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Original Article

Microbiology Section

Mupirocin Resistance in *Staphylococcus aureus* Isolates among Patients in a Tertiary Care Hospital in Central Kerala: A Cross-sectional Study

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ABSTRACT

Introduction: Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections, with mupirocin being a key agent for decolonisation. However, increasing resistance to mupirocin threatens its effectiveness. Understanding the prevalence and resistance mechanisms of mupirocin-resistant *S. aureus* is essential for infection control and antimicrobial stewardship.

Aim: The study aimed to determine the prevalence rate of mupirocin resistance in *Staphylococcus aureus* in a tertiary care hospital and to assess the association between mupirocin resistance and resistance to other classes of antimicrobial agents.

Materials and Methods: The present cross-sectional study was conducted at a tertiary care hospital in Central Kerala, India from September 2023 to February 2024. A total of 100 non duplicate *S. aureus* isolated from cultures of pus, wound swabs, tissues and biopsies from deep-seated sites and organs taken from patients admitted to surgical wards were included in the study. Identification and antimicrobial susceptibility testing were performed using standard microbiological methods. Mupirocin resistance was classified as low-level (Mup-LL) and high-level (Mup-HL) based on Kirby-Bauer disc diffusion (5 µg and 200 µg mupirocin discs), following Clinical Laboratory Standards Institute (CLSI) 2023 guidelines. Statistical analysis was performed using the Chi-square test and t-tests with significance set at $p < 0.05$.

Results: Mupirocin resistance was detected in 12 (12%) isolates. Of these, 9 (75%) isolates showed high-level resistance, predominantly in MRSA isolates ($p < 0.05$). High-level mupirocin resistance was significantly associated with resistance to clindamycin and tetracycline. Inducible clindamycin resistance was found in 2 out of 3 Mup-LL isolates and 2 out of 7 Mup-HL isolates.

Conclusion: The study highlights a concerning 12% prevalence of mupirocin resistance in *S. aureus* isolates, particularly in MRSA strains. Routine mupirocin susceptibility testing and antimicrobial stewardship are essential to mitigate the spread of Multidrug-Resistant (MDR) *S. aureus* in healthcare settings.

Keywords: Antimicrobial susceptibility, Community-acquired infections, Pneumonia, Bacteraemia

INTRODUCTION

Staphylococcus aureus is a major cause of hospital and community-acquired infections, particularly in developing countries [1]. It is associated with serious conditions such as pneumonia and bacteraemia, leading to extended hospital stays and increased healthcare costs, especially with MRSA. The increasing prevalence of MRSA further complicates treatment, as these strains are resistant to multiple antibiotics, limiting therapeutic options. MRSA poses a significant challenge in healthcare settings as it spreads rapidly among hospitalised patients and is responsible for prolonged hospital stays, increased morbidity, and higher mortality rates. Carriers of MRSA face a 10-30% risk of developing an infection. Mupirocin, an antimicrobial agent used topically for skin and soft-tissue infections caused by *S. aureus*, is also employed in a nasal formulation to eradicate MRSA carriage among healthcare workers and patients [2]. The drug inhibits bacterial protein synthesis by targeting Isoleucyl-Trna Synthetase (IRS) [3]. However, its widespread use has contributed to the emergence of resistance, with two primary types: low-level and high-level resistance [4].

