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Original Article

# MINIMUM INHIBITORY CONCENTRATION LEVELS OF MUPIROCIN BY E-TEST AMONG *STAPHYLOCOCCUS AUREUS* ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN

**UMAMAHESWARI JAGADEESAN, JAYAVARTHINNI MANAVALAN*, ROOBHINI SRI NSK, NANDITA BANAJI**

**Department of Microbiology, Indira Gandhi Medical College and Research Institute, Puducherry, India**
**Email: jayavarthinni@yahoo.co.in**

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**ABSTRACT**

**Objective:** This study was done to evaluate the susceptibility of *Staphylococcus aureus* to mupirocin and to determine the antimicrobial susceptibility pattern of *Staphylococcus aureus* among various clinical isolates.

**Methods:** All the consecutive, non-duplicative *Staphylococcus aureus* isolates collected during the year 2020 were subjected to the disk diffusion method to evaluate the antimicrobial susceptibility pattern and were stocked. Mupirocin susceptibility for all stocked *Staphylococcus aureus* was detected by Minimal inhibitory concentration (MIC) determination by Epsilometer test (E-test).

**Results:** The total number of *Staphylococcus aureus* was 52. The maximum number of *Staphylococcus aureus* was isolated from pus sample 40 (76.9%). Among the 52 isolates, 26 (50%) were found to be methicillin-resistant *Staphylococcus aureus* (MRSA). All the isolates were susceptible to tetracycline (100%), vancomycin (100%), teicoplanin (100%), and linezolid (100%). By E-test, the overall prevalence of mupirocin resistance was 63.5%. Low-level Mupirocin resistance (MupRL) of 8-256 µg/ml was 59.6% and high-level mupirocin resistance (MupRH) of ≥512 µg/ml was 3.9%.

**Conclusion:** The present study shows a high prevalence of mupirocin resistance (63.5%) which is a serious concern. Therefore, indiscriminate use of topical mupirocin in carriers is not advisable. It may be recommended only in case of an outbreak of skin and soft tissue infection attributed to *Staphylococcus aureus*.

**Keywords:** *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus* (MRSA), Mupirocin resistance, Minimum inhibitory concentration (MIC), Low-level mupirocin resistance (MupRL), High-level mupirocin resistance (MupRH)

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## INTRODUCTION

*Staphylococcus aureus* is an opportunistic pathogen found in the external environment and as a part of common flora in anterior nares, skin folds, vagina, perineum, and axilla [1]. *Staphylococcus aureus* is known to cause skin and soft tissue infections, infective endocarditis, osteoarticular infections, prosthetic infections, pleuropulmonary infections, and bacteremia [2]. Multidrug-resistant *Staphylococcus aureus* is one of the major organisms causing bloodstream infections which are associated with high morbidity and mortality, which warrants the development and introduction of newer antimicrobial agents [3]. Mupirocin is a topical antimicrobial used for the decolonization of anterior nares [1, 4].

Mupirocin has a unique chemical structure called pseudomonic acid A. The mechanism of action of mupirocin is the inhibition of the bacterial isoleucyl-tRNA synthetase, thereby inhibiting protein synthesis [5]. Mupirocin resistance was first reported in 1987 among *Staphylococcus aureus* [5]. Unrestricted use of mupirocin in the treatment of wounds and pressure sores and routine use in peritoneal dialysis with mupirocin is strongly associated with resistance [6].

Mupirocin-resistant strains are classified into two types: The strain with minimum inhibitory concentrations (MIC) of 8–256 µg/ml is called low-level mupirocin resistance (MupRL), which is due to the point mutation in the chromosomally encoded ileS-2 (mupA) gene. This MupRL can be managed with a higher dosage of mupirocin. The strains with MIC ≥512 µg/ml are called high-level mupirocin resistance (MupRH). There are two mechanisms for MupRH, one is the acquisition of plasmid encoding the mupA gene and another is due to the mupB gene. This resistance strain is associated with treatment failure [5, 7].

