

Prevalence pattern of MRSA from a rural medical college of North India: A cause of concern

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ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a global challenge. The shift of this organism from hospital settings to community setting and increasing resistance to non- β -lactams antibiotics have further aggravated the crisis. This trend in MRSA has necessitated the knowledge and sensitization about this agent among physicians in rural and peripheral settings. **Material and Methods:** Present study was undertaken at Department of Microbiology at rural medical college of North India from January 2017 to December 2019 (3 years). All the clinical samples collected with aseptic precautions were processed as per standard protocol. All the *Staphylococcus aureus* isolates cultured were subjected to antimicrobial susceptibility testing as per CLSI guidelines 2019. Screening for MRSA was done by CLSI recommended methods, such as cefoxitin disc (30 μ g), oxacillin disc (1 μ g), and oxacillin screen agar as per CLSI recommendation. **Results:** A steady increase in number of MRSA isolates was observed from year 2017 to 2019 with overall prevalence being 33.7%. Most MRSA isolates were obtained from pus samples. Cefoxitin disc diffusion method is a dependable detection method compared to oxacillin disc diffusion and oxacillin screen agar for identification of MRSA. **Conclusion:** The rising trend of MRSA impresses upon the acute need of stringent infection control practices namely strict compliance to hand hygiene, prevention of misuse and overuse of antibiotics and a continuous surveillance program for MRSA. Also sensitization about this agent among the primary health physician is the need of hour to implement the control measures and limit its spread in communities.

Keywords: Cefoxitin disc, Hand hygiene, Infection control, MRSA detection, Multi-drug resistant MRSA and Oxacillin disc diffusion

Introduction

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. Beginning with the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in UK within two years of Methicillin launch, this organism is notorious to develop resistance against majority of empirical

antibiotics targeted against it.^[1,2] Status of being methicillin resistant itself means that a *S. aureus* isolate will not be sensitive to Penicillins, Cephalosporins, β -lactamase inhibitors, and Carbapenems and can further exhibit resistance to other classes of antibiotics.^[3,4] MRSA have been implicated in serious skin infections, necrotizing fasciitis, deep tissue abscesses, and their hematogenous spread can result in bone and joint infections, sepsis and endocarditis.^[5] Drug of choice to treat these multidrug-resistant MRSA are glycopeptide antibiotics such as vancomycin.^[6]

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Methicillin resistance is due to harboring of mec-A gene, resulting in synthesis of an 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

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late eighties, this organism was reported mostly from hospital settings but after that more and more reports starting emerging from community settings. These strains were reclassified as community-associated MRSA (CA-MRSA) strains, spreading in general population with or without exposure to the health care environment.^[7-9]

The incriminating factors for emergence of MRSA include carriage of MRSA in nose, axilla, perineum and hands of patients and health care workers (HCWs), longer hospital stay, irrational use of antibiotics, presence of indwelling devices like catheter and cannulas, immunosuppression, elderly age, insulin-requiring diabetes and decubitus ulcers etc.^[10] These factors along with the diversity in *mecA* gene pose a major challenge to prevent the spread of this agent in community and hospitals.

As per European Antimicrobial Resistance Surveillance Network report 2018, the prevalence of MRSA range from 16% to 44% in various European countries along with increasing resistance to other antimicrobial groups.^[11,12] Similarly, prevalence of MRSA was reported to be 65% by Brog, *et al.* in 2006 in Jordan.^[13] According to National Nosocomial Infection Surveillance System (NNIS) report, in USA, 50% of hospital acquired infections in ICUs are due to MRSA.^[14] Studies conducted across various centers in Korea on CA-MRSA report it to be around 13–16%.^[8,9] In India, incidence of MRSA is increasing with time as reported by many studies spread across the country.^[15-17] According to Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India in 2008 and 2009 MRSA prevalence was reported to be 41%.^[18]

