

## Detection of Methicillin Resistance and $\beta$ -Lactamase Production in *Staphylococcus aureus* Isolates: A Cross Sectional Study from a Tertiary Care Hospital

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### Abstract

Nosocomial infections are proving to be a menace for the whole health care system, with methicillin resistant *Staphylococcus aureus* (MRSA) being a very notorious causative agent. Along with the role of *mecA* gene producing penicillin-binding protein (PBP2a), production of beta-lactamase enzyme by *Staphylococcus aureus* makes the organism resistant to all  $\beta$ -lactam agents. This study aims at phenotypic detection of methicillin resistance and  $\beta$ -Lactamase production in all *S. aureus* isolates by Cefoxitin disk diffusion test and Penicillin zone-edge test, respectively. In this prospective cross-sectional study, samples were obtained from patients admitted to different departments and were processed according to the standard laboratory protocols. As per the CLSI guidelines, phenotypic detection of resistance to methicillin was done by Cefoxitin Disk Diffusion test, whereas production of beta-lactamase enzyme was phenotypically detected by penicillin zone edge test. Among 179 isolates of *S. aureus*, 116 (64.8%) were MRSA, whereas the remaining 63 (35.2%) isolates were methicillin-sensitive *Staphylococcus aureus* (MSSA). *Staphylococcus aureus* infection among ICU and non-ICU patients were found to be 24(13%) and 155(86.6%) respectively. Among 24 ICU patients, 19 had MRSA infection, whereas among 155 non-ICU patients, 97 had MRSA infection. Out of 63 MSSA isolates, only 2 (3.17%) were found to be sensitive to Penicillin by Zone-edge test, 04 isolates showed presence of  $\beta$ -lactamase production, while remaining 57 isolates were resistant to Penicillin. At the same time, several class of antibiotics were found to be ineffective against these MRSA isolates. Cases of methicillin-resistant and  $\beta$ -lactamase producing *Staphylococcus aureus* infections are on the rise in our hospital settings.

**Keywords:** Methicillin Resistant *Staphylococcus aureus*, Beta Lactamases, Multidrug Resistance

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## INTRODUCTION

Methicillin resistant *Staphylococcus aureus* infections are the major contributors among different nosocomial infection worldwide, including India.<sup>1,2</sup> Penicillin-resistance among *Staphylococcus aureus* (*S. aureus*) strains have been known since 1942, which further paved the way for semisynthetic penicillins, including methicillin,<sup>3</sup> which is used in the treatment of infections caused due to penicillin resistant *S. aureus* strains. With the increasing number of infections due to *S. aureus*, the prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) infections has also increased in India.<sup>4</sup> Since 1980, about 33% of the world's population exhibiting increased incidence of MRSA infection in hospital settings belong to the Asia pacific region.<sup>5</sup> It has been reported from different parts of Indian subcontinent at a rate of about 25% to 50%.<sup>6</sup>

MRSA has been linked to the causation of several hospital acquired infections which are rapidly progressive and fatal such as pneumonia, endocarditis, necrotizing fasciitis, osteomyelitis, toxic shock syndrome and severe sepsis, whereas, various risk factors that are commonly associated with MRSA infection are prolonged hospitalization, intensive care admission, invasive procedures, hemodialysis, recent antibiotic use, HIV infection and discharge with long-term indwelling urinary catheter or long term central venous access. Earlier, MRSA was known to cause infection among hospitalized patients, commonly known as hospital-associated MRSA (HA-MRSA) infections. But recently, these infections are engulfing a large part of population which have never come in contact with the hospitals, health care workers or hospitalized patients, known as community-associated MRSA (CA-MRSA) infections.<sup>7</sup> CA-MRSA is generally known for causing skin and soft tissue infections like abscesses, furunculosis and cellulitis but it also causes severe morbidities like bacteremia, septic shock, necrotizing pneumonia and osteomyelitis.<sup>7,8</sup>

The Staphylococcal cassette chromosome mec (SCCmec) is a genomic island which when acquired, causes methicillin resistance. It expresses the *mecA* gene which helps in producing the penicillin-binding protein (PBP2a), making the organism resistant to all  $\beta$ -lactam antibiotics.<sup>9</sup>

Recently, several other related genes like *mecB*, *mecC*, and *mecD* have also been discovered.<sup>10</sup> The *mecA* mediated resistance can be detected in laboratory by using Oxacillin (broth microdilution or agar dilution technique) or Cefoxitin (broth microdilution or disk diffusion technique).<sup>11</sup> Cefoxitin (30 $\mu$ g) may be used as a surrogate marker for Oxacillin in detecting MRSA, if oxacillin MIC testing is not done and can prove to be a very effective method of detection of MRSA and MSSA (Methicillin sensitive *Staphylococcus aureus*) isolates in resource limited settings.

