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Original article

Comparative Assessment of Antibacterial Susceptibility Patterns of Methicillin Resistant Staphylococcus aureus (MRSA) Isolates at a Tertiary Care Hospital with Reference to ICMR Guidelines

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Abstract

Staphylococcus aureus is a significant pathogen associated with numerous hospital-acquired and community-related infections that result in high rates of illness and death. The rise of methicillin-resistant Staphylococcus aureus (MRSA) has escalated with a growing concern globally, including in India. Furthermore, MRSA has been known to show the highest resistance to most existing antimicrobial treatments. This study aims to assess the prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) and its susceptibility to antibiotics at a Tertiary Care Hospital in Indore (M.P.) according to ICMR guidelines. The research was conducted from May 2024 to April 2025 at the tertiary care hospital in Indore. A total of 611 clinical samples were analyzed for this study. The isolates were identified following standard laboratory protocols, which included staining, evaluating colony morphology, and conducting biochemical tests. All isolates underwent antibiotic susceptibility testing using the modified Kirby Bauer disk diffusion method. Out of the 611 clinical specimens, 608 Staphylococcus aureus isolates were identified and assessed for MRSA using the Cefoxitin disc diffusion test. Among these 608 Staphylococcus aureus isolates, 469 were identified as MRSA. The overall prevalence rate of MRSA isolates was 77.14%. The majority of MRSA was isolated from pus samples (69.72%), followed by blood infections (13.85%) and tissue swabs (6.82%). MRSA exhibited the greatest resistance to fluoroquinolones, with levofloxacin showing 97.7% resistance and ciprofloxacin at 94.3%, while there was no resistance detected to tigecycline, and only minimal resistance to nitrofurantoin. It is crucial to screen for MRSA and monitor its antimicrobial susceptibility patterns for early detection and management of the condition within hospitals. The antibacterial susceptibility profiles were also compared to the ICMR/NCDC-recommended anti-MRSA medications. This comparison indicates strong overall alignment between the antibiotic testing panel used in the study and the ICMR/NCDC-recommended anti-MRSA treatments, supporting the validity of the susceptibility results.

Keywords: *Staphylococcus aureus, MRSA, Resistance, Antibacterial susceptibility, Tigecycline, ICMR.*

Introduction

Staphylococcus aureus (S. aureus) is commonly present on the skin and in the nasal passages of healthy individuals. While it is harmless in these areas, it can enter the body through skin injuries such as cuts, abrasions, surgical wounds, or indwelling catheters, potentially causing infections ranging from mild to life-threatening sepsis in humans. [(1)]

In the past antibiotic era, invasive S. aureus infections were typically fatal. The advent of penicillin significantly enhanced the outcomes for serious infections; however, resistant bacterial strains emerged shortly after due to the production of β-lactamases. In 1960, methicillin was introduced as an alternative for treating penicillin-resistant infections, yet the first case of MRSA was documented in the United Kingdom in 1961. [(2)] Methicillin resistance in S. aureus arises from mutations in the mecA gene, which alters the penicillin-binding protein (PBP) on the S. aureus cell membrane to PBP2a. This modification causes the new penicillin-binding protein to bind beta-lactam antibiotics less effectively, resulting in resistance to all drugs in that class and consequently restricting treatment options for this pathogen. [(3)]

Antimicrobial resistance (AMR) poses a significant challenge within healthcare settings, with Methicillin-resistant Staphylococcus aureus being a growing global issue. The World Health Organization (WHO) has categorized MRSA as a 'High' priority pathogen. [(4)] MRSA strains are resistant to all currently available beta-lactam antibiotics, including penicillin, cephalosporins, and carbapenems, with the exception of ceftaroline & ceftobiprole. The global prevalence of MRSA is difficult to determine, whereas national surveillance data and publications from all World Health Organization (WHO) regions reported a prevalence ranging from 0% to 100%: African Region (0%–100%), Region of America (21%–90%), Eastern Mediterranean region (10%–53%), European region (0.3%–55%), South-east Asia region (10%–26%), Western Pacific region (4%–70%). [(5)]

