Mupirocin resistance in clinical isolates of methicillin-resistant Staphylococcus aureus from a tertiary care rural hospital

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ABSTRACT

Background and Aims: Mupirocin is a topical antibiotic that has been used extensively for treating methicillin-resistant Staphylococcus aureus (MRSA) associated infections. However, the prevalence of mupirocin-resistant MRSA has increased with the extensive and widespread use of this agent. The aim was to determine the rates of high-level and low-level mupirocin resistance in MRSA to study the antimicrobial resistance pattern and clindamycin resistance in mupirocin-resistant MRSA. Methods: A total of 267 non-duplicate clinical isolates of MRSA from various clinical specimens were tested for mupirocin resistance by the disk diffusion method using 5 and 200 μg mupirocin disks. MRSA isolates were tested for antibiotics by Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Erythromycin-resistant resistant isolates of MRSA were further studied for inducible clindamycin resistance by “D test” as per CLSI guidelines. Results: Of 267 MRSA isolates, high-level mupirocin resistance was observed in 5.99% and low-level resistance in 15.35%. Mupirocin-resistant MRSA isolates showed higher antibiotic resistance to fusidic acid (14.03% vs 7.14%), rifampicin (5.26% vs 2.38%), erythromycin (68.42% vs 58.57%), and clindamycin (52.63% vs 45.71%). No MRSA strains were found to be resistant to vancomycin and linezolid. Mupirocin-resistant MRSA isolates showed higher constitutive macrolide-lincosamide-streptogamin B (cMLSB; 51.28% vs 42.98%) and inducible macrolide-lincosamide-streptogamin B (iMLS B; 17.94% vs 13.15%) resistance, as compared to mupirocin-sensitive MRSA isolates. Conclusion: The emergence of mupirocin resistance could be limited by regular surveillance and effective infection control initiatives so to inform health care facilities to guide therapeutic and prophylactic use of mupirocin.

Key words: Antibiotic resistance, D test, methicillin-resistant Staphylococcus aureus, mupirocin resistance

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is emerging as the most significant pathogen because of the burden of serious diseases it causes and its multi-drug resistance nature.[1] MRSA infections range from those of the skin and surgical sites, to infections relating to catheters and prosthetic implants, to pneumonia.[2]

Mupirocin is a topical antibiotic used for treating MRSA-associated skin and soft-tissue infections, decreasing certain types of surgical site infections, and eliminating nasal colonization of MRSA among patients and medical staff.[3-5] As per the recommendations by the Infectious Disease Society of America (IDSA) Practice Guidelines for the Management of Skin and Soft-Tissue Infections,[6]

Mupirocin acts by inhibiting protein synthesis in bacteria by binding competitively to bacterial isoleucyl-tRNA
S. aureus wound infections and to eradicate nasal carriage of S. aureus including MRSA.\[8\] Within 2 years after its introduction, mupirocin resistance among MRSA isolates emerged in the UK\[9\] and since then in Ireland (2%),\[10\] New Zealand (12.4%),\[7\] the USA (24%),\[3\] and in Trinidad and Tobago (44.1%).\[11\]

Two mupirocin resistances are defined in staphylococci. Low-level resistance (minimum inhibitory concentrations [MICs], 8-256 \(\mu g/ml\)) is usually associated with point mutations in the chromosomally encoded \textit{ileS} gene, whereas high-level resistance (MICs, \(\geq 512 \mu g/ml\)) is generally due to a plasmid-mediated gene, \textit{mupA} (also referred to as \textit{ileS2}), which encodes an additional modified isoleucyl-tRNA synthetase.\[12\] The \textit{mupA} gene facilitates the dissemination of resistance mechanism and is of different sizes and restriction patterns.\[13,14\]

Detection and differentiation of both types has important clinical implications. The presence of high-level mupirocin resistance (MuH) excludes its clinical use; however, low-level mupirocin resistance (MuL) can be overcome by recommending higher than usual dosage.\[15\]

The risk of the emergence of resistance appears to be greater among MRSA than methicillin sensitive \textit{Staphylococcus aureus} (MSSA),\[16\] and is often associated with the widespread use of mupirocin.\[3\] Therefore, it is essential for clinical laboratories not only to discriminate between susceptible and resistant strains but also to determine the level of resistance.

