

Minimal Surveillance of MRSA in a Highly Polluted Region: A Cross-sectional Study on Prevalence in National Capital Region, India

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ABSTRACT

Introduction: In India, Methicillin Resistant *Staphylococcus aureus* (MRSA) causes both community infection as well as hospital acquired infection. The infection mostly is endemic in nature.

Aim: To find out the prevalence of MRSA in the NCR region, India.

Materials and Methods: The present study was a cross-sectional analysis in which 653 samples were screened for MRSA in the laboratory. Isolates were tested against cefoxitin using disk diffusion method from October 2022 to December 2022 in Department of Microbiology, Rama Medical College, Hospital and Research Centre Hapur, National Capital Region (NCR), India. A total of 46 isolates were found to be MRSA positive. All 46 specimens of MRSA were put to test for Antimicrobial testing to know the susceptibility of antibiotic individually. All positive specimens were segregated based on gender, type of specimen, indoor versus outdoor patients. The data of the study was analysed by excel software on different parameters as per objectives of the study.

Results: The positive MRSA specimens were 46 (7.04%) out of 653 different category of specimens tested. More positivity (8.56%) was recorded in female patients than 5.52% in male patients. The share of male positive was 39.13% and for female, the share among positive sample was 60.87%. Among male positive samples, 7.96% samples (maximum) were from 21-40 years age. The female patients above the age of 60 years had maximum (15.78%) share of positive cases. Inpatient Department (IPD) recorded more (54.35%) of MRSA positive cases. Vancomycin showed highest susceptibility (97.82%) to MRSA. The lowest susceptibility (4.34%) was shown by erythromycin.

Conclusion: This study establishes only 7.04% MRSA which was found on lower side comparing the average ratio of Delhi and Uttar Pradesh and perhaps the lowest in the country according to various studies carried out across India. Region-wise epidemiological study of MRSA is required periodically. The MRSA infection can be controlled by preventing its spread and minimising the emergence of drug resistance by following a robust antimicrobial stewardship.

Keywords: Antimicrobial susceptibility, Methicillin resistant *staphylococcus aureus*, Multidrug resistance, Nosocomial infection

INTRODUCTION

The MRSA is a type of bacteria that causes nosocomial infection as well as community infection and it is resistant to several antibiotics. In the community, MRSA most often causes skin infections. Lung infection and other infections are also reported in some cases. Untreated cases of MRSA may develop sepsis. Nosocomial infection of MRSA sometime may lead to blood stream infections, surgical site infection and pneumonia [1]. Nosocomial infection of MRSA in hospitals of United States of America (USA) and Europe ranges from 29-35% of all clinical isolates and therefore a major cause of nosocomial infection which causes morbidity and mortality worldwide [2,3].

Increasing antibiotic resistance is a worrisome trend being observed worldwide. Among Gram-positive cocci, *Staphylococcus aureus* is a well-known cause of community acquired as well as hospital acquired infections. MRSA started resistance against most of empirical antibiotics within two years of Methicillin launch in United Kingdom [4,5]. Being methicillin resistant itself means that a *Staphylococcus aureus* isolate will not be sensitive to penicillin, cephalosporin, β -lactamase inhibitors, and carbapenems and can further exhibit resistance to other classes of antibiotics [6,7].

Methicillin resistance is due to harbouring of *mec-A* gene; resulting in synthesis of altered Penicillin Binding Protein (PBP)-2a by the organism having low affinity for β -lactam antibiotics. The prevalence of MRSA strains has increased worldwide. Till late 80'S, MRSA was mostly nosocomial but later on started emerging as Community Associated-MRSA (CA-MRSA) [8-10].

Centres for Disease Control (CDC) is working on their structured program to prevent infection of MRSA by preventing spread of germs, restrictive antibiotic use to slowdown the process of resistance. CDC scientists track the number and kind of MRSA infections throughout the country with complementary systems. The tracking system of CDC is well-defined, well-coordinated with centres for medicare and Medicaid Services (CMS) and health departments to know where MRSA infection is happening. Then they deploy the resources to stop infections [11].

