DOI:10.5958/j.2319-5886.2.4.134

International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 2 Issue 4 Oct-Dec Coden: IJMRHS Copyright @2013 ISSN: 2319-5886

Received: 19th Aug 2013 Revised: 13th Sep 2013 Accepted: 24th Sep 2013

Research article

MUPIROCIN RESISTANCE IN CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* IN A TERTIARY CARE HOSPITAL SET UP IN NORTH INDIA

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ABSTRACT

Background: Mupirocin is a topical antibiotic used for nasal decolonisation of methicillin resistant *Staphylococcus aureus* (MRSA). While resistance to mupirocin has been described it is usually not tested for in most clinical laboratories. **Aim:** The present study was carried out to detect the occurrence of mupirocin resistance in clinical isolates of *Staphylococcus aureus* in a tertiary care hospital set up in northern India. **Materials and Methods:** *Staphylococcus aureus* isolates obtained from clinical samples received in the microbiology laboratory over a period of one year were included in this study. Mupirocin resistance was detected by three phenotypic methods; disk diffusion method using 5µg and 200µg mupirocin disk to determine low-level and high-level resistance, broth microdilution method and an agar dilution method for determining minimum inhibitory concentrations. Methicillin sensitivity was also determined in the study isolates. **Results:** Of 250 non-duplicate *Staphylococcus aureus* isolates obtained, 5 (2%) were found resistant to mupirocin. All mupirocin resistance isolates were shown to have high-level resistance (minimum inhibitory concentration > 512µg/ml). All mupirocin resistant isolates were also resistant to methicillin. Results obtained by all three phenotypic methods showed good correlation. **Conclusion:** High-level mupirocin resistance is present in the northern Indian population which may be of major concern to prevent the spread of MRSA in hospitals and community.

Keywords: Mupirocin resistance, MuH, MRSA

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major nosocomial pathogen in healthcare institutions and increasingly reported from hospitals and the community worldwide.[1, 2] Carriage of MRSA in nose, axilla, perineum and hands of patients and health care personnel is an important risk factor for MRSA acquisition and spread.[3] Decolonisation from the site of carriage is one of the modalities for prevention of MRSA infections in healthcare settings.[4] Mupirocin (pseudomonic acid A) derived from *Pseudomonas fluorescens* is an important topical antibiotic ointment for the nasal decolonisation of MRSA in carriers.[5-8] It acts by binding specifically to the bacterial isoleucyl-

tRNA synthetase (IRS) enzyme and inhibits its protein synthesis.[9] Along with its use as a decolonising agent in health care personnel and patients, it has also been used for treatment of staphylococcal skin and soft tissue infections.[10,11] Resistance to mupirocin is being increasingly found due to its irrational use, which leads to improper decolonisation of MRSA from the site of carriage and spread of infection.[12,13]

Although there is no such guidelines published for mupirocin susceptibility testing, traditionally susceptible strains have minimum inhibitory concentration (MIC) ≤2 µg/ml while those having a MIC of ≥4 µg/ml were designated as resistant, and by disk diffusion method those with zone diameter of ≥14 mm with a 5µg disk were taken as susceptible while zones of ≤14 mm were considered resistant[14]. However, recently mupirocin-resistant strains have been grouped into two distinct phenotypes: low-level resistance (MuL) with MICs of 8-256 µg/ml, and high-level resistance (MuH) with MICs ≥512 µg/ml. An isolate with MIC ≤4 µg/ml is considered as mupirocin-sensitive. With the previously used 5 µg mupirocin disk, MuL and MuH strains cannot be differentiated. However it can be performed by concomitant use of 5 µg and 200 µg mupirocin disks.[15]

MuH strains have been found to be associated with failure of mupirocin as a decolonising agent as well as for treatment of skin and soft tissue infections.[16] Plasmid-mediated *mupA* encoding a novel isoleucyl RNA synthetase is a major genetic mechanism seen in high-level mupirocin resistance isolates.[17,18] Whereas base pair changes in native isoleucyl RNA synthetase gene is seen in low-level mupirocin resistance isolates[18]. Various studies suggest that during mupirocin prophylaxis transfer of *mupA* gene from normal commensal flora of skin such as *Staphylococcus epidermidis* to MRSA is responsible for emergence of mupirocin resistance.[19]

Thus, this study was carried out to determine the rates of high-level and low-level mupirocin resistance in *Staphylococcus aureus* by disk diffusion and MIC methods and to evaluate its association with methicillin-resistant isolates.

