



## Bacteriophage types of methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital

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### RESEARCH

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### Abstract

#### Background

Phage typing had been utilised extensively to characterise methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak strains in the past. It is an invaluable tool even today to monitor emergence and dissemination of MRSA strains.

#### Aims

The aim of this study was to determine the prevalent phage types of MRSA in south India and the association between phage types, antibiotic resistance pattern and risk factors.

#### Method

A total of 48 non-duplicate MRSA strains recovered from various clinical samples during January to December, 2010 were tested against a panel of anti-staphylococcal antibiotics. Phage typing was carried out at the National Staphylococcal Phage Typing Centre, New Delhi. Out of 48, 32 hospitalised patients were followed up for risk factors and response to empirical and post sensitivity antibiotic therapy. The risk factors were compared with a control group of 30 patients with methicillin sensitive *Staphylococcus aureus* (MSSA) infection.

#### Results

Amongst the five prevalent phage types, 42E was most common (52%), followed by a non-typable variant (22.9%), 42E/47/54/75 (16.6%), 42E/47 (6.2%) and 47 (2%). Phage type 42E was the predominant strain in all wards and OPDs except in the ICU where 42E/47/54/75 was most common. Although not statistically significant, strain 42E/47/54/75 (n=8) showed higher resistance to all drugs, except ciprofloxacin and amikacin, and were mostly D-test positive (87.5%) compared to the 42E strain (32%). Duration of hospital stay, intravenous catheterisation and breach in skin were the most significant risk factors for MRSA infection.

#### Conclusion

We found MRSA strain diversity in hospital wards with differences in their antibiotic susceptibility pattern. The findings may impact infection control and antibiotic policy significantly.

#### Key Words

MRSA; phage types; risk factors

#### What this study adds:

1. Methicillin-resistant *Staphylococcus aureus* is a worldwide threat to infection control measures in hospitals. Phage typing had been successfully utilised to differentiate MRSA isolates.
2. Two major phage types had characteristic distribution in hospital wards. Four out of six ICU isolates of MRSA (66.7%) were 42E/47/54/75 phage type with higher levels of antibiotic resistance.
3. The unique distribution, resistance pattern of MRSA phage types and their association with risk factors can guide effective infection control measures and antibiotic policy.

#### Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a highly versatile pathogen with the potential to cause serious infections in both the community and hospitals. The epidemiology of MRSA is subjected to continual change worldwide and a significant change has been observed in



the last few decades. MRSA have emerged as successful pathogens in the community. Community-acquired MRSA (CA-MRSA) strains display an array of virulence factors *viz.* Panton-Valentine leukocidin (PVL), enterotoxins, phenol soluble modulins and secreted proteases and can be discriminated from hospital-acquired MRSA (HA-MRSA) by SCCmec typing.<sup>1,2</sup> Recently, CA-MRSA strains have also been reported in several hospital-acquired infections.<sup>3</sup> There has been a constant reduction in susceptibility of MRSA to several antibiotics including vancomycin over time.<sup>4,5</sup> It has been proposed that five major pandemic MRSA clones *viz.* Iberian, Brazilian, Hungarian, New York/Japan and paediatric clones emerged from methicillin sensitive *Staphylococcus aureus* (MSSA) at different time points and spread globally.<sup>6</sup> Later mutations accumulated in the genome and new clones emerged from these clones. Some of these strains, which had the potential to disseminate between healthcare facilities over a large area, have been traditionally designated as epidemic MRSA (EMRSA). The prevalent EMRSA strains vary in different parts of the world. MSSA phage type 80/81 was responsible for hospital epidemics and caused a serious health care crisis in 1950.<sup>7</sup>

Recently, a South West Pacific (SWP) clone of MRSA has drawn special attention due to its similarity of genomic background with MSSA phage type 80/81 and simultaneous acquisition of PVL.<sup>7</sup> Therefore, it is important to establish a distinction between closely related members of *S. aureus* for tracing the dissemination of a particular strain or the emergence of a new strain in a local region as well as to recognise evolutionary aspects. Apart from phage typing, genotypic methods *viz.* SCCmec typing, repetitive DNA sequence analysis of *spa*, *coa*, *clfB* genes, Internal Transcribed Spacer PCR, Pulse Field Gel Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP) and Multi Locus Sequence Typing (MLST) were also employed for strain differentiation. Genotypic methods are superior to phenotypic methods as they not only determine the genetic differences between strains, but can also be used to study their evolution or transformation over a period of time. However, they are not readily available in resource-limited settings.

