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Comparison of Disk Diffusion and Agar Dilution Method for the Detection of Mupirocin Resistance in Staphylococcal Isolates from Skin and Soft Tissue Infections

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Abstract

Aims and Objectives Mupirocin is a widely used topical antibiotic for the treatment of skin and soft tissue infections. This has resulted in resistance leading to treatment failure. Hence, the present study aimed to determine the prevalence of mupirocin resistance among staphylococcal isolates obtained from the skin and soft tissue infections. Also, comparison of disc diffusion and agar dilution method in detecting mupirocin resistance was done.

Materials and Methods This cross-sectional study was conducted in the Department of Microbiology of a tertiary health care center in Karnataka from January to December, 2018. Clinical samples such as wound swabs, tissues, and pus were included in the study. All staphylococcal isolates were screened for mupirocin resistance using 5 µg and 200 µg discs for low-level (MuL) and high-level mupirocin resistance (MuH), respectively. Minimum inhibitory concentration (MIC) was determined using the agar dilution method.

Results Out of 100 staphylococcal isolates, 68 were *Staphylococcus aureus* and 32 were CoNS. MuH was detected in 11 isolates. MuH was more common in CoNS (10/11) compared with *S. aureus* (1/11). MuL was not found in the study.

Discussion In our study, 10 out of 11 mupirocin-resistant isolates were methicillin resistant, which is statistically significant ($p < 0.05$). The correlation between results of disc diffusion and MIC were appropriate in this study.

Conclusion Judicial prescription of mupirocin after knowing the susceptibility report should become the standard practice. Screening for mupirocin resistance can be done by disc diffusion in resource-limited settings.

Keywords
- mupirocin
- low-level resistance
- high-level resistance
- disc diffusion
- agar dilution

Introduction

Mupirocin is a widely used topical antibiotic for the treatment of skin and soft tissue infections. It was first introduced in the United Kingdom (1985) and the use of mupirocin ointment has been progressively increasing worldwide.[1] Mupirocin (Pseudomonic acid A) derived from *Pseudomonas fluorescens* is effective against staphylococci, streptococci,

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certain gram-negative bacteria such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*.[2] In addition, nasal formulations are used in eradicating the nasal carriage of methicillin-resistant *S. aureus* (MRSA) in patients and health care persons.[3] Wide usage of mupirocin has resulted in resistance leading to treatment failure.

Mupirocin susceptibility is categorized into three types:

i. Mupirocin susceptible with minimum inhibitory concentration (MIC) of ≤ 4 µg/mL.
ii. Low-level mupirocin resistance (MuL) with MICs from 8 to 256 µg/mL and
iii. High-level mupirocin resistance (MuH) with MICs ≥ 512 µg/mL.

The resistance can be detected by the Kirby–Bauer disc diffusion testing using 5 µg and 200 µg discs. However, the dilution method is considered the gold standard for determination of mupirocin resistance levels.[4] Hence, the present study was done to compare disk diffusion and agar dilution methods for the detection of mupirocin resistance in staphylococcal isolates from the skin and soft tissue infections in a rural tertiary health care center.

Materials and Methods

Source of Data

The present cross-sectional study was conducted at the Department of Microbiology of a 1,000-bedded South Indian rural tertiary health care center from January to December, 2018. The institutional ethics committee clearance was obtained to conduct the study [IEC reference number-AIMS-SINGLE WORD/IEC/2688/2018–19].

Collection of Bacterial Isolates

A total of 100 staphylococcal isolates obtained from clinical samples such as pus, tissue, and wound swabs were included in the study. The isolates were identified as *S. aureus* and coagulase-negative staphylococci [CoNS] by standard laboratory techniques.[5] The pathogenic role of CoNS was established by repeated isolation of same species on two different occasions.

Antibiotic Susceptibility Testing

The antibiotic susceptibility testing was done as per the Clinical and Laboratory Standards Institute [CLSI M100-S28 document, 2018].[6] The test was done on the Mueller Hinton agar with the following discs obtained from HiMedia, Mumbai: penicillin (10 units). cotrimoxazole (1.27/23.75 µg), gentamycin (10 µg), ciprofloxacin (5 µg), and tetracycline (30 µg). Inducible and constitutive clindamycin resistance was determined by placing erythromycin (15 µg) and clindamycin (2 µg) discs 15 mm apart. Methicillin resistance was detected using a cefoxitin disc (30 µg) along with routine sensitivity testing. Quality control was achieved using *S. aureus* (ATCC 25923).