Determining the MIC levels helps to differentiate MupRL and MupRH. Studies conducted in different parts of India showed a varying percentage of mupirocin resistance among *Staphylococcus aureus*: Uttar Pradesh (13%), Madhya Pradesh (8.2%), and Karnataka (4.81%) [8, 9, 6]. The resistance in the Indian scenario is quite high [7]. The antimicrobial resistance of *Staphylococcus aureus* is on the rise and India, being a developing country, faces challenges in tackling antimicrobial resistance due to its geography and vast population, low healthcare spending, and inappropriate use of antimicrobials [10]. Mupirocin is not routinely tested; there is only limited knowledge about its resistance pattern. Hence the present study was designed and carried out to evaluate the susceptibility of *Staphylococcus aureus* to mupirocin by detecting the Minimal Inhibitory Concentration and to determine the antimicrobial susceptibility pattern of *Staphylococcus aureus* among various clinical isolates.

## MATERIALS AND METHODS

This study is a descriptive cross-sectional study, which was conducted in the Department of Microbiology IGMCRI, Puducherry. Fifty-two *Staphylococcus aureus* isolates obtained by routine sample processing and stocked by standard procedure [11] in the microbiology department in the year 2020 were included in the study. Repetitive duplicate samples were excluded from this study. The analysis was done after obtaining approval from the Institutional Ethics Committee (No. 340/IEC-32/IGMC and RI/PP-20/2021). The *Staphylococcus aureus* was identified based on Gram staining, catalase test, the tube coagulase test [1].

### Antimicrobial susceptibility testing

Routinely, Antimicrobial susceptibility testing (AST) for all *Staphylococcus aureus* isolates was done on Mueller-Hinton agar (HiMedia, Mumbai) by disc diffusion (Kirby–Bauer) technique as per the CLSI (Clinical and Laboratory Standards Institute) guidelines [12]. Isolate inoculum with a turbidity of 0.5 McFarland-standard ($1.5 \times 10^8$ CFU/ml) in peptone water was prepared and lawn culture

on Mueller Hinton agar (MHA) was made and allowed to dry. Then antibiotics discs with different potency were placed on MHA by sterile forceps. Determination of methicillin-resistant *Staphylococcus aureus* (MRSA) was determined by using cefoxitin 30 µg discs and incubated at 35 °C for 16-18 h. After incubation, the zone of inhibition was measured by an unaided eye, and a size of ≤21 mm was considered resistant and ≥22 mm as sensitive according to CLSI [12].

The antimicrobial profile of the stocked isolates done by Kirby Bauer disk diffusion method was obtained from the Microbiology laboratory register for the following antibiotics: penicillin (10 units); erythromycin (15µg); gentamicin (10µg); ciprofloxacin (5µg); cotrimoxazole (1.25/23.75µg); linezolid (30µg); clindamycin (2 µg); teicoplanin (30µg); tetracycline (30 µg); chloramphenicol (30µg); cefoxitin (30 µg) and vancomycin (using vancomycin screen agar-6 µg/ml). It was used for the final analysis.

**The minimum inhibitory concentration of mupirocin detection by Epsilometer (E) test**

E strips were used for the detection of MIC for mupirocin. Lawn culture was prepared on the MHA medium surface as per manufacturer instructions. Himedia E-strip with mupirocin antibiotic varied from 0.064-1240 µg/ml was placed on MHA by gently pressing using a sterile forceps. The plates were then incubated aerobically at 35 °C for 24 h. After incubation, plates were examined for the minimum inhibitory concentration (MIC).

Minimum inhibitory concentration (MIC) breakpoints for mupirocin susceptibility (MupS) isolates is ≤4 µg/ml, low-level mupirocin-resistant (MupRL) isolates are 8–256 µg/ml and high-level mupirocin-resistant (MupRH) isolates is ≥512 µg/ml, as per CLSI [13] (fig. 1). Results were interpreted accordingly.

