ORIGINAL ARTICLE

Need of Testing Mupirocin Susceptibility Pattern of Staphylococcus aureus from Clinical Isolates in a Tertiary Care Teaching Institute

Sumedh Sudhakar Lokapure[1], Chetana Joshi[1], Prasanna Nakate[1] and Yogendra Shelake[1]
[1]Department of Microbiology
[1]B.K.L. Walawalkar Rural Medical College and Hospital, Sawarde, Dist- Ratnagiri- 415606, Maharashtra, India

Abstract:

Introduction : *Staphylococcus aureus* is an important cause of a variety of infections, ranging from skin and soft tissue infections to serious, life-threatening bloodstream infections. Methicillin Resistant *Staphylococcus aureus* (MRSA) is involved in serious infections as well as nosocomial outbreaks. Mupirocin is drug used for decolonization of the carrier state as well as for topical treatment of MRSA infections. Nowadays, mupirocin resistance is increasing due to inappropriate usage. In the present study, we have demonstrated mupirocin resistance by simultaneous use of mupirocin discs with concentrations of 5 µg and 200 µg in *S. aureus* clinical isolates. *Material and Methods:* A prospective study for a period of six months was conducted in a tertiary care teaching hospital in Sawarde, Ratnagiri. *S. aureus* isolates obtained from 993 clinical specimens were processed further as per standard operating procedures in the Microbiology Laboratory for mupirocin resistance and interpreted with the help of Clinical and Laboratory Standards Institute (CLSI) guidelines. *Results:* In 993 clinical samples we got 100 isolates of *S. aureus*, 67% were MRSA and 33% were MSSA (Methicillin Sensitive *Staphylococcus aureus*). The maximum number of MRSA isolates were recovered from pus 32 (47.76%), followed by wound swab 17 (25.37%) and blood 9 (13.43%). Among 12 mupirocin-resistant MRSA isolates, 7 (10.44%) exhibited low-level resistance to mupirocin, and 5 (7.46%) isolates were found to be high-level mupirocin-resistant. In MSSA strains, no mupirocin resistance was observed. *Conclusion:* The use of topical ointment mupirocin is an effective modality for destroying MRSA in carriers. Differentiating between the two types of resistance in mupirocin (MuL and MuH i.e. Mupirocin Low Level Resistance and Mupirocin High Level Resistance) has a notable therapeutic impact. Due to the alarming rise in antimicrobial resistance, hospital laboratories should detect the susceptibility of *S. aureus* isolates to the drug mupirocin.

Keywords: Antimicrobial resistance, Mupirocin, MRSA, MSSA, MRSA carriers.

Introduction:

Staphylococcus aureus is one of the most commonly isolated gram-positive bacterial pathogens from community acquired and nosocomial infections. It is an important cause of the wide range of infections ranging from skin and soft tissue infections, pneumonia and osteomyelitis to severe bloodstream infections.[1] Methicillin resistant *S. aureus* (MRSA) is implicated in serious infections and nosocomial outbreaks. These strains show resistance to a wide range of antibiotics, thus limiting the treatment options to very few agents such as glycopeptides and linezolid. MRSA carriage in the nose, axilla, perineum and hands of patients and health care personnel is an important risk factor for MRSA acquisition and spread of infection to patient. [2] Decolonization from the site of carriage is one of the modalities for prevention of MRSA infections in healthcare settings. [3] Mupirocin, also known as Pseudomonic acid A, is the drug of choice to eradicate MRSA colonization which acts by inhibiting protein synthesis. It can be effectively used as topical antimicrobial to treat colonization with MRSA as well as MSSA (Methicillin sensitive *Staphylococcus aureus*). Along with its' use as a decolonising agent, it has also been used for treatment of skin and soft tissue infections. Irrational usage along with excessive accessibility has led to the resistance of this drug which causes incomplete decolonization of *S. aureus* and facilitates the spread of infection. Resistance to mupirocin can be detected by various methods viz. disc diffusion, Etest and polymerase chain reaction (PCR) etc. Initial screening of