Phage typing has been used for differentiating between MRSA outbreak strains. It is also invaluable in monitoring the emergence and dissemination of MRSA strains in hospitals and the community. However, recent data about phage types of MRSA strains from South India is inadequate. The last report was from Bangalore in 2002.<sup>8</sup> Therefore, this study was carried out to determine prevalent MRSA phage types in our hospital and the association between phage types, antibiotic resistance pattern and risk factors.

## Method

This prospective study was carried out in the Mahatma Gandhi Medical College and Research Institute, a tertiary care hospital catering to patients from Pondicherry and neighbouring districts of Tamil Nadu, India.

We included 48 MRSA clinical isolates recovered during January to December 2010 in this study. Consecutive isolates of MRSA from the same patient are excluded. Various clinical specimens (pus, blood, urine, respiratory and genital tract samples and body fluids) were cultured on blood agar and Mac Conkey agar aerobically for 24 hours. *S. aureus* isolates were identified in the laboratory, based on gram stain and colony morphology, mannitol fermentation, tube coagulase and DNase test. The disk diffusion test using 30µg cefoxitin disk, 1µg oxacillin disk and oxacillin screen agar (containing 6µg/ml oxacillin & 4% NaCl) tests were performed as per CLSI guidelines to detect MRSA stains. A panel of commonly used anti-staphylococcal antibiotics comprising of ciprofloxacin (5µg), tetracycline (30µg), gentamicin (10µg), amikacin (30µg), netilmicin (30µg), cotrimoxazole (1.25+23.75 µg), chloramphenicol (30µg), erythromycin (15µg), vancomycin (30µg), linezolid (30µg), clindamycin (5µg), mupirocin (5µg), rifampicin (5µg) were tested using the Kirby Bauer disk diffusion method for susceptibility pattern. *S. aureus* strains ATCC 25923 and ATCC 43300 were used as controls for antibiotic susceptibility test. The viability of test isolates was maintained by periodic subculture in semisolid nutrient agar. Erythromycin resistant MRSA isolates were subjected to D test for detection of inducible clindamycin resistance as per CLSI guidelines. A lawn culture of MRSA isolates was prepared on Mueller Hinton agar. Erythromycin (15µg) disk and clindamycin (2µg) disk are placed edge to edge 15mm apart and incubated overnight at 37°C. Inducible clindamycin resistant strains show sensitive zone around clindamycin disk (zone size ≥21mm) with a flattening towards erythromycin disk.

Phage typing was carried out at the National Staphylococcal Phage Typing Centre, Maulana Azad Medical College, New Delhi. A lawn culture of each test strain on nutrient agar plate was tested against 23 basic set of phage. After incubation at 30°C for 18 hours, the results were interpreted in terms of strong or weak reaction according to areas of lysis or plaques caused by routine test dilution (RTD) & 100X RTD of each phage. More than 50 plaques to semi-confluent or confluent lysis were regarded as strong reaction (++), while less than 50 plaques *i.e.*, 20 to 50 plaques (+) and less than 20 plaques (±) were considered as weak reaction. The phage type of the strain was described in terms of a set of phages that produced strong lytic



reaction of the strain. Isolates were considered indistinguishable if they did not differ by more than one strong reaction.

