ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 12, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

ANALYSIS OF ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN A TERTIARY CARE HOSPITAL

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Received: 24 September 2024, Revised and Accepted: 06 November 2024

ABSTRACT

Objective: The present study aimed to evaluate MRSA prevalence among *Staphylococcus aureus* isolates, identify demographic factors associated with infections, and evaluate susceptibility to various antibiotics.

Material and Methods: The study involved various clinically infectious samples. *S. aureus* was identified using a battery of tests and MRSA was identified utilizing the cefoxitin disk diffusion technique. At the same time, adherence to Clinical and Laboratory Standards Institute protocols guided the execution of the antibiotic susceptibility assay.

Results: Out of 28,239 culture samples, 569 *S. aureus* isolates were detected, with 308 (54.1%) being MRSA. Most samples originated from individuals aged 41–60 years (n=110, 36%), and males accounted for (n=205, 67%) of the isolates. Pus samples notably yielded the highest proportion of MRSA (n=249, 80.8%), primarily from the surgery ward (n=120, 38.9%). Remarkably, the strains demonstrated substantial sensitivity (>90%) to linezolid, vancomycin, chloramphenicol, and doxycycline.

Conclusion: In summary, MRSA strains were sensitive to drugs such as linezolid, vancomycin, chloramphenicol, and doxycycline. The emergence of resistant variants emphasizes the necessity for continuous surveillance and careful antibiotic use, informing antibiotic stewardship programs and clinical strategies for managing MRSA infections in health-care settings.

Keywords: MRSA, CA-MRSA, HA-MRSA, *Staphylococcus aureus*, Gram-positive cocci.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i12.52763. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major therapeutic issue worldwide. The prevalence of MRSA infections has reached epidemic levels worldwide, leading to fewer treatment options [1]. Extended hospital stays, higher rates of mortality and morbidity, and financial hardship are all linked to MRSA infections [2].

There is a broad spectrum of infections caused by MRSA, extending from minor scalded skin infections to, potentially fatal diseases such as endocarditis, severe sepsis, pneumonia associated with ventilator use, osteomyelitis, and fatal necrotizing fasciitis; additionally, toxicoses such as toxic shock syndrome, scalded skin syndrome, food poisoning, and fatal necrotizing pneumonia are also caused by MRSA [3].

The reports have pointed out novel MRSA strains that differ genetically, and these include community-associated MRSA (CA-MRSA) as well as livestock-associated MRSA (LA-MRSA) that were initially viewed as healthcare-related problems hence giving rise to the term hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) [4].

Staphylococcus aureus colonizes the nose and the skin of 30% of the population. Hospitals are the main place where MRSA gets transmitted. MRSA causes most nosocomial infections (25–50%). Transmission of MRSA is facilitated by a prolonged hospital stay, immunosuppression, overuse of antibiotics, indwelling catheters, invasive medical devices, drug addiction, and insufficient infection control protocols [5].

The pathogen is widespread nowadays and poses a substantial concern to public health because most anti-staphylococcal medications, such as penicillin, ciprofloxacin, gentamicin, erythromycin, cotrimoxazole, norfloxacin, tetracycline, chloramphenicol, and rifampin are not effective against these isolates [6].

METHODS

Study design

An unblinded retrospective study was initiated following approval from the Institutional Ethics Committee (SDUMC/KLR/IEC/652/2023-24) at the Department of Microbiology, Sri Devaraj Urs Medical College, Kolar, from 2021 to 2023.

Source of data

All clinically suspected infectious samples were submitted to the diagnostic microbiology laboratory. Patient demographics such as age, sex, and clinical sample site were documented in a proforma.

Study subjects

Inclusion criteria

Various specimens were included in the study which provides pus/wound swabs, tissue specimens, blood, urine, high vaginal swabs, cerebrospinal fluid, central venous pressure tips, throat swabs, and sputum.

