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# EMERGENCE OF MUPIROCIN RESISTANCE AMONG CLINICAL ISOLATES OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* — A CHALLENGING THREAT TO HOSPITAL CARE IN A RURAL MEDICAL COLLEGE OF EASTERN INDIA

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## ABSTRACT

Mupirocin is a topical antimicrobial agent utilized in treatment of methicillin resistant *Staphylococcus aureus*. Mupirocin resistance in *Staphylococcus aureus* is increasingly being reported in many parts of the world in the context of its widespread use. The objectives of this study were to determine the susceptibility of methicillin resistant *Staphylococcus aureus* clinical isolates to mupirocin to assess the prevalence of low and high level of mupirocin resistance among them, to detect their resistance to other antimicrobials and also to correlate their mupirocin susceptibility with resistance to other antimicrobials. Methicillin resistant *Staphylococcus aureus* isolates were tested by disc diffusion method using 5µg and 200µg mupirocin discs respectively to determine low and high level resistance to mupirocin. Susceptibility testing to other antimicrobials, including erythromycin, gentamicin, co-trimoxazole, ciprofloxacin, tetracycline, clindamycin, chloramphenicol, vancomycin and linezolid were also done. 54.12% *Staphylococcus aureus* isolates were found as methicillin resistant, of them 14.13% and 4.35% showed low and high level resistance to mupirocin respectively. No resistance were detected to vancomycin and linezolid. Mupirocin resistant isolates showed more susceptibility to clindamycin and tetracycline as compared to mupirocin sensitive methicillin resistant *Staphylococcus aureus* isolates.

**KEYWORDS:** **Staphylococcus aureus, Methicilline Resistance, Mupirocin**

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# INTRODUCTION

*Staphylococcus aureus* has become the single most frequently isolated bacterial pathogen in hospitals and continues to be the most common aetiological agent of nosocomial post- operative surgical wound infections.[1] In recent years it has also become an important community-acquired pathogen. Infections caused by this pathogen are often acute and pyogenic and range from skin and soft tissue infections (SSTI-s) to life threatening complications including pneumonia, foreign body infection, osteomyelitis, endocarditis and general sepsis.[2] The impact of *Staphylococcus aureus* (*S aureus*) infection on human health has dramatically increased as a result of the organism's remarkable ability to become resistant to antimicrobial agents. Soon after the introduction of methicillin in England in 1961, resistance of *S aureus* to this drug emerged in Europe and North America and then worldwide with prevalence increasing dramatically as 50% in USA, 40% in UK and 30-40% in France. Increased prevalence in India from 12% in 1992 to 81% in 1999 points to the seriousness of methicillin resistant *Staphylococcus aureus* (MRSA) infection in developing world.[1,2] Indiscriminate use of antibiotics, prolonged hospital stay, intravenous drug abuse, carriage of MRSA in nose, axilla, perineum are important risk factors for MRSA acquisition.[3] Nasal colonisation is a vital step in the pathogenesis of MRSA infection. In addition to self infection, colonised individuals are a potential MRSA reservoir for its spread. Hence, eradicating or suppressing MRSA colonisation has remained a cost effective strategy for prevention of infection as well as transmission.[4] The commonly used antibiotics for treatment of MRSA infection is vancomycin or linezolid, while mupirocin (derived from *Pseudomonas fluorescence*) is an effective topical antibiotic for the elimination of MRSA in carriers.[3] Mupirocin (pseudomonic acid A) is a topical antimicrobial agent with excellent antistaphylococcal and antistreptococcal activity.[4] It competitively inhibits bacterial isoleucyl tRNA synthatase, blocking the formation of isoleucyl tRNA, which in turn impairs bacterial protein synthesis.[5] It has already been recognised as the best and most effective topical antimicrobial agent for decolonisation. It is mainly used as an ointment (2% in paraffin base) and is very effective in eliminating MRSA strains from colonised nasal passages among patients and medical staff.[6] It has also been used to treat SSTI-s and to eradicate staphylococcal carriage in health care workers and patients. Intranasal mupirocin has also been used preoperatively to prevent surgical site infections.[7] Moreover topical application of mupirocin has also been proved to be effective in eradicating MRSA in cases of impetigo and burn wound infection.[4] But unfortunately, within two years after its first introduction in 1985 in UK, massive use of this agent has led to rapid emergence of resistance worldwide[6], most resistance being associated with extensive use and over prescribing of the drug.[2] Not only in hospital set up, but it has been reported among community associated MRSA strains also.[7] So the emergence of mupirocin resistance has led to cautions against its widespread and Institutional usages. Although no performance standards or interpretive criteria have been published for mupirocin susceptibility testing, mupirocin resistance in *Staphylococci* is commonly defined as low-level resistance (MuL, with MICs 8 to 256µg/ml) and high-level resistance (MuH, with MICs ≥512µg/ml). MuL results from spontaneous point mutations in the chromosomally encoded *ileS* gene, which is stable and non-transferrable, whereas, MuH is generally conferred by a novel gene, mupA (also referred to as *ileS2*), which encodes an additional modified isoleucyl tRNA synthatase found on an extra-chromosomal plasmid (plasmid mediated) and is transferrable.[7,8] There is also evidence that coagulase negative *Staphylococci* (CoNS) may also act as a reservoir for mupA, which may be transferred to *S aureus* in a clinical situation following mupirocin treatment; transfer of mupA from CoNS to *S aureus* has also been demonstrated in vitro.[9] So detection and differentiation of both types of resistance has important clinical implications. Although the agar dilution method considered to be the 'gold standard' for determination of mupirocin resistance levels and mupirocin E test with tetrazolium reduction is more accurate and closer to agar dilution, both these tests are too

