherlands [6]. Many different CA-MRSA clones are found, of which the ST80-SCCmec IV European clone is the most widely disseminated [6,7].

In India, the consumption of antibiotics is considerable, and the overall rate of MRSA in clinical specimens is significant. In a

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hospital located in eastern Uttar Pradesh, 54.8% of the *S. aureus* isolates were reported as MRSA [8]. These isolates were also resistant to multiple antibiotics: over 80% were resistant to penicillin, cotrimoxazole, ciprofloxacin, gentamicin, erythromycin and tetracycline [8]. In a study carried out in the neonatal intensive care unit in Amritsar, the rate of MRSA in blood cultures was 57.3% [9]. In India, the rate of MRSA carriage in the community is also high. Indeed, Chatterjee *et al.* [10] showed that 3.89% of children of an Indian community setting were MRSA carriers. In Delhi, 18.1% of healthy parents attending a well-baby clinic were shown to be MRSA carriers [11]. MRSA from India belong to a wide variety of clones, with the following predominant sequence types (ST): ST22, ST772, ST239 and ST30 [12,13].

In this work, we collected the clinical characteristics of 92 cases of *S. aureus* infections at St. John's hospital in Bangalore (Karnataka) and analysed bacteriologic characteristics and the antibiotic resistance phenotype of the corresponding isolates in order to determine the rate of MRSA in CA and HA infections and the different circulating clones.

Materials and methods

Patients

Cases of *S. aureus* infections diagnosed at the St. John's hospital from November 2011 to February 2012 were collected. For each case, a questionnaire was filled out by the physician in charge of the patient including the patient's sex, age and type of infection: skin and soft tissue infection (SSTI), urinary tract infection, respiratory infection, bone and joint infection and sepsis. CA *Staphylococcus aureus* (SA) infection was defined according to the US Centers for Disease Control and Prevention definition for CA-MRSA when patients did not meet any of the following criteria: (a) isolation of SA more than 48 hours after hospital admission; (b) history of hospitalization, surgery or dialysis within 1 year of the SA culture; and (c) presence of an indwelling catheter or a percutaneous device at the time of culture.

Identification and susceptibility testing

S. aureus strains were isolated from chromogenic agar medium (ChromAgar; BioMérieux, Marcy-L'Etoile, France). Gram staining was performed; the presence of catalase, coagulase and DNase was confirmed, as described elsewhere [14]. Antimicrobial susceptibility testing was performed for erythromycin, gentamicin, amikacin, tetracycline, chloramphenicol, cotrimoxazole and ciprofloxacin with the disk diffusion method according to recommendations of the Clinical Laboratory Standard Institute [15] on Mueller-Hinton agar plates (Himedia, Mumbai, India) at 37°C. Methicillin resistance was tested using a cefoxitin disk on Mueller-Hinton agar [16].

Molecular characterization

Genomic DNA was extracted from an overnight culture of cells in brain–heart infusion broth by using a standard phenol–chloroform procedure [17]. Detection of the Panton-Valentine leukocidin (*pvl*) encoding genes was carried out by PCR as previously described [18]. Multilocus sequence typing was performed as previously described [19]. Consensus sequences were assembled from both orientations and the allelic profile was matched using the Multi Locus Sequence Typing database (http://www.mlst.net/). Staphylococcal protein A (Spa) typing was performed according to the procedure of Shopsin *et al.* [20]. Consensus sequences were matched from both forward and reverse sequences, and the repeat units were identified using the Ridom database (http://spa.ridom.de/).

Statistical analysis

Chi-square test and Fisher's exact test were used for comparing proportions. Confidence intervals were calculated for proportions to allow sampling error.

Ethics committee approval

The Rural Development Trust Hospital Ethical Committee approved the study.

