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The emergence of Mupirocin resistance among the clinical isolates of Staphylococci in a rural tertiary health-care centre of South India

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Abstract:

INTRODUCTION: Mupirocin is mainly used for decolonisation of Methicillin-resistant *Staphylococcus aureus* among patients and health-care workers. However resistance originates from coagulase negative staphylococci (CoNS) and spreads to *S. aureus* by horizontal gene transfer. Hence, the present study was done to determine the overall prevalence of high- and low-level Mupirocin resistance among *S. aureus* and CoNS and correlate with the occurrence of Methicillin and inducible Clindamycin resistance.

MATERIALS AND METHODS: A total of 100 consecutive, non-repetitive, clinical isolates of Staphylococci were obtained from various samples in a rural tertiary care hospital of Karnataka between August and September 2016. Antibiotic susceptibility testing was done according to the Clinical and Laboratory Standards Institute (M100-S25, 2015) guidelines. Low- and high-level Mupirocin resistance was screened using 5 μg and 200 μg discs, respectively.

RESULTS: Of 100 staphylococcal isolates, high-level Mupirocin resistance (Mup^{RH}) was detected in 13 isolates and low-level Mupirocin resistance (Mup^{RL}) was found in 4 isolates. Of 13 HLMR isolates, majority were CoNS (12 out of 13, 92.31%). Even in LLMR isolates, 75% (3 out of 4) were CoNS. All the HLMR and LLMR isolates were Methicillin resistant ($P < 0.05$).

CONCLUSION: High prevalence of Mupirocin resistance was found in the present study. Hence, screening for Mupirocin resistance should be routinely done and the drug must be used judiciously.

Keywords:

High-level Mupirocin resistance, inducible Clindamycin resistance, low-level Mupirocin resistance, Methicillin resistance, Staphylococci

Introduction

Mupirocin (pseudomonic acid A) is one of the structurally related antibiotics of pseudomonic acids A, B, C and D. It is an analogue of amino acid isoleucine and it is derived from *Pseudomonas fluorescens*. The mechanism of its action is by inhibition of protein synthesis by competing with isoleucine-transfer RNA (tRNA) synthetase (IleS). Thus, preventing the formation of isoleucyl tRNA halts the incorporation of isoleucine into the nascent polypeptide chain. As Mupirocin is preferentially active against Gram-positive organisms, it is widely used as a topical antibiotic to treat *Staphylococcus aureus* infection and for decolonisation.[1] Irrational usage and over-the-counter availability has led to the development of resistance to this drug.

Mupirocin susceptibility is categorised into three types:

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- Mupirocin susceptible with minimum inhibitory concentration (MIC) of <4 μg/ml [Mup^S]
- Low-level Mupirocin resistance (Mup^{RL}) with MIC of >8–256 μg/ml
- High-level Mupirocin resistance (Mup^{RH}) with MIC of >512 μg/ml.

The resistance can be detected by Kirby–Bauer disc diffusion method, but dilution method is considered the gold standard for the determination of Mupirocin resistance levels.[2]

The high-level Mupirocin resistance is associated with Methicillin-resistant *S. aureus* (MRSA) decolonisation failure. In low-level Mupirocin resistance, eradication occurs initially but recolonisation is frequent.[2] 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.

Plasmids carrying mup A were detected in all major circulating MRSA clones, suggesting inter-clonal transfer of these plasmids. Intra-species transfer of mup A between *Staphylococcus epidermidis* and *S. aureus* occurs during Mupirocin prophylaxis. Hence, Mupirocin resistance in coagulase-negative Staphylococci (CoNS) may serve as a source for resistance in *S. aureus*.[3] Studies regarding the overall prevalence of Mupirocin resistance among staphylococcal isolates are few globally. Hence, the present study was conducted to determine the overall prevalence of Mupirocin resistance among the clinical isolates of *S. aureus* and CoNS. And also, correlation of Mupirocin resistance with Methicillin and inducible Clindamycin resistance was evaluated.