expensive and laborious than disc diffusion method for routine application.[10] Moreover, in determining mupirocin resistance, with only 5µg mupirocin disc, one cannot differentiate between MuL and MuH strains. The concomitant use of both 5 and 200µg mupirocin discs can easily differentiate them.[3] As MuH strains are more likely to be associated with clinical and microbiological failure, the presence of MuH excludes its clinical use; however MuL can be overcome by recommending higher than usual dosages.[4] The present study was planned to assess the prevalence of both high level and low level mupirocin resistance among clinical isolates of MRSA in a rural Medical College of West Bengal, India through a cost effective and convenient method, easily adopted by any clinical microbiology laboratory, to detect their susceptibility to other antimicrobial agents as well as to correlate their mupirocin susceptibility pattern with resistance pattern of those antimicrobials. The results of this study will provide local data to guide the treatment of MRSA infections as well as to shape the infection control policies necessary for effective control of spreading infections in a hospital set up.

## MATERIALS AND METHODS

The study was conducted in the laboratory of Department of Microbiology, Burdwan Medical College from 1st July, 2012 to 31st December, 2012. It was a hospital based cross-sectional prospective study. The study was performed with prior permission of Institutional Ethical Committee.

### *Collection of clinical isolates and subsequent processing*

Clinical samples such as pus/abscess from SSTI-s, respiratory tract specimens, urine and blood, submitted to the laboratory for culture and sensitivity during the six months' period, were screened first for the growth of *Staphylococci*. A total of 170 non-duplicate clinical isolates of *Staphylococci* were identified as *S aureus* by routine conventional microbiological procedures.[11] Detection of methicillin resistance was carried out by using cefoxitin discs (30µg) as per recent guidelines of Clinical and Laboratory Standards Institute (CLSI-2012).[12] Only the MRSA isolates were included in the study.

### *Antimicrobial susceptibility testing to mupirocin and other drugs*

Susceptibility to a panel of 10 antimicrobial agents was performed by the Kirby-Bauer Disc Diffusion method as per CLSI guidelines against the following antimicrobials– erythromycin (15µg), gentamicin (10µg), co-trimoxazole (1.25/23.75µg), ciprofloxacin (5µg), tetracycline (30µg), clindamycin (2µg), chloramphenicol (30µg), vancomycin (30µg), linezolid (30µg) and mupirocin (5µg & 200µg) by discs.[12] All isolates were tested by the disc diffusion method using a 5µg mupirocin discs as a first step in determining mupirocin resistance. Zone diameter breakpoints for susceptible and resistant isolates were set at ≥14mm and ≤13mm respectively, as recommended by Finlay et al.[13] High level resistance was confirmed using a 200µg mupirocin disc. Isolates that showed zone diameters of less than 14mm in 5µg disc but more than or equal to 14mm in the 200µg disc, were confirmed to be MuL strains whereas all isolates with zone diameters less than 14mm for both 5 and 200µg discs were considered to be MuH strains.[10] *Staphylococcus aureus* ATCC 25923 was used as control strain throughout the testing procedures. The collected data was analysed and evaluated on the basis of average and percentage values. The results were presented in the form of tables. Statistical analysis was done by Pearson Chi-square test using SPSS 2010 version 19.