Low-level resistance is typically linked to point mutations in the chromosomal ileS gene, while high-level resistance is plasmid-mediated by the mupA (ileS2) gene, which encodes a modified IRS. This plasmid, often located on mobile genetic elements, facilitates the spread of resistance [5,6]. High-level resistance usually predicts the failure of mupirocin-based decolonisation, while low-level resistance may still be overcome with higher dosages [2]. Although mupA does not confer resistance to other antibiotics, it is often associated with Multidrug Resistance (MDR), including clindamycin, tetracycline, and erythromycin. Geographic and patient group variations influence mupirocin resistance rates, underscoring the need for ongoing surveillance in healthcare settings [7,8]. Additionally, mupirocin susceptibility testing is recommended before decolonisation to mitigate the risk of spreading MDR strains. Several studies have reported an increasing prevalence of mupirocin-resistant MRSA isolates, particularly in healthcare environments where mupirocin is used extensively. Since mupirocin-resistant MRSA strains are often MDR, their presence raises concerns regarding the limited options available for MRSA decolonisation and infection control. Despite the global significance of this issue, data on mupirocin resistance in Kerala remains limited. Regional surveillance studies are necessary to understand the burden of resistance, risk factors, and the potential impact on infection control policies. Identifying resistance trends can guide hospital antibiotic stewardship programs and help in formulating appropriate infection prevention strategies. Therefore, this study was conducted with the aim to determine the prevalence rate of mupirocin resistance in *Staphylococcus aureus* in a tertiary care hospital. The study was done with the primary objective to determine the rates of high level Mupirocin resistance (Mup-HL) and low level Mupirocin resistance (Mup-LL) in *S. aureus.* The secondary objective was to estimate the association of mupirocin resistance and resistance to other classes of antimicrobial agents.

MATERIALS AND METHODS

The present cross-sectional study was conducted from September 2023 to February 2024 at a tertiary care hospital in Kerala. Ethical approval for the study was obtained from the Institutional Review Board before commencement (IRB No:266/2023).

Inclusion criteria: Consecutive, non-duplicate isolates of *Staphylococcus aureus* obtained from cultures of pus, wound swabs, tissues, and biopsies taken from deep-seated sites and organs were included in this study. These samples were collected from both inpatients and outpatients from various surgical departments.

Exclusion criteria: Duplicate isolates from the same patient, environmental or surveillance samples not linked to clinical specimens, and those with incomplete clinical data or unspecified specimen source were excluded from the study.

Sample size calculation: The sample size for this study was calculated using the formula $N=(Z\alpha)\ 2\ pq/d^2$, where N= required sample size, Zα=Z value at a 95% confidence level (1.96), p is the proportion of mupirocin resistance in *Staphylococcus aureus* (7%, based on previous studies), q is the complement of p (93%), and d is the desired precision (5%) [9]. Substituting these values into the formula, the calculated sample size was approximately 100.

Study Procedure

Clinical samples were processed in the microbiology laboratory. Each specimen was inoculated onto Blood agar, MacConkey agar, and mannitol salt agar plates, followed by incubation at 37°C for 18-24 hours to allow for the growth of isolated bacterial colonies. Standard biochemical tests like clumping factor, coagulase test, mannitol fermentation and deoxyribonuclease (DNAse (test) were employed to confirm the identification of *S. aureus* isolates [10].

Antimicrobial susceptibility testing: The antimicrobial susceptibility of the *S. aureus* isolates was determined using the Kirby-Bauer disc diffusion method. The following antibiotics were tested: Penicillin (10 units), clindamycin (2 µg), cotrimoxazole (1.25/23.75 µg), tetracycline (30 µg), erythromycin (15 µg), gentamycin (10 µg), linezolid (30 µg), and mupirocin (5 and 200 µg), following the Clinical Laboratory Standards Institute (CLSI) 2023 guidelines [11]. Detection of methicillin resistance was carried out by using cefoxitin (30 µg) discs as per CLSI 2023 guidelines. To detect inducible and constitutive clindamycin resistance, erythromycin and clindamycin discs were placed 15 mm apart. Quality control of antimicrobial susceptibility tests was ensured by using *S. aureus* ATCC 25923 as the control strain throughout the study. Mupirocin resistance was evaluated using 5 µg and 200 µg mupirocin discs (Hi-media Laboratories, Mumbai, India) to identify low and high-level resistance respectively. The plates were incubated at 350C for 24 hours of incubation. After incubation, the zone of inhibition was examined under transmitted light for light bacterial growth. Isolates with a zone diameter ≥14 mm for the 5 µg and 200 µg disc were considered susceptible. Isolates with a zone diameter <14 mm for the 5 µg disc but ≥14 mm for the 200 µg disc were classified as low-level mupirocin-resistant (Mup-LL), while those with diameters <14 mm for both discs were categorized as high-level mupirocin-resistant (Mup-HL) [12].