We followed up all 32 inpatients hospitalised during the period of study for risk factors and noted their response to empirical and post sensitivity antibiotic therapy. Positive responses to antibiotics were considered in cases of remission of fever, decrease in pus discharge, acceleration of wound healing and general improvement of health. The risk factors among inpatients with MRSA infection were compared with a control group comprising a randomly selected 30 inpatients with MSSA infection. The risk factors investigated were length of stay in hospital, past history of MRSA infection, previous hospitalisation, antibiotic exposure, breach in skin continuity, other comorbid conditions and invasive procedures such as intravenous catheters, urinary catheters, endotracheal tubes, nasogastric tubes and central lines. Since 7 out of 16 outpatients were lost in follow-up, we analysed risk factors in the remaining nine patients. We followed CDC Active Bacterial Core Surveillance, 2011 case definition to discriminate CA-MRSA from HA-MRSA.<sup>9</sup> Accordingly, all patients with positive MRSA culture on or before the fourth day of hospitalisation in the absence of HA-MRSA risk factors *viz.* history of hospitalisation, surgery, dialysis, or residence in a long-term care facility in the previous year, or the presence of a central vascular catheter within two days prior to MRSA culture, were classified as CA-MRSA. In addition to healthcare associated risk factors, we investigated risk factors such as intravenous drug addiction, immune suppression, homosexuality, involvement in contact sports and sharing of personal items, breach in skin and poor living conditions with overcrowding in this group.

All data was tabulated in Microsoft Excel 2007 and GraphPad InStat software version 3.00 (San Diego, CA, USA) was utilised for statistical analysis. The Chi-square test and odds ratio were used to compare two groups. All p values < 0.05 were considered statistically significant.

### Results

Of the 48 cases studied, 16 were outpatients, 32 were inpatients and were predominately male (70.83%). As per CDC Active Bacterial Core Surveillance, 2011 case definition, all 16 outpatients had CA MRSA and 32 inpatients had HA-MRSA. However, seven outpatients were lost to follow-up and we were unable to investigate them for MRSA risk factors. MRSA infection was most common in the 18-40 year age group (47.91%), followed by 41-65 years (31.25%), above 66 years and under-five age groups (10.41% each). MRSA stains were most prevalent in general surgery

(20.83%) and the orthopaedic ward (14.58%), followed by ICU, gynaecology, paediatric surgery and medicine wards in decreasing order of frequencies. The most common samples were pus swabs and aspirates (87.5%), followed by respiratory samples (6.25%), blood samples (4.16%) and vaginal swabs (2.08%).

**Table 1: Antimicrobial resistance pattern of MRSA phage types**

	42E (n=25)	42E/47/54/75 (n=8)	42E /47	47 (n=1)	Non- typable
D-test positive	8 32%	7 87.5%	0	1 100%	4 36.3%
Erythromycin	17 68%	7 87.5%	2 66.6%	1 100%	6 54.4%
Ciprofloxacin	20 80%	6 75%	2 66.6%	1 100%	10 90%
Tetracycline	12 48%	5 62.5%	0	1 100%	7 63.6%
Gentamicin	18 72%	8 100%	1 33.3%	0	7 63.6%
Amikacin	8 32%	2 25%	1 33.3%	0	3 27.2%
Netilmicin	2 8%	1 12.5%	0	0	0
Co-trimoxazole	23 92%	8 100%	2 66.6%	1 100%	10 91%
Chloramphenicol	1 4%	6 75%	0	0	0
Vancomycin	0	0	0	0	0
Linezolid	0	0	0	0	0
Mupirocin	0	0	0	0	0
Rifampicin	0	0	0	0	0

Out of 48 strains, 37 were typable by group III phages and belonged to four phage types: 42E, 42E/47/54/75, 42E/47 and 47. No statistically significant difference in distribution of these phage types was detected in outpatients and inpatients. All isolates were susceptible to vancomycin, linezolid, mupirocin, and rifampicin (Table 1). However, 42E strains were the most predominant in all wards except the ICU. The number of isolates belonging to 42E phage type recovered from surgery, orthopaedic, gynaecology, ICU, paediatric surgery and medicine wards was 6, 5, 4, 1, 1 and 1 respectively. In contrast, 42E/47/54/75 strains were most common in the ICU, had higher resistance to all drugs, except ciprofloxacin and amikacin, and were mostly D-test positive (87.5%) compared to 42E (32%). However, this may



not be significant owing to the small number of isolates. Out of six ICU strains, 42E/47/54/75, 42E and non-typable phage types were found in 4, 1 and 1 strains respectively. Whereas, among 10 isolates from patients in surgery wards, 42E, 42E/47/54/75, 42E/47, and non-typable phage types were 6, 2, 1 and 1 in number respectively.