In one of the recorded study from the data from 1999-2005, the annual number of hospitalisations associated with *S. aureus* and MRSA increased 62%, and the estimated number of MRSA-related hospitalisations became more than doubled [12]. The patients brought to hospital with history of either prior hospitalisation, OPD patients within previous six months visits to hospital or transfer from long-term care facility who develops MRSA within 48 hours of hospitalisation. In fact these pathogens are community strains but these pathogens can be hospital acquired also. The mean monthly patient colonisation rate is upto 23% which develops MRSA infection out of which 5-15% colonisation is seen in long-term care facility residents [13].

Region-wise prevalence of MRSA: The overall prevalence of MRSA was 37% (95% CI: 32-41) from 2015 to 2019. The pooled prevalence of MRSA zone-wise was 41% (95% CI: 33-50), 43% (95% CI: 20-68), 33% (95% CI: 24-43), 34% (95% CI: 26-42), 36% (95% CI: 25-47), and 40% (95% CI: 23-58) for Northern, Eastern,

Western, Southern, Central, and North-eastern region respectively. The state-wise stratified results showed a predominance of MRSA in Jammu and Kashmir with 55% (95% CI: 42-67) prevalence, 53% (30-75) in Uttar Pradesh, 52% (32-71) in New Delhi and the lowest was 21% (95% CI: 11-34) in Maharashtra [14].

Methicillin has resistance to β -lactam compounds because it is not hydrolysed by β -lactamase. This is termed as intrinsic resistance or methicillin resistance. The MRSA isolates and methicillin resistant Coagulase-Negative Staphylococci (CoNS) isolates are broadly resistant to penicillin and cephalosporins [15].

Infection control methods: The MRSA is an endemic infection the tracking of MRSA infection, preventing their spread, specific and time bound restrictive use of antibiotics and proved methods to treat colonisation are few steps to control MRSA as discussed in detail in a study carried out by Boyce JM [16].

Hence, present study was conducted with an aim to find out the prevalence of MRSA in the NCR region, drug resistance and to compare the prevalence with other regions.

MATERIALS AND METHODS

The present cross-sectional study was conducted on 653 samples brought to Microbiology Lab of tertiary care centre between October 1st, 2022 and December 31st, 2022 in Department of Microbiology, Rama Medical College, Hospital and Research centre Hapur, NCR, India. Ethical approval was obtained for the study with approval number- RMCH&RC/FMT/2022/13 dated 21-05-2022.

Inclusion criteria: Various clinical specimens of pus, urine, blood and swabs received in the microbiology department were included in the study.

Exclusion criteria: Duplicate samples and absence of informed consent were used as exclusion criteria.

Study Procedure

All the samples were cultured on blood agar and MacConkey agar except urine samples. For urine, Cysteine Lactose Electrolyte Deficient agar (CLED Agar) was used [17]. All the isolates were subjected to Cefoxitin Disk Diffusion (CFD) test using a 30 μ g cefoxitin disk (Hi-Media, India). A 0.5 McFarland standard bacterial suspension was prepared, the bacterial lawn was made on Muller-Hinton agar plate and the cefoxitin disk was placed. Plates were incubated at 35°C for 16-18 hours and then zone diameters were measured. ATCC 25923 and ATCC 43300 were used as negative and positive quality control strains, respectively.

Molecular detection of *mecA* gene is also possible by using Food and Drug Administration (FDA) approved assays. Chromogenic agars can also be used to detect MRSA. These chromogenic agars are commercially available. MRSA can also be detected by latex agglutination or immunochromatographic membrane tests for finding PBP2a [18,19].

The Clinical and Laboratory Standards Institute (CLSI) recommends incubation of isolates for testing against oxacillin at 33°-35°C (maximum of 35°C) for 24 hours before taking reading. Isolates tested against cefoxitin using either disk diffusion or broth micro dilution should also be incubated at 33-35°C but can be read after 16-18 hours and 16-20 hours, respectively. Cefoxitin should be used for disk diffusion testing in place of oxacillin which is not as reliable as cefoxitin [20,21].

