Research Article

Mupirocin-resistant methicillin-resistant *Staphylococcus aureus* – Are these strains wrongly reported and treated?

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ABSTRACT

Background: Mupirocin is an antibacterial drug and it is used for topical application either alone or along with other antiseptics, in areas where ever infections or colonization of methicillin-resistant *Staphylococcus aureus* (MRSA) are found. Enhanced use of mupirocin ointment these days for local application has led to the rise in data of its resistance. Many carriers and patients are treated inappropriately due to lack of sensitivity testing and improper reporting for mupirocin and development of different levels of resistance in these strains. Due to the lack of next level treatment possibilities, we need to report sensitivity accurately, record prevalence of resistant strains, and figure out the cause of resistance. **Objective:** We carried out this study to demonstrate levels of mupirocin resistance in MRSA strains isolated from patients samples and to check the resistance pattern of these strains to other antibacterial, in our hospital located in Delhi, North India. **Materials and Methods:** The study is framed as prospective type and performed on the strains of MRSA collected from the different samples from outpatient departments and inpatient departments from January 2017 to December 2017. Out of 221 *Staphylococcus aureus* collected from different clinical specimens, 113 isolates were confirmed as MRSA strains. Two of the suggested methods were selected to detect mupirocin resistance: Disk diffusion method by 5 μg disc and microbroth dilution method. **Results:** From our 113 MRSA isolates, resistance for mupirocin was noted in 16 (14.15%) isolates when subjected to disk diffusion and microbroth dilution test. These 16 strains showed varied level of resistance. High-level resistance was shown by 4 (3.5%) isolates and 12 (10.6%) isolates were found as low-level resistant (MuL). Mupirocin resistant MRSA isolates showed higher antibiotic resistance to erythromycin (81.26% vs. 78.76%), clindamycin (56.25% vs. 42.47%), linezolid (12.50% vs. 7.90%), and tigecycline (6.25% vs. 6.19%) as compared to MRSA strains. Not even single MRSA isolates were identified as vancomycin-resistant strain. **Conclusion:** Both high- and low-level mupirocin-resistant MRSA was found in high numbers from these patients. It is recommended that routine test must be performed to detect resistance for mupirocin subsequent to the detection of MRSA colonization among visitors, patients, and health care workers and its isolation from local sites. Treatment and decolonization of mupirocin-resistant strains are mandatory to reduce infection and spread in hospital after having done proper sensitivity testing only.

KEY WORDS: Methicillin-resistant *Staphylococcus aureus*; Mupirocin; Level of Resistance

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INTRODUCTION

Developing countries have been reporting Staphylococcus aureus as an important etiological agent of serious infections.[1-3] With constant rise in prevalence of *S. aureus* in health-care centers, it has emerged as common causative agent of nosocomial infection which may be the reason of its carriage in nose and

hands of health-care staff and patients.[4,5] Coinciding emergence of multidrug-resistant *S. aureus* (MRSA) infections is a challenge to the clinicians to prevent their spread in hospitals.[6] The close eye should be kept on use of antibiotics, duration of hospital stay, and nasal and hand carriage in health-care staff.[7,8] Infectious Diseases Society of America Practice Guidelines for the skin and soft-tissue infections recommend mupirocin for treating skin and soft-tissue infections, surgical site infections, and eliminating nasal colonization of MRSA among patients and medical staff.[9] Mupirocin distorts the synthesis of protein in these bacteria.[7] In 1985, mupirocin was launched in the UK to treat infections caused by *Staphylococcus* and *Streptococcus* and to clear the nasal carriage of MRSA.[8] Mupirocin resistance among MRSA isolates beginning to emerge in the UK[9] soon after 2 years and reported thereafter in Ireland (2%),[10] New Zealand (12.4%),[7] the USA (24%),[3] and in Trinidad and Tobago (44.1%).[11] Two types of mupirocin resistance has been defined in staphylococci. If minimum inhibitory concentrations (MICs) are in the range of 8–256 μg/ml, it is termed as low-level resistance said to be related with point mutations in the *ileS* gene, whereas high-level resistance is considered when MICs, ≥512 μg/ml, are supposed to be plasmid-mediated gene, *mupA* (*ileS2*).[12]