Presently, the Gold standard test for detecting MRSA is identification of the *mecA* gene using polymerase chain reaction (PCR).^[19] Among phenotypic methods, cefoxitin disc diffusion (CDD) test, oxacillin disc diffusion (ODD) test, and oxacillin screen agar are recommended by CLSI for detection of methicillin resistance.^[20] CHROMagar is another phenotypic method which utilizes a chromogenic medium for the rapid identification of MRSA. Cefoxitin is taken into consideration as it is a more potent inducer of *mecA* gene expression than oxacillin or methicillin and the results obtained are comparable with detection of *mecA* gene using PCR. Oxacillin is preferred than methicillin because of longer shelf life.^[21-24]

Knowledge of MRSA prevalence and their antimicrobial profile in a health care set up is crucial to implement control measures for these infections and minimize usage of second line antimicrobials. With increasing incidence of CA-MRSA, it becomes imperative to disseminate the awareness about this agent among physicians practicing in rural healthcare settings. Therefore, we planned this study to determine the prevalence of MRSA isolates and their antimicrobial susceptibility profile from rural medical college of North India with an objective to observe the trend of MRSA in consecutive 3 years and to study the antimicrobial profile of MRSA. The findings of the study

have helped us to recommend the empirical therapy schedule to clinicians and implement stringent hospital infection control program.

Material and Methods

This observational cross-sectional study was conducted between January 1st, 2017 and December 31st, 2019 in Department of Microbiology after obtaining permission of Institutional Ethics Committee wide letter no SHKM/IEC/2018/35 dated 29/10/2018. Various clinical specimens received in the microbiology department were included in the study. All specimens except urine were cultured on blood agar and MacConkey agar. For urine, Cysteine lactose electrolyte deficient agar (CLED Agar) was used.

Microbiological processing

Isolation and identification

S. aureus were isolated and identified as per standard microbiological methods, such as colony morphology on culture, Gram stain, catalase test, slide and tube coagulase, mannitol fermentation test, and DNase production.

Antimicrobial susceptibility pattern

The antimicrobial susceptibility of all *Staphylococcus aureus* isolates was determined by Kirby Bauer disc diffusion method as per CLSI guidelines 2019. The antibiotics tested were Cefoxitin (30 µg), Oxacillin, (1 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Co-trimoxazole (1.25/23.75 µg), Clindamycin (2 µg), Erythromycin (15 µg), Linezolid (30 µg), Teicoplanin (30 µg), and Vancomycin (30 µg).^[20]

Detection of methicillin resistance

Methicillin resistance was detected by various phenotypic methods, such as cefoxitin disc diffusion test, ODD method, and oxacillin screen agar method. The inhibition zone diameters was measured around each disc and interpreted as per CLSI guidelines 2019.^[20]

Cefoxitin disc diffusion

All the isolates were tested by CDD test using 30 µg disc after making a lawn culture of 0.5 Mc Farland suspension of isolates on Muller Hinton Agar (MHA) plate. Plates were read after incubating at 37°C for 18 h. Any zone diameter of ≤19 mm was reported as cefoxitin-resistant and therefore MRSA.

Oxacillin disc diffusion

With the help of a sterile swab, 0.5 Mc Farland suspension of isolates was lawn cultured on MHA plate. After that, oxacillin disc (1 µg) was applied using sterile forceps. Plates were read after incubating at 35°C for 24 h. Any zone diameter of <10 mm was reported as oxacillin resistant and hence MRSA.

Oxacillin screen agar

Using a sterile swab, 0.5 Mc Farland suspension of isolates was spot inoculated on Muller Hinton Agar with 6 µg/ml oxacillin

with 4% NaCl. Any visible growth on these plates after incubating at 35°C indicated oxacillin resistance.

Quality control strains – Methicillin sensitive *S. aureus* (MSSA) ATCC 25923 - negative control.

Results

Total 13,506 different clinical specimens like pus and wound swab, throat swab, ear swab, blood, body fluids, catheter tip were processed over a period of 3 years (January 2017 to December 2019). From these, 240 isolates were identified as *Staphylococcus aureus* of which 81 (33.7%) were methicillin-resistant *Staphylococcus aureus*. A continuing increase in MRSA prevalence was observed over 3 years. In the year 2017, out of 25 *Staphylococcus aureus* isolates MRSA were 7 (28%). The prevalence of MRSA increased to 14/44 (31.8%) in 2018 and 60/171 (35.1%) in 2019 as depicted in Figure 1.