There is also a second method which makes *S. aureus* resistant to all beta-lactams, which is by production of beta-lactamase enzyme by the organism, that hydrolytically destroy beta-lactams.<sup>12</sup> The gene responsible for encoding  $\beta$ -lactamase is *blaZ*, which develops such resistance.<sup>13</sup> Staphylococci, which are  $\beta$ -lactamase-positive, are resistant to penicillin, aminopenicillin, carboxypenicillin and ureidopenicillins. In the laboratory, penicillin disk diffusion zone-edge test and nitrocefin-based tests can be used for detecting  $\beta$ -lactamase production in *S. aureus*, phenotypically. However, penicillin zone-edge test has been found to be more sensitive and is recommended, if only one test is to be used for  $\beta$ -lactamase detection.<sup>11</sup> As per the Clinical and Laboratory Standards Institute (CLSI), penicillin zone edge test<sup>14</sup> has been recommended for screening *S. aureus* isolates for  $\beta$ -Lactamase production.<sup>11</sup>

This study aims at phenotypic detection of Methicillin resistance and  $\beta$ -Lactamase production in all *S. aureus* isolates by Cefoxitin disk diffusion test and Penicillin zone-edge test, respectively. *S. aureus* isolates were obtained from various clinical samples like pus, urine, blood, conjunctival swab, ET aspirate and synovial fluid.

## MATERIALS AND METHODS

A prospective cross-sectional study of one year duration from March 2020 to February 2021, was carried out in the Bacteriology laboratory, for the detection of Methicillin Resistance and  $\beta$ -Lactamase production in *Staphylococcus aureus*. Written and informed consent was taken from patients.

### Inclusion and Exclusion Criteria

All patients having a surgical site infection, skin ulcers or burn, skin abscess, diabetic foot ulcers and infected traumatic wound were included in the study. All patients who did not give their consent were excluded from the study.

### Sample Collection and Processing

Specimens were collected from various clinical departments. The specimens included in this study were swab/aspirate from soft tissue infection and abscess (pus), urine, blood, conjunctival swab, Endo-tracheal aspirate and synovial fluid. General culture media like Nutrient Agar and Blood Agar, whereas selective media like Mannitol salt agar (MSA) were used for inoculation purpose, which were subsequently incubated for 24 hours at 37°C, aerobically.

Detection of *S. aureus* was done following conventional methods of identification, like catalase test, tube coagulase test and mannitol salt agar. Furthermore, routine antimicrobial susceptibility test was carried out by Kirby Bauer disk diffusion method. Cefoxitin Disk Diffusion test was used for detection of methicillin resistance. Routine disk diffusion procedure was used to perform the Cefoxitin disk (30µg) test on *S. aureus* isolates, and isolates showing zone of inhibition of  $\geq 22$  mm were interpreted as MSSA or negative for *mecA* mediated resistance, whereas those showing zone of inhibition of  $\leq 21$  mm were interpreted as MRSA or positive for *mecA* mediated resistance. CLSI defines oxacillin resistance in *S. aureus* as an MIC  $\geq 4\mu\text{g}$  and all strains meeting this definition are

characterized as “MRSA” even though methicillin is no longer used either to test strains in the lab or as a therapeutic agent.<sup>11</sup>

### Penicillin Zone-Edge Test

For standard disk diffusion procedure, few bacterial colonies were used for making a normal saline suspension and the turbidity was matched up to 0.5 McFarland standard. It was further inoculated on Mueller–Hinton agar and 10-U penicillin G discs were applied, as per the CLSI guidelines. The plates were incubated at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , in ambient air, for 16 - 18 hours. Zone diameters were then measured and interpretation of susceptibility was based on CLSI (zone diameter of  $\geq 29$  mm) recommendations. All zone edges were assessed for sharp or fuzzy appearance. A sharp zone edge (“cliff”) corresponds to the presence of  $\beta$ -lactamase, whereas a fuzzy zone edge (“beach”) indicates the absence of  $\beta$ -lactamase.<sup>11</sup>

### Statistical Analysis

Data were entered into MS Excel spreadsheet and SPSS version 20 (IBM Corp) was used for data analysis purpose. Descriptive statistics were presented in the form of means, standard deviation was calculated for continuous variables and frequencies for categorical variables. Data were graphically presented using columns, graphs and pie charts. A chi-square evaluation was used for group comparison for categorical data. P value  $< 0.05$  was considered as statistically significant.

