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Mupirocin resistance patterns in staphylococcus isolates causing cutaneous and soft-tissue infections: An epidemiological study in a tertiary health-care facility, Dhule, Maharashtra, India

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ABSTRACT

Background: Skin and soft-tissue infections (SSI) caused by *Staphylococcus aureus* remain a significant concern in both community and hospital settings. Mupirocin resistance among these isolates poses challenges for infection management and control strategies. **Aims and Objectives:** The aim of this study was to determine the prevalence and patterns of mupirocin resistance among staphylococcus isolates responsible for cutaneous and soft-tissue infections in patients attending a tertiary health-care facility. Along with that, the study investigated into mupirocin resistance prevalence and identified risk factors. **Materials and Methods:** A prospective study was conducted at a medical college, including 256 non-consecutive staphylococcal isolates from SSI. Antibiotic susceptibility testing was performed using Clinical and Laboratory Standards Institute recommended methods. Mupirocin resistance was determined through disk diffusion testing using 5 µg and 200 µg Mupirocin disks for low-level and high-level resistance, respectively. **Results:** Among the samples, 16.4% were methicillin-resistant *S. aureus* (MRSA) and 9.37% were methicillin-resistant coagulase-negative staphylococci. Mupirocin high-level resistance was found in 16.6% of *S. aureus* isolates, and mupirocin low-level resistance in 19% of MRSA isolates. The prevalence of resistance was lower in inpatient departments compared to outpatient departments. Associations were observed between resistance and patient demographics, history of mupirocin use, surgical site infections, hospitalization history, and diabetes. **Conclusion:** Mupirocin resistance presents a multifaceted challenge in the context of both patient demographics and clinical settings. The prevalence of resistance was influenced by factors such as patient age, gender, and prior mupirocin usage.

Key words: *Staphylococcus aureus*; Skin and soft-tissue infections; Mupirocin resistance; Methicillin-resistant *Staphylococcus aureus*; Methicillin-resistant coagulase-negative staphylococci; Antibiotic susceptibility testing; Disk diffusion method

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INTRODUCTION

Staphylococcus aureus stands as a significant etiological agent for skin and soft-tissue infections (SSI), both in community and health-care settings, with a notable involvement in nosocomial infections.[1] Mupirocin, an antimicrobial compound derived from Pseudomonas fluorescens, is employed topically, either as a sole treatment or in conjunction with other antiseptics, to manage SSI and to eradicate methicillin-resistant *S. aureus* (MRSA)

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colonization in nasal passages.[2,3] Nevertheless, an escalating employment of mupirocin ointment for local application has contributed to an upsurge in resistance among health-care staff and patients, leading to a surge in mupirocin-resistant MRSA (MuRMRSA) strains, particularly those harboring plasmid-borne mupA (ileS2) genes, which not only endows high-level mupirocin (MuH) resistance but also associates with resistance to other antibiotics.[4-7]

The mechanism of action of mupirocin is predicated on its binding to the bacterial isoleucyl-tRNA synthetase enzyme, a product of the ileS gene, thus obstructing protein synthesis.[6] This resistance phenomenon manifests in two tiers: Low-level mupirocin (MuL) resistance, arising from alterations in the native ileS gene, and MuH resistance, facilitated by conjugative plasmids carrying mupA (ileS2) that can disseminate both clonally and horizontally. The latter has been implicated in cross-resistance with diverse antibiotics such as clindamycin, tetracycline, erythromycin, and levofloxacin.[7]

MuH resistance proves to be clinically challenging, as it hampers decolonization efforts, particularly in cases involving MRSA carriers.[7] Worldwide, mupirocin resistance prevalence among MRSA isolates is documented through studies conducted in various regions, including Ireland (2%), New Zealand (12.4%), the USA (24%), Trinidad and Tobago (44.1%), and India (ranging from 0% to 38.6%).[1,8] The agar dilution method, used for minimum inhibitory concentration determination, is the established benchmark for detecting mupirocin resistance. Nevertheless, the sensitivity and specificity of mupirocin disk diffusion susceptibility tests, using 5 μg and 200 μg mupirocin disks, respectively, show promise as a more economical and uncomplicated alternative method for discriminating between MuH and MuL strains.[9]

The prevalence of mupirocin resistance among staphylococcal isolates within the Dhule district is currently inadequately understood. To address this, a comprehensive study is proposed at JMF'S ACPM Medical College, Dhule, with the intention of gauging the extent of mupirocin resistance within staphylococcal isolates.