The prevalence was higher in males (55; 67.9%) than females (26; 32.1%) [Figure 2]. Maximum MRSA strains were isolated from patients >60 years of age which accounted for 34.6% of the total MRSA population followed by 0–15 years

age group patients (22.2%), 45–60 year of age group (16%), 30–45 year age group (14.8%), and the least was found in 15–30 year age group (12.3%) as depicted in Table 1. Maximum numbers of MRSA isolates were from inpatient (75.3%) than outpatient (24.7%). The maximum number of MRSA was isolated from the patients of surgery (25.9%) followed by intensive care units (24.6%), orthopedics (23.4%), medicine (11.1%), gynecology (9.9%), and pediatrics (4.9%) [Figure 3].

Among MRSA, the highest number was observed in pus samples (50; 61.7%) followed by urine (19; 23.4%), blood (10; 12.3%) and least was Catheter tip (1; 1.2%) followed by other body fluids (1; 1.2%) as depicted in Figure 4. When detection of MRSA by oxacillin disc is compared to cefoxitin (recommended by CLSI guidelines 2019) we observed that 8 isolates (9.9%) were missed by ODD method [Table 2].

The results of antibiotic susceptibility tests were studied [Table 3]. Out of 81 isolates, all isolates were resistant to Penicillin. 76.5% and 66.7% resistance was seen against Erythromycin and Clindamycin, respectively. Out of 81 isolates, 54 isolates were

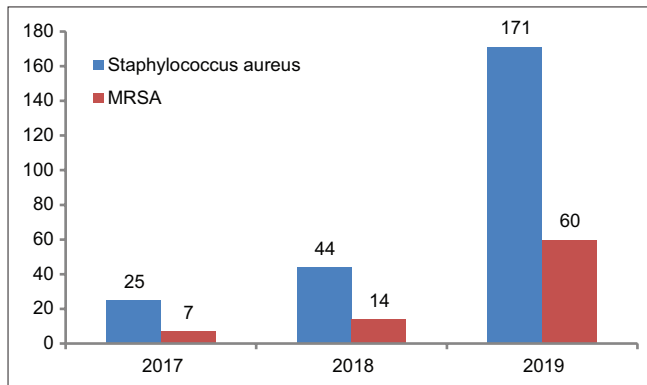


Figure 1: Trend of MRSA observed over 3 years (2017–2019)

