

**ABSTRACT** Irrational use of antibiotics and prolonged hospital stay leading to carriage of MRSA in nose, axilla, perineum are some risk factors for acquiring MRSA infections and its spread in community. Mupirocin is an effective antibiotic used topically for the elimination of MRSA in the carriers. But the long term use of antibiotics has led to emergence of mupitocin resistant strains. Material and methods: 300 S.aureus isolates were processed for antibiotic susceptibility testing and methicillin resistance using cefoxitin disc and mupirocin resistance using 5µg and 200µg disc. Results: In the present study, overall 4% LLM resistance and 3.33% HLM resistance was observed. 5% MRSA and 1.8% MSSA were high level mupirocin resistance while 7.2% MRSA and 2.4% MSSA were low level mupirocin resistance. Conclusion: Low level resistant strains need to be detected, so can be treated with higher concentration of the drug. Judicious use of mupirocin application needs to be done to avoid emerging its resistance.

KEYWORDS: MRSA, mupirocin resistance, Staphylococcus aureus

# INTRODUCTION:

MRSA (Methicillin resistant Staphylococcus aureus) strains which are now widespread, have complicated the treatment protocol and controlling staphylococcal infections. However it was earlier confined to hospitals and health care settings, now MRSA is a frequent causes of infection in community.<sup>1</sup>

Irrational use of antibiotics, intravenous drug use, prolonged hospital stay leading to carriage of MRSA in nose, axilla, perineum are some risk factors for acquiring MRSA infections and its spread in community. The most common antibiotics used for treatment of MRSA infection are vancomycin and linezolid. While mupirocin is an effective antibiotic used topically for the elimination of MRSA in the carriers.<sup>23</sup>

Mupirocin (i.e. pseudomonic acid A) is used as a topical ointment that can be used broadly for the treatment of MRSA skin and soft tissue infections and is highly effective for nasal decolonization of MRSA in carriers. Mupirocin acts by attaching to the bacterial isoleucyl-tRNA synthetase and interferes with the protein synthesis. But long-term use of the antibiotic led to emergence of mupirocin-resistant organisms.<sup>4</sup>

There are three types of mupirocin susceptibility described for S. aureus. First being mupirocin susceptibile with minimum inhibitory concentrations <4 mg/mL, second being low-level mupirocin resistant strains with MIC 8 - 64 mg/mL, and third high-level mupirocin resistance with MIC of as high as 512 mg/mL. The isolates that show high-level mupirocin resistance have acquired plasmid-mediated mupA. It encodes a novel isoleucyl RNA synthetase. The isolates presenting low-level mupirocin resistance have the acquired base changes in native isoleucyl RNA synthetase gene (ileS).5 Susceptible strains are those showing zone diameters of >14 mm around the 5 mg mupirocin disc. High-level mupirocin resistance is seen associated with failure to clear S.aureus harbour from patients. However, it is suggested that low level resistant nasal isolates can be controlled with mupirocin ointment, as it contains a much higher mupirocin concentration than its MIC. So, it is suggested to use two-disc system (5 and 200 mg) distinguish clearly the three groups of mupirocin resistant S. aureus isolates.<sup>6</sup>

The current study focussed on distinguishing all the three groups of mupirocin susceptibility in the S.aureus isolates of patients visiting our tertiary care hospital.

## Material and methods:

The study was conducted in Microbiology department at RNT medical college, Udaipur during the year Jan 2019-Dec 2019.

All the samples received in the bacteriology laboratory were further processed for staphylococcus aureus isolates. The samples included pus, wound swab, throat swab, sputum, blood, urine, vaginal swab and body fluids (pleural fluid, penitonial fluid, CSF, ascitic fluid)

300 S.aureus isolates was further confirmed by tube coagulase test, growth on mannitol salt agar and DNase agar. Antibiotic susceptibility testing of all the samples along with MRSA detection was done by cefoxitin disc using Kirby baeur disc diffusion method as per the CLSI guidelines.<sup>7</sup>

High level Mupirocin detection was done as per CLSI guidelines. A lawn culture of 0.5 Mc Farland was prepared on MHA plate and  $200\mu$ g mupirocin disc was placed on the inoculated plate and incubated for 24 hrs at ambient temperature at 37°C. Any zone of inhibition was considered as sensitive while no zone of inhibition was considered as resistant.<sup>7</sup>

Low level mupirocin resistance was detected using 5µg disc on the inoculated MHA plate and zone size  $<\!14$  was considered as resistant.  $^{\rm s}$ 

Statistical analysis was performed to observe significance correlation between mupirocin and methicillin resistance.

Fischer exact test was used for the above and p value  $\leq 0.05$  was considered significant correlation.

## **Results:**

300 staphylococcus aureus were subjected to antibiotic susceptibility testing with detection of MRSA strains using cefoxitin disc. Out of 300 isolates 138(46%) were found to be MRSA while 162(54%) were MSSA.

Vancomycin was completely sensitive and only one sample was found to be resistant to linezolid. 5-10% resistance was seen with gentamycin and tegicycline.

However, Ciprofloxacin (45%), cotrimoxazole (34%) and tetracycline (25%) were found to be highly resistant.

# 92.6% of isolates were found to be mupirocin sensitive in the present study with 4% LLM resistance and 3.33% HLM resistance.

5% of the MRSA isolates showed HLM resistance while 7.2% showed LLM resistance.