STATISTICAL ANALYSIS

All the data collected were coded and entered in Microsoft Excel sheet which was re-checked and analysed using Statistical Package for Social Sciences (SPSS) statistical software version 25. Quantitative variables were summarised using mean and Standard Deviation (SD). Categorical variables were represented using frequency and percentage. Pearson Chi-square test and Fisher's-Exact test were used for comparing categorical variables between groups. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 100 *S. aureus* isolates were included in the study. The majority of isolates were obtained from wound swabs 73 (73%) followed by aspirated pus 27 (27%). Among the samples, 62 (62%) were received from males and 38 (38%) from females. The mean age of the patients was 47 years. Among the 100 *S. aureus* isolates, 44 (44%) were MRSA and 56 (56%) were MSSA. Inducible clindamycin resistance was detected in 11 (13.1%) of the 84 *S. aureus* isolates in which D-test could be performed. Mupirocin resistance was detected in 12 (12%) isolates. Out of these 12 isolates, Mup-LL was detected in 3 (25%) isolates and Mup-HL in 9 (75%) isolates. The distribution of low-level mupirocin and high-level mupirocin resistance among MRSA and MSSA strains are shown in [Table/Fig-1]. The rate of mupirocin resistance was significantly higher in MRSA compared to MSSA isolates (p=0.021) by Chi-square test. Mup-HL was significantly more commonly associated with MRSA isolates (p=0.041) by Fisher's-exact test. The rates of Mup-HL among 12 mupirocin resistant *S. aureus* isolates and of Mup-HL among 9 mupirocin-resistant MRSA isolates was 75% and 77.8%, respectively.

Parameters	Mupirocin resistant	Mupirocin susceptible	p-value
MRSA (n=44)	9 (20.5)	35 (79.5)	*0.021, Chi-square value=5.32
MSSA (n=56)	3 (5.4)	53 (94.6)	
	Mup-HL	**Not Mup-HL**	
MRSA	7	37	# 0.041, Fisher's-exact test
MSSA	2	54	
	Mup-LL	**Not Mup-LL**	
MRSA	2	42	Not statistically significant
MSSA	1	55	

[Table/Fig-1]: Association of MRSA/MSSA with overall, high-level, and low-level mupirocin resistance.
*Statistically significant (Chi-square test applied); #Statistically significant (Fishers-exact test applied)

The rates of inducible clindamycin resistance among mupirocin-resistant, Mup-LL and Mup-HL strains are given in [Table/Fig-2]. A significant association was found between inducible clindamycin resistance and Mup-LL (p=0.044).

Inducible clindamycin resistance	Mupirocin resistant n=10	Mupirocin susceptible n=74	p-value
Detected	4 (36.4)	7 (63.6)	0.023* (Fishers-exact test)
Not detected	6 (8.2)	67 (91.8)	
	Low level mupirocin resistance detected (n=3)	**Low level mupirocin resistance not detected (n=81)**	
Detected	2 (18.2)	9 (81.8)	0.044*(Fishers-exact test)
Not detected	1 (1.4)	72 (98.6)	
	High level mupirocin resistance detected (n=7)	**Low level mupirocin resistance not detected (n=77)**	
Detected	2 (18.2)	9 (81.8)	Not statistically significant
Not detected	5 (6.8)	68 (93.2)	

[Table/Fig-2]: Association between type of mupirocin resistance and inducible clindamycin resistance.
*Statistically significant

S.aureus showed highest susceptibility to linezolid (100%), followed by tetracycline (90%) and cotrimoxazole (84.5%). The antimicrobial susceptibility patterns among MRSA and MSSA isolates are provided in [Table/Fig-3]. Most antimicrobial agents were effective against MSSA, while reduced susceptibility was noted among MRSA strains. In the MRSA group, linezolid (100%), tetracycline (88.6%), and cotrimoxazole (77.3%) demonstrated high levels of efficacy. The variation in the number of organisms tested for each antibiotic is due to factors such as isolate availability,

testing priorities, methodological constraints, and quality control measures. These considerations ensured that only reliable data was reported.