Currently, MRSA infections are most prevalent in India. According to a systematic review and meta-analysis on the prevalence of Methicillin-resistant S. aureus (MRSA) in the country, the overall MRSA prevalence was found to be 37%. The pooled prevalence by region was recorded as 41%, 43%, 33%, 34%, 36%, and 40%, respectively, in the north, east, west, south, central, and northeastern zones. Different states exhibited varying prevalence rates, with Jammu & Kashmir showing the highest rate at 55%, while Maharashtra had the lowest at 21%. [(6)]

Therefore, this study was conducted to investigate the prevalence and recent trends in antibiotic susceptibility of MRSA among clinical isolates from patients at a tertiary care hospital in Indore, Central India. This trend was also compared with the recommended anti-MRSA medications from ICMR/NCDC guidelines.

Methods

Study design and setting

The current research was retrospective study conducted in the department of Pharmacology & department of Microbiology of a tertiary care hospital. Prior to commencing the study, approval was obtained from the Institutional Ethical Committee (IEC). All Staphylococcus aureus isolates obtained from various clinical specimens during the May 2024 to Aprill 2025 were included in the study and duration of study is six months.

Sample

A total of 611 samples suspected of being infectious were collected and analyzed in the diagnostic microbiology laboratory during the study period. The study encompassed a variety of specimens, including pus/wound swabs, tissue samples, blood, urine, pleural fluid, central venous catheter/foleys tip, throat swabs, and sputum.

Identification of bacterial isolate

The gathered samples were inoculated onto two varieties of agar media: 5% sheep blood agar and MacConkey's agar followed by incubation at 37°C for a duration of 24 to 48 hours, after which the plates were examined for bacterial growth. Beta-hemolytic colonies from the blood agar plates were selected for Gram staining, and biochemical tests were conducted to confirm the presence of bacterial pathogens. S. aureus isolates were identified based on colony appearance and standardized biochemical tests, such as the catalase test and both slide and tube coagulase tests.

Identification of MRSA

All isolates of Staphylococcus aureus underwent testing for MRSA using the Cefoxitin disc diffusion method. Mueller Hinton Agar (MHA) plates were inoculated with a standardized inoculum (0.5 McFarland standard) of Staphylococcus aureus using a sterile swab. A 30 µg cefoxitin disc was positioned at the center of the plate. The plates were then incubated at 37º C for 24 hours, and the zone diameters were measured in accordance with CLSI guidelines. [(7)] It is essential to measure the zone diameter in reflected light. An inhibition zone diameter of ≤ 22 mm was classified as methicillin-resistant, while a diameter of ≥ 22 mm was deemed methicillin-susceptible.

Methicillin-sensitive S. aureus (MSSA) ATCC 25923 and methicillin-resistant S. aureus (MRSA) ATCC 43300 were utilized as quality control strains. All the MRSA cases were included.

Antibiotic susceptibility testing

A modified Kirby–Bauer disk diffusion technique was utilized to conduct antibiotic susceptibility testing on MRSA isolates. A sterile cotton swab was immersed in a bacterial suspension adjusted to a 0.5 McFarland turbidity standard. Following this, Muller–Hinton plates were inoculated using sterile cotton swabs, after which antibiotic discs were placed on them and incubated at 35°C for 24 hours. Antibiotic susceptibility testing and result analysis will be conducted in accordance with CLSI guidelines. [7] The antibiotics examined for MRSA strains included: Vancomycin, linezolid, clindamycin, doxycycline, gentamycin, ciprofloxacin, teicoplanin, tetracycline, tigecycline, daptomycin, rifampin, erythromycin, and cotrimoxazole. These results will then be compared with the recommended antibiotics for specific MRSA treatment as per ICMR guidelines.

Data analysis

Descriptive statistics was used for analysis. Data entry and analysis were conducted using Microsoft Excel® 2021 software. The findings concerning initial patient characteristics, microbial occurrence, and antibiotic resistance were displayed in the form of graphs and percentages.

Results

In total, 611 specimens were analyzed. Among these, staphylococci strains identified through standard methods numbered 608. Out of these, the strains that exhibited methicillin resistance (MRSA) accounted for 469 (77.14%). Consequently, cases of Methicillin-sensitive Staphylococcus (MSSA) were found to be 139 (22.86%).

