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Prevalence of Mupirocin Resistance in Methicillin Resistant *Staphylococcus aureus* Strains isolated from a Tertiary Care Hospital

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A B S T R A C T

Mupirocin is a topical antibiotic that has been used extensively for treating methicillin resistant *Staphylococcus aureus* (MRSA) associated infections. However, the prevalence of resistance to mupirocin is being increasingly found due to its irrational and widespread use. Retapamulin: a pleuromutilin antibacterial agent is an effective topical antibiotic against these resistant strains. This study was carried out to determine the rates of resistance to mupirocin in MRSA isolates in our patient population, and to estimate the association of such resistance and resistance to other classes of antimicrobial agents in order to consider the effect of the use of mupirocin on the selection of antimicrobial resistant strains. This prospective study was conducted in the Department of Microbiology at VIMS & RC, Bengaluru. 200 *Staphylococcus aureus* isolates were recovered from various clinical specimens from out patients and in patients admitted into various wards and intensive care units. Isolation and identification of isolates *S. aureus* isolated from clinical specimens were identified according to the standard laboratory operating procedures. Antimicrobial sensitivity was determined by the Vitek 2 K automated system. All tests and quality assurance procedures were performed and interpreted according to the standards set by the Clinical and Laboratory Standards Institute (CLSI). For fusidic acid and retapamulin The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria was used. For the macrolide-lincosamide streptogramin B (MLSB) phenotype (inducible clindamycin resistance encoded by the plasmid-borne gene, *erm*) was determined by the disc approximation test, clindamycin was reported to be resistant. Mupirocin resistance testing was done by disc diffusion with disc concentration of 5µg and 200µg. Among the 200 *Staphylococcus aureus* isolates 108 (54%) were MRSA, highest percentage of MRSA strains were from blood isolates (62%), followed by aspirated fluids and pus (59 % and 56 % respectively). Of the 108 MRSA strains, 26 (24%) were mupirocin resistant *Staphylococcus aureus* (MupRSA). Mupirocin resistance was not detected in methicillin sensitive *Staphylococcus aureus* (MSSA) isolates. High-level mupirocin resistance was observed in 11% and low-level resistance in 13% of the 108 isolates. Higher prevalence of high level mupirocin resistance was from blood isolates followed by pus (45% and 36.3% respectively), low level mupirocin resistance was maximum seen in the isolates from respiratory secretions (46%). MupRSA was more frequently isolated from ICUs and surgical wards. The mupirocin resistant MRSA strains exhibited resistance to other class of antibiotics also: ampicillin (90%), ciprofloxacin (88%), erythromycin (86%), co trimoxazole (73%). However fusidic acid, vancomycin and linezolid showed sensitivity of 99%, 96% and 96% respectively. All the strains were sensitive to retapamulin. Increase in mupirocin resistance among MRSA isolates is a matter of concern. Antimicrobial stewardship programmes are important to address excessive or inappropriate antimicrobial usage. Alternative agents to mupirocin should be considered to counteract the clinical failure of decolonization regimens and to prevent the selection of multiple resistant strains.

Introduction

Staphylococcus aureus is a major pathogen responsible for various community-acquired and nosocomial infections, including bacteraemia, pneumonia, skin and soft tissue infections, and osteomyelitis. Methicillin-resistant *S. aureus* (MRSA) are implicated in serious infections and nosocomial outbreaks. These strains show resistance to a wide range of antibiotics, thus limiting the treatment options to very few agents such as glycopeptides and linezolid (Xavier Malaviolle *et al.,* 2008).

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. Decolonization from the site of carriage is one of the modalities for prevention of MRSA infections in healthcare settings. Mupirocin (pseudomonic acid A) derived from *Pseudomonas fluorescens* is an important topical antibiotic ointment for the nasal decolonization of MRSA in carriers. It acts by binding specifically to the bacterial isoleucyl-tRNA synthetase (IRS) enzyme and inhibits its protein synthesis. 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 (Singh Amit *et al.,* 2013; Summiya Nizamuddin *et al.,* Hetem and Bonten, 2013).

Resistance to mupirocin is being increasingly found due to its irrational use, which leads to improper decolonization of MRSA from the site of carriage and spread of infection.

The mechanisms of mupirocin resistance have been elucidated, low-level resistance can be caused by an alteration in the isoleucyl t RNA sythetase gene, *ileS*. This mutation is stable and non-transferrable. High-level resistance is mostly associated with the presence of the *mupA* gene, which encodes an alternate isoleucyl-tRNA synthetase. High-level resistance in the absence of this gene has been encountered, suggesting resistance by other mechanisms furthermore, the *mupA* gene is associated with mobile genetic elements and is mostly plasmid borne, which may facilitate the spread of this resistance mechanism. These plasmids also carry resistance genes to other antimicrobial agents, such as the macrolides, gentamicin, tetracycline and trimethoprim. Although sensitivity to mupirocin is not affected by the same genetic elements as resistance to beta-lactam agents, such as cloxacillin, an association between MRSA and mupirocin resistance has been noted in the literature (Ravisekhar Gadepalli *et al.,* 2007; Wasserman *et al.,* 2014).