Usually, screening of MRSA is done in hospitals to keep check on its the spread in people in contact with hospital environment, but the blunder going on is not to check for sensitivity of this drug mupirocin. Hence, leading to therapy failure and development of resistance for this drug in MRSA strain. Documents have been provided by many studies that the treatment of strains having resistance of low level is still possible with normal dosage of 0.2% mupirocin ointment,[13] whereas trials have been reported to be failed for high-level resistant strains for decolonization as well as treatment.[14-16] Thus, it was aimed to assess the prevalence of high- and low-level resistance strains for mupirocin in our region. The sensitivity testing for mupirocin then can be included in screening and diagnostic policy.

MATERIALS AND METHODS

We conducted this study on samples collected from our patients attending outpatient departments or wards of HAHC Hospital, New Delhi, India. Duration of the study was 1 year from January 2017–December 2017. All *S. aureus* identified and found resistant to methicillin, from specimens such as pus, blood, urine, ear swab, sputum, ascetic fluid, tissue, cyst, and semen were included in this study. All the samples inappropriately collected were excluded from the study. This study is a part of project approved by the ethical committee of Hamdard Institute of Medical Sciences and Research and Jamia Hamdard.

MRSA Strains – Culture, Isolation, and Identification

All the samples received in bacteriology laboratory were processed as per standard operative procedures for isolation and identification of *S. aureus*. About 5% sheep blood agar and MacConkey agar media were inoculated with the sample and overnight incubation was done at 37°C aerobically. Confirmation of growth as *S. aureus* was done using biochemical tests and Vitek automated identification system.[17]

Bacterial Preservation and Storage

The strain after being confirmed was stocked in dimethyl sulfoxide (DMSO) for short-term storage. *S. aureus* can be stored for prolonged time at −80°C to avoid mutations. DMSO can also reduce freezing of the cells to diminish cellular damage at −80°C.[17]

Identification of MRSA

Clinical and Laboratory Standards Institute guidelines[18] recommend cefoxitin test for the identification of MRSA, using cefoxitin disc (30 μg).

Nutrient agar plate was inoculated and incubated at 37°C for 6 h. A broth of turbidity equivalent to 0.5 McFarland standards was made in test tube, from overnight growth in nutrient agar. A plate of Mueller-Hinton agar (MHA) was then lawn cultured and disc of cefoxitin (30 μg) was placed and incubated for 24 h aerobically at 35°C. Plates were examined for size of zone of inhibition. Strains with inhibition zone size of ≥22 mm were read as sensitive and excluded from the study, whereas zone size of ≤21 mm was interpreted as resistant and included in the study. These strains were identified and reported as a MRSA.

MRSA – Susceptibility Testing for Other Antimicrobials

Susceptibility testing was performed by Vitek 2 Compact System (BioMerieux) for the following antibiotics: Benzylpenicillin, oxacillin, clindamycin, cotrimoxazole, erythromycin, ciprofloxacin, linezolid, vancomycin, and tigecycline.[9] Suspension was prepared as manufacturer's instruction in normal saline.

Mupirocin Resistance – Disk Diffusion Method[17]

Modified Kirby–Bauer's disk diffusion method [Figure 1] which is considered to be the best test for evaluating mupirocin susceptibility in MRSA isolates was performed. MHA plates were inoculated with test strains and 5 μg of mupirocin discs were put to differentiate mupirocin-sensitive strains from resistant ones. Subsequent to proper incubation zone of inhibition was measured next day.