Materials and Methods

Source of data

This prospective study was conducted at the Department of Microbiology, Adichunchanagiri Institute of Medical sciences, a 1000-bedded tertiary health-care centre, B. G. Nagara, Karnataka, for a period of 2 months in August and September 2016. The institutional ethical committee clearance was obtained to conduct the study (IEC reference number-AIMS/IEC/412/2016-17, dated 10/06/2016).

Collection of bacterial isolates

A total of 100 consecutive, non-repetitive, clinical isolates of *S. aureus* and CoNS obtained from various samples such as pus, urine and blood were included in the study. Of 100 patients, 64 were male and 36 were female. Majority of the isolates were from inpatients (65), and the rest were from outpatients (35). Of these 100 isolates, 8 were from neonates, 8 from infants, 14 from age group 2–20 years, 30 in age group 21–40 years, 25 from 41–60 years' age group and 15 were above 60 years. The isolates were identified as *S. aureus* and CoNS by the standard laboratory techniques.[4] 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 by Clinical and Laboratory Standards Institute (CLSI, M100-S25, 2015)[5] recommended Kirby-Bauer disc diffusion method. The test was done on Meuller Hinton agar with the following discs obtained from Himedia, Mumbai: Penicillin (10 units), Co-trimoxazole (1.25/23.75 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Amoxicillin-Clavulanic acid (20/10 μg), Tetracycline (30 μg), Vancomycin (30 μg), Teicoplanin (30 μg), and Linezolid (30 μg). Quality control was achieved using *S. aureus* (ATCC 25923). Inducible and Constitutive Clindamycin resistance (iMLSB and cMLSB) was determined by placing Erythromycin (15 μg) and Clindamycin (2 μg) discs 15 mm apart. Methicillin resistance was detected by Cefoxitin disc (30 μg) along with routine sensitivity testing.

Detection of Mupirocin resistance by disc diffusion

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 h at 35°C + 2°C. The zone diameters were carefully examined with transmitted light for any light 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 Mup^{RL}. Isolates resistant for both the discs were considered high-level Mupirocin resistant [Figures 1-3].[6]

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 analyse variables. Chi-square test was done to determine the statistical significance. $P < 0.05$ was considered statistically significant.

Results

A total of 100 consecutive, non-duplicate *Staphylococcus* isolates (41 *S. aureus* and 59 CoNS) were included in the study. Among the 100 *Staphylococcus* clinical isolates, majority were obtained from pus (46), followed by

blood (31), urine (15), high vaginal swab (3), body fluids (2), throat swab (3), sputum (1) and drain tip from abdominal mesh (1).

MRSA was found in 19 (46.34%) isolates. MRCoNS was detected in 52 (88.14%) isolates. Overall, the total Methicillin resistance was 71% among staphylococcal isolates.

Of 100 staphylococcal isolates, 26 showed constitutive Clindamycin resistance (cMLSB) and 7 isolates were inducible Clindamycin resistant (iMLSB).

High-level Mupirocin resistance (Mup^{RH}) was detected in 13 isolates and low-level Mupirocin resistance (Mup^{RL}) was seen in 4 isolates. Of the 13 Mup^{RH} isolates, 9 (69.23%) were from blood and rest 4 were from pus (1), urine (1), and high vaginal swab (1). Among these three were from Neonatal Intensive Care Unit (NICU), eight from inpatient department (IPD) and two from outpatient department (OPD).