Aims and objectives

To determine the prevalence and patterns of mupirocin resistance among staphylococcus isolates responsible for cutaneous and soft-tissue infections in patients attending a tertiary health-care facility. And to find mupirocin resistance prevalence and identified risk factors.

MATERIALS AND METHODS

A prospective and observational investigation was undertaken within the Department of Microbiology at JMF'S ACPM Medical College, Dhule, spanning from October 2022 to April 2023, following the receipt of ethical committee clearance. The study encompassed a cohort of 256 consecutive staphylococcal species sourced from diverse SSI, comprising specimens such as pus, discharges, wound aspirates, and wound swabs, originating from both inpatient (IPD) and outpatient departments (OPD) settings.

The assessment of antibiotic susceptibility was executed using the Kirby-Bauer disc diffusion method, as endorsed by the Clinical and Laboratory Standards Institute (CLSI), performed on Mueller Hinton agar.[10] The isolation of MRSA strains was accomplished employing established microbiological protocols, utilizing a cefoxitin disk (30 μg) with the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar, following the guidelines stipulated by CLSI. Strains showcasing an inhibitory zone diameter of ≥22 mm were construed as sensitive, whereas those with a zone size of ≤21 mm were categorized as resistant. The procurement of discs for this purpose was sourced from HiMedia Laboratories.

Detection of resistance to mupirocin was effectuated through the utilization of the disk diffusion method, employing mupirocin disks with concentrations of 5 μg and 200 μg to delineate low-level and high-level resistance, correspondingly. Interpretative thresholds for susceptibility and resistance were defined based on zone diameter criteria, with diameters >14 mm and <13 mm signifying susceptibility and resistance, respectively. Isolates demonstrating resistance to both concentrations were classified as MuH resistant strains (MuH). Isolates manifesting resistance to the 5 μg concentration but retaining sensitivity to the 200 μg concentration were characterized as MuL strains.[11]

RESULTS

Sociodemographic profile

A total of 256 samples derived from patients afflicted with SSI attributed to staphylococcus were subjected to comprehensive scrutiny to discern the presence of mupirocin resistance (MuR) and its associated predisposing factors. Within this cohort, 148 individuals (57.8%) were male, while 108 (42.2%) were female. Notably, MuR was evident in seven male and 13 female patients. The age distribution demonstrated that the highest incidence of MuR, specifically nine instances, was observed within the 21–40 years age group. Among these samples, 13 emanated from OPD, while the remaining seven were procured from IPD. Within the 20 instances of Mur *Staphylococci* (MuRS) isolates, 12 were sourced

from rural populations and eight from urban settings. Moreover, seven instances of MuRS were derived from cases of surgical site infections.

Of the 20 individuals with MuRS, 12 reported a history of mupirocin utilization at the site of infection, with an additional six individuals acknowledging prior nasal application of mupirocin. It is worth noting that nine patients displaying mupirocin resistance were found to have a history of diabetes (Table 1 for details).

MuR among MRSA and total isolates

Among the entire sample set, *S. aureus* was cultured from 190 specimens, while coagulase-negative staphylococci (CoNS) were identified in 66 samples. Within the total of 256 samples, the prevalence of MRSA was determined to be 16.4%, representing 42 instances, while methicillin-resistant coagulase-negative staphylococci (MRCoNS) accounted for a prevalence of 9.37%, comprising 24 samples. Notably, the prevalence of these resistant strains was observed to be lower within the IPD when juxtaposed with the OPD, with percentages of 35% (7 isolates) and 65% (13 isolates) among the total MuR strains.