MATERIAL AND METHODS

Staphylococcus aureus isolates recovered from clinical specimens comprising pus, blood, various swabs and sterile body fluids received in the Postgraduate Department of Microbiology, King George Medical University, Lucknow, during a one year period from August 2011 to July 2012 from patients who attended the outpatient department (OPD) or were admitted to various inpatient departments (IPD) of Gandhi Memorial & Associated Hospitals were included in the study. Isolates from urine were not included.

Clinical specimens were processed and isolates were identified as *Staphylococcus aureus* by routine microbiological procedures. Non-duplicate *Staphylococcus aureus* isolates were tested for mupirocin resistance by disc diffusion method, broth microdilution method and agar dilution method.

In the disk diffusion method, mupirocin disks of 5µg (SD748, Himedia Labs, India) and 200µg (CT0523B, Oxoid, India) concentration were used. Zone diameter of ≥ 14 mm for both disks was taken as susceptible for mupirocin. Whereas, isolates that showed zone diameters < 14 mm in the 5 µg disk but ≥ to 14 mm in the 200 µg disk were considered to be low-level mupirocin resistant strains.[15] All isolates with zone diameters < 14 mm for both 5µg and 200µg disks were considered to be high-level mupirocin resistant strains[15] (Fig. 1).

The broth microdilution method was done for determination of Minimum Inhibitory Concentration (MIC) on Mueller-Hinton broth (MHB) with a final mupirocin concentration ranged from 0.25 µg/ml to 512 µg/ml (Fig. 2). Similarly agar dilution method was done for determination of MIC on Mueller-Hinton agar (MHA) with same concentration. Mupirocin MIC of ≤ 4µg/ml was taken as susceptible, that

of 8µg/ml to 256µg/ml as low-level resistance and ≥ 512 µg/ml as high level resistance (Fig. 3). Detection of methicillin resistance in *Staphylococcus aureus* isolates were performed as per Clinical Laboratory Standards Institute (CLSI) 2012 guidelines by using cefoxitin (30µg) disk[20]. Antimicrobial susceptibility testing was done as per CLSI guidelines by Kirby-Bauer disk diffusion method for the following antibiotics: ampicillin (10 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), linezolid (30 µg), tetracycline (30 µg), septran (1.25/23.75 µg), vancomycin (30 µg). Statistical test of significance applied z-test.

Fig.1: Demonstration of high-level mupirocin resistance and mupirocin sensitive phenotypes by disk diffusion method

Fig.2: Broth microdilution method for determination of MIC of mupirocin in Staphylococcus aureus isolates

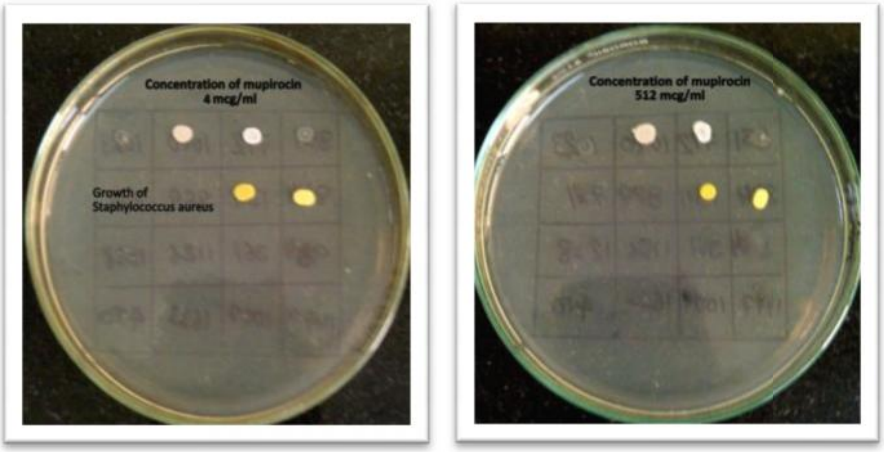

Fig.3: Agar dilution method for determination of MIC of mupirocin in *Staphylococcus aureus* isolates