Antibiotic	MRSA (n=total tested)	MRSA % Susceptible	MSSA (n=total tested)	MSSA % Susceptible
Penicillin	0/44	0.0%	7/56	12.5%
Erythromycin	3/34	8.8%	9/50	18.0%
Clindamycin	19/34	55.9%	29/50	58.0%
Tetracycline	39/44	88.6%	51/56	91.1%
Gentamycin	16/44	36.4%	22/53	41.5%
Cotrimoxazole	34/43	79.1%	48/54	88.9%
Linezolid	44/44	100.0%	56/56	100.0%

[Table/Fig-3]: Antimicrobial susceptibility patterns (%) among MRSA and MSSA strains.
MRSA: Methicillin-resistant *staphylococcus aureus*; MSSA: Methicillin-susceptible *Staphylococcus aureus*

The antimicrobial susceptibility patterns for high-level mupirocin-resistant and low-level mupirocin resistant strains are shown in [Table/Fig-4]. Tetracycline was highly effective against Mup-LL (100%) strains and moderately effective against Mup-HL (66.7%) strains, while clindamycin showed moderate activity against Mup-HL (57.1%) strains but none against Mup-LL strains. Gentamycin and cotrimoxazole were more effective against Mup-HL strains (66.7%) but had reduced efficacy against Mup-LL strains (33.3%).

Antibiotics	Mup-HL (n=9)	Mup-LL (n=3)
Penicillin	0 (0)	0 (0)
Erythromycin	0 (0)	0 (0)
Clindamycin	4 (44.4)	0 (0)
Tetracycline	6 (66.7)	3 (100)
Gentamycin	6 (66.7)	1 (33.3)
Cotrimoxazole	6 (66.7)	1 (33.3)

[Table/Fig-4]: Antimicrobial susceptibility patterns (%) in high-level mupirocin and low-level mupirocin resistant *S. aureus*.
Mup-HL: High level mupirocin resistance; Mup-LL: Low level mupirocin resistance

DISCUSSION

The study found that 44% of MRSA, a percentage like those reported in earlier studies from the same region. Shivanna V et al., reported a comparable MRSA rate of 46.34% in a rural tertiary healthcare center in Karnataka [13]. These findings confirm that MRSA continues to pose a significant challenge in hospital settings across India. Previous studies in similar settings have reported lower MRSA rates in South India, which may indicate geographic variability within the same country [14,15].

Mupirocin resistance in *S. aureus* isolates was found in 12% of cases, closely mirroring findings from global and regional studies [16-18]. For instance, studies by Ohadian Moghadam (12.8%) and Abbasi-Montazeri (11.5%) reported similar rates [16,18]. Furthermore, a study from the northern Indian state of Uttar Pradesh reported mupirocin resistance rates of 13%, suggesting that resistance is not confined to specific regions [19]. These comparable resistance rates emphasise the widespread nature of mupirocin resistance across India.

The study distinguished between high-level and low-level mupirocin resistance, each of which carries different clinical implications. High-level resistance was present in 9% of the isolates, which is similar to other studies in India [17]. This is particularly concerning because it predicts treatment failure in decolonization regimens. This finding is consistent with a study highlighting that Mup-HL strains are significantly associated with decolonization failure in MRSA carriers [20]. Low-level resistance (Mup-LL), while less prevalent (3% in this study), can still complicate treatment, especially if higher doses of mupirocin are not administered.

The presence of mupirocin-resistant strains was significantly higher among MRSA isolates compared to MSSA isolates, with Mup-HL observed in 20.5% of MRSA isolates versus 5.4% in MSSA (p=0.041). This finding is consistent with international reports which indicate that MRSA strains are more likely to carry plasmid-mediated resistance genes such as mupA, encoding for high-level mupirocin resistance [21]. The plasmid-mediated mupA gene, responsible for Mup-HL, can be easily transmitted between bacteria, potentially accelerating the spread of resistance in hospital environments.

A critical aspect of the present study is the association between mupirocin resistance and resistance to other antibiotics. Mupirocin-resistant strains, particularly Mup-HL isolates, were more likely to exhibit resistance to other antimicrobials such as clindamycin, tetracycline, and cotrimoxazole. This observation is consistent with studies by McNeil JC et al., who reported that mupirocin-resistant MRSA strains are often MDR, complicating the treatment of these infections [22].

