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The Emergence of Mupirocin Resistance among *Staphylococcus aureus* in a Tertiary Care Hospital in South India: The Necessity for Routine Susceptibility Testing

***Abirami lakshmy Jayachandran, Balan Kandasamy**

Department of Microbiology, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Chengalpet, Tamil Nadu India.
*Email: drabi3285@gmail.com
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Abstract: Methicillin-resistant Staphylococcus aureus (MRSA) is difficult to treat, causing considerable morbidity and mortality. Nasal carriage of MRSA can occur both in healthcare workers and patients. Mupirocin is used as a topical agent for the eradication of such isolates. The present study aims to study the prevalence of mupirocin resistance among the MRSA and MSSA (Methicillin-sensitive Staphylococcus aureus) isolates. A total of 148 *Staphylococcus aureus* isolates were tested. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method for amoxicillin, penicillin, cotrimoxazole, clindamycin, mupirocin(5 µg and 200 µg discs for low and high-level resistance), erythromycin, gentamicin and linezolid. MRSA isolates were detected by cefoxitin disc diffusion and Mec A detection by PCR (Polymerase Chain Reaction). MRSA was detected among 44 (29.7%) of the isolates. Among MSSA, good susceptibility was observed for cotrimoxazole 89 (85.5%) and clindamycin 92 (88.4%). An overall mupirocin resistance of 12(8.1%) was observed, with high-level resistance at 4 (2.7%) and low-level resistance at 8 (5.4%).The mupirocin resistance pattern between MRSA and MSSA was not statistically significant (p=0.1833). The emergence of mupirocin resistance highlights the necessity for creating cognizance among clinicians before prescribing mupirocin. In eradicating nasal carriage of MRSA, all the isolates should always be tested for mupirocin susceptibility to prevent the selection and spread of drug-resistant isolates.
Keywords: Infection control; Methicillin-Resistant Staphylococcus aureus; mupirocin resistance

INTRODUCTION

Staphylococcus aureus is a commonly encountered bacteria implicated in causing superficial and serious infections like hospital-acquired infections. Drug-resistant isolates like MRSA are difficult to treat, causing considerable morbidity and mortality when compared with the MSSA (Perumal G et al., 2022). Healthcare workers can also carry MRSA strains as colonizers, and antibiotics like mupirocin and chlorhexidine are used to eradicate the carrier state (Hayden et al., 2016). Multidrug resistance among MRSA is a serious situation. Mupirocin acts by interfering with protein synthesis, and prolonged usage can cause the emergence of drug resistance (Dadashia et al., 2020; Perumal G et al.,2022).

The prevalence of mupirocin resistance across various global studies is 6.6% to 26.6 % (Dadashia et al., 2020; Mahmoudi et al., 2019). Mupirocin is also used among individuals undergoing dialysis and surgical patients to reduce the carrier state of MRSA isolates (Fritz et al., 2013; Hayden et al., 2016). MRSA isolates can be

Corresponding Author: Abirami lakshmy Jayachandran, Associate professor
Department of Microbiology, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Chengalpet - 603308. Tamil Nadu, India.
Email: drabi3285@gmail.com

detected by both phenotypic and genotypic methods. The cefoxitin disc diffusion method and oxacillin screen agar are the commonly used phenotypic methods. Clinical microbiology labs with molecular facilities perform the detection of the Mec A gene by the Polymerase chain reaction method (Anand KB et al., 2009). Mupirocin resistance can be divided into High level and low-level resistance, and distinguishing both has implications while using the agent for therapy (Shivanna et al ., 2023). Susceptibility testing for mupirocin is not done in most cases. Empirically using mupirocin without performing susceptibility testing can lead to failure to eradicate the MRSA and treatment failure.

There have been studies of mupirocin resistance among MRSA and MSSA isolates, but they have had mixed results (Gader et al., 2020; Dadashia et al., 2020; Perumal G et al., 2022; Shivanna et al., 2023) More research is needed to support the success of MRSA treatment based on the pattern of mupirocin resistance. Hence this study aims to analyze the prevalence of mupirocin resistance among the *Staphylococcus aureus* isolates, along with the detection of MRSA by phenotypic and genotypic methods, and to determine the significance of differences in mupirocin resistance patterns among the MSSA and MRSA.