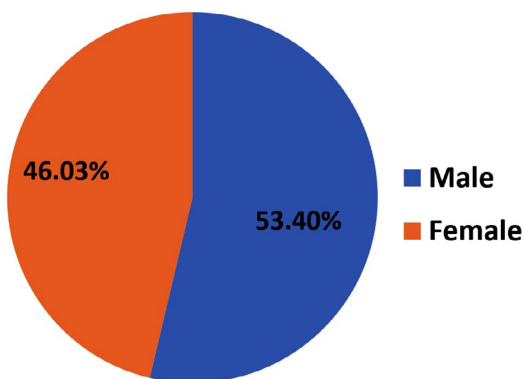


Figure 1. Gender distribution of MRSA isolates.

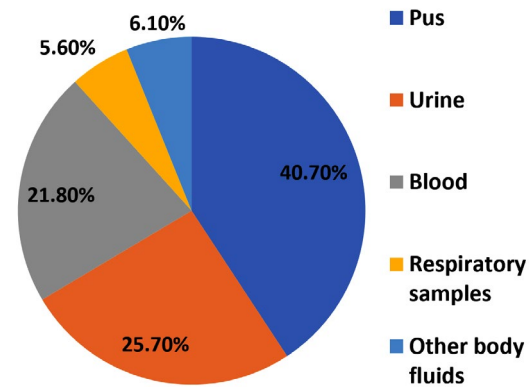


Figure 2. Sample distribution of MRSA isolates.

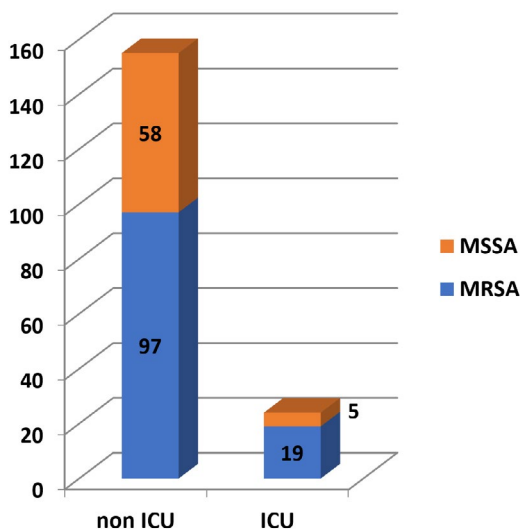
### Ethical Consideration

Mandatory ethical clearance was obtained for this study from the Institutional Ethical Committee (memo no.1602/IEC/IGIMS/2020)

### RESULTS

From March 2020 to February 2021, among 179 isolates of *S. aureus*, 116 (64.8%) were MRSA and 63 (35.2%) were MSSA. Among 116 MRSA isolates, 62 (53.4%) belonged to male patients [Figure 1]. The mean age of the patient was 35.08 with a standard deviation of 18.6.

*Staphylococcus aureus* was predominantly isolated from pus (73) 40.7% samples from skin and soft tissue infections, followed by urine (46) 25.7%, blood samples (39) 21.8% and respiratory samples (10) 5.6% including sputum, bronchoalveolar lavage, endotracheal aspirate, pleural fluid and throat swab [Figure 2]. Rest of the samples were corneal swab, ascitic fluid, conjunctival and corneal swab and CSF.



**Figure 3.** MRSA incidence in ICU and non-ICU patients.

**Table 1.** Zone edge test for detection of  $\beta$ -lactamase production in MSSA (n=63)

Pattern at edge	No. (%)
Fuzzy edge with >29 mm Zone diameter	2 (3.17 %)
Sharp edge with >29 mm Zone diameter	4 (6.34 %)
< 29 mm Zone diameter	57 (90.47 %)

*Staphylococcus aureus* infection was present in 24 (13%) ICU patients, whereas it was present in 155 (86.6%) non-ICU patients. Among 24 ICU patients, 19 (79.16%) had MRSA infection, whereas among 155 non-ICU patients, 97 (62.58%) had MRSA infection. [Figure 3].

Pearson Chi-square test was carried out to find any association of more incidence of MRSA infection among ICU patients. Value of Pearson's Chi-square was 2.507, degree of freedom was 1 and p value was 0.113. With a p value of  $p > 0.05$ , it is evident that there is no association between the incidence of MRSA infection among ICU patients.

As expected, all 116 MRSA isolates in our study were found to be Penicillin resistant (< 29 mm). Out of 63 MSSA isolates, only 2 (3.17%) were found to be sensitive to Penicillin by Zone-edge test having zone >29 mm with fuzzy edge at the zone indicating absence of  $\beta$ -lactamase production. Four of the MSSA isolates showed zone >29 mm with sharp edge at the zone indicating presence of  $\beta$ -lactamase production. Remaining 57 isolates had zone diameter < 29 mm [Table 1].

In our study, Antibiotic susceptibility testing data for other antibiotics revealed that MRSA isolates were resistant to many other antibiotics like Ciprofloxacin (71.8%), Erythromycin (70.6%), Clindamycin (59.3%), Gentamicin (36.8%) and Cotrimoxazole (34.1%). There was no resistance documented against vancomycin and linezolid [Table 2].