Though mupirocin resistance is often associated with methicillin resistance, the true extent of mupirocin-resistant MRSA in our area is unknown. Thus, this study was carried out to with the aim to determine the rates of high-level and low-level mupirocin resistance in MRSA, to study the antimicrobial resistance pattern of mupirocin-resistant MRSA and mupirocin-sensitive MRSA, and to determine inducible resistance to clindamycin in mupirocin-resistant MRSA.

Detection of mupirocin resistance was done by the disk diffusion method using 5 and 200 \(\mu g\) mupirocin disks (Mast Limited, UK) to determine low- and high-level resistance, respectively. Criteria of zone diameter breakpoints for susceptible and resistant isolates were set at >14 and <13 mm, respectively.\[17\] Three different phenotypes are:

- A zone diameter of greater than or equal to 14 mm for both 5 and 200 \(\mu g\) disks was considered to be susceptible for mupirocin.
- Isolates that showed zone diameters less than 14 mm in the 5 \(\mu g\) disk but more than or equal to 14 mm in the 200 \(\mu g\) disk were considered to be MuL strains.
- All isolates with zone diameters less than 14 mm for both 5 and 200 \(\mu g\) were considered to be MuH strains [Figure 1].

Erythromycin-resistant isolates of MRSA were further studied for inducible clindamycin resistance by “D test” as per Clinical and Laboratory Standards Institute (CLSI) guidelines.\[18\]

MRSA isolates were tested for antibiotic susceptibility by modified Kirby-Bauer’s disk diffusion method on Mueller Hinton agar as per CLSI guidelines,\[19\] except for fusidic acid where the French Society of Microbiology recommendations were used. The following antibiotics were tested: Amikacin (30 \(\mu g\)), erythromycin (15 \(\mu g\)), clindamycin (2 \(\mu g\)), linezolid (30 \(\mu g\)), gentamicin (30 \(\mu g\)), fusidic acid (10 \(\mu g\)), trimethoprim-sulfamethoxazole (25 \(\mu g\)), azithromycin (30 \(\mu g\)), ciprofloxacin (30 \(\mu g\)), rifampicin (10 \(\mu g\)), tetracycline (10 \(\mu g\)), vancomycin (30 \(\mu g\)), mupirocin (5 \(\mu g\)), and mupirocin (200 \(\mu g\)). (Disks were procured from Hi-media Laboratories, Mumbai, India and Oxoid and Mast group, UK.) Statistical analysis was done by using standard normal test (z test) for analyzing the difference between mupirocin-resistant and mupirocin-sensitive MRSA. Confidence interval \(P < 0.001^* = 99.9\) and \(P < 0.05^* = 95\).

![Figure 1](image)

**Figure 1:** Scanned ethical committee letter. (a) Mupirocin sensitive; (b) low-level mupirocin resistance; (c) high-level mupirocin resistance

**METHODS**

A prospective study was carried out in the Department of Microbiology from August 2012 to March 2014. Approval was obtained from the Institutional Ethics Committee, MIMER Medical College IEC/204 local review board for carrying out the study. A total of 267 non-duplicate MRSA isolated from various clinical specimens like pus, blood, urine, central venous catheters tips, tracheal aspirates, and sputum were randomly selected. MRSA isolates were identified by standard microbiological techniques. All MRSA isolates were included and repeat isolates were excluded.
RESULTS

Of 267 MRSA isolates, high-level mupirocin resistance was observed in 5.99% and low-level resistance in 15.35%. 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%). Mupirocin-resistant MRSA isolates showed higher constitutive macrolide-lincosamide-streptogramin B (cMLSb; 51.28% vs 42.98%) and inducible macrolide-lincosamide-streptogramin B (iMLSb; 17.94% vs 13.15%) resistance, as compared to mupirocin-sensitive MRSA isolates. No MRSA strains were found to be resistant to vancomycin and linezolid [Tables 1 and 2].