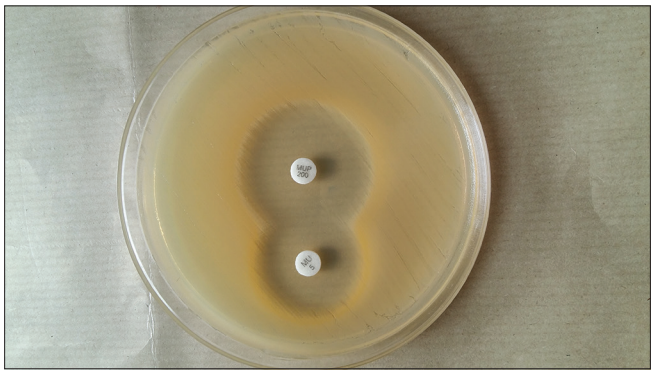

Figure 1: Mupirocin sensitivity

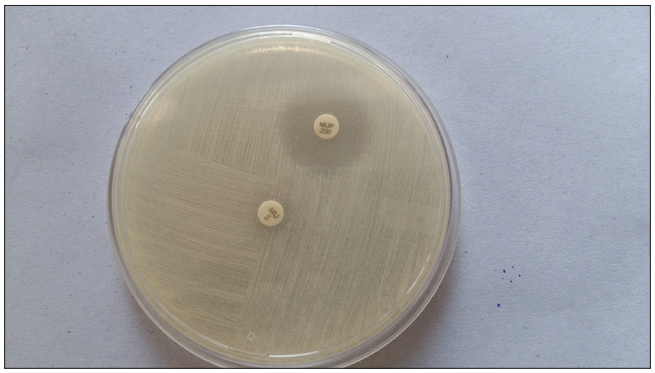

Figure 2: Low-level Mupirocin resistance

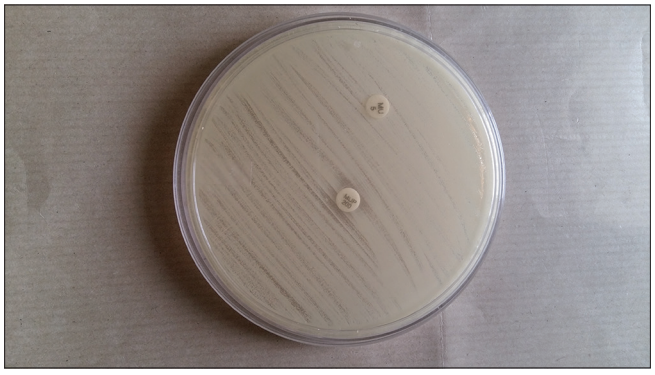

Figure 3: High-level Mupirocin resistance

Among the four Mup^{RL}, 2 (50%) from pus and 1 each (25% each) from blood and urine. Out of four Mup^{RL} isolates, two were from IPD and two from OPD.

Mup^{RH} was most commonly observed in age group <1 year (5, 38.46%) and 20–40 years age group (4, 30.77%). But, Mup^{RL} was seen in all age groups except in 1–20 years.

Table 1 shows the distribution of Mupirocin and Methicillin resistance among *S. aureus* and CoNS. All the 4 Mup^{RL} and 13 Mup^{RH} isolates were Methicillin resistant, which is statistically significant ($P < 0.05$).

None of the Mup^{R} isolates were associated with inducible Clindamycin resistance (iMLSB).

Table 2 shows the Comparison of antibiotic susceptibility with Mupirocin suceptibility. Out of 13 Mup^{RH}, 11 were multidrug resistant (>3 non-β-lactam antibiotics). All the 4 Mup^{RL} isolates were multidrug resistant.

Discussion

Mupirocin use can increase resistance through enhanced antibiotic pressure, more effectively selecting Mupirocin-resistant strains, and through facilitating cross-transmission. Emergence of Mupirocin resistance following the increased use has been reported extensively, though not consistently and previous exposure has been identified as a risk factor for Mupirocin resistance in MRSA.[7] Moreover, reducing Mupirocin use was associated with lower Mupirocin resistance levels over time.[2]

In the present study, the prevalence of MRSA and MRCoNS was 46.34% and 88.14%, respectively. However, in other studies,[8,9] conducted in South India

