

Prevalence of Mupirocin Resistance in *Staphylococci* Isolated from Skin and Soft Tissue Infections

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Abstract: Introduction: Mupirocin (pseudomonic acid A) is a topical antimicrobial agent with excellent antistaphylococcal activity. It inhibits protein synthesis by binding specifically to iso-leucyl-tRNA synthetase enzyme. It is used to treat superficial skin infections and to control spread of Methicillin Resistant *Staphylococcus aureus* (MRSA), which is now endemic in India. The increased pressure of MRSA infections among patients and its carriage in health care staff has led to the indiscriminate use of mupirocin which has resulted in emergence of its resistance. Mupirocin susceptibility is categorized into two types: low level mupirocin resistance with MIC of ≤ 4 $\mu\text{g/ml}$, and high level mupirocin resistance with MIC ≥ 200 $\mu\text{g/ml}$. Aim: The present study is aimed to determine prevalence of mupirocin resistance, the rates of high and low level mupirocin resistance and the antimicrobial susceptibility patterns of *Staphylococcus aureus* and Coagulase negative *Staphylococcus* (CONS) species isolated from skin and soft tissue infections. Materials and methods: Clinical isolates of *Staphylococcus aureus* and CONS, isolated from skin and soft tissue infections from patients of inpatient and outpatient departments were included in the study and identified as *Staphylococcus aureus* and CONS by standard laboratory techniques. The antibiotic susceptibility testing was done by CLSI guidelines. Methicillin resistance was detected by using cefoxitin discs (30 μg) along with routine sensitivity testing. Zone of inhibition $\geq 22\text{mm}$ was reported as Methicillin sensitive *Staphylococcus*, whereas $\leq 21\text{mm}$ was reported as Methicillin resistant *Staphylococcus*. Low and High level mupirocin resistance was screened by using 5 μg and 200 μg discs respectively. Results: Out of total 68 samples collected 49 (72.1%) were sensitive and 19 (27.9%) were resistant 5 μg mupirocin disc and 52 (76.5%) were sensitive and 16 (23.5%) were resistant to 200 μg mupirocin disc.

Keywords: Mupirocin, *Staphylococcus*

1. Introduction

Mupirocin (pseudomonic acid A) is a topical antimicrobial agent with excellent antistaphylococcal activity. Resistance to mupirocin is being reported worldwide. The increasing prevalence of mupirocin resistance among *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS) species could be a threat to the future use of mupirocin against methicillin resistant *Staphylococcal* species¹. *Staphylococcus aureus* continues to be a dangerous pathogen for both community-acquired as well as hospital-associated infections. *S. aureus* resistant to methicillin were reported soon after its introduction in October 1960. Methicillin resistant *S. aureus* (MRSA) is now endemic in India². Drug resistance seen in cases of *Staphylococcus aureus* infections is a great concern for the clinicians to prevent spread of infections. Methicillin an important drug of penicillin group was commonly used for these infections before the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains³. Coagulase-negative staphylococci (CoNS) are considered to be a part of the normal human flora. However in recent days, CoNS have emerged as a major cause of device associated infections, infections among immunocompromised and cancer patients. Multidrug-resistant phenotypes are more common among CoNS when compared to *S. aureus*. Many resistances have been proved to originate from CoNS and spread to *S. aureus* by horizontal gene transfer. Being the reservoir of conjugative plasmids CoNS might have transferred high-level mupirocin resistance to *S. aureus*. Mupirocin (pseudomonic acid A) an analogue of amino acid isoleucine derived from *Pseudomonas fluorescens*⁴ is a topical antibiotic used to treat superficial skin infections and to control spread of methicillin-resistant *Staphylococcus*

aureus (MRSA)⁵. It inhibits the protein synthesis by binding specifically to isoleucyl-tRNA synthetase enzyme. The increased pressure of MRSA infections among patients and its carriage in health care staff has led to indiscriminate use of mupirocin which has resulted in emergence of its resistance³. Mupirocin susceptibility is categorized into two types: Mupirocin susceptible with minimum inhibitory concentration (MIC) of ≤ 4 $\mu\text{g/ml}$ (Mup^S), and high level resistance (Mup^{RH}) with MIC of ≥ 512 $\mu\text{g/ml}$. The resistance can be detected in Kirby-Bauer disc diffusion testing by using 5 μg and 200 μg discs. The present study is aimed to determine the overall prevalence of high-and low-level mupirocin resistance and study the antimicrobial susceptibility pattern in *Staphylococci* isolated from skin and soft tissue infections.

Aims and objectives

- 1) To find the prevalence of mupirocin resistance in *S. aureus* and coagulase-negative *Staphylococcus* (CoNS) species isolated from skin and soft tissue infections.
- 2) To determine the rates of High level and Low level mupirocin resistance in *S. aureus* and CoNS.
- 3) To study the antimicrobial susceptibility patterns of *S. aureus* and CoNS from skin and soft tissue infections.