Among 16 outpatients with CA-MRSA infection, the majority of isolates belonged to 42E (43.7%), followed by non-typable (31.25%), 42E/47/54/75 (12.5%) and 42E/47 (6.25%) and 47 (6.25%) phage types. On the other hand, the prevalence of 42E, non-typable, 42E/47/54/75 and 42E/47 phage types among 32 HA-MRSA cases were 56.2%, 18.75%, 18.75% and 6.25% respectively. In comparison with 32 HA-MRSA strains, all 16 CA-MRSA showed higher sensitivity to gentamicin, amikacin, netilmicin, co-trimoxazole and chloramphenicol. Ciprofloxacin and tetracycline resistance were more common in CA-MRSA. (Table 2) These differences in antibiotic resistance patterns of HA-MRSA and CA-MRSA strains were not significant statistically (p value >0.05).

**Table 2: Comparison of antibiotic resistance of CA-MRSA & HA-MRSA**

	HA-MRSA (n=32)	CA-MRSA (n=16)	P value
D-test positive	16 50%	4 25%	0.127476
Erythromycin	24 75%	9 56.2%	0.206344
Ciprofloxacin	24 75%	15 93.7%	0.238048
Tetracycline	16 50%	9 56.2%	0.764813
Gentamicin	22 68.7%	9 56.2%	0.524201
Amikacin	10 31.2%	4 25%	0.745984
Netilmicin	2 6.2%	1 6.2%	1.00000
Co-trimoxazole	30 93.7%	13 81.2%	0.316383
Chloramphenicol	6 18.7%	1 6.2%	0.403635
Vancomycin	0	0	--
Linezolid	0	0	--
Mupirocin	0	0	--
Rifampicin	0	0	--

We conducted an analysis of risk factors and response to empirical and post-sensitivity antibiotic therapy in the 32 inpatients. In this group, 42E strains were most frequent (n=18) followed by non-typable (n=6), 42E/47/54/75 (n=6) and 42E/47 (n=2). Empirical antibiotics were given to 27 patients, of which the most commonly used were cephalosporins (44.4%) and quinolones (40.7%). In post-sensitivity antibiotic therapy, vancomycin (n=23) & linezolid (n=5) were preferred to other drugs, and the 14 patients (60.8%) treated with vancomycin and three patients (60%) treated with linezolid showed good outcome. Remission of fever, decrease in pus discharge, acceleration of wound healing, general improvement of health and negative repeat cultures were considered a good outcome. There were no significant differences in outcome between vancomycin and linezolid. While nine MRSA cases with lack of response to vancomycin had 42E (n=5), non-typable (n=2) and 42E/47/54/75 (n=2) phage types, all three cases treated with linezolid with good outcome were 42E. Best outcomes were noted in 42E/47 and 42E, while durations of hospital stay of these phage types were comparable (Table 3). The distribution of mean age and sex of patients with MRSA and MSSA infection shows no significant difference (Table 4).

**Table 3: Comparison of the treatment outcomes and duration of hospital stay in different phage types**

	42E (n=18)	42E/47/54/75 (n=6)	42E/47 (n=2)	47 (n=0)	Non typable (n=6)
Good outcome	13 77.3%	3 50%	2 100%	0	3 50%
Poor outcome	5 22.7%	3 50%	0	0	3 50%
Duration of hospital stay	11.33 ± 5.8 days	10.33 ± 8.9 days	8 ± 1.4 days	0	9.8 ± 2.7 days

**Table 4: Comparison of the characteristics of patients with MRSA & MSSA infection**

Characteristic	Patients with MRSA (n = 32)	Patients with MSSA (n=30)	P value
Mean ± SD age	37.69± 23.683	41.93 ± 17.463	0.290
Male	23 (71.8%)	25(83.3%)	0.43865
Female	9 (28.1%)	5 (16.7%)	