RESULTS

Among the 250 non-duplicate *Staphylococcus aureus* isolates included in study, 133 (53.2%) were MRSA. Of these, 5 i.e., 3.76% of MRSA were mupirocin resistant *Staphylococcus aureus* (MupRSA). Mupirocin resistance was not detected in methicillin sensitive *Staphylococcus aureus* (MSSA) isolates.

Table.1: MupRSA and MRSH strains among total *Staphylococcus aureus* isolates in different samples

Samples	*S. aureus*	MRSA (%)	MuH (%)	MuL (%)	95% CI	P-value
Pus	142	78 (54.93)	2 (1.4)	-	0.9-6.06	<0.0001*
Blood	48	27 **(56.25)**	3 **(6.25)**	-	0.7-22.96	<0.0001*
Genitourinary specimens	11	1 (9.09)	-	-		
Respiratory specimens	39	23 (58.97)	-	-	-	-
Miscellaneous	10	4 (40.0)	-	-	-	-
TOTAL	**250**	**133 (53.2)**	**5 (2.0)**		0.53-6.99	<0.0001*
MRSA= Methicillin resistant *Staphylococcus aureus*, MuH= High-level mupirocin resistance, MuL= Low-level mupirocin resistance, CI= Confidence Interval, * = Significant						

Table.2: Distribution of *S. aureus*, MRSA and MupRSA in different clinical wards

WARDS	*S. aureus*	MRSA (%)	MupRSA (%)	95% CI	P-value
Surgical	**115**	**69 (60)**	**2 (1.73)**	1.06-6.86	<0.0001*
• General surgery	54	23(42.59)	0	-	-
• Orthopaedics	29	21(72.41)	1(3.44)	4.35-13.87	<0.0001*
• Neurosurgical	32	20 (62.5)	1(3.12)	4.55-14.55	<0.0001*
Gynaecology	**11**	**5 (45.45)**	**0**	-	-
Paediatrics	**67**	**30 (44.77)**	**3 (4.47)**	0.74-20.74	<0.0001*
Medicine	**20**	**11 (55)**	**0**	-	-
OPD	**37**	**23 (62.16)**	**0**	-	-
Total	**250**	**133 (53.2)**	**5 (2.0)**	0.53-6.99	<0.0001*
MRSA= Methicillin resistant *Staphylococcus aureus*, MupRSA=Mupirocin resistance *Staphylococcus aureus*, CI= Confidence Interval, * = Significant					

Table.3: Antimicrobial sensitivity pattern of MRSA and MupRSA isolates

Antibiotics	Sensitive (%)		Intermediate (%)		Resistant (%)	
	MRSA	MupRSA	MRSA	MupRSA	MRSA	MupRSA
Ampicillin	7 (5.26)	-	7 (5.26)	-	119(89.47)	5 (100)
Ciprofloxacin	36 (27.06)	-	10(7.52)	-	87 (65.41)	5 (100)
Clindamycin	46 (34.58)	1 (20)	9 (6.77)	1 (20)	78 (58.65)	3 (60)
Erythromycin	44 (33.08)	1 (20)	6 (4.51)	1 (20)	83 (62.41)	3 (60)
Linezolid	133 (100)	5 (100)	-	-	-	-
Septran	44 (33.08)	3 (60)	12(9.02)	1 (20)	77 (57.89)	1 (20)
Tetracycline	68 (51.13)	3 (60)	9 (6.77)	1 (20)	56 (42.11)	1 (20)
Vancomycin	133 (100)	5 (100)	-	-	-	-
MRSA= Methicillin resistance *Staphylococcus aureus*, MupRSA= Mupirocin resistance *Staphylococcus aureus*						