resistance is done by disc with 5 µg concentration of the drug.[1] Simultaneous use of mupirocin discs with concentration of 5 µg and 200 µg is recommended to differentiate between low-level and high-level mupirocin resistant strains of *S. aureus*. [1,4] With this background, we have evaluated the susceptibility to mupirocin exerted by clinical isolates of *S. aureus* from various samples. The primary aim of this study was to identify mupirocin resistance in *Staphylococcus aureus* isolated from clinical
specimens. The other objective were, to calculate prevalence of MRSA & MSSA from different clinical specimens and to identify low-level and high-level mupirocin resistance in isolated *S. aureus* organism.

Material and Methods:

This prospective study was conducted in the Department of Microbiology at BKL Walawalkar Rural Medical College, Sawarde, Ratnagiri, from July 2023 to December 2023 for a period of 6 months. *Staphylococcus aureus* isolates were recovered from a total of 993 clinical specimens comprising pus, wound swabs, tissue, respiratory secretions, aspirated fluids, urine, and blood from out-patients and in-patients admitted to various wards and intensive care units are included in the study. All clinical isolates other than *Staphylococcus aureus* were excluded from the study.
The samples were initially subjected to microscopic observation and cultured on blood agar and MacConkey agar. A total of 100 non-duplicate Staphylococcal aureus isolates were identified using a Gram-positive identification card, and antimicrobial susceptibility was determined by the VITEK 2K automated system. All the tests and quality assurance procedures were performed and interpreted according to the standards set by the Clinical and Laboratory Standards Institute (CLSI). [5] Mupirocin resistance testing was determined by Kirby Bauer disc diffusion method with disc concentrations of 5µg and 200µg (Hi-Media Mumbai). The *S. aureus* isolates were lawned on Mueller-Hinton agar plates with these discs applied to the agar plates and incubated at 37°C overnight. The reading was noted for zone diameters surrounding both discs. A zone diameter of ≥ 14 mm for both discs was taken as susceptible to mupirocin. Whereas, isolates that showed zone diameters < 14 mm with a 5µg disc but ≥ 14 mm with a 200µg disc were considered to be lowlevel mupirocin-resistant strains. All isolates with zone diameters < 14 mm for both 5µg and 200µg discs were considered to be high-level mupirocin-resistant strains.

Results:

Fig 1: High level & Low-level Mupirocin Resistance

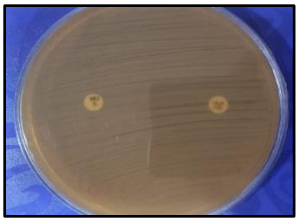


Fig 2: High level & Low-level Mupirocin Sensitive.

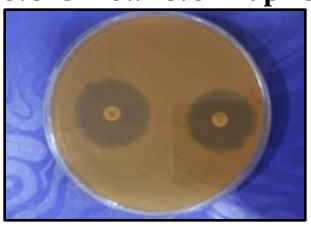
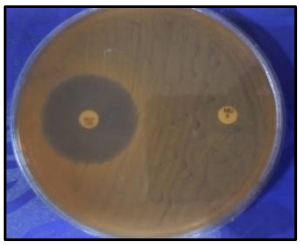


Fig 3: High level resistance but low-level mupirocin sensitive



Among the various samples processed during the study period, only 100 isolates were identified as *S. aureus*. Based on the cefoxitin susceptibility results, isolates were categorised as MRSA (67%) and MSSA (33%). In determining the sample-wise distribution, the majority of MRSA were isolated from pus 32 (47.76%), followed by wound swab 17 (25.37%) and blood 9 (13.43%). The isolation of MSSA was also done in a similar way, with pus 15 (45.45%) being the most common specimen, followed by wound swab 7 (21.21%) and blood 4 (12.12%). Sputum, sterile body fluids and urine showed the least isolation of *S. aureus*.