Microbiological Processing

Isolation and identification: On the basis of colony morphology on culture, Gram staining, catalase test, mannitol fermentation test, slide and tube coagulase test and DNase production, *Staphylococcus aureus* were identified and isolated.

Antimicrobial susceptibility pattern: The antimicrobial susceptibility of all *Staphylococcus aureus* isolates was ascertained by Kirby Bauer disc diffusion method as per CLSI guidelines 2019. The antibiotics

tested were cefoxitin (30 μ g), ciprofloxacin (5 μ g), gentamicin (10 μ g), co-trimoxazole (1.25/23.75 μ g), clindamycin (2 μ g), erythromycin (15 μ g), linezolid (30 μ g) and vancomycin (30 μ g) [20,22].

During the entire study, quality of specimens was maintained to avoid erroneous result. Proper inoculation of bacterial suspension was made to minimise the chances of laboratory infection to the extent possible. Reading was taken after incubation at 35°C for 18 hour. Any zone diameter of ≤ 21 mm was reported as cefoxitin-resistant and measured as MRSA. Interpretation criteria of (mm) for cefoxitin disc diffusion test with respect to *S.aureus* are as follows; susceptible ≥ 22 mm and resistant ≤ 21 mm. Marginal cases were not taken into consideration for susceptibility.

STATISTICAL ANALYSIS

The data of the study was analysed by excel software on different parameters as per objectives of the study.

RESULTS

In this study, total 653 suspected samples were brought to microbiology laboratory for finding MRSA and for further antimicrobial susceptibility pattern. A total of 46 specimens (7.04%) were found MRSA positive. A total of 607 specimens (92.96%) were negative for MRSA. Out of 46 samples detected for positive MRSA, the maximum 19 (41.30%) was from pus specimen and lowest was from swabs [Table/Fig-1]. Among the positive MRSA samples, 54.35% pertains to IPD and suspected to be nosocomial patients whereas 45.65% positive samples were found from OPD patients and were community acquired infections [Table/Fig-2].

Specimen	N (%)
Pus	19 (41.30)
Blood	18 (39.14)
Urine	8 (17.39)
Swabs	01 (2.17)
Total	46 (100)

[Table/Fig-1]: Distribution of positive MRSA based on type of specimen.

Specimen	IPD (n%)	OPD (n%)
Pus	11 (23.91)	08 (17.39)
Blood	10 (21.73)	08 (17.39)
Urine	04 (8.69)	04 (08.69)
Swabs	0	01 (02.17)
Total 46	25 (54.35)	21 (45.65)

[Table/Fig-2]: IPD versus OPD Distribution of positive specimens.

The age-wise distribution of total 653 samples describes that the maximum suspected samples 270 (41.35%) were from the patients of 21-40 years age group, 240 (36.75%) of total samples were from the patients of 41-60 years. The lowest 45 (6.89%) of total samples was from the age group having less than 20 years age group [Table/Fig-3]. In the study, 326 suspected samples from male and 327 from female patient (total 653 samples) were tested in Microbiology lab. MRSA was reported positive in 18 (5.52%) samples in male and 28 (8.56%) in female. It shows more MRSA are prevalent in female patients in this study. Out of total 46 positive MRSA patients, 39.13% of male and 60.87% of female patients have MRSA [Table/Fig-4,5].

Age group (years)	Male (n)	Female (n)	Total samples and percent value (%)
0-20	24	21	45 (6.89)
21-40	113	157	270 (41.35)
41-60	129	111	240 (36.75)
>60	60	38	98 (15.01)
Total	326	327	653 (100)

[Table/Fig-3]: Age-wise and gender-wise distribution of total samples.