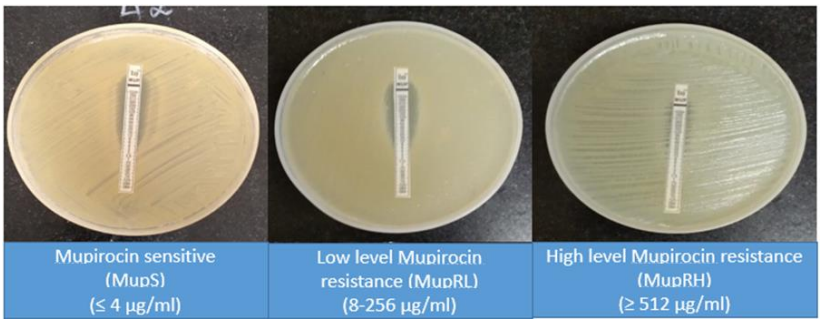
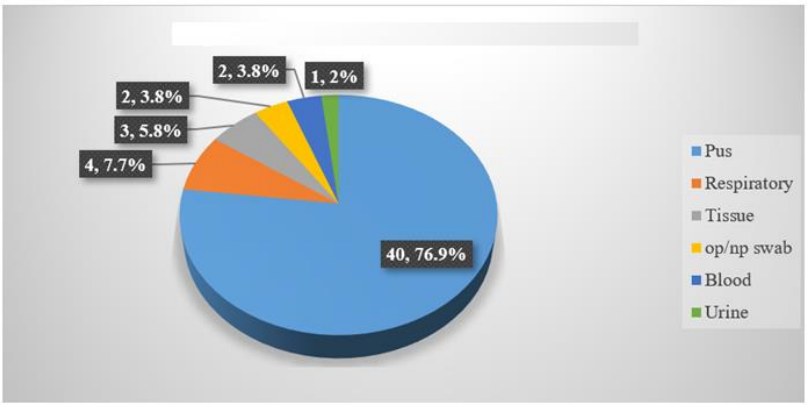



**Fig. 1: E-test showing mupirocin sensitivity (≤ 4 µg/ml), Low-level mupirocin resistance (8-256 µg/ml), and high-level mupirocin resistance (≥ 512 µg/ml) exhibited by *Staphylococcus aureus* on muller hinton agar plate**

**RESULTS**

The total number of non-duplicate *Staphylococcus aureus* included in the study was 52. The median age of the patient was 43.5, ranging from 0-85 y. Males were 28 (53.8%) and females were 24 (46%). 32 (61.5%) *Staphylococcus aureus* were isolated from inpatient wards and 20 (38.5%) were isolated from OPD patients. A maximum number of *Staphylococcus aureus* was isolated from the pus sample. The sample-wise distribution of *Staphylococcus aureus* is shown in fig. 2.




**(* op - oropharyngeal swab and np - nasopharyngeal swab)**

**Fig. 2: Sample-wise distribution of *Staphylococcus aureus***

Among the 52 *Staphylococcus aureus* isolates, 26 (50%) were found to be MRSA. All the isolates were 100% sensitive to tetracycline, teicoplanin, linezolid, and vancomycin. The overall mupirocin resistance was 33 (63.5%) among all *Staphylococcus aureus* isolates, with MSSA showing 14 (53.8%) and MRSA of 19 (73%) resistance to mupirocin. Of this, the percentage of MupRL was 59.6% and MupRH was 3.9%. Table 1 shows the distribution of MupS, MupRL, and MupRH among MSSA and MRSA isolates.