A hospital stay of more than eight days, intravenous catheters and breach in skin were the most significant risk factors in patients with MRSA infection compared to the control group (Table 5). Among the nine outpatients investigated, none had a history of contact with MRSA patients, admission to a healthcare facility within the last year, antibiotic exposure, medical procedures, intravenous drug use, immune suppression, homosexuality, involvement in contact sports and sharing of personal items. Six (66.7%) of them had breach in skin and three (33.3%) had poor living conditions with overcrowding. Except 42E/47/54/75, other phage types had no significant association with MRSA risk factors. Out of the six patients with 42E/47/54/75 strain, five had history of previous hospitalisation with antibiotic exposure and four had nasogastric intubation. These risk factors were statistically significant in patients with 42E/47/54/75 strain.

**Table 5: Distribution of risk factors of MRSA infection**

Risk factors	MRSA n = 32	MSSA n = 30	Odds ratio	P value
Hospital stay > 8d	22	13	2.87	0.04
Past MRSA infection	2	0	--	--
Previous hospitalisation	7	3	2.52	0.2
Past antibiotic exposure	7	2	3.92	0.08
Breach in the skin	18	6	5.14	0.003
Intravenous catheter	29	21	4.14	0.03
Urinary catheter	9	5	1.95	0.28
Endotracheal tube	4	1	4.14	0.18
Nasogastric tube	3	0	--	--
Central line	1	0	--	--
Impaired immunity	3	0	--	--

### Discussion

MRSA has an increased association with multidrug resistance, aggressive course, increased mortality and morbidity in both community and health care facilities.<sup>3,10</sup> Owing to significant differences in antibiotic prescription patterns, infection control measures and awareness among healthcare workers, the prevalence of MRSA varies regionally. In India MRSA has increased from 12% in 1992 to 40% in 2009. As per the Indian Network for Surveillance of Antimicrobial Resistance group report, the prevalence of MRSA varies from 22% to 68% in Indian hospitals.<sup>11</sup> Different MRSA strains show predisposition to particular age groups —old age is a risk factor for HA-MRSA but CA-MRSA are commonly known to infect healthy adults and children.<sup>12</sup> A paediatric strain of pandemic MRSA was

named according to its risk group. Adults (16-65 years), especially of Indian ethnicity were reported to have higher rates of infection in Malaysia.<sup>13</sup> In our study, we found the maximum number of MRSA cases in the 18-40 years age group and only 10.41% of cases constituted elderly patients above 66 years and children less than five years.

MRSA frequently causes skin and soft tissue infections, wound infections, burns, ulcers, pressure sores, lower respiratory and urinary tract infections, septicaemia and infections at sites of invasive devices.<sup>12</sup> In this study, we found MRSA infections are prevalent mainly in surgical wards and primarily associated with suppurative infections of skin, soft tissue and organs. In different Indian studies, pus sample was the most common source of MRSA isolation and it accounted for 61.5% to 76.3% of all clinical samples which recovered MRSA on culture.<sup>14,15</sup> Hospital workers with dermatitis, inadequate hand washing or asepsis, burns patients and patients in long-term care facilities are the main source of MRSA in hospitals.<sup>7, 11, 16</sup> In a recent study, 22.22% of healthcare workers were found to have *S. aureus* nasal colonisation and 11.8% of *S. aureus* displayed methicillin resistance.<sup>17</sup> Poor infection control can result in nosocomial outbreaks. Strain typing has been employed successfully for tracing the dissemination of a particular strain or emergence of a new strain in a local region as well as to recognise evolutionary aspects of MRSA. The basic international set of 23 phages (group I, II, III & miscellaneous) for *S. aureus* are recommended for typing. However, phage typing is mainly restricted to reference centres due to difficulties in maintenance of stocks of phages and the propagation of control strains. Furthermore, poor discrimination and reproducibility has limited its use. A significant proportion of MRSA strains have been reported non-typable by many workers.<sup>18,19</sup> These non-typable strains can be allocated into distinct groups by other typing methods. In order to achieve better discrimination of these strains, modifications like 48°C incubation before test, make use of different RTD and new phages were suggested.<sup>20</sup> Moreover, phage typing patterns are not always reproducible, as the same strains can show a variety of results when tested on separate occasions. Therefore, it is recommended that isolates with a single difference in lysis pattern should be considered indistinguishable.<sup>20</sup>