MATERIALS AND METHODS

The present study (cross-sectional) was conducted at the dept of Microbiology, Karpaga Vinayaga Institute of Medical Sciences and research center, Chengalpet, Tamil Nadu, India, from June 2018 to Feb 2019. Specimens from pus swabs, wound swabs, surgical wound infections, and pus aspirate from abscesses were included in the study. The study was approved by the institutional ethical committee (IEC Ref: KIMS/F/2019/02).

All the specimens were processed by standard microbiological techniques (Mackie & Mccartney., 2006). Direct gram stain was performed on the specimens. In the present study, all the samples showing the presence of polymorphs were included. *Staphylococcus aureus* isolated from the above-mentioned specimens was included in the study. These isolates were identified based on the colony appearance, Gram stain, catalase test, coagulase test, and biochemical tests (Mackie & Mccartney., 2006).

Antibiotic susceptibility testing was done by the Kirby-Bauer disc diffusion method. All the isolates were inoculated into peptone water, and the turbidity was adjusted to 0.5 Mcfarland standard (1.5x 108 CFU/ml). Using a sterile swab, lawn culture was done on Mueller Hinton agar. Amoxicillin (10 µg), penicillin (10U), cotrimoxazole(1.25/23.75 µg), clindamycin (2 µg), mupirocin (5 µg and 200 µg), erythromycin (15 µg), gentamicin(µg) and linezolid (30 µg) antibiotic discs (Himedia, Chennai, India) were placed on the lawn culture with a sterile forceps within 15 minutes. The plates were incubated at 35°C overnight. The zone sizes were measured with measuring calipers. The results were interpreted as per CLSI guidelines (CLSI, 2018; Mackie & Mccartney., 2006).

MRSA isolates were detected by testing with the cefoxitin disc diffusion method using cefoxitin 30 µg discs. The zone size of ≤21 mm was considered as resistant and ≥22 mm as sensitive and interpreted as per CLSI guidelines (CLSI, 2018; Shivanna et al., 2023; Mackie & Mccartney., 2006). Detection of mupirocin resistance was performed using 5 µg and 200 µg discs. Bacterial isolates showing no zone of inhibition were considered mupirocin resistant. Isolates showing resistance for 5 µg mupirocin disc and any zone size of inhibition for 200 µg disc was interpreted as MupRL (Mupirocin resistance low level) (CLSI, 2018; Rudresh et al., 2015; Shivanna et al.,

2023). As per CLSI guidelines absence of a Zone around the 200 µg disc is considered as High-level mupirocin resistance (CLSI, 2018).

Detection of MRSA by Polymerase Chain Reaction

The presence of the Mec A gene was detected in all the isolates by Polymerase chain reaction (PCR). Himedia Genomic purification kit and MRSA uniplex PCR amplification kit were used for DNA extraction and amplification. The PCR programming was set as follows 94 degrees C - 10 minutes for Initial denaturation,94 degrees C for 1 minute for denaturation, 60 degrees C - one minute for the process of annealing, 72 degrees C - one minute for extension (30 cycles) and 72 degrees C - 10 minutes for a final extension. The products were visualized by agarose gel electrophoresis with ethidium bromide.100 bp DNA ladder was used as a control for the size of the products, and the band at 533 bp was identified as Mec A positive (Anand KB et al., 2009)

Statistical Analysis

The significance of the association for mupirocin resistance among MRSA and MSSA was analyzed by statistical software Graph pad Quick Calcs by chi-square test with a significance level at p-value less than 0.05.

RESULTS AND DISCUSSION

A total of 148 *Staphylococcus aureus* isolates were obtained from the specimens. Out of the 148 isolates, MRSA was detected among 44 (29.7%) by cefoxitin disc diffusion method and Mec A gene detection by PCR. All 44 isolates were positive for MRSA by the above two methods (Figure 1; Figure 2).