Table 1: Distribution of Mupirocin and Methicillin resistance among *Staphylococcus aureus* and coagulase-negative Staphylococci

Mupirocin	MSSA	MSCoNS	MRSA	MRCoNS	Total
Mup^{S}	22	7	17	37	83
Mup^{RL}	0	0	1	3	4
Mup^{RH}	0	0	1	12	13
Total	22	7	19	52	100

MRSA: Methicillin-resistant *Staphylococcus aureus*; MRCoNS: Methicillin-resistant coagulase-negative Staphylococci; MSCoNS: Methicillin-sensitive coagulase-negative Staphylococci; MSSA: Methicillin-sensitive *Staphylococcus aureus*; Mup^{RL}: Low-level Mupirocin resistance; Mup^{RH}: High-level Mupirocin resistance; Mup^{S}: Mupirocin susceptible

with similar study cohort, the prevalence rates were much lower. The prevalence of Methicillin resistance varies from place to place and also depends on local antibiotic policy, infection control strategies, duration of study, number of samples and biological characteristics of staphylococcal strains.

In this study, $Mup^{RH}$was seen in 13 isolates, whereas Mup^{RL} was seen in 4 isolates. This is higher than those observed in other studies.[8,10-13] But, Rudresh *et al*.[9] found a higher prevalence of Mup^{RL} (14.7%). This is attributed to over-the-counter availability of Mupirocin in community settings and more frequent usage in general population for skin infections than for eradicating carriage or treating MRSA outbreaks. This increased usage of Mupirocin has led to the development of resistance.[14]

We report an increase in the frequency of high-level Mupirocin resistance in Staphylococci compared to other studies done in India [Table 3].

Majority of the Mup^{RH} isolates (9 out of 13, i.e., 69.23%) were from blood which correlated with the report of Desroches *et al*.[15] Among the overall Mupirocin-resistant isolates, majority were from IPD and NICU (13/17) than OPD, which was similar to other reports.[8,9]

In our setting, Mupirocin resistance was more common in CoNS (15/17) compared to *S. aureus* (2/17). There are not many studies documenting Mupirocin resistance in CoNS, which may be the reservoir of Mupirocin-resistant genes. The presence of comparatively higher rates of Mupirocin resistance in CoNS is also a cause for concern. Studies suggest that mupA gene which is known to code for mupirocin resistance can be transferred from *S. epidermidis* to MRSA during Mupirocin prophylaxis, which could be an important threat to the future use of Mupirocin against MRSA.

In our hospital, both high-level and low-level Mupirocin-resistant isolates were Methicillin resistant, which is statistically significant ($P < 0.05$). These results suggest that Mupirocin resistance may be linked to a MRSA epidemic in the hospital.

Our study showed no correlation between the existence of inducible Clindamycin resistance and Mupirocin resistance, as none of the Mupirocin-resistant isolates showed inducible Clindamycin resistance. This was in contrast to the observations of Abimanyu *et al*.[10] and Rudresh *et al*.[9]

High-level Mupirocin-resistant *S. aureus* were more likely to be susceptible to Cotrimoxazole, Gentamycin and Tetracycline. They were more likely to be resistant to