## RESULTS

Among the 170 non-duplicate *S aureus* isolates included in this study, 92 (54.12%) were MRSA. The distribution of these MRSA isolates among different samples was shown in Table 1. 61 (66.30%) strains were isolated from pus/abscess from SSTI-s, 18 (19.57%) from respiratory tract specimens like sputum, bronchial aspiration, bronchoalveolar lavage etc. Urine accounted for 9 (9.78%) isolates whereas 4 (4.35%) MRSA were obtained from blood culture.The overall prevalence of mupirocin resistance among the MRSA isolates was 18.48% (17/92), the frequency of MuL and MuH were 14.13% (13/92) and

4.35% (4/92) respectively. Among 13 MuL-MRSA isolates, 8 were obtained from pus/abscess, 3 from respiratory tract specimens and 2 from urine; no MuL isolates were obtained from blood whereas blood accounted for highest percentage i.e. 25% (1/4) of MuH isolates. Apart from this single isolate from blood, among the other three MuH-MRSAs, two were from pus/abscess and one from respiratory tract specimen respectively. (Table 1)

**Table 1**

***Mupirocin susceptibility pattern of MRSA isolates obtained from different clinical samples***

| Type of clinical samples (%) | No of MRSA Isolates (%) | No of Mupirocin sensitive MRSA Isolates (MuS) (%) | No of Mupirocin resistant MRSA isolates (MuR) | |
|---|---|---|---|---|
| | | | Low level resistant Isolates (MuL) (%) | High level resistant Isolates (MuH) (%) |
| Pus /Abscess from SSTI-s | 61 (66.30) | 51 (83.61) | 8 (13.11) | 2 (3.28) |
| Respiratory Tract specimens | 18 (19.57) | 14 (77.78) | 3 (16.67) | 1 (5.55) |
| Urine | 9 (9.78) | 7 (77.78) | 2 (22.22) | 0 (0) |
| Blood | 4 (4.35) | 3 (75) | 0 (0) | 1 (25) |
| Total | 92 (100) | 75 (81.52) | 13 (14.13) | 4 (4.35) |

***[Total no of S aureus isolates:170, no of MRSA isolates: 92 (54.12% of S. aureus isolates)]***

Table 2 showed the susceptibility pattern of MRSA isolates against other antimicrobials used in the study as well as correlation in resistance pattern of the mupirocin sensitive (MuS, n=75) and resistant (MuR, n=17) strains separately with resistance to those antimicrobials. Comparatively higher resistance values were found against erythromycin (82.61%), gentamicin (81.52%), co-trimoxazole (66.30%) and ciprofloxacin (56.52%); resistance to tetracycline was 43.48%. Lower percentages of resistance were documented against clindamycin (31.52%) and chloramphenicol (23.91%). Each isolate observed fully sensitive to vancomycin and linezolid. Susceptibility test results of MuS (n=75) and MuR (n=17) MRSA isolates, when compared, found that MuR isolates were more likely to be susceptible to clindamycin (23.52% vs. 33.33%), tetracycline (41.18% vs. 44%) and co-trimoxazole (64.71% vs. 66.67%) than the MuS ones. Almost similar pattern of resistance was found against erythromycin (82.35% & 82.67%), though the level of resistance was high. However MuR isolates were more likely to be resistant to gentamicin (88.23% vs. 80%) and ciprofloxacin (70.59% vs. 53.33%). Although relative resistance to chloramphenicol (29.41% vs.22.67%) was also higher but the overall value was within accepted limit. Resistance to vancomycin and linezolid, as previously stated, was not observed. Statistical analysis of resistance pattern of MuS and MuR MRSA isolates done by Pearson Chi-square test using SPSS 2010 version 19 revealed that it was not statistically significant ($p < 0.05$).