DISCUSSION

Mupirocin (pseudomonic acid A) derived from Pseudomonas fluorescens is a topical antibiotic widely used for the treatment of MRSA-associated skin and soft-tissue infections.[3] Prolonged, widespread, or uncontrolled and multiple courses of mupirocin are all associated with the development of mupirocin resistance. Resistance to mupirocin among clinical isolates of MRSA has been reported worldwide.[3,7,10,11]

The prevalence of high-level mupirocin-resistant MRSA in our study was 5.99% and low-level resistance was 15.35%. This is comparable to reports in the literature of 1-13% for low-level and 24-14% for high-level resistance.[19] Similar were the findings of John et al., who found high-level resistance of 9.3% and low-level mupirocin resistance of 12% in MRSA.[19]

Nicholson et al., Orrett et al., and Vasquez et al. observed the higher prevalence of low-level and high-level resistance to mupirocin to the tune of 30% and 24%, 26.1% and 44.1%, and 58% and 42%, respectively.[5,11,20]

In a study which first documented the extent of mupirocin resistance in an Indian hospital, it was found that 1% of 200 S. aureus isolates (including 0.9% of MRSA and 1.1% of MSSA) showed low-level resistance and 5% showed high-level resistance (8.2% of MRSA and 1.1% of MSSA isolates).[21] Summiya et al. reported the overall frequency of low-level and high-level resistance to mupirocin as 1% and 0%, respectively. Similar were the findings of Oomen et al. –2% of MRSA and 28% of methicillin resistant coagulase negative Staphylococcus aureus (MRCoNS) were mupirocin resistant.[22,23] Various studies suggest that during mupirocin prophylaxis, transfer of mupA gene from normal commensal flora of skin as in Staphylococcus epidermidis to MRSA is responsible for the emergence of mupirocin resistance.[24]

There was some variation in the antibiotic susceptibility pattern of mupirocin-susceptible MRSA isolates compared with mupirocin-resistant MRSA isolates. Mupirocin-resistant MRSA isolates showed higher antibiotic resistance to fusidic acid (14.03% vs 7.14%), rifampicin (5.26% vs 2.38%), erythromycin (68.42% vs 58.57%), and clindamycin (52.63% vs 45.71%), compared with mupirocin-susceptible isolates. No statistically significant difference was observed between mupirocin-resistant and mupirocin-sensitive MRSA.

Whereas increased susceptibility to tetracycline (15.78% vs 36.19%), cotrimoxazole (19.29% vs 44.76%),

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**Table 1: Antimicrobial susceptibility pattern of mupirocin-resistant MRSA isolates and mupirocin-sensitive MRSA isolates**

| Antibiotic tested | Mupirocin resistant MRSA (%) (n = 57) | Mupirocin sensitive MRSA (%) (n = 210) | Z value | P value |
|-------------------|--------------------------------------|---------------------------------------|---------|---------|
| Ciprofloxacin     | 42 (73.68)                           | 179 (85.23)                           | 1.8     | NS      |
| Erythromycin      | 39 (68.42)                           | 123 (58.57)                           | 1.4     | NS      |
| Azithromycin      | 38 (66.66)                           | 178 (84.76)                           | 2.69    | *P<0.001* |
| Clindamycin       | 30 (52.63)                           | 96 (45.71)                            | 0.93    | NS      |
| Fusidic acid      | 8 (14.03)                            | 15 (7.14)                             | 1.39    | NS      |
| Amikacin          | 22 (38.59)                           | 76 (36.19)                            | 0.33    | NS      |
| Gentamicin        | 19 (33.33)                           | 102 (48.57)                           | 2.13    | *P<0.05* |
| Cotrimoxazole     | 11 (19.29)                           | 94 (44.76)                            | 4.07    | *P<0.001* |
| Tetracycline      | 9 (15.78)                            | 76 (36.19)                            | 3.48    | *P<0.001* |
| Rifampicin        | 3 (5.26)                             | 5 (2.38)                              | 0.91    | NS      |
| Linezolid         | 0                                   | 0                                     | 0       | NS      |
| Vancomycin        | 0                                   | 0                                     | 0       | NS      |