**Table 1: Distribution of mupirocin resistance among *Staphylococcus aureus***

| Isolates | Mupirocin sensitive (MupS) (≤ 4 µg/ml) | Low level mupirocin resistance (MupRL) (8-256 µg/ml) | High level mupirocin resistance (MupRH) (≥ 512 µg/ml) |
|---|---|---|---|
| MSSA (26) | 12 (46.1%) | 13 (50%) | 1 (3.8%) |
| MRSA (26) | 7 (26.9%) | 18 (69.2%) | 1 (3.8%) |
| Total (52) | 19(36.5%) | 31(59.6%) | 2(3.9%) |

A comparison of antibiotic sensitivity patterns was done for three groups (MupS, MupRL, and MupRH). Overall, all three groups were 100% sensitive to tetracycline, teicoplanin, linezolid, and vancomycin. All antibiotics showed good sensitivity against all group isolates except penicillin, ciprofloxacin, and erythromycin, as described in table 2.

**Table 2: Antibiotic sensitivity (%) pattern of mupirocin sensitive and mupirocin resistant *Staphylococcus aureus* isolates**

| Antibiotics | *MupS* isolates (n=19) % | *Mup*RL isolates (n=31) % | *Mup*RH isolates (n=2) % | *Staphylococcus aureus* isolates (n=52) % |
|---|---|---|---|---|
| Penicillin (10 units) | 2(10.5%) | 3 (9.7%) | 0 | 5 (9.6%) |
| Cefoxitin (30 µg) | 12 (63.1%) | 13(41.9%) | 1(50%) | 26 (50%) |
| Ciprofloxacin (5 µg) | 4(21%) | 18(58%) | 0 | 22 (42.3%) |
| Erythromycin (15 µg) | 9(47.3%) | 11(35.4%) | 1 (50%) | 21 (40.3%) |
| Clindamycin (2 µg) | 14(73.6%) | 23(74.1%) | 2 (100%) | 39 (75%) |
| Gentamicin (10 µg) | 16(84.2%) | 27(87%) | 2 (100%) | 45 (86.5%) |
| Cotrimoxazole (1.25/23.75µg) | 15(78.9%) | 22(70.9%) | 2 (100%) | 39 (75%) |
| Chloramphenicol (30 µg) | 15(78.9%) | 28(90.3%) | 2 (100%) | 45 (86.5%) |
| Tetracycline (30 µg) | 19(100%) | 31 (100%) | 2 (100%) | 52 (100%) |
| Teicoplanin (30 µg) | 19(100%) | 31(100%) | 2 (100%) | 52 (100%) |
| Linezolid (30 µg) | 19(100%) | 31(100%) | 2 (100%) | 52 (100%) |
| Vancomycin (6 µg/ml) | 19(100%) | 31(100%) | 2 (100%) | 52 (100%) |

## DISCUSSION

In this study, the median age of the patient is 43.5 y, *Staphylococcus aureus* was isolated from a wide age range, from 0-85 y. Out of the total 52 isolates, 28 (53.8%) isolates were obtained from males and 24 (46%) were from females. Male to female ratio is 1.17:1.

In the present study, a maximum number of *Staphylococcus aureus* were isolated from pus sample 40 (76.9%), followed by respiratory 4 (7.7%) and tissue 3 (5.8%) which is consistent with other similar studies by Kumar *et al.*, [8] and Mohanty *et al.*, [14] where 60% and 61.6% of *Staphylococcus aureus* were mainly isolated from pus samples respectively followed by other samples in varying frequency. This finding is also correlating with a study done in south India by Chavadi *et al.*, which isolated the maximum number of MRSA (38%) from the pus sample [15].

In the current study, the majority 32 (61.5%) of *Staphylococcus aureus* were isolated from inpatient wards, and the remaining 20 (38.5%) were isolated from the outpatient department (OPD), which is similar to the study done by Mohanty *et al.*, [14] in East India in which 72.2% were from admitted patients, whereas 27.8% isolates were from the OPD patients.