Exclusion criteria

The following criteria were excluded from the study:
Patients on antibiotics
Grossly contaminated samples
Incomplete patient details

Identification of bacterial isolate

All samples were inoculated on blood, chocolate, and MacConkey agar. Identification of *S. aureus* was done by colony morphology, Gram

staining, and catalase test. Strains positive for coagulase were identified as *S. aureus* and methicillin susceptibility was determined using cefoxitin disk.

A modified Kirby–Bauer disk diffusion method was employed to perform antibiotic susceptibility testing. A total of 3–5 colonies isolated from the blood agar plates were inoculated in normal saline using a sterile wire to make the bacterial suspension.

The turbidness of suspension was then standardized to 0.5 McFarland turbidity standard, after which Muller–Hinton plates were inoculated using sterile cotton swabs, and then antibiotics were placed and incubated at 35°C for 24 h

The zones of inhibition around the antibiotic disk were measured and reported according to the Clinical and Laboratory Standards Institute guidelines. The cefoxitin-resistant strain was labeled as MRSA and the cefoxitin-sensitive strain was labeled as methicillin-sensitive *S. aureus* MSSA.

The tested antibiotics reported for MRSA strains were: Vancomycin, linezolid, gentamycin, clindamycin, tetracycline, penicillin, doxycycline, erythromycin, ciprofloxacin, levofloxacin, chloramphenicol, and nitrofurantoin (for urine culture only).

Statistical analysis
A Chi-square test was utilized for statistical analysis.

RESULTS

A total of 569 *S. aureus* bacteria were isolated from 28,239 culture samples. Of these, MRSA were (n=308, 54.1%), MSSA (n=233, 40.9%), and CoNS (coagulase-negative *Staphylococcus*) [n=28, 4.9%] isolates.

According to Table 1, the predominant age group was 41–60 years (110, 36%), followed by 21–40 years (85, 28%), 61–80 years (72, 23%), and < 1–20 years (41, 13%). MRSA is more prevalent among the elderly due to weakened immunity, underlying conditions, frequent health-care exposure, and longer hospital stays, necessitating stringent infection control measures. The p-values for the age groups < 1–20 years and 41–60 years are < 0.0001 which is statistically significant.

According to Fig. 1, Out of a total of 308 isolates of MRSA, (n=103, 33%) were from females, and (n=103, 33%) were from males. MRSA is more common in males due to biological differences in immune response, behavioral patterns favoring higher-risk activities, and potentially delayed healthcare-seeking behavior.

MRSA was predominantly found in pus samples (n=249, 80.8%), followed by blood (n=23, 7.5%) and ET aspirates (n=9, 2.9%). Surgical wards had the highest MRSA isolation rate (n=120, 38.9%), trailed by orthopedics (n=55, 17.9%) and medicine wards (n=36, 11.7%). This preference for pus samples aligns with MRSA's association with skin and soft-tissue infections, where pus formation is common.

The MRSA were susceptible to vancomycin (n=308, 100%) trailed by linezolid (n=296, 96%), chloramphenicol (n=279, 90%), and doxycycline (n=271, 88%). Only three MRSA strains were isolated from urine samples; among these, only one strain was sensitive to urine-specific drugs such as nitrofurantoin and nalidixic acid (n=1, 33%).

DISCUSSION

Methicillin-resistant *S. aureus* (MRSA) represents a substantial contribution to mortality and morbidity in both nosocomial and community-acquired infections [7]. The rise of MRSA demonstrating resistance to non-β-lactam antibiotics has exacerbated the gravity of this pervasive worldwide issue. This phenomenon significantly complicates the management and containment of MRSA infections,

Table 1: Distribution of MRSA isolates by age group

Age-wise distribution	Number and Percentage	p-value
<1–20	41 (13)	<0.0001
21–40	85 (28)	0.362
41–60	110 (36)	<0.0001
61–80	72 (23)	0.572
Total	308 (100)	