NS = Not significant and *significant, Z > 1.96, P < 0.05 and **Z > 2.46, P < 0.01 for difference between mupirocin-resistant and mupirocin-sensitive MRSA, Confidence interval P < 0.001 = 99.9% and P < 0.05 = 95. This table depicts the difference of resistance in mupirocin-resistant and mupirocin-sensitive MRSA isolates by using standard normal test (z test).

**Table 2: MLS* resistance in mupirocin-resistant and mupirocin-sensitive MRSA**

| Isolates            | Constitutive MLS* resistance (%) | Inducible MLS* resistance (%) | MS phenotype (%) | Erythromycin susceptible (114) | Total |
|---------------------|----------------------------------|-------------------------------|-----------------|--------------------------------|-------|
| Mupirocin-resistant | 20 (51.28)                       | 7 (17.94)                     | 12(30.76)       | 18                             | 57    |
| MRSA                |                                  |                               |                 |                                |       |
| Mupirocin-sensitive | 49 (42.98)                       | 15 (13.15)                    | 50(43.85)       | 96                             | 210   |
| MRSA                |                                  |                               |                 |                                |       |
| Total               | 69 (46)                          | 22 (14.37)                    | 62(40.52)       | 114                            | 267   |

NS = Not significant, No statistically significant difference in constitutive MLS* resistance and inducible MLS* resistance in mupirocin-resistant and mupirocin-sensitive MRSA. However, statistically, it was marginally higher (P < 0.1) in mupirocin-susceptible MRSA isolates as compared to mupirocin-resistant MRSA isolates in MS phenotypes.
gentamicin (33.33% vs 48.57%), and azithromycin (66.66% vs 84.76%) was observed among mupirocin-resistant isolates compared with mupirocin-susceptible isolates, statistically significant difference (P < 0.001) was observed between mupirocin-resistant and mupirocin-sensitive MRSA.

Nicholson et al. observed resistance of 12% to tetracycline, rifampicin, and cotrimoxazole in the mupirocin-resistant MRSA isolates, whereas in our study we found resistance to cotrimoxazole, gentamicin, tetracycline, and rifampicin to the tune of 19.29%, 33.33%, 15.78%, and 5.26%, respectively.\[20] O’Neill et al. reported that MRSA strains with mupirocin resistance were often more susceptible to other antimicrobial agents, such as tetracycline and trimethoprim-sulfamethoxazole. In contrast, mupirocin-resistant isolates were more likely to be resistant to fusidic acid. It is speculated by the author that the fisB determinant, which is responsible for fusidic acid resistance,\[25] is on the same plasmid as the mupA gene in isolates with high-level mupirocin resistance.