Among MSSA, good susceptibility was observed for cotrimoxazole 89 (85.5%), clindamycin 92 (88.4%), and ciprofloxacin 87 (83.6%). Mupirocin showed a susceptibility of 40 (90.9 %) and 96 (92.3%) among MSSA and MRSA isolates (Table 1). The least susceptibility was observed for penicillin and amoxicillin among MSSA. All isolates were susceptible to linezolid. For MRSA isolates it was observed that reduced susceptibility was observed for penicillin, amoxicillin, ciprofloxacin, and gentamicin as opposed to MSSA (Table 1).

An overall mupirocin resistance of 12(8.1%) was observed, with high-level resistance at 4(2.7%) and low-level resistance at 8(5.4%) among the isolates (Figure 3). There was no difference in mupirocin resistance between MRSA and MSSA (Table 2). The mupirocin resistance pattern between MRSA and MSSA was not statistically significant (p=0.1833).

Table 1. Antibiotic Susceptibility Pattern among MRSA and MSSA

No	Antibiotic disc	MRSA N=44	MSSA N=104
1	Cotrimoxazole	26 (59%)	89 (85.5%)
2	Amoxicillin	9 (20.45%)	10 (9.61%)
3	Penicillin	01 (2%)	0
4	Clindamycin	38 (86.36%)	92 (88.4%)
5	Ciprofloxacin	22 (50%)	87 (83.6%)
6	Mupirocin	40 (90.9%)	96 (92.3%)
7	Erythromycin	31 (70.45%)	85 (81.7%)
8	Gentamicin	19 (43.18%)	81 (77.88%)
9	Linezolid	44 (100%)	104 (100%)

Table 2. Mupirocin Resistance among the *Staphylococcus aureus* Isolates

No	Mupirocin resistance	Overall n=148	MRSA n=44	MSSA n=104
1	Total mupirocin resistance	12(8.1%)	6(13.6%)	6(5.7%%)
2	High level resistance	4(2.7%)	2(33.3%)	2(1.9%)
3	Low level resistance	8(5.4%)	4(66.6%)	4(3.84%)

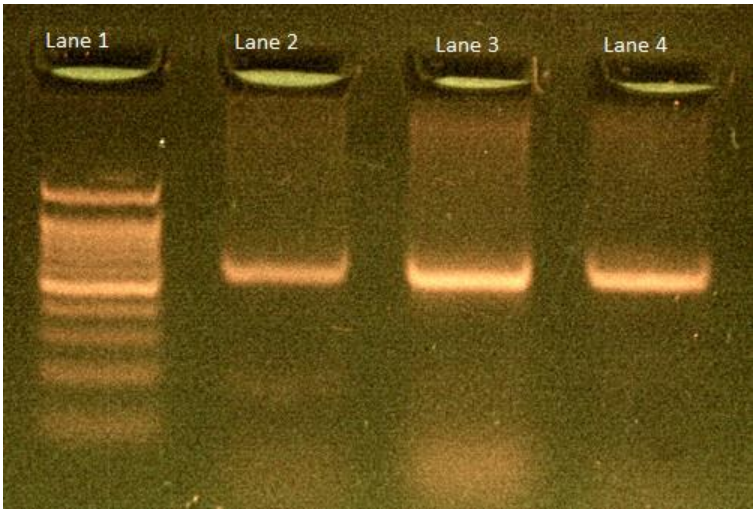

Figure 1. PCR - Gel Electrophoresis for the Detection of Mec A Gene. Lane 1- DNA ladder(100 bp). Lane 2to 3-Mec A positive band (533 bp)