Results

Patient characteristics and types of infections

Over the study period, 92 *S. aureus* clinical isolates were collected and further analysed. Patients' median age was 43 years (range, 7 days to 91 years), and 16 patients were younger than 18 (17.4%). Thirty-four patients (37.2%) were hospitalized in medical units, 41 (44.6%) in surgical units and 17 (18.2%) in intensive care units. Male/female sex ratio was 2.29. Most of the cases comprised SSTIs (n = 63, 68.5%), bone and joint infections (n = 11, 12.0%) and respiratory tract infections (n = 13, 14.1%) (Table 1). Three patients had septic shock; one had a urinary

TABLE 1. Types of *Staphylococcus aureus* infections collected in Bangalore between November 2011 and February 2012

Site	CA infections	HA infections	Total, *n* (%)
Skin and soft tissue infections	43	20	63 (68.5)
Bone and joint infections	10	1	11 (12.0)
Respiratory infections	5	8	13 (14.1)
Sepsis	2	2	4 (4.3)
Urinary tract infection	1	0	1 (1.0)
Total	61	31	92

CA, community acquired; HA, hospital acquired.

tract infection. CA infections accounted for 66.3% of the cases (n = 61) and HA infections for 33.7% (n = 31).

Strain characteristics

The overall MRSA rate was 52.2% and the major associated resistances of the 92 isolates concerned erythromycin (n = 50, 54.3%) and ciprofloxacin (n = 65,70.6%) (Table 2). The percentage of resistance to erythromycin was not significantly different between MSSA and MRSA. The percentage of resistance to ciprofloxacin was extremely high for MRSA (n = 41, 85.4%) and also for MSSA (n = 24, 54.5%). MRSA were significantly more resistant to gentamicin (p <0.001), cotrimoxazole (p <0.001) and ciprofloxacin (p 0.001) than MSSA (Table 2). Of note, all the isolates were susceptible to vancomycin. MRSA were detected in 47.5% (n = 29) of the CA infections and 61.3% (n = 19) of the HA infections (p 0.27). A comparison of the antibiotic resistance profiles of CA- vs. HA-MRSA and those of CA- vs. HA-MSSA showed no significant differences (Table 3).

Spa types STs were determined for all the 92 isolates. Sequence type 772 was the most frequently detected ST (n = 18, 19.6%), either in MRSA or MSSA isolates, either in CA or HA infections (Table 4). Specific *spa* types differentiated ST772 isolates as MSSA (t345 and t11383) or MRSA (t3387 and t657) (data not shown); all were Panton-Valentine leucocidin (*PVL*) positive. The second most prevalent ST was ST217, detected in 13 isolates (14.1%), mostly in MRSA isolates (n = 12) and in both CA and HA infections; all were *PVL* positive. Both major STs ST772 and ST217 were highly resistant to erythromycin (50% and 69.2%, respectively) and ciprofloxacin (83.3% and 76.9%, respectively). However, ST772 was significantly more resistant to cotrimoxazole (77.8%) than ST217 (15.4%, p 0.001). MRSA and MSSA were also detected in isolates belonging to ST1208 and ST239, both in CA and HA infections. ST22 was detected for MRSA isolates associated with HA infections only. ST5, ST30 and ST291 were detected for MSSA only, associated with HA and CA infections. ST1, ST7, ST9, ST45, ST88, ST672, ST2371 and ST2849 were detected for MSSA isolates associated with CA infections only.

PVL encoding genes were detected in 49 of the 92 strains (53.3%). The *PVL* positive strains were associated with either HA (54.8%) or CA (52.5%) infections. However, *PVL* were found to be significantly more prevalent in MRSA isolates (68.8%) than in MSSA isolates (36.4%, p <0.05).

Discussion

In this study, we showed that from a prospective analysis of 92 cases of *S. aureus* infections from Bangalore, the rate of MRSA was 52.2%: in CA infections 47.5% and in HA infections 61.3%. Most of the specimens were isolated from SSTI, which is a major cause of *S. aureus* disease in the community as well as in hospitalized patients. Previous studies performed in both northern and southern India have reported rates of CA-MRSA of 9.4% and 10.9%, r; however, another study in a rural area of Andhra Pradesh showed a CA-MRSA prevalence of 64.7% [21–23]. This is consistent with a study performed in and around Bangalore, where Goud *et al.* [24] reported a rate of MRSA nasal carriage of 72.7% in healthy individuals. CA-MRSA emergence has been described from every continent, even though prevalence varies considerably between different parts of the world [25]. Hence, compared to European countries, CA-MRSA infection rate is particularly high in India, as well as the United States [4,26]. Besides CA-MRSA prevalence, we confirmed that the proportion of HA-MRSA was also important in our study (61.3%; 70.7% in the Alvarez-Uria study [23]).