Table 2: Comparison of antibiotic susceptibility with Mupirocin suceptibility

Antibiotic	*Staphylococcus aureus* (%)			CoNS (%)		
	Mup^{S} (*n*=39)	Mup^{RL} (*n*=1)	Mup^{RH} (*n*=1)	Mup^{S} (*n*=44)	Mup^{RL} (*n*=3)	Mup^{RH} (*n*=12)
Penicillin	4 (10.26)	0	0	3 (6.28)	0	2 (16.67)
Co-trimoxazole	32 (82.05)	1 (100)	0	24 (54.55)	1 (33.33)	6 (50)
Amoxicillin-Clavulanic acid	5 (12.82)	0	0	12 (27.27)	0	2 (16.67)
Gentamicin	35 (89.74)	1 (100)	1 (100)	41 (93.18)	3 (100)	5 (41.67)
Ciprofloxacin	11 (28.21)	1 (100)	0	22 (50)	0	2 (16.71)
Tetracycline	37 (94.81)	1 (100)	0	34 (77.27)	3 (100)	11 (91.67)
Vancomycin	39 (100)	1 (100)	1 (100)	44 (100)	3 (100)	12 (100)
Teicoplanin	39 (100)	1 (100)	1 (100)	44 (100)	3 (100)	12 (100)
Linezolid	39 (100)	1 (100)	1 (100)	44 (100)	3 (100)	12 (100)
Erythromycin	27 (69.23)	1 (100)	0	22 (50)	1 (33.33)	3 (25)
Clindamycin	34 (87.18)	1 (100)	0	29 (65.91)	3 (100)	3 (25)

CoNS: Coagulase-negative Staphylococci; Mup^{RL}: Low-level Mupirocin resistance; Mup^{RH}: High-level Mupirocin resistance; Mup^{S}: Mupirocin susceptible

Table 3: Comparison of Mupirocin resistance from various studies from India

Author (year)	Mup^{RH} (%)				Mup^{RL} (%)			
	MSSA	MRSA	MSCoNS	MRCoNS	MSSA	MRSA	MSCoNS	MRCoNS
Gadepalli *et al*., (2007 in New Delhi)[11]	1.1	8.2	-	-	1.1	0.9	-	-
Oommen *et al*., (2010 in Calicut and Coimbatore)[8]	0	2.08	0	28.2	0	0	0	0
Abimanyu *et al*., (2012 in Chennai)[10]	-	32	-	-	-	-	-	-
Jayakumar *et al*., (2013 in Kancheepuram dist)[12]	1.5	2.17	0	7.1	1.5	0	0	7.1
Chaturvedi *et al*., (2014 in Barabanki, UP)[13]	-	9.8	-	-	-	8.5	-	-
Rudresh *et al*., (2015 in Bengaluru)[9]	9.2	4.5	5.5	55.5	17.1	18.2	11.1	0
Present study (2016 in B.G.Nagara)	0	5.26	0	23.08	0	5.26	0	5.77

MRSA: Methicillin-resistant *Staphylococcus aureus*; MRCoNS: Methicillin-resistant coagulase-negative Staphylococci; MSCoNS: Methicillin-sensitive coagulase-negative Staphylococci; MSSA: Methicillin-sensitive *Staphylococcus aureus*; Mup^{RL}: Low-level Mupirocin resistance; Mup^{RH}: High-level Mupirocin resistance

Penicillin and Amoxicillin-Clavulanic acid. All the isolates were sensitive to Vancomycin, Teicoplanin and Linezolid. Hence, the judicial use of these antibiotics is a must.

The Mup^{RH}CoNS showed decreased susceptibility to Penicillin, Amoxicillin-Clavulanic acid, Ciprofloxacin, Erythromycin and Clindamycin. The co-occurrence of Mupirocin resistance and resistance to other antibiotics may indicate the organism carrying plasmid with multiple resistance genes.

In the present study, a strong association of Mup^{RH} with resistance to >3 non-β-lactam antimicrobial classes (multidrug resistance) in Staphylococci was observed, which was in agreement with the findings of Cadilla *et al*.[16]

Our study defines the overall Mupirocin resistance rates among *Staphylococcus* species, while most of the previous studies have focused only on Mupirocin resistance in MRSA outbreaks. Since ours is a tertiary care hospital catering to the patients from different geographical areas, the information we report may also apply to other hospitals, where data are lacking.

The limitation of this study was relatively small sample size, since it is a short-term research project. Also, speciation of CoNS, MIC and gene detection for Mupirocin-resistant isolates were not done due to technical and financial constraints.

