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Original Research Article

Pattern of methicillin resistant *Staphylococcus aureus* (MRSA) in clinical isolates from a tertiary care hospital

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ABSTRACT

Background: Methicillin-resistant *S.aureus* (MRSA) is one of the leading causes of hospital acquired infections and is commonly associated with significant morbidity and mortality.
Aim and Objective: This study was undertaken to understand the prevalence, recent pattern of antibiotic susceptibility of MRSA in clinical isolates from patients attending a tertiary care hospital in Northern India.
Materials and Methods: The 460 coagulase positive Staphylococcal isolates were obtained from various samples from 460 patients attending different OPDs and also from patients admitted in IPDs and ICUs from January 2023 to June 2023. Antibiotic susceptibility testing was performed from the isolates and interpreted as per standard CLSI guidelines. MRSA isolates were identified by cefoxitin (30μg) disk testing, E-test and by VITEK 2 system.
Results: A total of 140 (30.43%) were found to be methicillin resistant. Out of 140 MRSA isolates, 109 (77.85%) were MDRSA (Multi drug resistant *Staphylococcus aureus*). 10 (7.14%) isolates were also resistant to Vancomycin and 5(3.57%) of the MRSA isolates were also resistant to linezolid.
Conclusion: The overall prevalence of MRSA during the study period was 30.43%. Prevalence of MRSA isolates was found to be significantly higher among isolates from indoor patients (57.1%) than OPD (31.4%) and ICU patients (12.8%). Antibiotic policies based on continuous surveillance of antibiotic resistance profiles of local strains is one of the most effective intervention to prevent MRSA infections.

1. Introduction

Methicillin-resistant *S. aureus* (MRSA) is well known to commonly cause infections of skin and soft tissue but may also involve surgical wounds and bone and joints while less commonly infections of urinary tract and respiratory tract. MRSA is also implicated in life-threatening infections of prosthetic devices like indwelling catheters. MRSA can thrive for months in a hostile environment which facilitates the transmission from surfaces long after its initial deposition.[1]

As per systemic review and meta-analysis done on prevalence of Methicillin-resistant *S. aureus* (MRSA) in India, overall prevalence of MRSA was reported as 37%. Zone-wise pooled prevalence of MRSA was found as 41%, 43%, 33%, 34%, 36%, and 40% respectively for north, east, west, south, central and north-east zones.[2] The results across different states showed a predominance of MRSA in Jammu & Kashmir with 55% prevalence while the lowest prevalence was 21% in Maharashtra.[2] Owing to its ability to cause variety of serious health complications, MRSA has emerged as one of the well-known etiologic agents for a wide variety of infections in both hospital and community

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setting. Colonized healthcare workers (HCWs) and patient-contaminated surfaces act as reservoirs and promote cross-infection to other patients, healthcare workers and may also involve the community.[3]

S. aureus possesses various potent virulence factors which contribute in its ability to act as pathogen or to persist as a commensal and also in developing antimicrobial resistance.[4] WHO in 2014 published a data where 80% of *Staphylococcus aureus* infections were to be of multidrug resistant *Staphylococcus aureus* (MRSA).[5]

Presence of mecA gene sequence in MRSA generates transpeptidase PB2a which lowers the binding affinity of MRSA to beta-lactam antibiotics and makes it resistant to this group of antibiotics.[1,6]

This study was undertaken to study the prevalence, recent pattern of antibiotic susceptibility of MRSA in clinical isolates from patients attending a tertiary care hospital in Northern India.

2. Materials and Methods

2.1. Study design

This study was retrospective, conducted from Jan 2023 to June 2023 in Department of Microbiology of a tertiary care centre in North India.

2.2. Sample collection method

The 460-coagulase positive Staphylococcal isolates were obtained from various samples of the patients (N=460) attending different OPDs and also from patients admitted in IPDs and ICUs. Staphylococcal isolates were taken mainly from samples such as respiratory fluids (sputum, bronchial washings, Endotracheal aspirates etc), endotracheal catheter tips and pus samples (aspirates and swabs). Other swabs received were collected from ear, nasal swabs and throat. Genital specimens such as High vaginal swabs were included in the study as well.