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. 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. Plasmid-mediated *mupA* encoding a novel isoleucyl RNA synthetase is a major genetic mechanism seen in high-level mupirocin resistance isolates. Whereas base pair changes in native isoleucyl RNA synthetase gene is seen in low-level mupirocin resistance isolates (Singh Amit *et al.,* 2013).

The presence of the *mupA* gene can be confirmed by polymerase chain reaction (PCR), but this may be prohibitively expensive when large numbers of isolates are to be screened, and the other mechanisms of

resistance also cannot be excluded (Wasserman *et al.,* 2014).

Retapamulin: a pleuromutilin antibacterial agent is an effective topical antibiotic against these resistant strains. Currently it is used against a variety of Gram positive pathogens associated with secondarily-infected traumatic lesions and secondarily-infected dermatoses. The pleuromutilins are potent inhibitors of protein synthesis in bacteria through the interference of peptide bond formation by binding to the peptidyl transferase center of the 50S ribosomal subunit1 Due to the unique pleuromutilin mode of action, retapamulin shows no target specific cross-resistance to other classes of antibacterials (Dhingra *et al.,* 2013; Ronald N. Jones *et al.,* 2006).

Though mupirocin resistance is often associated with methicillin resistance, however the true extent of mupirocin resistance in our area is unknown.

Thus, this study was carried out to determine the rates of high-level and low level resistance to mupirocin in MRSA isolates in our patient population, and to estimate the association of such resistance and resistance to other classes of antimicrobial agents in order to consider the effect of the use of mupirocin on the selection of antimicrobial resistant strains.

Materials and Methods

This prospective study was conducted in the Department of Microbiology at VIMS & RC.

Staphylococcus aureus isolates were recovered from clinical specimens comprising of pus, respiratory secretions, catheter tip, aspirated fluids and blood from out patients and in patients admitted into various wards and intensive care units.

Isolates from urine were not included.

Isolation and identification of isolates *S. aureus* isolated from clinical specimens was identified according to the standard laboratory operating procedures. Antimicrobial sensitivity was determined by the Vitek 2 K automated system. All tests and quality assurance procedures were performed and interpreted according to the standards set by the Clinical and Laboratory Standards Institute (CLSI). For fusidic acid and retapamulin The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria was used (EUCAST and CLSI potency NEO-SENSITABS). For the macrolide-lincosamide streptogramin B (MLSB) phenotype (inducible clindamycin resistance encoded by the plasmid-borne gene, *erm*) was determined by the disc approximation test, clindamycin was reported to be resistant. Moxifloxacin was used as a marker for fluoroquinolone resistance. Mupirocin resistance testing was done by disc diffusion with disc concentration of 5µg and 200µg. 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. All isolates with zone diameters < 14 mm for both 5µg and 200µg disks were considered to be high-level mupirocin resistant strains.

Results and Discussion

In this study 200 *Staphylococcus aureus* isolates were obtained from various clinical specimens such as pus (n=88), respiratory secretions (n=51), aspirated fluids (n=27), blood (n=21) and others (n=13) (Table 1).

Among the 200 *Staphylococcus aureus* isolates 108 (54%) were MRSA, highest percentage of MRSA strains were from blood isolates (62%), followed by aspirated fluids and pus (59 % and 56 % respectively) (Table 1).

Table.1 Isolation of *Staphylococcus aureus* and MRSA from various clinical samples

Sl. no	Clinical samples	*Staphylococcus aureus* isolates	MRSA strains	%
1	Pus	88	49	56
2	Respiratory secretions	51	27	53
3	Aspirated fluids	27	16	59
4	Blood	21	13	62
5	Others	13	3	23
	Total	**200**	**108**	**54**

Table.2 Prevalence of mupirocin resistance in MRSA strains

MRSA strains	Mupirocin sensitive n (%)	Mupirocin resistance n (%)
108	82 (76)	26 (24)

Table.3 Isolation of MuH and MuL in MRSA strains from various clinical samples

Sl.no	Clinical samples	MRSA strains	MuH n (%)	MuL n (%)
1	Pus	49	4 (33.3)	3 (21.4)
2	Respiratory secretions	27	2 (17)	6 (43)
3	Aspirated fluids	16	1 (8.3)	3 (21.4)
4	Blood	13	5 (42)	1 (7)
5	Others	3	0	1 (7)
	Total	**108**	**12 (11)**	**14 (13)**

Table.4 Antimicrobial resistance pattern of MRSA and MupRSA against various antibiotics tested

Sl.no	Antibiotic	MRSA (%)	MuRSA (%)
1	Erythromycin	80	86
2	Clindamycin	51	65
3	Co-trimoxazole	65	73
4	Linezolid	2	4
5	Tetracycline	60	82
6	Vancomycin	1	4
7	Rifampicin	10	24
8	Chloramphenicol	21	36
9	Ciprofloxacin	53	88
10	Gentamycin	63	76
11	Ampicillin	86	90
12	Fusidic acid	0	1
13	Retapamulin	0	0

Of the 108 MRSA strains, 26 (24%) were mupirocin resistant *Staphylococcus aureus* (MupRSA) (Table 2). Mupirocin resistance was not detected in methicillin sensitive *Staphylococcus aureus* (MSSA) isolates. High-level mupirocin resistance was observed in 11% and low-level resistance in 13% of the 108 isolates.