Total 326 samples were tested for male patients. MRSA positive samples were 18 (5.52%). The maximum positivity 9 (7.96%) was observed in 21-40 years age group. The lowest positivity 1 (4.16%) was found in patients below 20 years age [Table/Fig-4]. Of 327 female patient, 28 samples (8.56%) were found positive MRSA. The maximum cases (15.78%) were detected from the samples belonging to patients in more than 60 years of age followed by 12.61% in 41-60 years age bracket. The lowest (3.82%) MRSA was detected in 21-40 years age group of female patients [Table/Fig-5]. It shows that female more than 40 years age suffer maximum with MRSA. It was observed that female are more susceptible for MRSA as their age advances. All 46 MRSA positive samples were tested to 11 different antibiotic for ABST. The highest 45 (97.82%) sensitivity was observed with Vancomycin and lowest 2 (4.34%) with Erythromycin. Doxycycline showed 84.78% sensitivity against bacteria, which was second highest in this study followed by Linezolid 36 (78.26%) [Table/Fig-6].

Age group (years)	Total male samples (n)	Positive male samples n (%)
0-20	24	01 (4.16)
21-40	113	09 (7.96)
41-60	129	05 (3.87)
>60	60	03 (5.0)
Total	326	18 (5.52)

[Table/Fig-4]: Age-wise distribution of MRSA positive samples of male patients.

Age group (years)	Total female samples (n)	Positive female samples n (%)
0-20	21	02 (9.52)
21-40	157	06 (3.82)
41-60	111	14 (12.61)
>60	38	06 (15.78)
Total	327	28 (8.56)

[Table/Fig-5]: Age-wise distribution of positive samples of female patients.

S. No.	Antibiotic	Sensitive ratio (%)	Resistance ratio (%)
1	Vancomycin	45 (97.82)	01 (2.18)
2	Gentamicin	30 (65.22)	16 (34.78)
3	Linezolid	36 (78.26)	10 (21.74)
4	Doxycycline	39 (84.78)	07 (15.22)
5	Amoxyclav	03 (06.52)	43 (93.48)
6	Tetracycline	28 (60.86)	18 (39.14)
7	Erythromycin	02 (04.34)	44 (95.65)
8	Cotrimoxazole	13 (28.26)	33 (71.74)
9	Clindamycin	11 (23.91)	35 (76.09)
10	Ciprofloxacin	06 (13.04)	40 (86.96)
11	Cefoxitin	0	46 (100)

[Table/Fig-6]: Antibiotic sensitivity pattern of positive MRSA samples.

DISCUSSION

The MRSA has increased many fold throughout the world, India is no exception for the increase of emergence of this pathogen. There was no systematic review on prevalence of MRSA in India at one place. One meta-analysis was done in one of the study available on record which was based on results of 98 eligible articles published from 2015 to 2020 in India. This meta-analysis has evaluated state-wise, zone-wise and year-wise prevalence of MRSA in India, which is reproduced here as such. The analysis shows that in 2015, 27 articles showed the prevalence of MRSA as 38%. In 2016, 27 articles showed the prevalence of MRSA as 39%. In 2017, 20 articles showed the prevalence of MRSA as 31%. In 2018, seven articles showed the prevalence of MRSA as 35%. In 2019, 16 articles showed the prevalence of MRSA as 37%. In 2020, a single article showed prevalence of MRSA as 69% [Table/Fig-7] [23].

Year	Pooled prevalence, % (95% CI)	I ² (%)	τ ²	p-value
2015	38 (30-45)	97	0.0414	<0.01
2016	39 (29-50)	99	0.0797	<0.01
2017	31 (20-44)	99	0.0835	<0.01
2018	35 (26-43)	62	0.0091	0.02
2019	37 (28-46)	95	0.0343	<0.01
2020	69 (64-74)	-	-	-

[Table/Fig-7]: Year-wise data from meta-analysis [23].

Before start of study, the emphasis was given on accuracy in collecting samples, observing protocol and SOP'S strictly, reading the literature and history of MRSA and Lab procedures to be used.

If oxacillin and cefoxitin are tested, why are the isolates called "MRSA" instead of "ORSA"?

Since Methicillin was used in the beginning to test and treat infection caused by *Staphylococcus aureus*, the acronym methicillin Resistant *Staphylococcus aureus* (MRSA) still continue to be used though oxacillin and cefoxitin were used later in place of methicillin [24].