2.3. Processing of samples

The collected samples were inoculated on two types of agar media, 5% sheep blood agar and MacConkey's agar (HiMedia, New Delhi, India), and subsequently incubated at 37°C for 24-48 h, and then the plates were observed for bacterial growth. Beta haemolytic colonies in blood agar plates were taken for gram's staining and biochemical tests were performed for confirmation of bacterial pathogens. *S. aureus* isolates were identified depending upon colony morphology and standard biochemical tests like catalase, coagulase test (slide and tube), etc.

2.4. Antibiotic susceptibility testing

Kirby Bauer's' disc diffusion technique was used to detect the antibiogram of the isolates.[7] Clinical and Laboratory Standards Institute (CLSI) 2022 recommendations were followed. Co-trimoxazole (1.25/23.75μg), clindamycin (2μg), vancomycin (30μg), ciprofloxacin (5μg), gentamicin (10μg), erythromycin (15μg), tetracycline (30μg), linezolid (30μg), and penicillin (10 Units) were also tested for sensitivity. All the discs were obtained from Hi-media (Mumbai HiMedia, Maharashtra, India). Inoculum with a turbidity of 0.5 McFarland standard was prepared by making a saline suspension of isolated colonies from blood agar plate and as per CLSI guidelines five discs were applied on a 100mm Mueller Hinton agar plate.[8] For quality control of antimicrobial susceptibility for disc diffusion *S. aureus* ATCC 25923 strain was used. MRSA isolates were identified by cefoxitin (30μg) disc testing and MIC by E (Epsilometer)-test (Liofilchem, Italy). Cefoxitin Disc diffusion test was performed by making inoculum of test isolate. Subsequently test isolate was incubated for 2-3 hours and then isolate turbidity was matched to 0.5 McFarland standard. After standardization of inoculum, MHA plate was inoculated for lawn culture and cefoxitin disc 30 μg was placed and plate was incubated for 35°C for 18-24 hour. The < 22mm cefoxitin disk (30μg) zone was considered as Methicillin resistance as per CLSI 2022 guidelines. E-test was performed on Mullar-Hilton Agar plate. Test stain was lawn cultured on plate. Oxacillin strip was applied on the inoculated plate further plates were incubated at 35°C for 18-24 hour. MIC observed where ellipse of zone of resistance was intersected. MRSA is considered when MIC of ≥4μg/ml. All MRSA isolates were also tested by VITEK 2 system (bioMérieux, Marcy-l'Étoile, France) as per manufacturer's instructions.

3. Results

The < 22mm cefoxitin disk (30μg) zone was considered as Methicillin resistance as per CLSI 2022 guidelines. Among the total 460 coagulase positive Staphylococcus, 140 (30.43%) were found to be methicillin resistant. Out of 140 MRSA isolates, 80 (57.1%) were from indoor wards (non-ICU), 44 (31.4%) from OPD and 16 (12.8%) were from ICU patients. (Figure 1)

Maximum isolation of MRSA was obtained from pus swabs and tissue samples (55%) followed by respiratory samples (17.14%) and catheter tips (12.85%). Less isolation was observed from throat and ear swabs (7.8%), pus aspirates (5%) and genital swabs (2.14%) (Table 1).