## Table 1: Three categories of mupirocin sensitivity among all the isolates of S.aureus.(HLM=high level mupirocin resistance, LLM= low level mupirocin resistance)

MUPIROCIN	HLM	LLM	MUP SENSITIV	TOTAL
NO. OF ISOLATES	10	12	278	300
PERCENTAGE	3.33	4	92.66	100%

Table 2: Category wise distribution of mupirocin resistance among Methicillin resistant (MRSA) and methicillin sensitive (MSSA) strains.

MUPIROCIN	SENSITIVE	HLM	LLM	TOTAL
MRSA	121(87.6%)	7(5%)	10(7.24%)	138(100%)
MSSA	155(95.6%)	3(1.8%)	4(2.46%)	162(100%)

Statistical analysis conducted in the present study showed p value < 0.05 meaning the correlation between methicillin resistance and mupirocin resistance was found to be statistically significant.

# DISCUSSION:

Topical preparations of mupirocin became available in 1985, since then it is widely used for management of infection and colonization of MRSA in both patients and medical personnel. The first report of mupirocin resistant S. aureus came shortly after its introduction (1987) from UK. In recent days, there is a worldwide increase in mupirocin resistance among S. aureus.<sup>8</sup>

Mupirocin demonstrates higher efficacy with significant duration for clearance of MRSA in carriers. If resistance to mupirocin is observed, especially HLM resistance, it offers a very few topical treatment options.

In the present study, the high-level resistance was 3.33% of the total isolates. 5% MRSA isolates were seen to have HLM resistance while 1.8% were MSSA. Statistically, significant correlation was found between methicillin resistance and mupirocin resistance.

A study by Jaykumar et al9 showed lower prevalence of mupirocin resistance in MRSA as compared to our study. The overall HLM was 2.2% while only one isolate of MRSA was HLM resistant. Oommen et al10 also showed 2% HLM resistance in MRSA which is lower as compared to our study. However, 9.7% HLM resistance was shown in study conducted by Chaturvedi et all1 which is higher as compared to our study.

In the present study, 4% overall isolates were LLM resistant. MRSA isolates with LLM resistance were 7.24% while in MSSA it was found to be 2.46%. other studies like Jaykumar et al9 and Oomman et al10 show no LLM resistant strains in MRSA. While study of Chaturvedi et all 1 shows 8.5% LLM resistance. The above study concludes that 7.24% MRSA and 2.46% MSSA can be treated by higher concentration of mupirocin present in the ointments as they show low level mupirocin resistance. But the isolates showing high level need a different treatment modality. Routine detection for mupirocin resistance is important so as to retain the usefulness of the antibiotic agent for the treatment and the prevention of staphylococcus aureus infections. Judicial use of mupirocin should be done by the clinicians after antibiotic susceptibility testing. Prolonged or widespread use of mupirocin in community must be regulated.

#### **REFERENCES:**

- Kateete, D.P., Bwanga, F., Seni, J. et al. CA-MRSA and HA-MRSA coexist in community and hospital settings in Uganda. Antimicrob Resist Infect Control 8, 94 (2019). https://doi.org/10.1186/s13756-019-0551-1
- Klutymans J, van Balkum A, Verbrughi H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms and associated risk. Clin Microbiol Rev 1997;10:505-20.
- Cookson D. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. J Antimicrob Chemother 1998;41:11-8.
- Mahmoudi S, Mamishi S, Mohammadi M, Banar M, Haghi Ashtiani MT, Mahzari M, Bahador A, Pourakhari B. Phenotypic and genotypic determinants of mupirocin resistance among Staphylococcus aureus isolates recovered from clinical samples of children: an Iranian hospital-based study. Infect Drug Resist. 2019;12:137-143
- Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin Resistance. Clin Inf Dis.2009; 49(6).935–941.
- Moreira de Oliveira NE, Cardozo APCM, Marques EA, Netto Dos Santos KR, deMarval MG. Interpretive criteria to differentiate low- and high-level mupirocin resistance in Staphylococcus aureus. J Med Microbiol. 2007;56(Pt 7):937-939. doi:10.1099/jmm.0.46965-0
- Performance standards for antimicrobial testing.30th ed. CLSI supplement M100. Wayne, PA. clinical and laboratory standard institute.2020.
- Rudresh MS, Ravi GS, Motagi A, Alex AM, Sandhya P, Navaneeth BV. Prevalence of Mupirocin Resistance Among Staphylococci, its Clinical Significance and Relationship to Clinical Use. J Lab Physicians. 2015 Jul-Dec;7(2):103-7.
- S J, M M, A S SB, Mathew R, M K, Lal Y B. Prevalence of High and Low Level Mupirocin Resistance among Staphylococcal Isolates from Skin Infection in a Tertiary Care Hospital. J Clin Diagn Res. 2013 Feb;7(2):238-42
- Oommen SK, Appalaraju B, Jinsha K. Mupirocin resistance in clinical isolates of staphylococci in a tertiary care centre in south India. Indian Journal of Medical Microbiology, (2010) 28(4): 372-5
- Chaturvedi P, Singh AK, Singh AK, Shukla S, Agarwal L. Prevalence of Mupirocin Resistant Staphylococcus aureus Isolates Among Patients Admitted to a Tertiary Care Hospital. N Am J Med Sci. 2014 Aug;6(8):403-7. doi: 10.4103/1947-2714.139293. PMID: 25210674; PMCDI: PMC4158649.