Out of the total 52 *Staphylococcus aureus* isolates, 26 (50%) were found to be MRSA. Our study shows a high prevalence of MRSA, which is in agreement with Antimicrobial resistance research and surveillance network report by ICMR [16]. According to this report, MRSA prevalence was 42.6% by 2021. On the contrary, other studies, Chaturvedi *et al.*, [17]. Rajaduraipandi K *et al.*, [18] conducted in India with a similar study cohort, the prevalence rates were much lower 22.7% and 31.1%, respectively. ICMR surveillance study conducted in Jipmer in 2017 by Rajkumar *et al.*, [10] also showed an overall low prevalence of MRSA at 37.3%. This could be due to the varied prevalence of methicillin resistance from one place to another, the number of samples as well as local antibiotic policy.

Among the 26 MRSA isolates, 19 (73%) were resistant to mupirocin and among the 26 MSSA isolates, 14 (53.8%) were resistant to mupirocin. A maximum percentage of resistance was observed among MRSA isolates when compared to MSSA isolates in our study. This finding is similar to a study done in Ghaziabad, in which 19% of mupirocin resistance was seen with MRSA and 9% of mupirocin resistance was observed among MSSA isolates [8]. Overall mupirocin resistant *Staphylococcus aureus* isolates were found to be 33 (63.5%). Of these, the majority were resistant to penicillin, followed by erythromycin, clindamycin, and ciprofloxacin. All the *Staphylococcus aureus* isolates were fully (100%) susceptible to teicoplanin, vancomycin, and linezolid and this finding was found to be consistent with other similar studies [8, 9]. However, the antimicrobial susceptibility profile of mupirocin-sensitive *Staphylococcus aureus* isolates with mupirocin-resistant *Staphylococcus aureus* isolates did not show many variations.

The overall prevalence of mupirocin resistance in *Staphylococcus aureus* was found to be 63.5% in our study. The observed resistance was quite higher than that reported from an earlier study done in Uttar Pradesh, India, which showed 13% of mupirocin resistance in *Staphylococcus aureus* [8]. Our study showed a higher percentage (59.6%) of low-level resistance to mupirocin (MupRL). In contrast, studies done by Kumar *et al.*, [8] and Rudresh MS *et al.*, [9] showed 9% and 17.3% of MupRL, respectively. Also, a study done by Shukla *et al.*, in 2019 did not encounter low-level mupirocin resistance among *Staphylococcus aureus* [19]. However, the prevalence of high-level resistance to mupirocin (MupRH) in our study was only 3.9%, which is similar to the studies done by Kumar *et al.*, [8], Tiewsoh JB *et al.*, [6] and Bhavana *et al.*, [7] who reported 4%, 4.16% and 4.81% of high-level resistance to mupirocin respectively. The increased usage of mupirocin in the general population for treating nonspecific skin and soft tissue infections rather than eradicating the carrier state of MRSA has probably led to a higher percentage of low-level resistance in this study.

Low-level mupirocin resistance (MupRL) can be managed with a normal dosage schedule of mupirocin i.e., 2%, whereas high-level mupirocin resistance is associated with failure of mupirocin as a decolonizing agent. Fortunately, our study documented a very less percentage of MupRH which is a favorable finding.

## CONCLUSION

The present study shows a high prevalence of mupirocin resistance (63.5%) which is a serious concern. Therefore, indiscriminate use of topical mupirocin in carriers is not advisable. It may be recommended only in case of an outbreak of skin and soft tissue infection attributed to *Staphylococcus aureus*.

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## AUTHORS CONTRIBUTIONS

Dr. Umamaheswari Jagadeesan was involved in the planning, writing, and execution of the study. Dr. Jayavarthinni Manavalan was involved in the writing and reviewing of the manuscript. Dr. Roobhini Sri NSK was involved in the planning and execution of the study. Dr. Nandita Banaji was involved in the planning and reviewing of the manuscript.

## CONFLICT OF INTERESTS

The authors have no known conflicts of interest to declare.

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