Table 1: Sample wise distribution of MRSA and MSSA clinical isolates

Sample Type	MRSA, n (%)	MSSA, n (%)
Pus	32 (47.76)	15 (45.45)
Wound swab	17(25.37)	7(21.21)
Blood	9(13.43)	4(12.12)
Sputum	4 (5.97)	4(12.12)
Body fluid	3 (4.47)	3(11)
Urine	2 (2.98)	-
Total	67 (100)	33(100)

All *S. aureus* isolates were screened for mupirocin susceptibility. About 12 (17.91%) MRSA isolates showed mupirocin resistance. Among these resistant isolates, 7 (10.44%) MRSA isolates exhibited low-level resistance to mupirocin, whereas 5 (7.46%) isolates were found to be high-level mupirocin resistant. All MSSA isolates obtained in the study were found to be susceptible to mupirocin.

Table 2: Comparison of Mupirocin resistance in MRSA and MSSA clinical isolates

S. aureus isolates	Mupirocin sensitive n (%)	Mupirocin resistant n (%)	MuL n (%)	MuH n (%)
MRSA	55 (82.08)	12 (17.91)	7 (10.44)	5 (7.46)
MSSA	33(100)	0	0	0

Mupirocin resistance was seen in MRSA isolates from suppurative samples such as pus 5 (15.62%), followed by wound swab 3 (17.64%). Among these isolates, 5 (8.16%) were found to be low-level mupirocin resistant and 3 (6.12%) isolates were found to be highlevel mupirocin resistant. Among the sputum sample, out of 4 isolates, 2 (50%) showed high-level mupirocin resistance and none of them showed lowlevel mupirocin resistance, whereas out of 3 MRSA isolated from body fluids, only 1 isolate showed lowlevel mupirocin resistance with no high-level resistance. The isolates from urine samples failed to demonstrate any mupirocin resistance.

Table 3: Distribution of low-level and high- level Mupirocin resistance in MRSA isolates

Sample Type	MRSA n (%)	Low level mupirocin resistant n (%)	High level mupirocin resistant n (%)
Pus	32 (47.76)	3 (9.37)	2 (6.25)
Wound swab	17(25.37)	2 (11.76)	1 (5.88)
Blood	9(13.43)	1 (11.11)	-
Sputum	4 (5.97)	-	2 (50)
Body fluid	3 (4.47)	1 (33.34)	-
Urine	2 (2.98)	-	-
Total	67 (100)	7(10.44)	5 (7.46)

Discussion:

Mupirocin is extensively used to control the colonisation and infection caused by *S. aureus* in healthcare workers as well as in patients. The first mupirocin-resistant S. aureus isolate was reported from the UK in 1987, two years after the introduction of mupirocin for treatment purposes. The population of mupirocin-resistant MRSA isolates started rising worldwide due to the absurd, unregulated and longterm use of this drug.[6] This study highlights the importance of determining low-level and high-level mupirocin resistance in *S. aureus* isolates from clinical samples. In the current study, around 100 isolates of *S. aureus* were obtained during the study period of 6 months. These isolates were further processed for mupirocin susceptibility testing, of which 67 (67%) and 33 (33%) were MRSA and MSSA respectively. Perumal PG et al. showed 51 % & 49% of MRSA & MSSA isolation in their study [7]. Another study by C. Senthilvadivu et al with 96 clinical isolated of *S. aureus*, 76 (79%) were found to be MRSA and 20 (21%) isolates were found to be MSSA.[8] Majority of the studies mention MRSA as a significant pathogen as compared to MSSA. In our study, *S. aureus* (MRSA & MSSA) was most commonly isolated from pus sample (47%) followed by wound swab (24%) and blood (13%). Sputum (8%) and urine (2%) were the least common samples which showed isolation of *S. aureus.* A similar study from Bhavana et al. showed *S. aureus* isolation was most commonly from pus sample (70%) followed by wound swab (18%) and blood (5%). The higher percentage of isolation from pus may be higher number of samples enrolled in the study. [9] The study done by Nada KK et. al. observed a lower percentage of *S. aureus* isolates from deep wounds (13.5%), which is discordant with the current study.[10] In the present study, among MRSA isolates, the percentage of high-level mupirocin resistance was 7.46% and that of low-level mupirocin resistance was 10.44%. The studies performed by Dardi CK and Rudresh MS et. al. revealed that the percentage of high-level mupirocin-resistant MRSA was 5.99% and 14.7%, respectively, whereas the percentage of low-level mupirocin-resistant isolates was found to be 15.35% and 10.5%, respectively. [11, 12] Tiewsoh JBA and Dias M. found that high-level mupirocin resistance among MRSA isolates was 4.16%, which is closer to the present study. [13] In other studies conducted by Orrett FA and Vasquez JE et. al., it was found that the percentage of lowlevel and high-level mupirocin resistance was to the extent of 26% and 44%, 58% and 42%, respectively.[14, 15]The variation in the percentage of resistance was attributed to factors such as demographic conditions, local antibiotic guidelines and the number of samples. In the present study, among various samples, MRSA isolates from sputum and body fluids chiefly exhibited mupirocin resistance. Considering sample-wise distribution, higher

resistance. Considering sample-wise distribution, higher percentages of mupirocin resistance were obtained from sputum isolates (50%), followed by isolates from body fluids (33.34%) and wound swabs (MuL 11.76% and MuH 5.88%). A study by Dardi CK showed a higher prevalence of mupirocin resistance (MuL and MuH) was from pus (26.92% and 10.25%), followed by blood (17.14% and 5.71%), sputum (15.38% and 6.15%), miscellaneous (15.78% and 10.52%) and the lowest was in urine (1.42% and 0%). [11] B. Madhumati et al identified higher prevalence of high-level mupirocin resistance from blood isolates followed by pus (45% and 36.3% respectively), low level mupirocin resistance was maximally seen in the isolates from respiratory secretions (46%).[16] The difference in mupirocin resistance in clinical samples may be related to local epidemiological factors such as prevalence of MRSA isolation, use of mupirocin in the hospital and community settings. Mupirocin is an ointment that is effective in destroying MRSA in carriers. [1] It is accepted therapeutically for superficial skin and soft tissue infections. It has been observed that using this drug on a large scale in the community for this objective is accelerating the development of resistance. [2, 7] The application of mupirocin in the anterior nares of MRSA carriers can lead to the presence of a low concentration of this drug in the pharynx, which might be responsible for the appearance of mupirocin-resistant MRSA. Determining and differentiating between the two types of resistance in mupirocin (MuL and MuH) has a notable therapeutic impact. High-level mupirocin resistance (MuH) prohibits its' use as a treatment option, whereas low-level mupirocin resistance (MuL) can be dealt with a higher dosage of mupirocin. [2, 7] The threat of developing resistance seems to be more prevalent among MRSA, which is frequently connected with illicit use of the drug.[17]Therefore, laboratories in healthcare settings should differentiate between susceptible and resistant *S. aureus* isolates. Also, laboratories should detect the level of resistance to mupirocin (MuL and MuH). [18] The "gold standard" method for detection of mupirocin resistance is minimum inhibitory concentration (MIC) determination by the agar dilution method. [19] In the present study, we incorporated the disc diffusion method to determine low and high-level mupirocin resistance. The study performed by Malaviolle X et al. calculated the sensitivity and specificity of this method by using 5 µg and 200 µg mupirocin discs simultaneously. The sensitivity and specificity of using 5 µg disc were 100% and 98.1%, respectively, whereas those of 200 µg disc were 100% and 92.3%, respectively, in their study.[20] Hence, disc diffusion for susceptibility testing is an inexpensive and easy method for repeated use.