Conclusion

The increasing prevalence rates of Mupirocin resistance leads to loss of the major treatment option in MRSA carriers. Hence, Mupirocin must be used cautiously. Hence, a Mupirocin stewardship programme should be developed to monitor resistance in both *S. aureus* and CoNS, as CoNS is a huge reservoir of mupA gene capable of transferring resistance to other species of Staphylococci. Infection control and antibiotic policies have to be developed, audited and reviewed regularly. In addition, Mupirocin-resistant strains may be treated with other alternatives such as Chlorhexidine,[8] Neomycin, Fusidic acid and newer agents such as Retapamulin.

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Conflicts of interest

There are no conflicts of interest.

References

1. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. Clin Infect Dis 2009;49:935-41.
2. Hetem DJ, Bonten MJ. Clinical relevance of mupirocin resistance in *Staphylococcus aureus*. J Hosp Infect 2013;85:249-56.
3. Hurdle JG, O'Neill AJ, Ingham E, Fishwick C, Chopra I. Analysis of mupirocin resistance and fitness in *Staphylococcus aureus* by molecular genetic and structural modeling techniques. Antimicrob Agents Chemother 2004;48:4366-76.
4. Collee JG, Marr W. Culture of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone; 2006. p. 113-29.
5. Clinical and Laboratory Standards Institute. M100-S25. Performance Standards for Antimicrobial Susceptibility Testing: 24th Informational Supplement. Wayne, PA: CLSI; 2015.
6. Clinical and Laboratory Standards Institute. M100-S25. Screening Test for Detection of High-Level Mupirocin Resistance in *Staphylococcus aureus*: 24th Informational Supplement. Wayne, PA: CLSI; 2015.
7. Caffrey AR, Quilliam BJ, LaPlante KL. Risk factors associated with mupirocin resistance in meticillin-resistant *Staphylococcus aureus*. J Hosp Infect 2010;76:206-10.
8. Oommen SK, Appalaraju B, Jinsha K. Mupirocin resistance in clinical isolates of *Staphylococci* in a tertiary care centre in South India. Indian J Med Microbiol 2010;28:372-5.
9. Rudresh MS, Ravi GS, Motagi A, Alex AM, Sandhya P, Navaneeth BV, *et al*. Prevalence of mupirocin resistance among staphylococci, its clinical significance and relationship to clinical use. J Lab Physicians 2015;7:103-7.
10. Abimanyu N, Murugesan S, Krishnan P. Emergence of methicillin-resistant *Staphylococcus aureus* ST239 with High-level mupirocin and inducible clindamycin resistance in tertiary care center in Chennai, South India. J Clin.Microbiol 2012;50:3412-3.
11. Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das BK, Chaudhry R, *et al*. Mupirocin resistance in *Staphylococcus aureus* in an Indian hospital. Diagn Microbiol Infect Dis 2007;58:125-7.
12. Jayakumar S, Meerabai M, Banu SA, Mathew R, Kalyani M, Lal LY, *et al*. Prevalence of high and low level mupirocin resistance among staphylococcal isolates from skin infection in a tertiary care hospital. J Clin Diagn Res 2013;7:238-42.
13. 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;6:403-7.
14. Bathoorn E, Hetem DJ, Alphenaar J, Kusters JG, Bonten MJ. Emergence of high-level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin use. J Clin Microbiol 2012;50:2947-50.
15. Desroches M, Potier J, Laurent F, Bourrel AS, Doucet-Populaire F, Decousser JW, *et al*. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant *Staphylococcus aureus* (MRSA) in France: Emergence of a mupirocin-resistant MRSA clone harbouring mupA. J Antimicrob Chemother 2013;68:1714-7.
16. Cadilla A, David MZ, Daum RS, Boyle-Vavra S. Association of high-level mupirocin resistance and multidrug-resistant methicillin-resistant *Staphylococcus aureus* at an academic center in the Midwestern United States. J Clin Microbiol 2011;49:95-100.