The majority of MRSA isolates were derived from pus samples (69.72%), followed by blood infections (13.85%) and tissue swabs (6.82%). Minimal isolation was detected from ear discharge, and nasal swabs (0.21%). (Table 1) Among the 139 MSSA cases, 95 (80%) were isolated from pus, 21 (10.6%) from urine, 7 (8.2%) from sputum, and 1 (1.2%) from a throat swab.

Table 1: Percentage distribution of MSSA and MRSA in various clinical specimens

Clinical Specimen	*S. aureus* (608)	MSSA (139)	%	MRSA (469)	%
PUS	423	96	69.06	327	69.72
BLOOD	86	21	15.1	65	13.85
TISSUE	43	11	7.91	32	6.82
URINE	11	3	2.15	8	1.7
SPUTUM	7	0	0	7	1.49
PLEURAL FLUID	11	4	2.87	7	1.49
BRONCHOALVEOLAR LAVAGE	5	1	0.71	4	0.85
NASAL SWAB	1	0	0	1	0.21
EYE DISCHARGE	2	0	0	2	0.42
EAR DISCHARGE	1	0	0	1	0.21
TIP FOLEYS/CENTRAL LINE CATHETER	15	3	2.15	12	2.55
TRACHEAL CULTURE	3	0	0	3	0.63

MRSA displayed the highest levels of resistance to fluoroquinolones, including levofloxacin (97.7%) and ciprofloxacin (94.3%), as well as to benzylpenicillin (96.2%) and erythromycin (68.1%). Moderate resistance is also observed in MRSA for trimethoprim-sulfamethoxazole (46.8%), gentamicin (26.7%), and clindamycin (21%) as shown in table 2. Conversely, MSSA shows reduced resistance rates across all antibiotics when compared to MRSA.

Table 2: Antibiotic sensitivity pattern of MRSA and MSSA

Antibiotics	MRSA showing resistance (Number)	%	MSSA showing resistance (Number)	%
Vancomycin	15	3.2	0	0
Linezolid	8	1.7	1	0.7
Clindamycin	100	21	3	2.2
Gentamycin	127	26.7	7	5.2
Ciprofloxacin	449	94.3	114	84.4
Teicoplanin	4	0.8	0	0
Tetracycline	42	8.8	3	2.2
Tigecycline	0	0	0	0
Daptomycin	40	8.4	0	0
Rifampicin	27	5.7	0	0
Erythromycin	324	68.1	47	34.8
Levofloxacin	465	97.7	121	89.6
Nitrofurantoin	3	0.6	0	0
Benzylpenicillin	458	96.2	117	86.7
Trimethoprim-Sulfamethoxazole	223	46.8	37	27.4

Table 3 provides a comparative analysis of antimicrobial susceptibility patterns of MRSA isolates collected from various clinical specimens with the recommended Indian Council of Medical research (ICMR)/NCDC antimicrobial agents for anti-MRSA

Table 3: Comparison of antibiotic susceptibility patterns of MRSA isolates with ICMR/NCDC Recommended Anti-MRSA Drugs

Clinical Specimen	MRSA isolates (n)	Anti MRSA Drugs by ICMR/NCDC	Susceptibility (%)
Pus	327	Teicoplanin	99.1
		Vancomycin	96.9
Blood	65	Teicoplanin	98.5
		Vancomycin	96.9
Tissue	32	Teicoplanin	100
		Vancomycin	96.9
Urine	8	Teicoplanin	100
		Vancomycin	87.5
Sputum	7	Vancomycin	100

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		Linezolid	100
		Teicoplanin	100
Pleural Fluid	7	Vancomycin	100
		Linezolid	100
		Teicoplanin	100
Bronchoalveolar Lavage	4	Teicoplanin	100
		Vancomycin	100
Nasal Swab	1	Vancomycin	100
		Linezolid	100
Eye Discharge	2	Vancomycin	100
		Linezolid	100
Ear Discharge	1	Teicoplanin	100
		Vancomycin	100
Tip foleys/central line catheter	12	Teicoplanin	100
		Vancomycin	83.3
Tracheal culture	3	Vancomycin	100
		Linezolid	100
		Teicoplanin	100

Discussion

In India, the importance of Methicillin-resistant Staphylococcus aureus (MRSA) was acknowledged relatively late, becoming a concern in the 1980s and 1990s. The rise in MRSA cases in India has been attributed to factors such as lack of awareness, excessive use of antimicrobial drugs, insufficient sanitation and hygiene, and the absence of strict regulations regarding antibiotic use. [6] MRSA has now become endemic in India and poses a significant threat as a pathogen responsible for hospital-acquired infections.