The establishment of mupirocin resistance among MRSA isolates is alarming, as mupirocin-resistant strains have hardly any treatment alternatives. Though the use of polysporin triple ointment has been made in practice, research work related to its' potency is lacking. If antibiotics such as vancomycin and fusidic acid are used in combination, it shows a favourable outcome in the systemic infections caused by MRSA, but not as monotherapy.[21]Hydrogen peroxide cream can be used instead of mupirocin as a topical agent. [22] It is usual practice to treat a healthcare worker with MRSA colonisation by using chlorhexidine baths for a week, topical 2% mupirocin ointment in the anterior nares and discontinuation from routine hospital duty until two culture reports obtained are negative. Hence, every isolate recovered from nasal carriers must be screened with mupirocin (with 5 µg discs and 200 µg discs simultaneously) prior to any treatment so that high-level mupirocin-resistant isolates can be managed with better alternatives like fusidic acid, neomycin or even retapamulin.[21,23] The rising incidence of mupirocin resistance can be curtailed with some prompt action. More research work is required to determine the potency and incidental outcomes of mupirocin when used as a preventive strategy. If a decision is made to make use of mupirocin on a constant basis, a technique to record the rate of resistance must be generated and implemented. More details are essential to direct healthcare workers on how to utilize these techniques to manage the use of mupirocin for prevention as well as for treatment.

Sources of supports: Nil

Conflicts of Interest: Nil

References

1. Rajkumari N, Mathur P, Bhardwaj N, Gupta G, Dahiya R, Behera B, Misra MC. Resistance pattern of mupirocin in methicillin-resistant *Staphylococcus aureus* in trauma patients and comparison between disc diffusion and E-test for better detection of resistance in low resource countries. *Journal of Laboratory Physicians* 2014 Jul;6(2):91-95.
2. Hetem DJ, Bonten MJ. Clinical relevance of mupirocin resistance in *Staphylococcus aureus*. *Journal of Hospital Infection* 2013 Dec;85(4):249-256.

