Original Article

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Microbiology Section

Prevalence of High and Low Level Mupirocin Resistance among Staphylococcal Isolates from Skin Infection in a Tertiary Care Hospital

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ABSTRACT

Background: Mupirocin has been used for the treatment of skin infections and for the eradication of the nasal carriage of Methicillin -resistant *Staphylococcus aureus* (MRSA). The increased use of this antibiotic has been accompanied by its resistance, resulting in treatment failures.

Objective: This study was aimed at determining the prevalences of low and high level Mupirocin resistance among the clinical isolates of Staphylococcus species which were obtained from pyogenic infections.

Material and Method: Clinical samples such as wound swabs, tissues and pus which were submitted to the microbiology laboratory during a period of six months were screened for the growth of *Staphylococcus* species, which were identified as *Staphylococcus aureus* and Coagulase negative *Staphylococcus* species by the routine microbiological procedures. All the isolates were tested for their Mupirocin susceptibilities by using 5 and 200 µg discs and their resistance was confirmed from their Minimum Inhibitory Concentrations (MICs).

Result: Out of 400 samples, 150 samples grew *Staphylococcus species,* of which 113 were *Staphylococcus aureus* and 37 were Coagulase negative *Staphylococcus* (CoNS). Only 5(3.3%) mupirocin resistant *Staphylococcus* species*:* three high level and two low level strains were detected. The MICs for the two low level and three high level Mupirocin resistant strains were 256 mg/L and ≥512mg/L each respectively.

Conclusion: We conclude that the screening for mupirocin resistance, in terms of high-level and low-level resistance among the *Staphylococcus* species from patients with skin and soft tissue infections is warranted and that it is important for the clinicians in selecting the appropriate, empirical, topical, antimicrobial therapy. It also provides useful information about the prevalence of these resistant pathogens.

Key Words: Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase negative *Staphylococcus* (CoNS), High and Low level mupirocin resistance

INTRODUCTION

The soft-tissue infections are common, they are generally of mild to modest severity, and they are easily treated with a variety of agents. *Staphylococcus aureus* and *Streptococcus pyogenes* are the common organisms which cause a variety of skin and soft-tissue infections and the emerging antibiotic resistance among these isolates is problematic [1]. The minor skin and soft-tissue infections may be empirically treated with topical agents such as mupirocin, which has been widely available for many years. It has been approved for use in ointment formulations which are used for the topical treatment of impetigo and secondary wound infections which are caused by *Staphylococcus aureus.* In addition, it is used in a nasal formulation which is used in eradicating the nasal carriage of Methicillin -resistant *Staphylococcus aureus* (MRSA) in adult patients and health care personnel [2].

Mupirocin, which was derived from *Pseudomonas fluorescens,* is an effective topical antibiotic for treating skin and soft tissue infections. It was introduced into the clinical practice in 1985 and the use of the mupirocin ointment has been progressively increasing worldwide [3]. The first report on the *Staphylococcal* resistance to mupirocin came 2 years after its introduction. Since then, varying rates of resistance have been reported among the *Staphylococcal* species [4]. The resistance was found to be more common among MRSA than among the Methicillin- Sensitive *Staphylococcus aureus* (MSSA) [5]. Mupirocin resistances has been reported widely in various countries, mainly because of the widespread use of Mupirocin. Another factor that may lead to an increasing mupirocin use is the growing interest in the preoperative eradication of the *Staphylococcus aureus* colonization as a strategy for preventing postsurgical infections [6]. There is an emerging body of evidence that suggests that the preoperative eradication of the *Staphylococcus aureus* colonization can reduce the number of postsurgical *Staphylococcal* infections [7,8]. This evidence will most likely lead to an increased use of mupirocin for this purpose.

The application of mupirocin to the patients who are colonized with *Staphylococcus aureus* may eliminate the carriage of this organism and it is important in preventing the spread and the development of *Staphylococcal* infections [9]. In order to prevent the MRSA infections, it is possible that there will be an increased use of mupirocin for the decolonization of the MRSA. The increased use of this antibiotic has been followed by the reports of outbreaks which were caused by MRSA with both low and high-level resistanc.