Resistance to erythromycin was observed in 54.3% of *S. aureus,* with no significant difference between MSSA and MRSA or CA and HA isolates. Another study reports slightly lower rates in a rural setting in India (41.4% of CA-SA and 38% of HA-SA) [23]. However in Indian tertiary-care hospitals, Anupurba *et al.* [8] reported that more than 80% of HA-MRSA were resistant to erythromycin, and Kini *et al.* [27] found a resistance rate to erythromycin of 67% and 83% in CA-MSSA and CA-MRSA, respectively. When considering European countries, data differ depending on the area. When only 2% and

TABLE 2. Antibiotic resistance profiles of 92 isolates of *Staphylococcus aureus* collected between November 2011 and February 2012 in Bangalore

Antibiotic	*S. aureus* (95%) (95% CI)	MSSA (44%) (95% CI)	MRSA (48%) (95% CI)	p, MSSA vs. MRSA	CA *S. aureus* (61%) (95% CI)	HA *S. aureus* (31%) (95% CI)	p, CA *S. aureus* vs. HA *S. aureus*
Penicillin	91.3 (91.2–91.4)	81.8 (70.4–93.2)	100	0.002	86.9 (78.4–95.4)	100	0.04
Oxacillin	52.2 (42–62.4)	0	100	NA	47.5 (35–60)	61.3 (44.2–78.4)	0.27
Gentamicin	34.7 (25–44.4)	9.1 (0.6–17.6)	58.3 (44.4–72.2)	<0.001	36.1 (24–48.2)	32.3 (15.8–48.8)	0.82
Tetracycline	19.6 (11.5–27.7)	15.9 (5.1–26.7)	22.9 (11–34.8)	0.44	18.0 (8.4–27.6)	22.6 (7.9–37.3)	0.60
Erythromycin	54.3 (44.1–64.5)	45.5 (30.8–60.2)	62.5 (48.8–76.2)	0.14	52.5 (40–65)	58.1 (40.7–75.5)	0.66
Chloramphenicol	3.3 (0–7)	2.3 (0–6.7)	4.2 (0–9.9)	1.00	3.3 (0–7.8)	3.2 (0–9.4)	1.00
Cotrimoxazole	40.2 (30.2–50.2)	20.5 (8.6–32.4)	58.3 (44.4–72.2)	<0.001	37.7 (25.5–49.9)	45.2 (27.7–62.7)	0.51
Ciprofloxacin	70.6 (61.3–79.9)	54.5 (39.8–69.2)	85.4 (75.4–95.4)	0.001	67.2 (55.4–79)	77.4 (62.7–92.1)	0.34

CI, confidence interval; MSSA, methicillin-susceptible *Staphylococcus aureus;* MRSA, methicillin-resistant *Staphylococcus aureus;* CA, community acquired; HA, hospital acquired; NA, not applicable.

TABLE 3. Comparison of antibiotic resistance profiles of *Staphylococcus aureus* samples collected between November 2011 and February 2012 in Bangalore

Antibiotic	CA MRSA (29%) (95% CI)	HA MRSA (19%) (95% CI)	p, CA MRSA vs. HA MRSA	CA MSSA (32%) (95% CI)	HA MSSA (12%) (95% CI)	p, CA *S. aureus* vs. HA *S. aureus*
Penicillin	100	100	NA	75.0 (60–90)	100	0.08
Gentamicin	69.0 (52.2–85.8)	42.1 (19.9–64.3)	0.08	6.3 (0–14.7)	8.3 (0–23.9)	1.00
Tetracycline	20.7 (6–35.4)	26.3 (6.5–46.1)	0.73	15.6 (3–28.2)	16.7 (0–37.8)	1.00
Erythromycin	62.1 (44.4–79.8)	63.1 (41.4–84.8)	1.00	43.8 (26.6–61)	41.7 (13.8–69.6)	1.00
Chloramphenicol	6.9 (0–16.1)	0	0.51	0	8.3 (0–23.9)	0.27
Cotrimoxazole	58.6 (40.7–76.5)	57.9 (35.7–80.1)	1.00	18.8 (5.3–32.3)	16.7 (0–37.8)	1.00
Ciprofloxacin	79.3 (64.6–94)	94.7 (84.6–100)	0.22	56.3 (39.1–73.5)	50 (21.7–78.3)	0.75