A study done on Canadian MRSA isolates also showed increased susceptibility to tetracycline (7% vs 23% resistance) in the mupirocin-resistant isolates compared with the mupirocin-susceptible isolates, as well as to cotrimoxazole (10% vs 40%) and ciprofloxacin (75% vs 90%).\[26] A study from Pakistan observed the proportion of the MRSA strains resistant to other antibiotics was as follows: Amikacin 20%, chloramphenicol 9%, gentamicin 78.5%, clindamycin 72%, erythromycin 84%, fusidic acid 15%, cefoxitin 100%, ofloxacin 83.5%, penicillin 100%, cotrimoxazole 56%, tetracycline 72%, vancomycin 0%, and tegicycline 0%.\[22]

In our study, there was no resistance to vancomycin and linezolid; similar was the observation reported by Simor.\[26] Antibiotic resistance pattern of the mupirocin-resistant MRSA isolates showed 78.94% and 68.42% being resistant to erythromycin and clindamycin, respectively. Similar were the findings of Nicholson who reported resistance of 88% and 63% to erythromycin and clindamycin, respectively.\[20]

In vitro, S. aureus isolates with constitutive resistance are resistant to erythromycin and clindamycin, while isolates with inducible resistance are resistant to erythromycin but appear susceptible to clindamycin. It has been demonstrated that clindamycin treatment in patients with MLSB may lead to cMLS (clindamycin-resistant MLSB) and therapeutic failure; this is due to modification of ribosomal target encoded by various erm (erythromycin ribosome methylase) genes. This mechanism can be constitutive (cMLS), always producing the RNA methylase, or inducible (MLS), that is producing methylase only in the presence of an inducer.\[27] The MLS resistance mechanism is not recognized by using standard susceptibility test methods. The best way to detect inducible clindamycin resistance is disk approximation test or D test. The D-test is an easy, sensitive, and reliable means for detection of MLSB strains in a clinical laboratory setting without specialized testing facilities.

Mupirocin-resistant MRSA isolates showed higher cMLS (51.28% vs 42.98%) and MLS (17.94% vs 13.15%) resistance as compared to mupirocin-sensitive MRSA isolates. Statistically no significant difference was noted in constitutive MLS resistance and inducible MLS resistance in mupirocin-resistant and mupirocin-sensitive MRSA. However, statistically it was marginally higher (P < 0.1) in mupirocin-susceptible MRSA isolates as compared to mupirocin-resistant MRSA isolates in MLS phenotypes.

The mupA gene is typically located on mobile genetic elements. These plasmids typically carry resistance determinants to other antimicrobial agents, including macrolides, gentamicin, tetracycline, and trimethoprim,\[28] suggesting that mupirole use could lead to increased drug resistance in S. aureus.

The “gold standard” method for detection of mupirocin resistance is MIC determination by the agar dilution method. In our study, we used the disk diffusion method for detection of low- and high-level mupirocin resistance. The sensitivity and specificity of this method have already been evaluated by Malaviolle et al., previously.\[29] The most accurate disk diffusion test results were obtained with the concomitant use of 5 µg mupirocin and 200 µg mupirocin disks.\[29] They found that the sensitivity and specificity of 5 µg mupirocin disk was 100% and 98.1%, respectively, whereas that of 200 µg mupirocin disk was 100% and 92.3%, respectively, to differentiate MuH from MuL. This makes the disk diffusion susceptibility test a cheaper and simple alternative method for its routine use.

Genotypic methods such as PCR can be used as the final confirmatory test for detection of mupirole-resistant MRSA isolates. The lack of a confirmatory test is a limitation of our study.

The existence of mupirocin resistance among MRSA isolates is a cause for concern since there are not many effective alternatives for mupirocin-resistant strains. Polysporin triple ointment has been used empirically, but no studies on its efficacy have been done. For systemic treatment of MRSA, fusidic acid has proven useful when combined with agents such as vancomycin, but not as monotherapy.\[29] Hydrogen peroxide cream also has been recommended as a topical alternative to mupirocin\[30] or perhaps the newer reptapamulin.
CONCLUSION

The prevalence of high-level mupirocin resistance (5.99%) and low-level resistance (15.35%) in MRSA is a cause for concern. Hence, it is recommended that routine testing of MRSA for mupirocin resistance be conducted even in facilities where mupirocin is not administered. This will facilitate the early detection of resistance and assist in the control and spread of mupirocin-resistant MRSA.

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