3. Parenti MA, Hatfield SM, Leyden JJ. Mupirocin: a topical antibiotic with a unique structure and mechanism of action. *Clinical Pharmacy* 1987 Oct;6(10):761-770.
4. Sanju AJ, Kopula SS, Palraj KK. Screening for Mupirocin Resistance in *Staphylococcus*. *Journal of Clinical and Diagnostic Research* 2015 Oct;9(10): DC09-10.
5. CLSI. 2023. Performance standard for antimicrobial susceptibility testing. M100 standard, 33rd edition Clinical and Laboratory Standard Institute, Wayen, PA.
6. Kumar D, Bisht D, Faujdar SS. Incidence of mupirocin resistance in *Staphylococcus aureus* isolated from rural population: A new emerging challenge. *International Journal of Current Research and Review* 2020;12(22):82-85.
7. Perumal PG, Kannan S, Appalaraju B.Detection and Distribution of Low-Level and High-LevelMupirocin Resistance among Clinical Methicillin Resistant *Staphylococcus aureus* Isolates. *Journal of Clinical and Diagnostic Research* 2022; 16(5):DC06-DC10.
8. Senthilvadivu C, Stalin M. Mupirocin Resistance among Clinical Isolates of *Staphylococcus aureus* from Skin and Soft Tissue Infection in Tertiary Care Centre, Tamil Nadu. *International Journal of Pharmaceutical and Clinical Research* 2023; 15(7); 59-62.
9. Bhavana MV, Joshi SA, Adhikary RA, Beena HB. Mupirocin Resistance in *Staphylococcus aureus* in a tertiary care hospital of South India–a prospective study. *Asian Journal of Pharmaceutical and Clinical Research* 2019 Jan 7;12(1):98-100.
10. Nada KK, Shaimaa OH, Shatha KK. Bacteriological study of urinary tract infections with antibiotics susceptibility to bacterial isolates among honeymoon women in Al-Qassim hospital, Babylon Province, Iraq. *British Biotechnology Journal* 2013;3:332-340.
11. Dardi CK. Mupirocin resistance in clinical isolates of methicillin-resistant *Staphylococcus aureus* from a tertiary care rural hospital. *International Journal of Advanced Medical and Health Research* 2014;1(2):52-56.
12. Rudresh MS, Ravi GS, Motagi A, Alex AM, Sandhya P, Navaneeth BV. Prevalence of mupirocin resistance among *Staphylococci* and its clinical significance and relationship to clinical use. *Journal of Laboratory Physicians* 2015;7:103-107.
13. Tiewsoh JBA, Dias M. Screening of methicillin-resistant *Staphylococcus aureus* in healthcare workers and students and its susceptibility to mupirocin in a tertiary care teaching hospital in South India. *Journal of Laboratory Physicians* 2017 Oct-Dec;9(4):239-242.
14. Orrett FA. The emergence of mupirocin resistance among clinical isolates of MRSA in Trinidad- A First report. *Japanese Journal of Infectious disease* 2008:61:107-110.
15. Vasquez JE, ES Walker, BW Franzus, BK Overbay, DR Reagan, FA Sarubbi.The epidemiology of mupirocin resistance among methicillin-resistant Staphylococcus aureus at a Veterans' Affairs hospital. *Infection control & Hospital Epidemiology* 2000;21(7):459-464.
16. Madhumati B, Vijaya Rajendran and K. Ashwin. Prevalence of Mupirocin Resistance in Methicillin Resistant *Staphylococcusaureus*Strains isolated from a Tertiary Care Hospital. *International Journal of Current Microbiology and Applied Sciences* 2018; 7(5): 329-336.
17. Adhikari RP, Shrestha S, Barakoti A, Amatya R. Inducible clindamycin and methicillin resistant *Staphylococcus aureus* in a tertiary care hospital. *BMC Infectious Diseases* 2017;17:483.
18. Ghosh S, Banerjee M. Methicillin resistance & inducible clindamycin resistance in Staphylococcus aureus. *Indian Journal of Medical Research* 2016;143(3):362-364.
19. McDougal LK, Fosheim G, Patel JB. Emergence of resistance amongUSA300 methicillin-resistant Staphylococcus aureus isolates causing invasive disease in the United States. *Antimicrobial agents and chemotherapy* 2010 Sep;54(9):3804-3811.
20. Malaviolle X, Nonhoff C, Denis O, Rottiers S, Struelens MJ. Evaluation of disc diffusion methods and Vitek 2 automated system for testing susceptibility to mupirocin in *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 2008;62(5):1018-1023.
21. 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):372-375.
22. Christensen OB, Anehus S. Hydrogen peroxide cream: An alternative to topical antibiotics in the treatment of impetigo contagiosa. *Acta Dermato Venereologica* 1994;74(6):460-462.
23. Simor AE, Phillips E, McGeer A, Konvalinka A, Loeb M, Devlin HR, et al. Randomised controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Journal of Clinical Infectious Diseases* 2007;44(2):178-185.

Address for correspondence:
Dr Yogendra Shelake
Associate Professor of Microbiology,
B.K.L. Walawalkar Rural Medical College and Hospital, Sawarde, Dist- Ratnagiri- 415606, Maharashtra, India.
Mobile no: +91 9284938672
Email: dr.yogendra007@gmail.com

How to cite this article:
Sumedh Sudhakar Lokapure, Chetana Joshi, Prasanna Nakate and Yogendra Shelke. Need of Testing Mupirocin Susceptibility Pattern of *Staphylococcus aureus* from Clinical Isolates in a Tertiary Care Teaching Institute. Walawalkar International Medical Journal 2024; 11(1):37-42. http://www.wimjournal.com.

Received date:18/06/2024 Revised date:25/07/2024 Accepted date: 27/07/2024