The MRSA prevalence identified in this study was 77.14%, reflecting a strikingly elevated level of resistance that ranks among the highest documented rates in existing literature. This percentage is significantly above the average prevalence, which is typically between 30% to 60% in many regions of India. [8-15]. Gupta also reported similar findings to our study, with MRSA prevalence cited at 80.8%. [8] The increased rate observed in our research could be linked to the study being carried out at a tertiary care multispecialty facility, which attracts more patients from remote areas and smaller nursing homes, where the misuse of antibiotics and poor infection control measures are common. Extended use of broad-spectrum antibiotics makes patients more susceptible to infections caused by antibiotic-resistant pathogens such as MRSA.

The significant differences in prevalence rates could result from various factors such as study design, inclusion and exclusion criteria, types of specimens, laboratory methods, duration of the study, population demographics, and the phenotypic or genotypic traits being analyzed. These factors can occasionally lead to an exaggerated estimation of MRSA prevalence based on a single center study, and this inflated rate is then applied nationwide.

Most MRSA was isolated from pus samples (n=327, 69.72%). A similar observation was made by Ramesh, who reported the highest MRSA isolation from pus samples (81%). [16] Notably, MRSA has no resistance to tigecycline and demonstrates very low resistance to nitrofurantoin, indicating that these agents remain effective. This underscores the considerable therapeutic challenge that MRSA presents due to its wider resistance profile and highlights the importance of selecting antibiotics carefully in clinical treatment.

In most specimen types, essential antibiotics like vancomycin, teicoplanin and linezolid were consistently featured in both the study and national guidelines, suggesting methodological consistency. However, there were some discrepancies, as certain agents like clindamycin, cotrimoxazole, tetracycline, or fluoroquinolones were included in this study but are not universally part of standardized MRSA treatment protocols.

Samples such as blood, tissue, sputum, pleural fluid, bronchoalveolar lavage (BAL), and others were entirely comparable, indicating that the testing strategy employed in the study was well-aligned with national guidelines. There was partial comparability observed in urine specimens, mainly attributed to the use of vancomycin and nitrofurantoin, which are not part of the guideline-recommended anti-MRSA treatment. No isolates were found in abscess samples during the study period, rendering comparison irrelevant. These results collectively suggest that the antimicrobial panel utilized accurately reflects national treatment standards, thereby reinforcing the validity of the study's susceptibility findings.

Study limitations

The research on MRSA distribution has various limitations. The sample size might not accurately reflect the population and could lead to sampling bias. Factors like the length of hospital stay and antibiotic utilization were not taken into account, and the absence of comparison groups restricts interpretation. The retrospective design does not consider long-term trends, and the accuracy of the data relies on proper documentation. Assuming a consistent distribution for expected frequencies might not be suitable, and different sample types have varying effectiveness in detecting MRSA. The findings may have restricted external validity and lack comprehensive clinical context.

Conclusion

Our research indicates the frequency and antimicrobial susceptibility trends of MRSA. We found that MRSA strains were prevalent and exhibited multidrug resistance. Nevertheless, some antibiotics like tigecycline and nitrofurantoin were still effective against MRSA and should be reserved for confirmed MRSA cases in the studied population. The emergence of resistant strains highlights the necessity of ongoing monitoring of antibiotic resistance patterns among local strains and careful antibiotic usage to effectively address this global health concern. Moreover, further confirmatory research should be conducted to detect the mecA gene using the PCR method, which is considered the gold standard. The comparison of the antibiotics examined in this study with those recommended by ICMR/NCDC for Anti-MRSA treatment shows strong agreement for managing MRSA infections.

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