Zone size of <12 mm was interpreted as mupirocin-resistant strain and zone of ≥14 mm size was considered as mupirocin-sensitive strain. The intermediate strains were confirmed by microbroth dilution method. Having the confirmation done for resistance, resistant strains were retested by microbroth dilution method to assign them level of resistance either high or low.

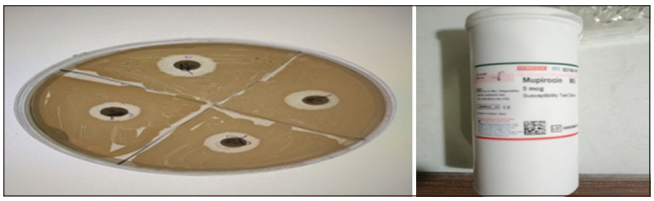

Figure 1: Disk diffusion method for mupirocin sensitivity

Microbroth Dilution Method Used for the Determination of MICs for Mupirocin

Resistant strains were tested with different dilution of mupirocin antibiotic. Strains were inoculated in different concentrations of antibiotics and incubated for 18–24 h at 37°C. Microtiter plates were incubated for 6–8 h and readings for the MIC were noted. Strains showing MICs >512 μg/ml were interpreted as highly resistant and reported to be MuH. On the other hand, lower MICs of 8–256 μg/ml were interpreted as strains having low resistance reported as MuL. Only strains with MIC <4 μg/ml were documented as mupirocin sensitive.

Statistical Analysis

We recorded the data of our study in Microsoft Excel sheet. SPSS software was used to analyze the data statistically. Chi-square test was used to evaluate significant levels. Statistically significance criteria is taken as $P < 0.05$.

RESULTS

Quantitative Distribution of MRSA in Clinical Samples

From all the clinical samples processed during this study, only 221 strains were identified as *S. aureus*. These strains were then subjected to cefoxitin test and 113 isolates were confirmed as MRSA. Maximum number of MRSA strains were isolated from blood (68.90%), followed by ear swab (60%), pus (51%), and urine (31.03%). Table 1 shows the isolation rate of *S. aureus* and MRSA from different samples collected in bacteriology laboratory.

Isolation Rate of *S. aureus* and MRSA from Different Samples

Among all samples yielding *S. aureus*, the prevalence of MRSA isolation was almost same in inpatient department (IPD) and outpatient department (OPD) with percentage of 52.17% and 48.33%.

Resistance Against Mupirocin [Table 2]

A total of 113 MRSA strains were subjected to test, 16 (14.15%) isolates showed mupirocin resistances by both disk diffusion method and microbroth dilution method. Sixteen strains were confirmed as mupirocin resistant. Level of resistance reported was as documented, 4 (25%) were MuH isolates and 12 (75%) were interpreted as MuL.

Table 1: MRSA isolates from different clinical samples

Sample	*Staphylococcus aureus (n)*	MRSA (*N*) (*N*/*n*×100)(%)	Total MRSA
Pus	149	76 (51.00)	113/221×100=51.13%
Blood	29	20 (68.90)	
Urine	29	9 (31.03)	
Ear swab	5	3 (60)	
Sputum	3	3 (100)	
Ascitic fluid	2	1 (50)	
Tissue	2	0 (0)	
Cyst	1	1 (100)	
Semen	1	0 (0)	

MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 2: Resistance pattern of 113 MRSA isolates for mupirocin

Mupirocin^{R}	Percentage (%)	Total
Sensitive	97 (85.8)	113
LLMR	12 (10.6)	
HLMR	04 (3.5)	

MRSA: Methicillin-resistant *Staphylococcus aureus*

Antimicrobial Susceptibility of MRSA Isolates[18]

The resistance pattern of MRSA isolates was studied and important facts were noted in this study. MRSA isolates revealed resistance to 100%, 91.10%, 78.76%, 73.45%, and 42.47% of benzylpenicillin, ciprofloxacin, erythromycin, cotrimoxazole, and gentamycin, respectively. Few MRSA isolates were reported resistant to tigecycline and linezolid and none for vancomycin.