Higher prevalence of high level mupirocin resistance was from blood isolates followed by pus (45% and 36.3% respectively), low level mupirocin resistance was maximum seen in the isolates from respiratory secretions (46%) (Table 3).

MupRSA was more frequently isolated from ICUs and surgical wards.

The mupirocin resistant MRSA strains exhibited resistance to other class of antibiotics also: ampicillin (90%), ciprofloxacin (88%), erythromycin (86%), co trimoxazole (73%). However fusidic acid, vancomycin and linezolid showed sensitivity of 99%, 96% and 96% respectively (Table 4).

All the strains were sensitive to retapamulin.

200 *Staphylococcus aureus* isolates were obtained from various clinical specimens, among them 108 (54%) were MRSA, highest percentage of MRSA strains were from blood isolates (62%), followed by aspirated fluids and pus (59 % and 56 % respectively).

Of the 108 MRSA strains 26 (24%) were mupirocin resistant and 82 (76 %) were mupirocin sensitive

Higher prevalence of high level mupirocin resistance (MuH) was from blood isolates followed by pus (42% and 33.3% respectively), low level mupirocin resistance (MuL) was maximum seen in the isolates from respiratory secretions (43%).

The mupirocin resistant MRSA strains exhibited resistance to various classes of antibiotics: ampicillin (90%), ciprofloxacin (88%), erythromycin 86% and co - trimoxazole 73%. However fusidic acid, vancomycin and linezolid showed sensitivity of 99%, 96% and 96% respectively.

All the strains were sensitive to retapamulin.

Mupirocin (pseudomonic acid A) derived from *Pseudomonas fluorescens* is widely used topical antibiotic for the treatment of MRSA-associated infections. In healthcare institute it is used for nasal decolonization of health care personnel to prevent the spread of MRSA among co-workers and the patients. 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. This could be a threat to irrational use of mupirocin as it may lead to the development and spread of mupirocin resistance (Singh Amit *et al.*, 2013; Charan Kaur Dardi, 2014). The prevalence of high-level mupirocin-resistant MRSA in the present study was 11% and low-level resistance was 13%.

This is comparable to reports in the literature of 1-13% for low-level and 2.4-14% for high-level resistance by various authors (Vasquez *et al.*, 2000; Banerjee John *et al.*, 2013; Banerjee John *et al.*, 2013). None of the MSSA were mupirocin resistant.

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 (Nonika Rajkumari *et al.*, 2014; Hurdle *et al.*, 2005). Therefore the sensitivity to mupirocin should be confirmed before it can be used as a decontaminating agent and

should be factored into local infection control policies.

The majority of the resistant isolates were from the surgical disciplines, ICUs and in patients as they deal with the majority of complicated soft tissue and skeletal infections from which *S. aureus* is the most frequently isolated pathogen.

The link between antimicrobial consumption and antimicrobial resistance profiles is well established. Therefore, collections of isolates representing invasive infection (blood culture, pus isolates) and nosocomial infection have a denser antimicrobial history, resulting in the resistance to antimicrobial agents.

Mupirocin-resistant MRSA isolates showed higher antibiotic resistance to several classes of antimicrobial agents, i.e. MLSB phenotypes, quinolones, co-trimoxazole, and rifampicin, however they showed sensitivity to ritapamulin, vancomycin, linezolid and fusidic acid, which is in comparison with the various authors around the world (Banerjee John *et al.,* 2013; Banerjee John *et al.,* 2013).

This is biologically plausible as genetic material encoding for resistance to different classes of antimicrobial agents may be carried on the same mobile genetic elements. It is a cause for concern as it implies that mupirocin use will lead to environmental pressure for the selection of resistance to other classes of antimicrobial agents, and vice versa (Wasserman *et al.,* 2014).

For systemic treatment of MRSA, fusidic acid has proven useful when combined with agents such as vancomycin, but not as monotherapy (Malaviolle *et al.,* 2008). Hydrogen peroxide cream also has been recommended as a topical alternative to mupirocin or perhaps the newer ritapamulin has also seen in this study (Christensen and Anehus, 1994).

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

Increase in mupirocin resistance among MRSA isolates is a matter of concern. The better understanding of the mechanisms, clinical significance and epidemiology of mupirocin resistance is important for predicting how changes in mupirocin use may affect the emergence of mupirocin resistance. Hence it is recommended that routine testing of MRSA for mupirocin resistance be conducted which facilitates the early detection of resistance and assists in the control and spread of mupirocin-resistant MRSA.

Antimicrobial stewardship programmes are important to address excessive or inappropriate antimicrobial usage. It is important to look beyond the usage of systemic antimicrobial agents and to adopt a more comprehensive approach to decolonization and environmental stewardship.

Alternative agents to mupirocin should be considered to counteract the clinical failure of decolonization regimens and to prevent the selection of multiple resistant strains.

Approved by ethical committee

Conflict of interest

The authors declare that there are no conflicts of interest

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