**Table 2**
***Antimicrobial susceptibility (resistance) pattern of MRSA isolates and the correlation of mupirocin susceptibility with resistance pattern of these antimicrobials***

| Antimicrobials (µg) | No of MRSA Isolates resistant to particular antimicrobial (n=92) (%) | No of mupirocin sensitive (MuS) MRSA resistant to Particular antimicrobial (n=75) (%) | No of mupirocin resistant (MuR) MRSA resistant to Particular antimicrobial (n=17) (%) |
|---|---|---|---|
| Erythromycin (15µg) | 76 (82.61) | 62 (82.67) | 14 (82.35) |
| Gentamicin (10µg) | 75 (81.52) | 60 (80.00) | 15 (88.23) |
| Co-trimoxazole (1.25/23.75µg) | 61 (66.30) | 50 (66.67) | 11 (64.71) |
| Ciprofloxacin (5µg) | 52 (56.52) | 40 (53.53) | 12 (70.59) |
| Tetracycline (30µg) | 40 (43.48) | 33 (44.00) | 7 (41.18) |
| Clindamycin (2µg) | 29 (31.52) | 25 (33.33) | 4 (23.52) |
| Chloramphenicol (30µg) | 22 (23.91) | 17 (22.67) | 5 (29.41) |
| Vancomycin (30µg) | 0 (0) | 0 (0) | 0 (0) |
| Linezolid (30µg) | 0 (0) | 0 (0) | 0 (0) |

***[Pearson chi-square test: Not-significant, P- value-1.902, df-12]***

# DISCUSSION

The need for antimicrobial susceptibility testing of *S aureus* isolates is becoming more urgent in day by day as because it is the most frequently isolated pathogen from nosocomial infections and due to an increased number of infections caused by multidrug resistant MRSA strains, chemotherapy has become difficult.[10] So the accurate diagnosis of MRSA in the laboratory is vital for patients' management. It is also essential for the meaningful interpretation of surveillance data.[14] Mupirocin is a topical antibiotic used particularly to eradicate methicillin resistant *Staph aureus* (MRSA) carriage and to prevent infection.[8] So clearance of *S aureus* nasal colonisation can reduce the subsequent risk of development of infection by MRSA in addition to reducing the spread of these micro-organisms. In the past few years, mupirocin resistance has been increasing among *Staphylococci* in many parts of the world. The risk of emergence of such resistance appears to be greater among MRSA than among MSSA strains and is often associated with the widespread use of mupirocin.[7] In our study, the prevalence MRSA in various clinical samples was 54.12% (92/170), which is of great concern in this era of increasing prevalence rate of multidrug resistant MRSA in health institutions worldwide. Moreover most of these MRSA isolates (66.30%) were recovered from pus/abscess from skin and soft tissue infection sites, which is less than the finding of Nizamuddin S et al where 78% of MRSA isolates were recovered from pus/abscess.[4] Respiratory tract specimens, in our study, accounted for 19.57% of MRSA followed by urine (9.78%) and blood (4.35%). The above study however showed 20% isolates from tracheal aspirates followed by blood (1.5%) and then urine (0.5%). Fitzroy A Orrett, in another study, reported that most (74%) of their MRSA isolates were recovered from infected surgical sites and burn wounds followed by blood (8%).[1] The prevalence of mupirocin resistance among MRSA during the study period was 18.48% (17/92) with overall frequency of low (MuL) and high (MuH) level resistance were14.13% (13/92) and 4.35% (4/92) respectively. These resistance rates vary from region to region and country to country. Gadepalli R et al from India, in 2007, reported MuL and MuH in 1% and 5% of *S aureus* strains respectively.[15] JW Banerjee John et al from South India reported less high level (9.3%) and more low level (12%) Mupirocin resistance.[6] Another study from South India by Oommen SK revealed MuL as

