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MUPIROCIN RESISTANCE AMONG METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM THE CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL IN SOUTH-KERALA

Dona Susan Mathew¹, Ashish Jitendranath², Ramani Bai³

Department of microbiology, Sree Gokulam Medical College and Research Foundation,
Venjarammood, Trivandrum, Kerala, India.

Structured abstract

Background: Mupirocin is an antimicrobial that inhibits synthesis of bacterial proteins by competitive inhibition of bacterial isoleucyl-tRNA synthetase. Emergence of high level mupirocin resistance among MRSA in patients without a past history of mupirocin exposure is a cause for concern.

Objectives: The study aimed to determine the prevalence of mupirocin resistance among MRSA and characterization using molecular study.

Materials and Methods: Consecutive, non repetitive clinical isolates of methicillin resistant Staphylococcus aureus from skin and soft tissue infections, blood, and urine samples between January 2020 and October 2022 were studied. Antibiotic susceptibility testing was done according to Clinical and Laboratory Standards Institute guidelines. Mupirocin resistance was screened by using 5 µg and 200µg disc and confirmed by E – strip and by Polymerase Chain Reaction.

Results: Prevalence of Mupirocin resistance was found to be 13.3%. 31 isolates among MRSA was found to be mupirocin resistant, in which 14 isolates (45.2%) were positive to *mupA* and 19 isolates (61.3%) were negative to *mupA*. Besides, It was observed that n=31 (13.34%) were mupirocin resistant MRSA, in which there is a significant association ($p<0.05$) is found with resistance to Cotrimoxazole (n=11), Erythromycin (n=14), Clindamycin (n=12), Gentamicin(n=27), and Ciprofloxacin (n=31).

Conclusion: Emerging prevalence of high level mupirocin resistance among methicillin resistant *Staphylococcus aureus* (MRSA) was found in the study. Recent studies suggested the presence of non-*mupA* mediated high level mupirocin resistance. The identification of *mupB* would explain those observations. Further studies are required to be performed to determine prevalence of *mupB*.

Keywords: Methicillin-resistant *Staphylococcus aureus*, mupirocin resistance, mupirocin.

INTRODUCTION

Staphylococcus aureus has become the single most frequently isolated gram-positive bacterial pathogen in hospitals and one of the most common etiological agents of nosocomial post-operative surgical wound infections. Owing to the remarkable ability of *Staphylococcus aureus* to become resistant to antimicrobial agents, its impact on hospital-acquired infections has dramatically increased.¹ Soon after the introduction of Methicillin, the resistance of *Staphylococcus aureus* to this family emerged in Europe and North America and then worldwide.¹

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the emerging causes of hospital-acquired infections. There is an increase in MRSA carrier rate among healthcare workers and patients, with resistance to various classes of beta-lactam antibiotics.² Mupirocin is the drug of choice used to eradicate Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization.³

Mupirocin was first introduced in the United Kingdom in 1971 by Sutherland et al, for primary and secondary skin disorders caused by gram-positive bacteria.³ Since then it has been widely used to treat various Staphylococcal and Streptococcal skin infections, in patients requiring peritoneal dialysis, and to eradicate nasal carriage of Methicillin Susceptible *Staphylococcus aureus*(MSSA) and MRSA. This has decreased nosocomial infections in hospitalized patients.¹

Mupirocin 2% topical antimicrobial ointment is used as a part of MRSA decolonization. Nasal carriers with increased mupirocin use lead to mupirocin resistance, which is contributing to persistent MRSA carriage.⁴

Recent studies showed two types of resistance to mupirocin, high-level resistance (MUPHR) and low-level resistance to mupirocin (MUPLR).³ Its underlying factor was the previous exposure to mupirocin. This is significant for infection prevention and control strategies as these are used for the decolonization of MRSA carriers, especially healthcare workers.⁵

Therefore mupirocin use is limited for infection control measures only.² Delay in administering appropriate treatment for these patients would result in an ineffective decolonization regimen. This increases nosocomial infection or MRSA transmission within the hospital. Therefore mupirocin should be used judiciously.⁴ A rise in resistance to mupirocin in MRSA necessitates the discovery of new antimicrobial therapy like polyhexanide, lysostaphin, ethanol, chlorhexidine, naseptin, omiganan pentahydrochloride, tea tree oil, bacteriophages, and honey.⁵

MATERIALS AND METHODS

Collection of bacterial isolates

A total of 233 consecutive, non-repetitive clinical isolates of Methicillin-Resistant Staphylococcus aureus (MRSA) from skin and soft tissue infections, blood, urine and peritoneal fluids from patients of Intensive Care Unit (ICU), Inpatients and Outpatient departments between January 2020 and October 2021 were included in the study. The isolates were identified as MRSA by standard laboratory techniques.