Screening for Mupirocin Resistance by Disc Diffusion Method

Mupirocin discs (5 µg and 200 µg) were purchased from HiMedia laboratories Pvt. Ltd., (Mumbai, India). Both the discs were included in the routine sensitivity testing and plates were incubated for 24 hours at 35°C $\pm$ 2°C. The zone diameters were carefully examined with transmitted light for any growth within the zone of inhibition. Isolates with no zone of inhibition were interpreted as mupirocin resistant. Isolate resistant to 5 µg disc and any zone for 200 µg disc was considered MuL. Isolates resistant for both the discs were considered MuH[7] [►**Fig. 1**].

Detection of MIC by Agar Dilution Method

MIC was detected by CLSI-recommended agar dilution method using the Mueller Hinton agar with mupirocin concentrations ranging from 0.016 to 1024 µg/mL.[8] Staphylococci with MIC of ≤ 4 µg/mL were considered mupirocin sensitive,

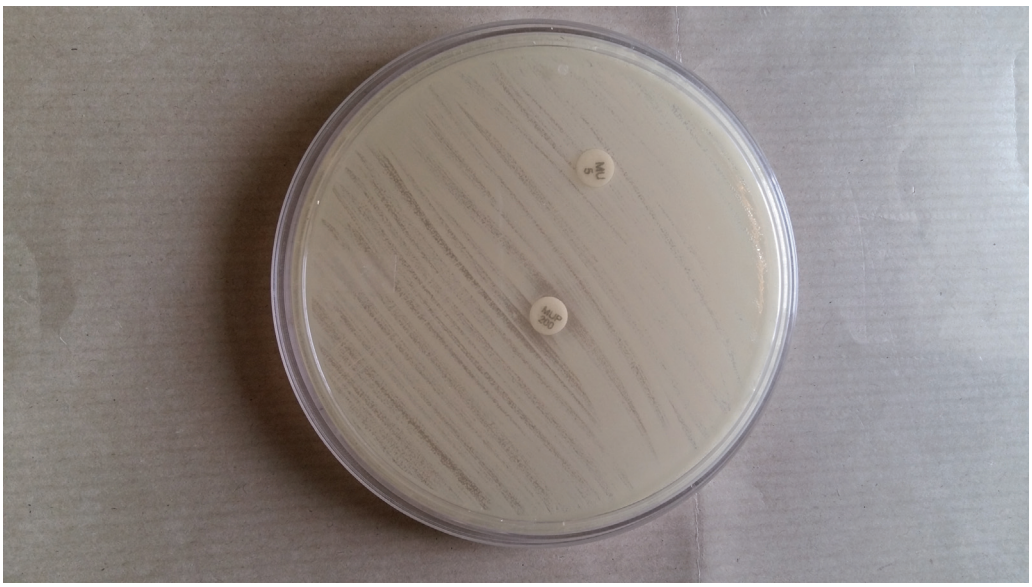

Fig. 1 Disc diffusion test showing high-level mupirocin resistance.

Fig. 2 Agar dilution method for detection of mupirocin MIC.

those with 8 to 256 µg/mL were considered MuL and isolates with $\geq$ 512 µg/mL were considered MuH [►**Fig. 2**]. Quality control was achieved by *S. aureus* ATCC 25923.

Statistical Analysis

Statistical analysis was done using Microsoft Excel. The data analysis involved transcription, preliminary data inspection, content analysis and interpretation. Percentages were used in this study to analyze variables. Chi-square test was done to determine the statistical significance. A p-value < 0.05 was considered statistically significant.

Results

Out of 100 staphylococcal isolates, 68 were *S. aureus* and 32 were CoNS. Among 68 *S. aureus* isolates, 41 were methicillin-resistant (MR) and 27 were methicillin-sensitive (MS). Out of 32 CoNS isolated, 29 were MRCoNS and 3 were MSCoNS. Inducible clindamycin resistance was detected in six isolates. The majority of staphylococcal isolates were sensitive to tetracycline (83%), followed by 74% sensitivity to gentamycin and 62% sensitivity to clindamycin. Minimal sensitivity was seen to penicillin (8%), followed by ciprofloxacin (19%), erythromycin (41%), and cotrimoxazole (48%). ►**Table 1** shows the distribution of mupirocin resistance among *S. aureus* and CoNS with methicillin resistance.

A total of 100 staphylococcal isolates were subjected to disc diffusion and agar dilution method for detecting mupirocin resistance. Eleven isolates showed MuH by both disc diffusion and agar dilution methods. Among 11 MuH, 7 (63.64%) were male patients and 4 (36.36%) were females; 7 samples from inpatient department (IPD) and 4 were out patient department (OPD) cases. ►**Table 2** shows the age-wise distribution of mupirocin resistant isolates. The majority of MuH isolates were seen in age group $>$ 60 years, i.e., 5 (45.45%) out of 11.