Present study positivity ratio was certainly encouraging in terms of prospective health of people in this region. In one study carried out in Northern India, a continuous increase in number of MRSA isolates was observed from year 2017-2019 with overall prevalence being 33.7% [25]. In one other study conducted by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, the overall prevalence of methicillin resistance during the study period was 41% [26].

Pus specimen was found having highest positive MRSA in other studies also [25] which was almost similar to present study. In one other study carried out in 2019, the isolates were mostly from the pus specimen in burn, diabetic and surgical wound patients [27]. When authors compared with one study in US, 12% were community-associated (likely OPD) and 85% were healthcare associated (likely Nosocomial); 3% could not be classified due to lack of information [28].

In one other study, 73% males and 27% females were found positive MRSA contrary to the results of present study where females had more share of positive MRSA [29]. In study elsewhere isolation of MRSA was maximum among age group of 21-30 years and 31-40 year which was almost similar to present study [30]. MRSA isolates showed greater resistance to multiple drugs than methicillin sensitive *Staphylococcus aureus* MSSA isolates. Inducible clindamycin resistance was 18.8% in MRSA as against 3.5% in MSSA. About 40-50% of MRSA were resistant to erythromycin, gentamicin, and chloramphenicol, while less than 30% were resistant to ciprofloxacin and amikacin. However, all strains were sensitive to vancomycin [31]. The higher price of vancomycin, its unavailability in many parts of the country, and also the possibility of emergence of resistance to the drug should atleast make the clinicians look into the alternatives. The regular surveillance for hospital acquired MRSA infection is required to be carried out to know the prevalence of this pathogen. The hospitals should also develop a viable and effective antibiotic policy to reduce the burden of MRSA [31]. A study from Maharashtra has reported that more than 90% isolates from Southern Maharashtra have been found resistant to penicillin, ampicillin, erythromycin, gentamycin, and tobramycin, whereas only 39.1% were resistant to methicillin [32].

The present study reports that antibiotics other than vancomycin, for instance, doxycycline, linezolid, gentamicin, can be promising if a susceptibility testing is done, reserving vancomycin for life-threatening infections. The findings have been reported different in different zones as reported in other studies. In some studies for instance, clindamycin, amikacin, ciprofloxacin, and netilmycin have shown promising on susceptibility testing basis, reserving vancomycin for life-threatening infections [33].

The MRSA nasal screening has emerged as a powerful antibiotic decision-making tool. With its high Negative Predictive Value (NPV) regardless of the method and infection type, negative MRSA nasal

swabs are more useful than positive results; given its low Positive Predictive Value (PPV), a positive MRSA nasal swab cannot be used to diagnose MRSA infection. Given consistently high NPVs, a negative MRSA nasal swab can be used to rule out MRSA pneumonia in many circumstances and avoid anti-MRSA therapy initiation or facilitate de-escalation. For infections in which MRSA is an uncommon pathogen (e.g., UTI, community-acquired intra-abdominal infection) and empiric anti-MRSA therapy is not typically indicated, MRSA nasal screening is unlikely to affect management. Finally, in patients with septic shock or other severe infections, empiric anti-MRSA therapy is often recommended, and additional factors, beyond MRSA nasal screening results, should be used to guide antibiotic decision-making [34].

Limitation(s)

The main limitation in the study was the samples collected from the patients living in and around five kilometre (approx.) radius of Medical College location. The Medical College is located in semi-urban area and patients in the study mostly belong to rural area. The outcome of the line of treatment could not be measured in the study. The test accuracy to detect MRSA may be another limitation for exactness of results.

CONCLUSION(S)

This study establishes only 7.04% MRSA which was lower side comparing the average ratio of Delhi and Uttar Pradesh and perhaps the lowest in the country according to various studies carried out across India. It was also observed that female were more susceptible for MRSA as their age advances than males. The present study reports that antibiotics other than vancomycin, for instance, doxycycline, linezolid, gentamicin, can be promising if a susceptibility testing is done, reserving vancomycin for life-threatening infections.

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