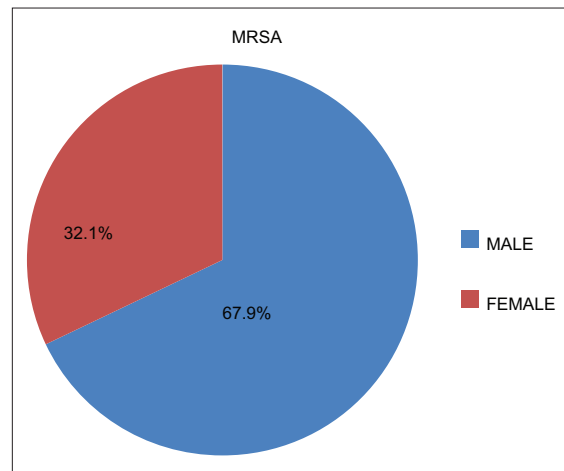


Figure 2: Gender distribution of MRSA isolates

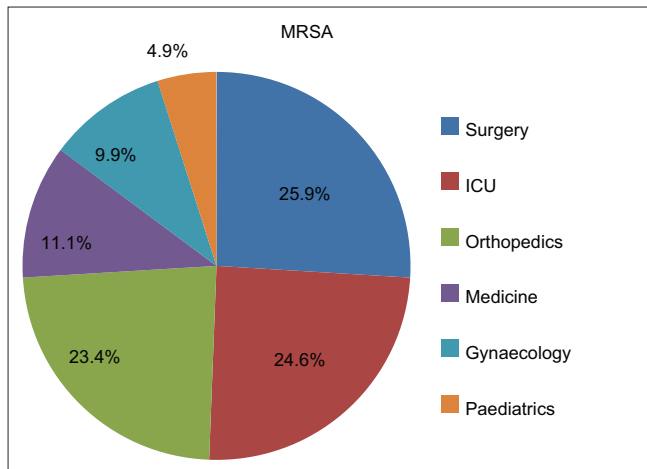


Figure 3: Department-wise distribution of MRSA isolates

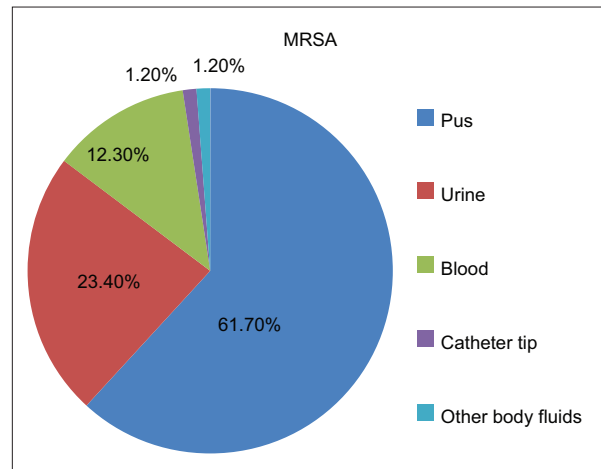


Figure 4: Sample distribution of MRSA isolates

Table 1: Age wise distribution of MRSA isolates

Age group	MRSA (n=81)
0-15	18 (22.2%)
15-30	10 (12.3%)
30-45	12 (14.8%)
45-60	13 (16%)
>60	28 (34.6%)

Table 2: Comparison of Oxacillin disc and Oxacillin screen agar with Cefoxitin disc method for detection of MRSA

Method	MRSA detected	Sensitivity (%)	Specificity (%)
Cefoxitin disc diffusion method	81	100	100
Oxacillin disc diffusion method	73	90.1	100
Oxacillin Screen Agar	78	96.3	100

Table 3: % Resistance of MRSA isolates to Antibiotics tested using Kirby Bauer Diffusion

Antimicrobials Tested	Resistance pattern of MRSA (n=81)
Penicillin	81 (100%)
Erythromycin	62 (76.5%)
Clindamycin	54 (66.7%)
Ciprofloxacin	48 (59.2%)
Gentamicin	38 (46.9%)
Cotrimoxazole	43 (53.1%)
Vancomycin	10 (12.3%)
Linezolid	6 (7.4%)
Teicoplanin	10 (12.3%)

resistant to Clindamycin (66.7%). Among 54 isolates, 25 isolates had inducible Clindamycin resistance as confirmed by D- test.^[16] Resistance to Ciprofloxacin, Co trimoxazole, and Gentamicin was 59.2%, 53.1%, and 46.9%, respectively. Majority isolates were sensitive to Vancomycin, Linezolid, and Teicoplanin.

Discussion

In the present study, from 2017 to 2019; continuous increase in MRSA isolates in the clinical specimen was observed. The number of MRSA increased from 28% in 2017 to 35.1% in 2019 [Figure 1]. The increase in trend of methicillin-resistant *Staphylococcus aureus* can be attributed to multiple risk factors like carriage of MRSA by HCWs and patients, poor compliance to hand hygiene, lack of active surveillance programs for MRSA, misuse and abuse of antimicrobials, prolonged hospitalization, and lack of bundle approach. Also, the institute is relatively new (6 years old) whereby an explanation could be that it took time for MRSA flora to localize hence lower number in early years.