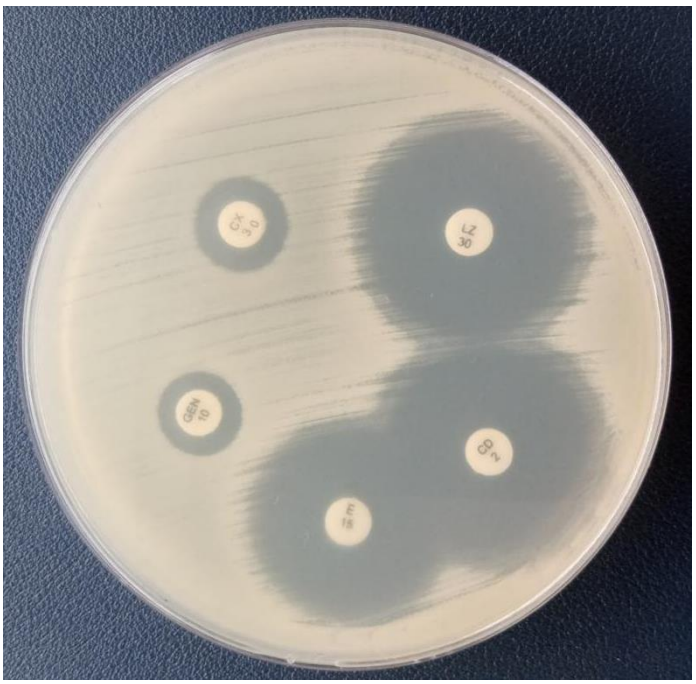

Figure 2. Antibiotic Susceptibility Testing by Kirby Bauer Disc Diffusion Method. MRSA Isolate Showing Cefoxitin (CX) Zone Size Less Than 21 mm

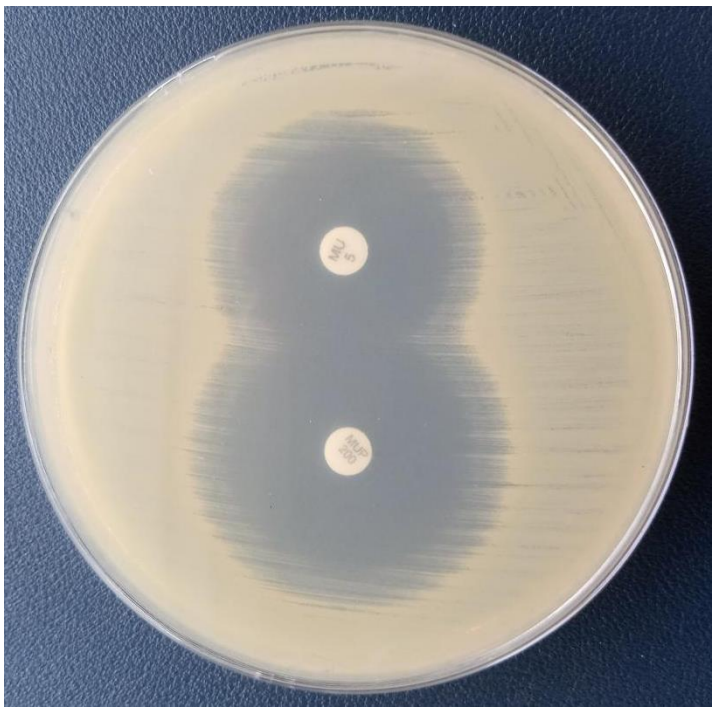

Figure 3. Antibiotic Susceptibility Testing for Mupirocin by Kirby Bauer Disc Diffusion Method. *Staphylococcus aureus* Isolate Showing Susceptible Zones of Inhibition with Both Mupirocin (5 and 200 Microgram Discs)