Further evaluation was conducted on a selection of 42 MRSA strains and 24 methicillin-resistant CoNS, assessing their susceptibility to mupirocin. Of the total 20 methicillin-resistant isolates, all exhibited mupirocin resistance as determined through the disk diffusion method. Among these, 15 isolates were MRSA and five were MRCoNS. MuH resistance was detected in seven MRSA strains and one MRCoNS strain, while MuL resistance was observed in eight MRSA strains and four MRCoNS strains (Table 2 for detailed findings).

DISCUSSION

Within the encompassing array of samples, *S. aureus* was isolated from 190 specimens, while CoNS were discerned in 66 instances. Among the aggregate 256 samples, the prevalence of MRSA stood at 16.4%, manifesting itself in 42 occurrences, whereas MRCoNS accounted for a prevalence of 9.37%, comprising 24 samples. Remarkably, the frequency of these recalcitrant strains appeared notably diminished within the confines of the inpatient department (IPD) in contrast to the OPD, demonstrating proportions of 35% (seven isolates) and 65% (13 isolates), respectively, among the total spectrum of mupirocin resistant (MuR) strains.

Subsequent meticulous assessment targeted 42 MRSA strains and 24 methicillin-resistant CoNS, scrutinizing their susceptibility to mupirocin. In this endeavor, the entirety of the 20 methicillin-resistant isolates evinced resistance to mupirocin as delineated by the disk diffusion methodology. This contingent encompassed 15 MRSA isolates alongside five MRCoNS isolates. Noteworthy is the revelation that seven MRSA strains and one MRCoNS strain displayed MuH resistance, while eight MRSA strains and four MRCoNS strains demonstrated MuL resistance. For a detailed summary of above mentioned results, please see Table 2.

Table 1: Sociodemographic profile of patients from whom mupirocin-resistant staphylococci were isolated

Socio-demographic and clinical profile of study participants	MuSSA (n=25)	MuRSA (n=15)	MuSCoNS (n-19)	MuR CoNS (n=5)	Total MUSS (n=44)	TotalMURS (n=20)
Age (years)	6	2	5	1	11	3
0–20	13	7	10	2	23	9
21–40	4	3	5	2	9	5
41–60	2	3	4	0	6	3
>61						
Gender						
Female	18	10	13	3	31	13
Male	7	5	6	2	13	7
Residence						
Rural	16	9	11	3	27	12
Urban	9	6	8	2	17	8
OPD	17	10	12	3	29	13
IPD	8	5	7	2	15	7
Surgical site infection	11	5	6	2	17	7
Infection other than surgeries	14	9	13	4	27	13
Prior hospitalization	13	10	9	0	22	10
Prior history of mupirocin use at infection site	18	10	13	2	31	12
Previous history of nasal application of mupirocin	6	4	2	2	8	6
Diabetes	4	8	3	1	7	9

OPD: Outpatient department, IPD: Inpatient department

Table 2: Sensitivity of mupirocin among methicillin-resistant staphylococcal isolates

Mupirocin	MRSA (n=42)	MSSA (n=148)	MRCoNS (n=24)	MSCoNS (n=42)	Total (n=256), n (%)
High-level resistance	7	0	1	0	8 (3.12)
Low-level resistance	8	0	4	0	12 (4.68)
Sensitive	27	148	19	42	236 (92.18)

MRSA: Methicillin-resistant *Staphylococcus aureus*, MRCoNS: Methicillin-resistant coagulase-negative staphylococci

Table 3: The comparison of mupirocin resistance among total staphylococcal isolates in different studies