►**Table 3** shows a comparison between zone diameters in disc diffusion method and the MIC distribution of

Table 1 Distribution of mupirocin resistance among *S. aureus* and CoNS with methicillin resistance

Mupirocin	MSSA	MSCoNS	MRSA	MRCoNS	Total
Sensitive	27	02	40	20	89
MuL	00	00	00	00	00
MuH	00	01	01	09	11
Total	27	03	41	29	100

Abbreviations: MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MSCoNS, methicillin-sensitive CoNS; MRCoNS, methicillin-resistant CoNS.

Table 2 Age-wise distribution of mupirocin-resistant isolates

Age (y)	MuH
1–20	2
20–40	3
40–60	1
$>$ 60	5
Total	**11**

Table 3 Comparative evaluation of zone size in disc diffusion (5 µg and 200 µg mupirocin discs) and MIC levels by agar dilution

Number of isolates	Zone diameter (mm)	MIC (µg/mL)	Interpretation
9	25–30	0.5	Sensitive
23	25–20	1	Sensitive
37	20–15	2	Sensitive
20	15–07	4	Sensitive
4	<6	512	Resistant
7	No zone	>1024	Resistant

Table 4 Comparison of mupirocin resistance in staphylococci isolated from the skin and soft tissue infections in various studies from India

Author (y)	Mupirocin resistance
Gadepalli et al, 2005 in New Delhi[11]	6%
Jayakumar et al, 2012 in Kancheepuram Dist[14]	3.3%
Rajkumari et al, 2012 in New Delhi[13]	0%
Rudresh et al, 2014 in Bengaluru[9]	25.18%
Sanju et al, 2018 in Tamil Nadu[12]	19.6%
Present study 2018 in Karnataka	11%

staphylococcal isolates. The MIC of MuH isolates ranged from 512 µg/mL to ≥1024 µg/mL. the MIC of other isolates ranged from 0.5 µg/mL to 4 µg/mL. The zone diameters matched with the MIC values and did not affect the final interpretation of results.

Discussion

In recent days, there is a worldwide increase in mupirocin resistance among staphylococcal isolates.[9] The genetic basis of mupirocin resistance is defined. Most isolates with high-level mupirocin resistance have acquired plasmid-mediated mup A, which encodes a novel isoleucyl RNA synthetase. Isolates with low-level mupirocin resistance usually have acquired base changes (point mutation) in the native isoleucyl RNA synthetase gene *IleS*.[10]

The present study showed 11% isolates as high-level mupirocin resistant. Out of 11 MuH, 10 were methicillin resistant, which is statistically significant ($p < 0.05$). These findings were similar to that of other studies.[10,11] These results suggest that mupirocin resistance may be linked to the spread of MRSA clones rather than the topical use of mupirocin. This highlights the importance of methods to control the spread of hospital clones.

In the present study, mupirocin resistance was more common in CoNS (10/11) compared with *S. aureus* (1/11). The presence of comparatively higher rates of mupirocin resistance in CoNS is a cause of concern. Because CoNS may be the reservoir of mupirocin-resistant genes, it can be transferred from them to MRSA during mupirocin prophylaxis.[12]

The prevalence of mupirocin resistance is reported from many parts of the world viz, USA-13.2%, China-6.6%, Spain-11.3%, Turkey-45%, and Korea-5%.[13] We report an increase in the frequency of high-level mupirocin resistance in staphylococci isolated from the skin and soft tissue infections, compared with other similar studies done in India.[11,13,14] ►**Table 4** shows the comparison of mupirocin resistance in staphylococci isolated from the skin and soft tissue infections in various studies from India.

The results of disc diffusion test for detection of mupirocin resistance correlated very well with those of the agar dilution method. The same findings were obtained in other studies also.[9,12–15] A few studies suggest that disc diffusion may miss low-level mupirocin resistance and MIC has to be determined.[12,13] Our study showed only high level resistance and we did not encounter such situation. Moreover, disc diffusion method is convenient and cost-effective compared with the agar dilution method.

Conclusion

The results of the study indicate that the rate of mupirocin resistance has been increasing constantly. So, continued surveillance for mupirocin resistance is important in the treatment of skin and soft tissue infections. Screening for mupirocin resistance can be done by disc diffusion, which is easier to perform and cost effective in resource-limited settings.

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Conflict of Interest
None declared.

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