To overcome the limitations of phage typing, several genotypic methods were introduced. However, no strain typing method has been implemented as the internationally recognised standard method. Furthermore, none of these methods achieves all criteria to be recommended as the best method having a highly discriminatory, standardised technique which is reproducible, widely available,



economical and works satisfactorily in epidemiological investigations.<sup>20</sup> Among various methods available, PFGE and MLST have gained popularity. PFGE distinguishes bacterial strains by their patterns of restriction enzyme digested genomic DNA fragments separated as individual bands on electrophoresis.<sup>21,22</sup> In contrast to conventional electrophoresis, PFGE changes electric field orientation after each pulse to efficiently separate larger fragments of DNA. PFGE results are reproducible, providing good strain differentiation. Efficient detection of minor genetic changes makes it particularly suitable for local or short-term investigations. The drawbacks of PFGE include poor reproducibility, lack of standardisation, data storage and long turnaround time.<sup>21</sup> MLST is a nucleic acid sequence-based typing method which characterises bacterial population based on the allelic variations in several housekeeping genes resulting from slow accumulation of single-nucleotide polymorphisms (SNP) generated spontaneously. Unlike PFGE, it is not suitable for short-term investigations, rather it allows better strain relatedness, reconstruction of evolutionary relationships, provides unambiguous, portable data stored in internet database that can easily be compared among laboratories without using reference isolates for each important clone for standardisation.<sup>23,24</sup> Recently, Suzuki et al. developed phage open-reading frame (ORF) typing (POT).<sup>25</sup> It is based on PCR amplification of phage ORFs lysogenised in MRSA. POT has shown reproducibility of results and discriminatory power comparable to PFGE.<sup>26</sup>

Resistance patterns of MRSA differ widely within India. In a nationwide study, erythromycin, gentamicin, cotrimoxazole, ciprofloxacin and clindamycin resistance were significantly higher in MRSA compared to MSSA isolates. MRSA displayed highest resistance to penicillin (100%), ciprofloxacin (79.3%) and erythromycin (70.8%).<sup>11</sup> The antibiotic resistance patterns and distribution of prevalent phage types in our hospital were consistent with other Indian studies which identified a significant proportion of isolates as non-typable and the majority of isolates belonged to phage group III.<sup>6, 27, 28</sup> Although several workers reported most MSSA belong to phage group II, Mehndiratta et al. found phage type 81 of the non-allocated group was the predominant phage type among MSSA and 9.4%, 2.7%, 14.8% and 40.5% MRSA strains belonged to phage group I, II, III and the non-typable group, respectively.<sup>18</sup> In another study, only 35.6% were typable and maximum strains belonged to phage group III (49.3%) followed by phage group of mixed phages (32.8%), group I (16.4%) and group II (1.5%).<sup>27</sup> Compared to national data, our isolates showed more resistance to gentamicin and ciprofloxacin.<sup>18</sup> We found that 42E/47/54/75 and 42E strains constitute the

majority of MRSA isolates from ICU and non-ICU wards, respectively. The predominance of 42E/47/54/75 strains (four out of six strains) in the ICU may be attributed to risk factors for MRSA colonisation such as long hospital stay, indwelling medical devices and indiscriminate antibiotic use. Although poor outcome was less in patients harbouring 42E MRSA strains (22.2%) compared to 42E/47/54/75 strains (50%), they had comparable mean duration of hospital stay (11.33±5.8 days and 10.33 ±8.9 days respectively) (Table 3). Apart from higher antibiotic resistance, 42E/47/54/75 strains (n=8) also showed a greater proportion of inducible clindamycin resistance. However, further studies are needed to confirm this finding.