In the present study, the mupirocin resistance pattern among the *Staphylococcus aureus* isolates (MSSA and MRSA) was analyzed. In clinical microbiology, direct Gram stain examination of the clinical specimens will provide crucial information as to whether the bacteria isolated is a true pathogen or a mere colonizer. The presence of polymorphs indicates infection. Direct Gram staining examination of the clinical specimens should always be performed and correlated with the culture results for correct interpretation. MRSA isolates were detected by both cefoxitin disc diffusion and by PCR method (Mec A gene detection). In most scenarios, mupirocin is prescribed as a topical agent for staphylococcal skin and soft tissue infections without performing susceptibility testing. In the current study, Mupirocin susceptibility was performed for all the isolates since mupirocin resistance can have serious implications like the failure to eradicate MRSA and treatment failure, especially among patients in dialysis units and surgical ICU (Fritz et al., 2013; Hayden et al., 2016; Khan et al., 2020). Worldwide the prevalence of mupirocin resistance is in the range of 5% to 26.6% (Hogue et al., 2010; Pereira et al., 2014; Jung et al., 2015; Cavalcante et al., 2015; Emaneini et al., 2011). Studies across India have documented mupirocin resistance varying from 4 % to 25.5% (Perumal et al., 2022; Kumar et al., 2020; Kavitha et al., 2019; Rudresh et al., 2015). Mupirocin resistance was identified among 12 (8.1%) of the *Staphylococcus aureus* isolates in the present study. High-level and low-level mupirocin resistance was detected among 4 (2.7%) and 8 (5.4%), respectively, in the present study. Studies have reported high-level mupirocin resistance in the range of 2 to 9% and low-level resistance in the range of 4 to 17% among the isolates (Kumar et al., 2020; Gader et al., 2020; Rudresh et al., 2015).

Among MSSA, good susceptibility was observed for cotrimoxazole 89 (85.5%), clindamycin 92 (88.4%), and ciprofloxacin 87 (83.6%). Studies have shown the least

resistance to vancomycin, linezolid, clindamycin, ciprofloxacin, and cotrimoxazole, similar to the present study (Jung et al., 2015).

The prevalence of MRSA was 44 (29.7%) along with multidrug resistance, similar to Pourakbari et al. (2011) and Wu et al. (2010). Studies have demonstrated a high antibiotic resistance rate among MRSA, similar to the present study. Higher rates of MDR among MRSA isolates make it difficult to treat and select appropriate therapy for eradication. In the case of therapy for eradication of nasal carriage of MRSA, all the isolates should always be tested for resistance before using mupirocin to prevent the selection and spread of drug-resistant isolates (Perumal G et al., 2022).

A study by Dadashia et al. (2020) has reported mupirocin resistance among MRSA at 15.2% and High-level mupirocin resistance at 6.8% (Dadashia et al., 2020). Gader A et al. have reported high-level mupirocin resistance at 4% and low-level mupirocin resistance at 18% in MRSA (Gader et al., 2020). High-level resistance to mupirocin might be associated with decolonization failure, and low-level resistance can be treated with a higher dosage (Hetem et al.,2013). Few studies have demonstrated higher resistance to mupirocin among both MRSA and MSSA (Antonov et al., 2015; Mcneil et al., 2011). Vázquez NM et al. (2019) reported a lower percentage of mupirocin resistance among MRSA in a study conducted in the pediatric population in Argentina (Vázquez et al., 2019) in contrast to Khan A et al. (2020) where higher rates of mupirocin resistance were reported among MRSA (Khan et al., 2020). Mupirocin resistance can be due to injudicious prior drug usage for a long period (Kumar D et al., 2020).

In the present study, no difference was observed statistically between MRSA and MSSA for mupirocin resistance. Baek et al. (2016) showed statistically significant differences between mupirocin-resistant MRSA and MSSA (Baek et al., 2016). Since the alternative therapies for treating MRSA infections are limited and also in the wake of the emergence of resistance, looking into the trends of mupirocin resistance is of paramount importance for optimizing therapy as well as infection control activities. The limitation of the study is that the agar dilution method for MIC (Minimum inhibitory concentration) was not performed for the isolates.

CONCLUSION

Strict implementation of guidelines for infection control and prevention measures for MRSA should be followed. The emergence of mupirocin resistance can be attributed to prescribing practices without assessing the Antibiotic susceptibility and also extensive usage of mupirocin for the treatment of superficial staphylococcal infections due to easy availability and over-the-counter accessibility. Routine susceptibility testing should be performed before prescribing mupirocin for therapy as well as eradication of MRSA isolates. The pattern of mupirocin resistance in the present study highlights the necessity for creating cognizance among the clinical and infection control and prevention team before prescribing mupirocin to perform susceptibility before decolonization and therapy. Strict implementation of guidelines for infection control and prevention measures for MRSA should be followed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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