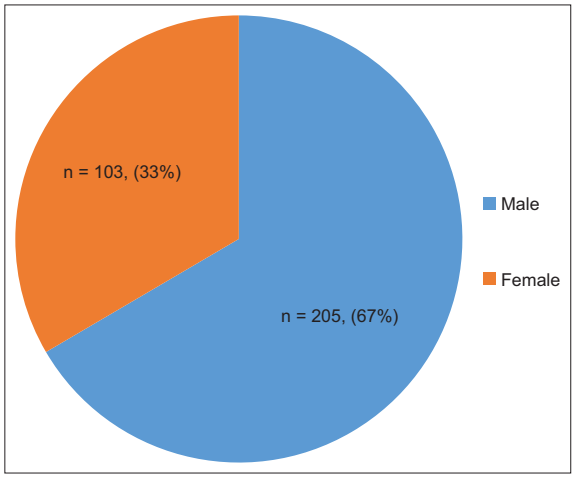

Fig. 1: Distribution of MRSA isolates by gender.

especially considering the limited availability of effective antimicrobial options [8].

The current investigation reveals a notable prevalence of MRSA infection (n=308, 54.1%), consistent with findings from Mao *et al.*'s study on risk factors associated with HA-MRSA, which reported a 56% incidence of MRSA. However, Garoy *et al.* reported a higher incidence of 72% in this multicentric study on MRSA prevalence [3,5]. This discrepancy underscores the significant geographical variability observed in MRSA infection frequencies globally, influenced by factors such as comorbidities, length of hospital stays, and adherence to standard precautions [9].

Our study observed a higher prevalence of MRSA among males, which aligns with the observations documented by Adhikari *et al.* [10] In addition, comparable outcomes were detected in a study conducted by Choudhury *et al.* [11] focusing on MRSA antibiotic susceptibility analysis. This male predominance may be attributed to factors such as occupational exposure, behavioral practices, and potential differences in the immune response.

According to Table 1, the largest population of the samples were obtained from the age group 41–60 years (n=110, 36%), followed by 21–40 years (n=85, 28%), 61–80 years (n=72, 23%), and < 1–20 years (n=41, 13%). Our study is concordant with a study by Garoy *et al.* on MRSA antibiotic susceptibility analysis, a multicentric study. *Staphylococcus* infections are more commonly observed in individuals aged 41–60 due to factors such as underlying health conditions, health-care exposure, occupational/lifestyle factors, and age-related changes in skin integrity and hygiene practices.

Most MRSA was isolated from pus samples (n=249, 80.8%), followed by blood (n=23, 7.5%) and ET aspirates (n=9, 2.9%). These results are concordant with research conducted by Choudhury *et al.*, Al-Zoubi *et al.*, and Upreti *et al.*, which also testified pus is the most common source of MRSA isolation, followed by blood [11-13]. *S. aureus* is frequently found in pus samples and blood due to its pyogenic properties, the tendency to induce skin and soft-tissue infections, the capacity to cause systemic infections such as bacteremia, and nosocomial infections, and the rise of resistance to antibiotics.

Our research indicates that the maximum number of MRSA originated from the surgery ward (n=120, 38.9%) followed by orthopedics (n=55, 17.9%) and medicine ward (n=36, 11.7%) [Table 2]. This observation can be attributed to the colonization of MRSA on the skin, with increased chances of invasion due to invasive procedures commonly performed in surgical departments and the use of indwelling devices in intensive care units. Comparable patterns were noted by Sanjana *et al*. [14].

Most MRSA strains were all susceptible to vancomycin, linezolid, chloramphenicol, doxycycline, tetracyclines, and cotrimoxazole, according to our findings. In addition, most of the isolates showed gentamycin and clindamycin sensitivity. However, the number of isolates that demonstrated erythromycin and ciprofloxacin susceptibility is small. Only one isolate exhibited nitrofurantoin sensitivity from urine specimens (Table 3). Our study results were comparable to a similar study conducted by Adhikari *et al*. The majority of isolates also showed sensitivity to gentamicin and clindamycin, as per Nabi *et al*. and Naimi *et al*. with little variations in linezolid susceptibility [10,13,15].