Risk factors of HA-MRSA are essentially different from those of CA-MRSA. Overcrowding, poor hygiene, sharing of personal items such as razors, nail clippers, toothbrushes, breach in skin (as in athletes, male homosexuals, prisoners, soldiers) and travel to countries with a higher prevalence of CA-MRSA are associated with significant risk of CA-MRSA infection.<sup>2,9</sup> On the other hand, prior hospitalisation or admission in long-term care centres, ICU and burn units, elderly patients, immune-suppression or previous antibiotic, indwelling medical devices, dermatological conditions, long duration of hospital stay, inadequate environmental cleaning, frequent transfers of patients and staff between wards or hospitals are the most eminent risk factors for HA-MRSA.<sup>2,9</sup> Sarma et al. found undergoing surgery was a significant risk factor and duration of hospital stay, while exposure to quinolones, aminoglycoside, cephalosporins was not associated strongly with MRSA infection.<sup>6</sup> However, in a meta-analysis an association between antibiotic exposure and MRSA isolation was established.<sup>29</sup> Nasal colonisation was reported to be related to post-operative infections by MRSA.<sup>30</sup> In a recent multicentric study, Huang et al. documented the benefit of universal decolonisation over targeted decolonisation strategy.<sup>31</sup> Decolonisation of all ICU patients irrespective of colonisation status reduced MRSA infection in the ICU. It highlights the importance of admission screening and decolonisation strategies for control of MRSA. In this present study, all patients had at least one independent risk factor. Out of the 32 patients only two had past MRSA infection and seven had previous hospitalisation with antibiotic exposure, while most had breach in the skin 56.2% (n=18) and health care associated risk factors of MRSA infections, such as; intravenous catheters 90.6% (n=29), urinary catheters 28.1% (n=9), endotracheal tubes 12.5% (n=4), nasogastric tubes 9.3% (n=3) and central line 3.1% (n=1). In comparison with the control group, longer hospital stay, intravenous catheters and breach in skin were the most significant risk factors. Previous hospitalisation, antibiotic exposure and nasogastric



intubation were significantly associated with 42E/47/54/75 strains ( $p < 0.05$ ) and may be attributed to its distribution in ICU patients. The existence of a distinct MRSA phage type with unique resistance pattern in ICUs may be of great concern, since it may mandate substantial changes in antibiotic policy for ICUs. However, further studies are required for confirmation.

In this study, seven out of 16 outpatients were lost in follow-up. The remaining nine patients had no history of contact with known MRSA cases, previous hospitalisation, antibiotic exposure, medical procedures, intravenous drug addiction, immune suppression, homosexuality, involvement in contact sports and sharing of personal items. However, six (66.7%) had breach in skin (skin ulcer and wound) and three (33.3%) had poor living conditions with overcrowding. Therefore, it is essential that MRSA is suspected in patients with skin and soft tissue infection especially if they come from poor living and overcrowded conditions. Furthermore, studies or active surveillance should be carried out to estimate the disease burden of CA-MRSA in the neighbouring community of our hospital. CA-MRSA causes mainly skin and soft-tissue infections ranging from furuncles to necrotising fasciitis in otherwise healthy persons without comorbidities outside healthcare facilities.<sup>2</sup> An increasing number of cases of neonatal sepsis and breast abscess in lactating mothers from rural and urban areas of India respectively has been reported.<sup>11</sup> Overcrowding and lack of personal hygiene have been found to have a vital role in community-acquired infections in India. Unlike Western countries, reports of CA-MRSA infections in homosexuals, athletes and prisoners are infrequent. However, epidemiology of CA-MRSA can show regional variation. Hence, CA-MRSA risk factors need to be studied optimally in an Indian scenario to direct precautionary measures.

The main limitation of our study is the small sample size. However, the findings of our study are in accordance with other studies from various parts of the country. We have not studied isolates from the same patient after a period of time. This method could be employed to detect if any transformation has taken place in the strains.

## Conclusion

Phage typing revealed two major MRSA strains in our hospital. These findings add to the knowledge that the ICU and non-ICU strains of MRSA differ in resistance mechanism, antibiotic susceptibility pattern and distribution.

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