Antibiotic Susceptibility testing

The antibiotic susceptibility testing was done by Clinical and Laboratory Standards Institute (CLSI) recommended Kirtby-Bauer disc diffusion testing on Muller-Hinton agar.

The antibiotics used in this study included Penicillin (10units), Cefoxitin (30µg), Cotrimoxazole (25µg), Erythromycin (15µg), Clindamycin (2µg), Vancomycin (30µg), Teicoplanin (30µg), Linezolid (30µg), Rifampicin (5µg), Tetracycline (30µg), Ciprofloxacin(5µg), Gentamicin (10µg). (Himedia laboratories Pvt Ltd Mumbai, India). The growth inhibition zones were measured and interpreted according to the CLSI guidelines. Quality control was achieved by using S.aureus ATCC 25923.⁶

Disc diffusion method

Pure form of mupirocin was purchased from Himedia laboratories Pvt Ltd (Mumbai, India). Mupirocin disc of 5µg and 200µg strength were included in the routine susceptibility testing and plates were incubated for 24hr at 35±2°C. The zone diameters were carefully examined with transmitted light for light growth within the zone of inhibition. Isolates with no zone of inhibition were interpreted as mupirocin resistant. Isolate resistant to 5µg disc and any zone for 200µg disc was considered low level mupirocin-resistant (MUP^{RL}). Isolates resistant for both the discs were considered high-level mupirocin resistant (Mup^{RH}).⁶

Determination of minimal inhibitory concentration (MIC)

The mupirocin MIC is assessed by using E-test by using E-strips of mupirocin (Himedia laboratories Pvt Ltd (Mumbai, India) according to manufacturer's guidelines. Strains were considered susceptible if MIC was ≤ 4 mg/L and levels of mupirocin resistance were defined as low-level with MIC 8-256mg/ and high level with MIC ≥ 512 mg/l

Molecular characterization of isolates

DNA was extracted using a CyBio Felix DNA extraction kit as recommended by manufacturer. MupA gene was detected in DNA extracts by Real-time PCR assay. The gene was amplified on an Eppendorf (Hamburg, Germany) thermocycler.

Statistical analysis

Data was analyzed using SPSS statistical software version 21. The categorical data were compared using the Chi-square test. Statistical significance was set at 0.05 levels.

RESULTS

Among 233 isolates of MRSA, 31 isolates (13.34%) were found to be mupirocin-resistant MRSA. Out of these, 29 isolates (12.4%) were showing High-level Mupirocin resistant MRSA (Mup^{RH} MRSA) with MIC > 1024 and 2 isolates (0.85%) showed Low-level mupirocin resistance (MUP^{RL} MRSA) with MIC > 1024 . 202 isolates (86.6%) were susceptible to mupirocin among MRSA. 31 isolates among MRSA was found to be mupirocin resistant, in which 14 isolates (45.2%) were positive to *mupA* and 19 isolates (61.3%) were negative to *mupA*. Among the mupirocin-resistant MRSA isolates (n=31), it was noted that most of the samples were from males (n=19). It was also noted that samples obtained from mupirocin-resistant MRSA were predominantly from pus (12%) followed by blood (1%) and aspirates (n=0.4%).

Table 1

Results of high- level mupirocin resistance (200µg) & low-level mupirocin resistance(5 µg) in MRSA by mupirocin disc diffusion and E-test (N=233)

PATTERN OF RESISTANCE	NO. OF LLMupR MRSA (5µg) CASES	NO. OF HLMupR MRSA (200 µg)	E- TEST	PERCENTAGE
SUSCEPTIBLE	231	204	202	86.6%
RESISTANT	2 (0.85%)	29 (12.4%)	31	13.3%
TOTAL	233	233	233	100%

MUPIROCIN SUSCEPTIBILITY TESTING – DISC DIFFUSION



MUP S MRSA



MUP R MRSA

DISCUSSION

The rise in skin infections has been a risk factor for persistent nasal carriage of MRSA, resulting in person-to-person transmission and these strains cause difficulty in the management. Therefore, it is necessary to identify these organisms for the specific antimicrobial therapy. Elimination of MRSA carriage is done by using topical antimicrobials like mupirocin which can control the outbreaks and prevent recurrences. However, there is an emergence in the prevalence of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* due to increased use of topical application of mupirocin. Genetic basis of MUP^{RL} is due to point mutations in native isoleucyl-TRNA synthetase (ileS) gene. Mup^{RH} is due to plasmid mediated gene, MupA, that encodes modified, ileS, which has less affinity for mupirocin.