Selection of subjects

- **Study Population:** This study will include all clinical isolates of *S. aureus* and CoNS isolated from skin and soft tissue infection from patients of inpatient, and outpatient departments of tertiary care hospital.
- **Sample size:** 100 consecutive Clinical isolates of *S. aureus* and CoNS isolated from skin and soft tissue infection from patients of inpatient, and outpatient departments.

- **Study Place:** This study will be carried out in the Department of Microbiology, Tertiary Care hospital.
- **Study Period:** This study shall be carried out from May 2016 to May 2017.
- **Inclusion criteria:** Clinical isolates of *S. aureus* and CoNS isolated from skin and soft tissue infection from patients of inpatient, and outpatient departments of tertiary care hospital.
- **Exclusion criteria:** Patients who are not willing to be a part of this study.

Design

This is a hospital based cross sectional study.

2. Materials and Methods

Clinical isolates of *S. aureus* and CoNS isolated from skin and soft tissue infection from patients of inpatient, and outpatient departments between April 2016 and September 2016 will be included in the study. The isolates will be identified as *S. aureus* and CoNS by standard laboratory techniques⁶. The antibiotic susceptibility testing will be done by Clinical and Laboratory Standards Institute (CLSI). Quality control will be achieved by using *S. aureus* ATCC 25923. Inducible clindamycin resistance will be determined by D-test. This test will be performed during the antimicrobial susceptibility testing. Erythromycin disk will be placed in close proximity (20 mm) to clindamycin disk. After 16-18 hours of incubation the plates will be observed for flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) which will indicate inducible clindamycin resistance. These isolates will be reported as resistant to clindamycin. Hazy growth within the zone of inhibition around clindamycin will also be considered as clindamycin resistance, even if no D-zone is apparent. Methicillin resistance will be detected by using cefoxitin disc (30 µg) along with routine sensitivity testing. Zone of inhibition ≥ 22 mm will be considered as sensitive and reported as Methicillin sensitive *Staphylococcus*, whereas ≤ 21 will be considered as resistant and reported as a Methicillin resistant *Staphylococcus*. Low- and- high level mupirocin resistance will be screened by using 5 µg and 200 µg discs respectively and confirmed by agar dilution. The dilution method will be considered the gold standard for the determination of mupirocin-resistance levels⁴.

E-test will be performed by Kirby Bauer disc-diffusion method as per CLSI guidelines by using HiComb mupirocin strip. Lawn culture will be made on the surface of MHA medium. HiComb strip with mupirocin antibiotic ranges from 0.1-240 µg/ml will be applied perfectly by gently pressing using a sterile forceps. The plates will then be incubated aerobically at 35°C for 24 hours. After incubation plates will be examined for the minimum inhibitory concentration (MIC). Isolates with MICs > 512 µg/ml will be considered as MuH, and with < 4 µg/ml will be considered as mupirocin sensitive. Agar dilution method will be performed by doubling dilution of mupirocin incorporated in MHA plate. A suspension of turbidity equivalent to 0.5 MacFarland Standards will be prepared from overnight growth of *Staphylococcus aureus* on nutrient agar. The surface of each MHA plate will be inoculated with 1 µl of suspension and plate will be incubated at 37°C

aerobically for 24 hours. Plates will be examined for growth and compared with positive growth control plate without the antibiotic agent, more than one colony or light film of growth will be considered as mupirocin resistant. By this method, more than one bacteria can be tested per plate. MIC values will be same as used for E-test.

3. Results

Total 68 samples were collected out of which 37 were *Staphylococcus aureus* and 31 were CoNS. Out of the total, 16 (23.5%) showed both low and high level mupirocin resistance and 3 (0.04%) showed only low level mupirocin resistance. 19% of *Staphylococcus aureus* showed both low and high level resistance to mupirocin, 22% of CoNS showed both low and high level mupirocin resistance and 0.9% showed only low level mupirocin resistance.

4. Discussion

Topical preparations of mupirocin first 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*. Genetic basis of mupirocin resistance is defined. Low-level mupirocin resistance is due to point mutations in native isoleucyl-tRNA synthetase (IRS) gene (ileS). High-level resistance is due to a plasmid-mediated gene, *mupA* (ileS2), which encodes an additional modified IRS, which has less affinity for mupirocin. Recently, a novel *mupB* gene is also identified for high-level mupirocin resistance.^{7,8}

Mupirocin demonstrates superior efficacy with a significant duration of nasal clearance of MRSA in carriers. Resistance to mupirocin, especially high-level resistance, offers fewer topical treatment options. 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 encode 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.⁹

The present study showed that Mupirocin resistance was more frequent in CoNS compared to *Staphylococcus aureus*. Out of the total, 16 (23.5%) showed both low and high level mupirocin resistance and 3 (0.04%) showed only low level mupirocin resistance.

5. Conclusion

The presence of mupirocin resistance in *Staphylococcus* species is a cause for concern. It can be limited by regular surveillance and effective control in the prophylactic use of mupirocin. Judicial prescription of mupirocin after understanding the susceptibility report should be made a standard practice by clinicians. Prolonged or widespread use of mupirocin in hospital or community must be stopped.

Infection control and antibiotic policies have to be developed, audited, and reviewed regularly.

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