CI, confidence interval; CA, community acquired; HA, hospital acquired; MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-susceptible *Staphylococcus aureus;* NA, not applicable.

15% of *S. aureus* collected from nursing home residents in the Netherlands and Germany, respectively, were resistant to macrolides [28], Adaleti *et al.* [29] reported a prevalence of 54.6% of erythromycin-resistant *S. aureus* in a hospital in Istanbul, Turkey. In France, 21.2% of MSSA and 46.3% of MRSA were resistant to erythromycin according to national survey networks reports in 2008 (Rapport d'activité ONERBA, 2009, http://www.onerba.org/IMG/pdf/ONERBA_rap2009-10_CH6-2.pdf). Increasing resistance to erythromycin is of great concern because macrolides are useful in cases of intolerance to β-lactams or resistance to methicillin and exert beneficial antitoxin effects.

Finally, we found a high overall resistance to ciprofloxacin (70.6%). Although resistance to fluoroquinolones has been widely described among MRSA, we report here that MSSA, and especially CA-MSSA, are also strikingly resistant (54.5% and 56.3%, respectively). Ciprofloxacin resistance in MSSA has been rarely described except in India, where a nationwide study reported a prevalence of 46.6% of MSSA resistant to ciprofloxacin in tertiary-care centres [30]. In another study in India, 48% of CA-MSSA isolated from children with CA bone and joint infections were resistant to ciprofloxacin [27]. Between 2011 and 2012 in a rural hospital in Andhra Pradesh, another area close to Bangalore, the rate of CA-MSSA resistant to ciprofloxacin was 57.1% [23]. The increase of ciprofloxacin resistance among MSSA has also been observed in other parts of the world [31,32], but very few data are available. In France, 6.2% of MSSA were resistant to fluoroquinolones in 2008 (Rapport d'activité ONERBA, 2009).

We characterized *S. aureus* isolates, which we found to belong to a wide variety of STs. Indeed, the 92 analysed isolates fell into 19 different STs, and MSSA isolates were the most diverse. ST772 was the most predominant clone and was detected in MSSA and MRSA associated with CA and HA infections. Among MRSA isolates, ST772 was the predominant clone, along with ST217, ST239, ST22 and ST1208. The occurrence of ST772, ST22 and ST239 in India has already been reported in global epidemiologic trials [12,33]. Subsequently,

TABLE 4. Sequence types (ST) and *spa* types of *Staphylococcus aureus* isolates from Bangalore according to origin of acquisition of infection