Hospital environments, HCWs, and colonized patients were the main sources of MRSA in hospitals. The principal mode of patient-to-patient spread occurs through the transient carriage of the organism by healthcare workers. Numerous infection regulatory practices, including routine hand hygiene training, compliance evaluations, and heightened MRSA scrutiny of healthcare workers, were put in place to address this problem. In addition, strict adherence to the antimicrobial policy was desired. We anticipate a future in which our institute's MRSA numbers will decline as a result of these actions.

Table 2: Distribution of MRSA isolates based on sample type and ward (n=308)

	MRSA	
Distribution based on sample type	Pus/Wound swab	249 (80.8%)
	Blood	23 (7.5%)
	ET	09 (2.9%)
	HVS	07 (2.3%)
	Sputum	07 (2.3%)
	Tissue bits	06 (1.9%)
	Urine	05 (1.6%)
	Fluids	02 (0.6%)
	Others	00
	Total	308
Ward-wise distribution of MRSA	Surgery	120 (38.9)
	Orthopedics	55 (17.9%)
	ENT	39 (12.7)
	Medicine	36 (11.7)
	OBG	16 (5.2%)
	ICU	13 (4.2)
	Others	12 (3.9)
	Pediatrics	11 (3.6%)
	Neurosurgery	06 (1.9)
	Total	308

HVS: High vaginal swabs

Table 3: Antimicrobial susceptibility profile of MRSA (n=308)

S. No.	Antibiotic	Sensitive and percentage	Resistance and percentage
1	Vancomycin	308 (100)	0 (0)
2	Linezolid	296 (96)	12 (4)
3	Chloramphenicol	279 (90)	29 (10)
4	Tetracycline	278 (90)	30 (10)
5	Doxycycline	271 (88)	37 (12)
6	Cotrimoxazole	250 (81)	58 (19)
7	Clindamycin	189 (61)	119 (39)
8	Gentamycin	167 (54)	141 (46)
9	Erythromycin	57 (18)	251 (82)
10	Ciprofloxacin	23 (7)	285 (93)
11	Penicillin	0 (0)	308 (100)
12	Amoxicillin and clavulanic acid	0 (05)	308 (100)
13	Nitrofurantoin ([only for urine] n=3)	1 (33)	2 (67)
14	Nalidixic acid ([only for urine]) n=3)	1 (33)	2 (67)

Limitation of study

The study on MRSA distribution has several limitations. The sample size of 308 may not be representative and could introduce sampling bias. Confounding variables such as hospital stay duration and antibiotic use were not controlled, and the lack of comparative groups limits context. The cross-sectional nature does not account for trends, and data accuracy depends on documentation. Assuming a uniform distribution for expected frequencies may not be appropriate, and sample types vary in detecting MRSA. Findings may have limited external validity and lack detailed clinical context.

CONCLUSION

Our study reveals the antimicrobial susceptibility patterns of MRSA. We discovered that MRSA strains were common and were multidrug-resistant. However, certain antibiotics such as linezolid, vancomycin, chloramphenicol, and doxycycline remained effective against MRSA. Despite this, the rise of resistant strains underscores the importance of continued surveillance and cautious antibiotic use to effectively combat this global health threat. These findings offer valuable insights for antibiotic stewardship programs and guide clinical strategies to formulate empirical therapy for managing MRSA infections in health-care settings.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS CONTRIBUTION

Gowri R: Conceptualization, methodology, investigation, data curation, writing, and original draft. Pasha S A R: Data curation, formal analysis, investigation, methodology, software, validation, visualization, and writing – review & editing, project administration. S. Parimala: Investigation, validation, visualization, methodology, and writing – review & editing. Natarajan A: Investigation, validation, visualization, methodology, and writing – review & editing.

AUTHORS FUNDING

We acknowledge that no financial support was granted for this research.

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