In a similar study conducted in Wardha, India increasing trend was observed by Mallick and Basak.^[25] Similar rising trend were observed from Germany, United Kingdom, and Greece by Tiemersma *et al.* between 1999 and 2002.^[12]

The present study showed an average prevalence of MRSA to be 33.7% in different clinical samples over 3 years. Many studies showed similar trend of MRSA from across India ranging from 26.14% to 43%.^[26,27] However, a higher MRSA prevalence of 54.85% and 59.3% was observed by Anupurba *et al.* and Tiwari *et al.*, respectively.^[28,29] The factors responsible for rate of variations seen with different studies could be the different geographical area, variation in sample sizes and length of study, specimens, methods used for testing, antibiotic policies, and status of infection control.

While comparing gender distribution of MRSA infections, males (67.9%) were more affected than female patient (32.1%). The similar trends were observed by Rao *et al.* in 2012.^[30] The affected age group was of elderly patients >60 years in our study. Similar trends were observed by Sharma and Mall.^[31] MRSA was observed more in admitted patients (56.8%) as compared to outpatients (43.1%) cases which could be attributed to presence of MRSA in hospital environment.

In the present study, highest number of MRSA came from surgery followed by intensive care units, orthopedics, and other departments. An obvious reason for this observation is colonization of skin by MRSA and the chances of invasion increasing with use of invasive approach as with surgical departments and indwelling devices in intensive care units. Similar trends were observed by Mallick and Basak and Sanjana *et al.*^[25,32]

Among all clinical samples the highest rate of MRSA isolation (61.7%) was from pus swabs and aspirates. The predominance in pus could be due to exposure of wound to microorganism in the environment and *S. aureus* present on skin as commensal makes the wound more prone for infection. Similar findings were reported by Dar *et al.* in Aligarh (35.5%), Mallick and Basak in Maharashtra (61.4%), Tiwari *et al.* in Varanasi (42%), and Rao and Srinivas *et al.* in Andhra Pradesh (64%).^[25,29,30]

The methicillin resistance was detected using recommended phenotypic methods such as CDD, ODD, and oxacillin screen agar.^[20] Many studies indicated that disc diffusion testing employing cefoxitin disc is superior to other phenotypic methods like ODD and oxacillin screen agar testing and is now an accepted method for the detection of MRSA as per CLSI.^[19-24] So while comparing the results of oxacillin disc and oxacillin screen agar with Cefoxitin disc the sensitivity was found to be 90.1% and 96.3%, respectively, in the present study. Anand *et al.* reported similar finding from Pune, Maharashtra.^[21]

Considering the antibiogram pattern of MRSA, all isolates were resistant to antibiotic Penicillin. Out of 81 MRSA isolates, 25 had induced Clindamycin resistance (30.9%). More than half MRSA isolates were resistant to Cotrimoxazole and Ciprofloxacin. Gentamicin resistance was seen in 46.9%. Most MRSA were sensitive to second line of antibiotics like Vancomycin, Linezolid, and Teicoplanin. 12.3% isolates resistant to Vancomycin were later found to be intermediate sensitive by MIC. Similar pattern was obtained in other studies.^[32-35]

The continuous rising trend of MRSA in our institute was perceived with great concern. The major reservoir of MRSA in hospitals are colonized patients, Hospital environment and HCWs. Transient hand carriage of the organism by HCWs account as the major factor for patient to patient transmission. To address this issue, many infection control practices were implemented such as regular hand hygiene training and compliance assessment and increased MRSA surveillance of HCWs. Also stringent adherence to antibiotic policy was sought to. After these measures we look forward to decreasing MRSA numbers in our institute in future.

Conclusion

Present study stress upon the need of continuous monitoring of MRSA and their antibiogram in tertiary care setting as well as hospital located in periphery. The most effective way to prevent MRSA infection is by performing regular MRSA surveillance of HCWs, strict compliance to hand hygiene, and formulation of antibiotics policies with effective infection control practices. The message needs to spread loud and clear: We are running out of antibiotic armamentarium against *Staphylococcus aureus*, if we do not stop antibiotic abuse, we will be left with no drug to deal with this dreaded organism. Every HCW need to take hand hygiene seriously. These control measures if implemented can help control spread of this dreaded organism in hospitals as well as community.

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Conflicts of interest

There are no conflicts of interest.

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