Study (year)	Total staphylococcal isolates	Overall MupR, n (%)	MupRSA, n (%)	MupRCoNS, n (%)
Jayakumar *et al*. (2013)[19]	150	5 (3.3)	39 (2)	2 (1.33)
Sanju *et al*. (2015)[12]	100	28 (28)	7 (7)	21 (21)
Rudresh *et al*. (2015)[8]	143	36 (25.17)	25 (17.48)	11 (4.29)
Arularasu *et al*. (2016)[21]	100	7 (7)	7 (7)	-
Shivanna *et al*. (2018)[18]	100	17 (17)	2 (2)	15 (15)
Bhavana *et al*. (2019)[20]	187	9 (4.81)	9 (4.81)	-
Khan *et al*. (2020)[1]	221	16 (7.23)	16 (7.23)	-
Present study (2023)	256	22 (8.59)	17 (6.64)	5 (1.95)

CoNS: Coagulase-negative staphylococci

Table 4: The comparison of mupirocin resistance among methicillin-resistant staphylococcal isolates in different studies

Study (year)	MRSA, n (%)	MuH in SA, n (%)	MuL in SA, n (%)	MRCoNS, n (%)	MuH in CoNS, n (%)	MuL in CoNS, n (%)
Jayakumar *et al*. (2013)[19]	46	1 (2.17)	0	14	1 (7.14)	1 (7.14)
Rudresh *et al*. (2015)[8]	22	1 (4.54)	4 (18.18)	9	5 (55.55)	0
Sanju *et al*. (2015)[12]	35	7 (20)	4 (11.42)	40	19 (47.5)	7 (17.5)
Arularasu *et al*. (2016)[21]	21	2 (9.52)	0	-	-	-
Shivanna *et al*. (2018)[18]	19	1 (5.26)	1 (5.26)	52	12 (23.07)	3 (5.76)
Bhavana *et al*. (2019)[20]	70	4 (5.71)	0	-	-	-
Khan *et al*. (2020)[1]	113	4 (3.53)	12 (10.61)	-	-	-
Present study (2023)	42	7 (16.6)	8 (19)	24	1 (4.16)	4 (16.6)

SA: *Staphylococcus aureus*, MRSA: Methicillin-resistant SA, MuH: Mupirocin high-level resistance, MuL: Mupirocin low-level resistance, CoNS: Coagulase-negative staphylococci, MRCoNS: Methicillin-resistant CoNS

Our investigation revealed a MuH resistance occurrence of 16.6% within *S. aureus* isolates, a prevalence lower than that observed in the study conducted by Sanju et al.,[12] yet comparatively higher than other investigations.[1,8,13-21] Mupirocin low-level resistance (MuLRSA) was documented in eight MRSA instances, representing 19%, a finding congruent with the observations made by Rudresh et al.,[8] In the context of mupirocin resistance among CoNS, our study reported a solitary case of MuH, accounting for 4.16% of MRCoNS isolates, a lower incidence compared to other studies.[8,12,18,19] However, our MuLRCoNS prevalence of 16.6% approximated the findings of Sanju et al.,[12] The scarcity of data on Mur CoNS in India highlights the need for further research, given that resistance rates appear influenced by diverse factors, including the study population's characteristics, isolate origins, and geographical locales.[22]

Among the 20 Mur isolates, a notable 60%[23] were derived from individuals below the age of 40 and 65%[24] were from female patients. While scant literature examines the relationship between age, gender, and mupirocin resistance, our findings resonate with the observations made by Guthridge et al.,[25] This concurrence might arise from the heightened incidence of skin infections such as boils, carbuncles, and impetigo, leading to the topical use of mupirocin in both children and adults. However, a more dedicated study would be requisite to solidify this hypothesis. In addition, the relatively higher prevalence among female patients might stem from gender-associated asymmetries in child-rearing practices.[25]

To facilitate a more comprehensive comparison between patients in community and hospital environments, our study deliberately encompassed individuals from both outpatient (OPD) and inpatient (IPD) settings. Among the 20 Mur isolates, the majority — 13 in total — emanated from OPD, a pattern mirrored in the findings by Rudresh et al.,[8] and Bali et al.,[26] This trend may be attributed to the unrestricted accessibility of mupirocin

over-the-counter in community pharmacies, coupled with its widespread usage for skin infections, rather than its intended application for MRSA eradication and the management of outbreaks within health-care facilities.[27,28]