### DISCUSSION

In this study, analysis of 179 *S. aureus* isolates from different clinical samples revealed

**Table 2.** Resistance of MRSA isolates to other Antibiotics by Kirby Bauer Disc Diffusion method (n=116)

Antibiotics tested	Resistance pattern of MRSA (%)
Penicillin	100
Erythromycin	70.6
Clindamycin	59.3
Ciprofloxacin	71.8
Gentamicin	36.8
Cotrimoxazole	34.1
Vancomycin	0
Linezolid	0

that the overall rate of MRSA infection was as high as 64.8%, which is definitely alarming. Similar high prevalence rates have been registered by Gupta N. et al.,<sup>15</sup> and Tiwari et al.<sup>16</sup> A similar study from Andhra Pradesh showed a CA-MRSA prevalence of 64.7%.<sup>17</sup> Other reports on CA-MRSA in India were by Shenoy et al.,<sup>18</sup> from Mangalore, D'Souza et al.<sup>19</sup> from Mumbai and Bouchiat et al. from Bangalore.<sup>20</sup> A nasal carriage rate of 72.7% for MRSA was reported by Goud et al among healthy individuals in Bangalore, India.<sup>21</sup>

There are also studies from India showing lesser prevalence of MRSA, ranging from 26.14% to 43%.<sup>22,23</sup> An overall 41% prevalence of MRSA was reported by a study undertaken by the Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India, from January 2008 to December 2009, among 15 tertiary care centers.<sup>4</sup> Reason behind such variation could be many like different geographical area, varied sample size, methods used for testing and infection control status etc.

Most of the isolates (40.7%) were obtained from Skin and soft tissue infection (SSTI), proving it to be a major cause of *S. aureus* disease in the community, as well as among hospitalized patients. Similar findings were reported by Tiwari et al. from Varanasi (42%) and Mallick and Basak from Maharashtra (61.4%).<sup>24,25</sup> The predominance in pus sample could be due to over-exposure of wound to environmental microorganisms. At the same time, presence of *S. aureus* as skin commensals makes the wound more prone for infection.

When gender distribution was compared, MRSA was found to be more prevalent among male patients (53.4%). Similar observations were reported by Rao et al.<sup>26</sup> There were no significant differences in the clinical presentations between both genders, as regards to the prevalence of MRSA.

Antimicrobial resistance pattern for other antibiotics in this study shows that MRSA strains present maximum resistance against Ciprofloxacin (71.8%), Erythromycin (70.6%), Clindamycin (59.3%), Gentamicin (36.8%) and Cotrimoxazole (34.1%). Similar findings were reported by Raj Kumar et al.<sup>27</sup>

Almost all MRSA isolates were discovered to be sensitive to Linezolid and Vancomycin in our

study, which is at par with the study done by Abbas et al.<sup>28</sup>

In this study, no association was found between increased incidence of MRSA infections and patients admitted in ICU (p value 0.113). This suggests that MRSA infection is not confined to severely ill patients nowadays and is getting more prevalent among communities.

Among 63 isolates of MSSA, only two isolates (3.17 %) were found to be Penicillin susceptible (absence of  $\beta$ -lactamase) by Penicillin zone edge test. Both the isolates were from pus sample and belonged to male patient. Simultaneously, four isolates (6.34 %) were found to be  $\beta$ -lactamase producers. SS Richter et al<sup>29</sup> found around 4.46%  $\beta$ -lactamase producers among 448 MSSA, which is slightly lower than that observed in this study. Also, very large proportion of MSSA isolates (90.47 %) were found to be resistant to Penicillin, which is really worrisome.

## CONCLUSION

High prevalence of  $\beta$ -lactamase production and methicillin-resistance among *Staphylococcus aureus* is a cause of concern. Along with the prevalence of HA-MRSA infection, there is an increase in the incidence of CA-MRSA infection as well. A patient with MRSA infection is exhibiting increased resistance to many classes of antibiotics, except Vancomycin and Linezolid, a fact which is more challenging for the health care providers and also for the clinical researchers. We recommend effective surveillance of MRSA at hospital and community level to know the exact burden and to follow all standard measures to reduce the spread of MRSA, including strict infection control and hand hygiene practices.

## Limitations

Major limitations of this study are small sample size, lack of molecular detection of blaZ gene and smaller study duration. Moreover, the study involves only clinical isolates from a single tertiary care center.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

### AUTHORS' CONTRIBUTION

NS, KS contributed in conceptualization, study design, literature review, data collection, data analysis, interpretation, writing original draft and editing. NK contributed in study design, data analysis, writing review and editing. All authors read and approved the final manuscript for publication.

### FUNDING

None.

### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in manuscript.

### ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India with reference number 1602/IEC/IGIMS/2020.

### INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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