All (100%) isolates were found resistant to Penicillin. Majority of the MRSA isolates exhibited resistance to ciprofloxacin (95.7%) while 75.7% isolates were found resistant to erythromycin. Resistance to gentamicin, clindamycin, cotrimoxazole and tetracycline were 43%, 35.7%, 35.7% and 31.4% respectively. (Figure 2)

10 (7.14%) isolates out of 140 MRSA were also resistant to Vancomycin. These included 7 isolates from pus swabs, 2 from catheter tips and 1 from ETA. 5 (3.57%) MRSA

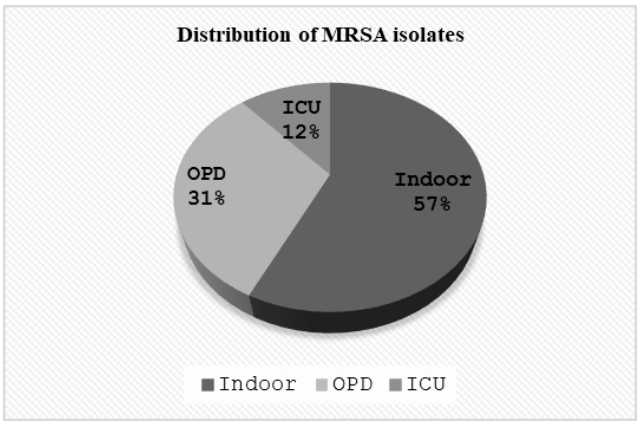

Figure 1: Distribution of MRSA isolates as per hospital setting

Table 1: Percentage distribution of MRSA as per respective samples

Sample type	Percentage distribution of MRSA as per samples (N-140)
Pus Swabs (SSTIs) and tissues	77 (55%)
Respiratory (BAL, sputum, ETA)	24 (17.14%)
Catheter tips	18 (12.85%)
Throat swabs and ear swabs	11 (7.8%)
Pus aspirates	7 (5%)
Genital swabs	3 (2.14%)

SSTIs: Skin and soft tissue infections; BAL: Bronchoalveolar lavage; ETA: Endotracheal aspirate

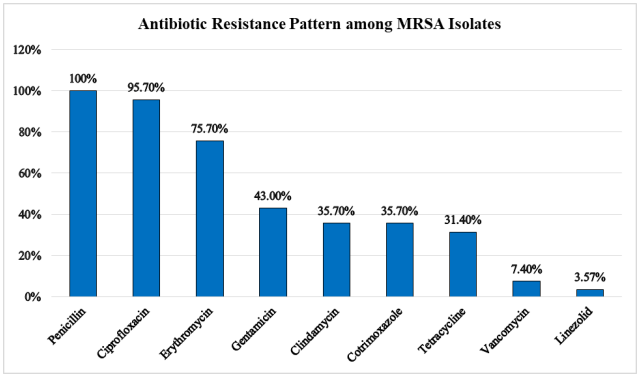

Figure 2: Antibiotic resistance pattern among MRSA isolates

isolates were also resistant to linezolid. All the linezolid isolates were obtained from pus swabs, collected from superficial skin infections.(Figure 2)

Out of 140 MRSA isolates, 109(77.85%) were MDRSA (Multi drug resistant *Staphylococcus aureus* i.e. non-susceptible to ≥ 3 classes of antimicrobials).

4. Discussion

Staphylococcus spp. remains the leading cause of skin and soft tissue infections within the community. Moreover, its endemicity has made it an important nosocomial pathogen in Indian hospital settings. The prevalence of MRSA in our study population was observed as 30.43%. This correlates with the reports from other studies stating the incidence of MRSA around 25% in western India to around 50% in Southern India.[9,10] In a study conducted in north India, the prevalence of MRSA was found to be 46% and MRSA isolates were observed to be more resistant to other antibiotics than MSSA.[11]

According to ICMR 2022 report the overall prevalence of MRSA was found to be 44.5%.[12] The majority of MRSA isolates (55%) were obtained from cases of skin and soft tissue infections. The findings align with a study published by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, which included observations from 15 Indian tertiary care centres over the period of two years. The proportion of MRSA from skin and soft tissue infections was also higher (51.5%) as per ICMR report 2022.[12,13] Throat swab and genital specimens were predominantly received from outpatients, while *S. aureus* isolates from tissue, catheter tips and fluid from sterile body sites were predominantly received from inpatients.