Given the predominantly rural patient demographic of our hospital, it is noteworthy that 60%[12] of Mur isolates originated from rural populations. Similarly, seven MuRS isolates were associated with surgical site infections, and intriguingly, 50% of the patients had a history of previous hospitalization. Studies have indicated the potential involvement of biofilm-forming MuRMRSA strains in hospital-acquired resistance.[29] Countermeasures such as rigorous infection control practices encompassing proper hand hygiene, effective handling of MRSA carriers, and targeted topical antibiotic application could mitigate resistance within hospital settings.

Our findings further unveiled that 60%[23] of the isolates were linked to prior mupirocin use, aligning with the observations reported by Bali et al.,[26] which underscore the substantial role of prior mupirocin usage as an independent predictor of mupirocin resistance in staphylococci.

Another notable discovery was that nine Mur isolates were obtained from patients diagnosed with diabetes. This finding aligns with earlier reports by Bali et al.,[26] possibly attributed to recurrent SSI and the consequent application of mupirocin ointment among diabetic individuals.

The emergence of mupirocin resistance within methicillin-resistant staphylococci raises concerns, as mupirocin is a key topical agent employed for MRSA elimination. This resistance could potentially perpetuate MRSA infections. While Fusidic acid serves as a topical and oral alternative, reports have noted the coexistence of MuH resistance and Fusidic acid resistance within the same isolates. Notably, some studies have showcased the successful use of a hydrogen peroxide cream as an alternative to mupirocin.[8] The scarcity of studies in Maharashtra investigating Mupirocin resistance alongside sociodemographic variables underscores the necessity for expanded case–control investigations with larger sample sizes to facilitate more comprehensive evaluations.

Limitations of the study

The scarcity of studies in Maharashtra investigating Mupirocin resistance alongside sociodemographic variables

underscores the necessity for expanded case–control investigations with larger sample sizes to facilitate more comprehensive evaluations.

CONCLUSION

Our study delved into the prevalence of mupirocin resistance among staphylococcal isolates from patients with SSI, shedding light on the intricate interplay of various factors that contribute to this phenomenon. The prevalence of MRSA and MRCoNS underscored the persistence of resistance concerns in both community and hospital settings. Notably, MuH and MuL resistance were identified, indicating the potential for significant challenges in decolonization efforts and therapeutic strategies.

Our findings unveiled intriguing patterns in terms of patient demographics, with a higher incidence of resistance in younger individuals and an overrepresentation of female patients. These trends, while requiring further exploration, could be attributed to various factors including the over-the-counter availability of mupirocin and gender-specific health-care practices. In addition, the prevalence of mupirocin resistance in rural populations, history of prior mupirocin usage, and its association with diabetes highlighted the multifaceted nature of this issue.

Moreover, the correlation between mupirocin resistance and methicillin resistance adds a layer of complexity to the challenge of managing staphylococcal infections. The emergence of resistance within health-care facilities emphasizes the crucial role of infection control practices in mitigating its spread.

In light of the growing concern, our study underscores the imperative for a comprehensive and systematic approach toward addressing mupirocin resistance. This encompasses promoting judicious use of antibiotics, raising awareness among health-care providers, implementing infection control measures, and exploring alternative therapeutic strategies. While this study provides valuable insights, further extensive investigations with larger sample sizes are warranted to gain a more holistic understanding of the factors driving mupirocin resistance and to formulate effective interventions to combat its escalation.

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KA- Definition of intellectual content, Literature survey, Prepared first draft of manuscript, implementation of the study protocol, data collection, data analysis, manuscript preparation, and submission of the article; **RW-** Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **BP-** Design of study, statistical analysis and interpretation, review manuscript, literature survey, and preparation of Figures; and **DS-** Coordination and manuscript revision.

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