Amongst the mupirocin resistant isolates, all the 5 isolates were high-level mupirocin resistant. Percentage of methicillin resistant and mupirocin resistant Staphylococcus aureus isolates among different samples is documented in the Table 1. Distribution of samples according to the wards from where they were received is documented in Table 2. Amongst other antibiotics, percentage resistance is documented in Table 3, with a maximum resistance seen for ampicillin. Vancomycin and linezolid were found to be the most sensitive drugs across all staphylococcal species.

DISCUSSION

Staphylococcus aureus is one of the most frequently isolated pathogen from both nosocomial and community associated infections causing a wide range of infections from abscesses, impetigo and cellulitis to deep seated pyogenic lesions, pneumonias, meningitis and septicaemias.[1] Increasing number of infections caused by MRSA strains has led to poorer treatment outcome.[2] Mupirocin is an important topical antibiotic widely used for treatment of skin and soft tissue infections caused by Staphylococcus aureus.[11] In healthcare institute it is used for nasal decolonisation of health care personnel to prevent the spread of MRSA among co-workers and the patients.[10] Emergence of resistance to mupirocin is likely to worsen the problem. Studies suggest mupA gene which encodes mupirocin resistance is transferred from commensal flora of skin to MRSA during mupirocin therapy.[19] This could be a threat to irrational use of mupirocin as it may lead to the development and spread of mupirocin resistance.

In this study, out of total 250 Staphylococcus aureus isolates, 5 i.e. 2% showed mupirocin resistance by disk diffusion method. All the mupirocin resistant Staphylococcus aureus isolates are high-level resistant strains as determined by disk diffusion method and two different MIC methods: broth microdilution method and agar dilution method.

The percentage rate of high-level mupirocin resistance in this study is consistent with other studies conducted in different regions of India.[2,21] Low-level resistance is not found in this setup which is in agreement with the study conducted in Chennai by Oommen et al., but in contrast Krishnan, et al., and Gadepalli, et al., has shown 1.5% and 1% low-level resistance, respectively.[2,21,22] In this study, none of MSSA isolates showed resistance to mupirocin (either high-level or low-level), and it is seen only in MRSA isolates. Also, there was no significant association seen between mupirocin resistance with resistance to other antibiotics in this study, which is in contrast to studies conducted by MCDougal etal and Cadilla etal.[23,24]

Efficacious nasal clearance of MRSA for a significant duration in carriers is shown in mupirocin sensitive isolates. Emergence of high-level mupirocin resistance has shown to be associated with the failure of decolonisation therapy among carriers and patients and offers fewer topical treatment options16. However, studies has suggested that low-level mupirocin resistance strains can still be controlled with normal dosage schedule of mupirocin ointment, as it contains a higher concentration of mupirocin (2000 µg/ml) than the MICs of low-level mupirocin resistance strains.[25]

CONCLUSION

The present study has demonstrated the presence of high-level mupirocin resistance in a major tertiary care setup of northern India which is a concern to prevent the spread of MRSA in hospitals and community. This may be attributed to irrational use of antibiotics as well as over the counter sale of drugs. Nasal decolonisation of MRSA in healthcare personnel is performed by using 2% mupirocin ointment along with absence from duty till culture reports are documented negative and since low-level mupirocin resistance can be treated with the normal dosage

of mupirocin ointment, thus detection of high-level mupirocin resistance seems to be mandatory. Hence, it would be advisable to detect mupirocin resistance by using both 5 µg and 200 µg mupirocin disks from carriers before starting mupirocin decolonisation therapy so that alternatives may be used.

ACKNOWLEDGEMENTS

Our thanks to Ms. Shubhra Mishra, Msc, PhD for technical help during the study

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