0% and MuH as 2% among the MRSA strains.[3] The first study from Pakistan, in this context, also stated 1% and 0% of MuL and MuH respectively.[4] One literature from Trinidad reported high percentage (44.1% & 26.1%) of them among the MRSA isolates.[1] The Jones JC et al study report from United States with a range of rates of resistance varying from 4.6% to 17.8% corresponded well with our study findings.[5] One recent study from Jamaica, West Indies by Nicholson AM et al revealed 7% of MRSA strains, among which 30% and 24% showing low and high level resistance to mupirocin respectively.[2] One Canadian study reported 8% MuL and 4% MuH among MRSA isolates which were slightly lower than our finding values.[7] Prolonged widespread or uncontrolled use and multiple courses of mupirocin are all associated with the development of mupirocin resistance. In New Zealand where mupirocin was available without prescription in the 1990s, the rate of MuH in *S aureus* was 14.2% in 1999 occurring mainly among community acquired isolates.[16] In present study 25% (1/4) of MuH-MRSA were recovered from blood which was of great concern in relation to blood stream infections caused by *Staphylococcus.* Exposure of CoNS on skin surfaces during prolonged or repeated topical application of mupirocin may lead to the development of a reservoir of high level resistance determinant in CoNS which may be transferred to *S aureus* in patients on mupirocin therapy.[16] Mupirocin in our institution is used mostly post-operatively to prevent infections and also in out-patients department in limited number and not as a routine decolonising agent. Moreover high cost and non availability in hospital pharmacy also accounted for its restricted use in a rural Medical College like ours. In spite of this, the prevalence of 18.48% of mupirocin resistance poses a challenging threat to hospital infection control measures to revise the strategies and to introduce regulated decolonisation of the hospital staffs, health care workers and infected patients colonised with MRSA strains, with mupirocin. Since alternatives to mupirocin for eradicating MRSA carriage is limited (fusidic acid may be an alternative), it is important to have the knowledge of prevalence of mupirocin resistance among MRSA as it will facilitate effective decolonisation. Therefore it is essential for clinical laboratories not only to discriminate between susceptible and resistant strains but also to determine the level of resistance.[4] Antimicrobial susceptibility pattern of all MRSA isolates detected a high level of resistance against erythromycin (82.61%) and gentamicin (81.52%); indicating its therapeutic failure against multidrug resistant MRSA infection. Such high percentage of resistance also reported in other literatures,[1,2,4] however resistance to gentamicin reported by Nicholson et al was 33%.[2] Resistance to ciprofloxacin and tetracycline were 56.52% and 43.48% respectively. Lower resistance rate were found against clindamycin (31.52%) and Chloramphenicol (23.91%). Here resistance to clindamycin was lower than other studies where it was found to be 52%, 69.1% and 72% respectively.[1,2,4] This was probably due to limited use of clindamycin in our institution. However the D test might be performed routinely to determine the existence of inducible resistance to clindamycin before its therapeutic use.[17] Chloramphenicol, though not routinely used in MRSA infection due to its bacteriostatic action and toxic potentiality, it was important that organisms are regaining sensitivity to this drug now-a-days. However empiric antibiotic choices for mild to moderate MRSA infection may include tetracycline and clindamycin but erythromycin and gentamicin should not be used. In our institution, the two antimicrobials that can be used for serious systemic infections caused by multidrug resistant MRSA are vancomycin and linezolid, to which all the isolates showed full sensitivity. So these two drugs should be used judiciously. If the prevalence of MRSA in hospital environment is contained, then only the emergence of mupirocin resistance may also be contained. The antimicrobial susceptibility test results for MuS and MuR isolates revealed that, in compared to MuS isolates, MuR ones were more resistant to gentamicin (88.23% vs. 80%) and ciprofloxacin (70.59% vs. 53.33%) and nearly equal in resistance to erythromycin (82.35% vs.82.67%), indicating therapeutic failure of these drugs in MuR-MRSA infections. On the contrary MuR isolates were more likely to be susceptible to clindamycin (23.52% vs. 33.33%), tetracycline (41.18% vs. 44%) and co-trimoxazole (64.71% vs. 66.67%). But co-

trimoxazole should not be used because of high percentage value of resistance. Vancomycin and linezolid should be used as reserve drug in this situation. Such pattern of antimicrobial resistance in relation to mupirocin susceptibility was detected in some other studies also.[2,4,7] Keeping in view that the mupirocin resistance strains were also found to be multidrug resistant, it would be essential to eradicate these strains by decolonisation rather than treatment with the limited and expensive therapeutic options available.

## CONCLUSION

The emergence of mupirocin resistance among MRSA signals the potential loss of a major drug against these organisms. Detection of low frequency of resistance in endemic isolates does not advocate its indiscriminate and widespread use. To keep the resistance in check, 'Blanket' use of mupirocin must be stopped and eradication strategies must be carefully designed, as proposed by Cookson.[18] Targeted prophylaxis rather than general prophylaxis, only in cases where isolate is sensitive to mupirocin, should be recommended by nasal eradication in patients and health care workers under selective circumstances, such as in MRSA outbreaks. Other valid uses are in high risk patient population such as those with diabetes mellitus, peripheral vascular disease, in-dwelling tubes, decubitus ulcers or multifunctional disabilities.[4] Lastly, it would be prudent, therefore, that institutions monitor the use of mupirocin to ensure that misuse, including inappropriate, prolonged or repeated use be avoided, especially among long-term patients so that this most valuable antimicrobial drug is not lost to therapeutic practice.

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