The association between Mup-LL and inducible clindamycin resistance is another important finding. Inducible clindamycin resistance, detected in 66.7% of Mup-LL strains and 28.6% of Mup-HL strains, presents a challenge in selecting appropriate treatment options. This finding aligns with other reports which documented similar associations between mupirocin and clindamycin resistance, particularly in MRSA isolates [17].

Additionally, tetracycline showed high efficacy against Mup-LL strains (100%), while Mup-HL strains exhibited only moderate susceptibility (66.7%). This differential susceptibility pattern suggests that Mup-HL strains may possess additional resistance mechanisms, potentially involving efflux pumps or other mobile genetic elements that confer resistance [23].

The study underscores the limited therapeutic options for treating *S. aureus*. Linezolid was the most effective antibiotic, with 100% susceptibility in both MRSA and MSSA isolates. This finding is consistent with global trends, as linezolid remains one of the few antibiotics with consistent efficacy against MDR *S. aureus*, including mupirocin-resistant strains [24]. Tetracycline also showed high efficacy (90% susceptibility overall), particularly against Mup-LL strains, indicating its potential use as a secondary option when mupirocin resistance is encountered [23].

However, other commonly used antibiotics, such as gentamycin and cotrimoxazole, were less effective against Mup-LL strains, with susceptibility rates of 33.3% for both antibiotics. This is concerning given the limited number of antibiotics that remain effective against MDR *S. aureus*. Similar studies have reported reduced efficacy of these antibiotics against resistant strains, particularly in settings with high MRSA prevalence [17].

The findings of this study have significant implications for infection control and treatment strategies, particularly in hospitals where MRSA colonisation is prevalent. Mupirocin resistance, especially high-level resistance, jeopardizes the effectiveness of decolonisation protocols, which are critical for preventing the spread of MRSA in healthcare settings. Given the high association between Mup-HL and MRSA strains, screening for mupirocin resistance by 5 µg disk scan be considered, especially before decolonisation regimens are initiated.

Moreover, the potential for mupirocin resistance to be linked to other resistance determinants suggests that the indiscriminate use of mupirocin could contribute to the broader problem of MDR in *S. aureus*. As noted by Patel JB et al., the co-selection of resistance genes due to the use of topical antibiotics like mupirocin can amplify the spread of MDR pathogens [2]. Therefore, antimicrobial stewardship programs should include guidelines for the prudent use of mupirocin to limit the emergence and spread of resistant strains.

Limitation(s)

This study has some limitations that should be acknowledged. Firstly, the sample size of 100 isolates may not fully represent the

wider population or account for variations across different healthcare settings. Conducting the study across multiple institutions with a larger sample could offer more generalised and robust findings. Secondly, the study was restricted to a single tertiary care hospital in Kerala, limiting the applicability of the results to other regions where antibiotic prescribing practices and resistance patterns may differ. The limited range of antibiotics tested in this study is another constraint, testing a broader spectrum could have provided a more comprehensive picture of antimicrobial resistance. Furthermore, the use of the Kirby-Bauer disc diffusion method for antimicrobial susceptibility testing, though widely accepted, may lack the sensitivity and specificity of agar dilution method which is considered to be the gold standard for detecting mupirocin resistance. Finally, the study did not include genetic analysis of the resistant strains, which could have offered deeper insights into the mechanisms of mupirocin resistance, particularly for strains exhibiting high-level resistance.

CONCLUSION(S)

In conclusion, the study highlights the growing challenge of mupirocin resistance in *Staphylococcus aureus*, particularly in MRSA strains. High-level mupirocin resistance is significantly associated with MRSA and is linked to resistance to other antibiotics, complicating the treatment of these infections. Routine mupirocin susceptibility testing and careful antimicrobial stewardship are essential to mitigate the spread of resistance and preserve the efficacy of existing treatment options. The findings underscore the need for ongoing surveillance of antimicrobial resistance patterns in healthcare settings, particularly as MDR *S. aureus* continues to pose a significant threat to public health.

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