In the present study, the overall prevalence of mupirocin resistance among MRSA among 233 isolates of MRSA received during the study period at the laboratory was 13.3% which is higher than the study done by Rajkumari *et al* in 2014 at a tertiary care hospital in Andhra Pradesh, India, which was found to be 0%.

Rudresh *et al* also reported a high prevalence of mupirocin resistance of 22.5% among MRSA from Karnataka in 2015.⁷ The study of AE Simor *et al*, done in Canada in 2015, found the high- level mupirocin resistance has increased in MRSA from 1.5% to 7%, in the surveillance of 5 years.³

Table 2.

Comparison of various studies in India

Author	(year)	Mupirocin resistance (%)	
		MupHL	MupLL
Oommen et al. Tamilnadu	2010	2.08	0
Abimanyu et al Tamilnadu	2012	32	-
Jayakumar et al Tamilnadu	2013	2.17	0
Rajkumari et al Andhra Pradesh	2014	0	0
Rudresh et al. Karnataka	2014	4.5	18.2
Present study Kerala	2021	13.34	0

In this study genotypic test was also done for all the isolates (n=31) of mupirocin-resistance among MRSA that are tested by disc diffusion and E-test method. It was observed that 14 isolates were positive for the *mupA* gene and 19 isolates were negative for *mupA* with aMIC>1024.

This study showed a similar pattern with the study conducted by Solmaz *et al* among healthcare workers in Iran in 2015. He had noticed out of 5 isolates of mupirocin resistance of MRSA, tested by E-test, with MIC >1024, 2 were shown to be *mupA* positive by PCR, with the remaining strains of high-level mupirocin resistance negative to *mupA*.²

However, there was an exception of 2 isolates that showed low-level mupirocin resistance, with MIC≥1024µg/ml, and was found to be *mupA* negative. This observation was also seen in the study of Ramsey

et al in Michigan, USA 1996 in which low-level mupirocin-resistant MRSA with MIC>1024 was found to have *mupA* on a chromosome that was negative in plasmid.⁸ So, in the present study, it could be suggestive of the chromosomal location of *mupA*.

In the present study, the distribution of CA-MRSA and HA-MRSA differed between *mupA* positive and *mupA* negative isolates of mupirocin-resistant MRSA. Most of the *mupA* negative isolates were HA-MRSA and *mupA* positive isolates were present both in CA-MRSA and HA-MRSA. These findings showed a similar pattern with the study done by Cadilla *et al* in an academic center in the Midwestern United States in 2010.⁹ Besides, it was observed in the study that antibiotic susceptibility patterns of all high-level mupirocin-resistant methicillin-resistant *Staphylococcus aureus* strains have a significant association with resistance to many other antimicrobial classes.⁹

In our study, Cotrimoxazole resistance was 35.5 %, Erythromycin resistance was 45.16%, Clindamycin resistance was 38.70%, Gentamicin resistance was 87.1% and Ciprofloxacin resistance was 100%. No resistance was found to vancomycin and Teicoplanin.

Studies by Nicholas *et al* did in a tertiary care children's hospital in Argentina in 2019 showed resistance to Erythromycin, Clindamycin, and Gentamicin (27.6%, 25%, 15.2% respectively) and low-level resistance to Ciprofloxacin and Cotrimoxazole (6.9% and 0.5% respectively).¹⁰

CONCLUSION

Determining the mupirocin resistance is important in our study as there is a high prevalence of mupirocin resistance noticed among community-acquired than hospital-acquired strains. Continued surveillance for mupirocin is important in order to retain the usefulness of this agent since mupirocin is the topical antimicrobial that is used for the decolonization of methicillin-resistant *Staphylococcus aureus*.⁷

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