The antimicrobial resistance of mupirocin-resistant MRSA isolates was quite high in few antibiotics in comparison to mupirocin susceptible MRSA isolates. Table 3 and graph depict that resistance to other antibiotics has no direct correlation. Few drugs were found more resistant in mupirocin-sensitive drugs than others.

DISCUSSION

In the present study, we collected 221 *S. aureus* from different samples. One hundred and thirteen were confirmed as MRSA. Out of these 113, 16 (14.1%) strains were found to be resistant to mupirocin.

It is higher than some other studies but many studies conducted in this region have documented as high as our data.[9,10] Increase spread of resistance could be due to an aggravated use of mupirocin ointment for soft tissue and skin infections and not doing proper sensitivity testing before its application. In our set up, 4 (3.5%) MuH and 12 (10.6%) MuL MRSA isolates were identified by microbroth dilution method. Normal dosage schedule is found effective for patients with MuL isolates, but treatment and decolonization in MuH were

Table 3: Difference in resistance pattern between mupirocin-resistant and mupirocin-sensitive strain

Antibiotics	Mupirocin-resistant strains (*n*=16) (%)	Mupirocin-sensitive strains (*n*=97) (%)	Total strain (*n*=113) (%)
Benzylpenicillin	100	100	100%/113
Oxacillin	100	100	100%/113
Gentamycin	25	29.89	29.2%/33
Ciprofloxacin	87.50	91.70	91.1%/103
Erythromycin	81.25	78.35	78.76%/89
Clindamycin	56.25	41.48	42.47%/48
Linezolid	12.50	7.21	7.9%/9
Vancomycin	0	0	0%/0
Tigecycline	6.25	6.18	6.19%/7
Trimethoprim/sulfamethoxazole	62.50	75.25	73.45%/83

discovered to be related with failure.[13-16] Combination of antibiotics was used in these types of patients to clear MRSA. Carriers of MRSA having high-level mupirocin resistance should instead be given chlorhexidine/neomycin or, where neomycin resistance also exists, polyhexamethylene biguanide for nasal decolonization.[19]

Health-care staff and hospitalized patients are easily exposed to MRSA and harbor it as pathogen or commensal.[20-22] In this study, we have tried to include all patients coming from OPD as well as IPD so that better comparison can be made between patients of community stings and hospital settings. We observed where infection control practices were better performed rate of isolation significantly get reduced in those wards of hospitals MRSA infections are on continuous rise because of insufficient hand hygiene and improper handling of MRSA carrier patients. Unfortunately, we could not involve health care workers in these studies, also time limitation was there otherwise better preview could have obtained for better understanding of treatment results in heath staff also.

Many hospitals use rapid MRSA detection methods for screening and use mupirocin as ointment for decolonization of MRSA in carriers and for the treatment of infections due to MRSA.[23,24] The topical antibiotic could be applied only where needed, this reduces resistance and because of topical nature systemic adverse effects can be avoided.[25] However, sensitivity of mupirocin is not checked before its use for decolonization. Hence, strategy should be reconsidered as high-level resistance has been reported to mupirocin, leading to therapy failure and rise in resistance due to its availability as over-the-counter drug which is further worsening the problem of MRSA infections.

CONCLUSION

Higher isolation rate of both MuH and MuL from MRSA and its carriage in nose has been recognized as a serious threat for clinicians to deal with MRSA and its transmission in the hospital. This may be due to an increased prevalence of MRSA infections in the health-care setup, carelessness of control measures, and over-the-counter sale of drugs. Mupirocin resistance which is considered to be associated with multidrug resistance cannot be easily treated and eliminated. Thus, it is advisable for infection control team to routinely perform nasal decolonization of health care workers to prevent spread of infections among admitted patients and to detect mupirocin resistance in MRSA strains isolated from the carriers so that proper dosage may be given and alternative decolonization methods may be used.

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