		MSSA			MRSA			
Sequence type (*n*)	*spa* type (*n*)	*n* (%)	CA infection, *n* (%)	HA infection, *n* (%)	*n* (%)	CA infection, *n* (%)	HA infection, *n* (%)	*PVL* genes
ST772 (18)	t11383 (1), t3387 (2), t345 (1), t657 (14)	2 (4.4)	2 (5.9)	0 (0)	16 (34.8)	11 (40.7)	5 (26.3)	18/18
ST217 (13)	t852 (9), t11682(1), t5(1), fail (2)	1 (2.2)	0 (0)	1 (8.3)	12 (26.1)	8 (29.7)	4 (21.1)	13/13
ST239 (11)	t037 (10), t852 (1)	2 (4.4)	1 (2.9)	1 (8.3)	9 (19.6)	5 (18.5)	4 (21.1)	0/11
ST1208 (8)	t064 (3), t008 (1), t2658 (2), t304 (2)	5 (10.9)	4 (11.8)	1 (8.3)	3 (6.5)	1 (3.7)	2 (10.5)	0/8
ST30 (8)	t021 (6), t4109 (1), t318 (1)	8 (17.4)	6 (17.6)	2 (16.7)	0 (0)	0 (0)	0 (0)	8/8
ST5 (8)	t442 (7), t491 (1)	8 (17.4)	5 (14.7)	3 (25.0)	0 (0)	0 (0)	0 (0)	2/8
ST291 (5)	t1149 (2), t3096 (1), t2313 (1), t937 (1)	5 (10.9)	4 (11.8)	1 (8.3)	0 (0)	0 (0)	0 (0)	0/5
ST22 (4)	t852 (4)	0 (0)	0 (0)	0 (0)	4 (8.7)	0 (0)	4 (21.1)	4/4
ST2371 (3)	t852 (3)	2 (4.4)	2 (5.9)	0 (0)	1 (2.1)	1 (3.7)	0 (0)	3/3
ST672 (2)	t3841 (2)	2 (4.4)	2 (6.5)	0 (0)	0 (0)	0 (0)	0 (0)	0/2
ST7 (2)	t091 (1), t1243 (1)	2 (4.4)	2 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0/2
ST88 (1)	t186 (1)	1 (2.2)	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	0/1
ST2849 (1)	t878 (1)	1 (2.2)	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	0/1
ST121 (1)	t159 (1)	1 (2.2)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	1/1
ST689 (1)	t91(1)	1 (2.2)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	0/1
ST580 (1)	t12355 (1)	1 (2.2)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	0/1
ST3049 (1)	Fail (1),	0 (0),	0 (0),	0 (0),	1 (2.1),	1 (3.7),	0 (0)	0/1
ST9 (1)	t547 (1),	1 (2.6),	1 (2.9),	0 (0),	0 (0),	0 (0),	0 (0)	0/1
ST1 (2)	t127 (2),	2 (5.1),	2 (5.9),	0 (0),	0 (0),	0 (0),	0 (0)	0/2
ST45 (1)	t015 (1)	1 (5.1)	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	0/1
Total		46	34	12	46	27	19	49/92

MSSA, methicillin-susceptible *Staphylococcus aureus;* MRSA, methicillin-resistant *Staphylococcus aureus;* CA, community acquired; HA, hospital acquired.

D'Souza *et al*. [13] described the emergence of ST772 and ST22 replacing ST239 in Indian hospitals. ST772 has now been also reported in England, Hong Kong, Germany, Abu Dhabi and Ireland [33–35]. The second most frequent ST was ST217 and was mainly detected in MRSA isolates, either CA or HA. In our study, the MRSA strains belonging to the ST217 clone harboured a group I *agr* and a class B *mec* complex with a *ccrA2B2* (type 2) *ccr* complex, consistent with a type IV SCC*mec* cassette (data not shown), as previously described [36,37]. ST217 has been identified as a single locus variant of epidemic MRSA-15 within CC22 [38], and it has been detected only rarely in humans in Italy and Switzerland [36], as well as in one animal [37]. The presence of ST217 has not yet been reported in India, suggesting the emergence of this clone in this area. Finally, a large diversity of other STs were characterized, some of them being common to MRSA and MSSA (ST1208, ST239 and ST291). ST1208, accounting for almost 12% of our isolates, was reported as a novel clone in 2012 in India [12].

In conclusion, we collected 92 *S. aureus* isolated from CA and HA infections in a tertiary-care hospital in Bangalore. A worrying proportion of MSSA isolates was resistant to fluoroquinolones, even among CA-MSSA. MRSA occurrence was also important in both hospital and community settings, and MRSA isolates were multidrug resistant. However, the distinction between CA- and HA-MRSA is blurring because the CA-MRSA resistance profile is no longer significantly different from that of HA-MRSA. ST772 was the predominating clone, as is usually reported in India these days. We report here the emergence of ST217 in Bangalore in CA and HA infections, with a high rate of resistance to erythromycin and ciprofloxacin. This clone has rarely been detected elsewhere in the world.

Conflict of interest

None declared.

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