The majority of isolates were obtained from IPD patients (57.1%) followed by OPD (31.4%) patients in our study. Overall MRSA rates among *S. aureus* isolates were lowest in OPD (40.1%), moderate among ward isolates (47.3%) and highest among isolates obtained from ICU patients (50.1%) as per ICMR report 2022.[12]

In India, Hospital-acquired MRSA (HA-MRSA) has always been a major cause of nosocomial infections but cases of Community acquired MRSA (CA-MRSA) are also being reported with increased frequency. A study reported 70% cases of CA-MRSA having SCCmec IV and SCCmec V genes, among 412 cases of MRSA isolated mainly from skin and soft tissue infections.[14] The prevalence of MRSA in our ICU setting (12%) was much lower when compared to other studies.[15,16] The prevalence of MRSA in ICUs is recognized as a benchmark for hospital infection-control practices.

Glycopeptide antibiotics are commonly used to treat MRSA and MDRSA infections and vancomycin is considered as drug of choice. Indiscriminate use of vancomycin results in increasing drug resistance and as a risk factor for developing VRSA. So, glycopeptide

antibiotics should be used only as reserve drug for life-threatening MDR MRSA infections.[1,11] The prevalence of LR-MRSA was 3.57% in this study. MDRSA strains having additional resistance to linezolid (Linezolid-resistant MRSA, LR-MRSA) and Tigecycline are also being reported from India.[17,18]

The emergence of multiple drug resistance among *Staphylococcus aureus* isolates is a significant concern, though the exact prevalence and clinical implications have yet to be determined. The prevalence of MDRSA in this study was 77.85%. Similar findings were observed by a recent study published from another northern India hospital, stating the MDRSA prevalence as 73%.[11] The association of multidrug resistance with MRSA increases the problem as very limited options are available in terms of antibiotic treatment to be given to the patient. It is noteworthy to mention here that, as per our study, co-trimoxazole and tetracycline has re-emerged as a sensitive group of drugs, when compared to other class of drugs, which can be incorporated as appropriate empirical treatment as per antibiotic policy of the hospitals, sparing the higher groups of antibiotics. The similar finding was published by another study, where percentage resistance of co-trimoxazole and tetracycline were both reported to be 25%.[19] As per ICMR report 2022, the susceptibility to most antibiotics was observed least among ICU isolates and highest among OPD isolates of *S. aureus* including MRSA and coagulase-negative Staphylococcus (CONS). Methicillin-sensitive *S. aureus* (MSSA) isolates were found to be more susceptible to tetracycline, clindamycin, co-trimoxazole, erythromycin and ciprofloxacin compared to MRSA. Excellent in-vitro activity (100% against MRSA isolates) was observed for anti-MRSA antibiotics such as vancomycin and tigecycline.[12] Resistance to Linezolid among MRSA, CONS and MSSA isolates showed rates of 2.1%, 0.9% and 0.6% respectively. The variation in MRSA prevalence across different regions may be observed due to the different antibiotic usage depending upon areas, hospital antibiotic policies and infection control policy.[12]

5. Conclusion

The overall prevalence of MRSA during the study period was 30.43%. Maximum Isolation of MRSA was obtained from pus swab and tissue samples (55%). Prevalence of MRSA was found to be more among indoor patients (57.1%) than outdoor patients (31.4%) and ICU (12.8%).

Antibiotic selection as per antibiotic protocols based on continuous surveillance of antibiotic resistance profiles of local strains is one of the most effective intervention to prevent MRSA infections. If culture sensitivity results in a MSSA isolate the treating physician should de-escalate the antibiotic to beta-lactams. Linezolid and glycopeptide antibiotics should only be recommended to be used in confirmed cases of MRSA. A local antibiogram should be available with the clinicians to start with the therapeutic drug, while awaiting for the AST report. Ours is a country where rampant use and prescription of antibiotic is very usual. With no check on antibiotic use in humans, poultry and livestock, surveillance is our only last hope.

6. Conflict of Interest

None.

7. Source of Funding

None

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