The high-level and the low level Mupirocin resistance was identified by using 200 and 5 µg discs [10] and the concomitant use of the 5 and 200µg mupirocin discs could easily differentiate the mupirocin

low level (MuL) and the mupirocin high level (MuH) strains [11]. The Minimum Inhibitory Concentration (MIC) for the low-level (MuL) resistant strains was 8-256mg/L and that for the high level (MuH) resistant strains was ≥512mg/L, while the mupirocin-sensitive isolates were those with an MIC of ≤4mg/ml [12].

A high-level resistance (>1,000mg/L) to mupirocin has been reported in Coagulase-positive and Coagulase-negative *Staphylococci,* together with an evidence that the resistance was present within the *Staphylococcal* population before mupirocin was used therapeutically. In many instances, the high-level mupirocin resistance in *Staphylococci* is mediated by plasmids which are transferred into susceptible recipient strains [13]. Studies which were done on the mupirocin resistance in the *Staphylococcus aureus* populations indicated that nearly all the *Staphylococcus aureus* isolates with a high-level mupirocin resistance were *mupA* positive and that this gene was located in the plasmids. Moreover, the isolates with a low level mupirocin resistance had the *mupA* gene in the chromosomes rather than in the plasmids. A high-level mupirocin resistance has been associated with a failure to clear the organism from the patients after giving a mupirocin therapy [14].

Now and then, as various percentages of the mupirocin resistant strains were reported, it was ideal to screen for their prevalence in their own hospital setup. In our hospital, mupirocin was widely used as an empirical topical antibiotic for superficial skin infections. Hence, we studied the prevalences of the low-level and the high-level mupirocin resistances among all the *Staphylococcal* isolates which were obtained from skin and soft tissue infections.

MATERIALS AND METHODS

This study was done during a period of six months, from August 2011 to January 2012 in Saveetha Medical College and Hospital, which is located at Thandalam in the Kancheepuram district, India. This study was conducted in the hospital after obtaining the ethical committee's clearance and the university scientific review board's approval. All the samples such as pus and wound swabs were subjected to gram staining and culturing on basic media such as Blood agar and MacConkey's agar. The suspected Staphylococal colonies were processed further for their species level identification.

Identification of the Staphylococcus species [15]

The isolates were identified as *Staphylococcus aureus* and other Coagulase negative *Staphylococcus* species by using a panel of basic biochemical reactions such as the coagulase test, sugar fermentation tests, the ornithine decarboxylase test, the urease production test, acetoin production, the nitrate reduction test and the disc sensitivity test (novobiocin 5µg and polymixin B 300 units)

Antibiotic Susceptibility testing

The Kirby Bauer disc diffusion test which is used for studying the susceptibility of the organisms to various antibiotic discs, was performed by using Mueller Hinton agar. The discs which were used were as follows; mupirocin 5 µg from (Hi-Media) and 200µg from BD for detecting the MuL and the MuH strains, cefoxitin 30µg (Hi- Media) for detecting the methicillin resistance and the novobiocin 5µg and the polymixin B 300 units differentiation discs were used as per the Clinical Standards Laboratory Institute (CSLI) 2010 guidelines [16]. The zone diameters were read by using both reflected and transmitted light after 16 to 18 hrs and 24 hrs of incubation. *Staphylococcus aureus* ATCC 25923 was used for the quality control in the disc diffusion testing.

Detection of the Minimum Inhibitory Concentration (MIC) of the mupirocin resistant isolates: [17]

The MIC for mupirocin was determined by the broth dilution method. *Staphylococcus aureus* ATCC 29213 was used for the quality control in the MIC testing.

The statistical analysis was done by using the Chi-square test and the Student's t test (unpaired).

RESULT

A total of 400 samples were received in the Clinical Microbiology Laboratory, among which 150 samples which grew *Staphylococcus* species were used for the study purpose. The samples were categorized as pus [103 (68.7%)], wound swabs [41 (27.3%)], and tissue samples [6 (4.0%)]. More than two-third of the samples were collected from pus.

Among the total samples, 92 (61.3%) were from male patients and 58 (38.7%) were from female patients. The male and female representation of the sample collection was in the ratio of 3:2. A higher incidence of the infection was commonly found in males than in females. Among the 150 samples, 14 were received from the paediatric age group. Age wise, 90 (60.0%) patients were below 40 years of age and the remaining 60 (40.0%) were above 40 years of age. Among the total 150 samples, the maximum number of cases were in the age group of 21-30 years (22.7 %), followed by the age group of 31-40 years (16.0%).

Different species of *Staphylococcus* were observed; among the 150 isolates, the predominant isolate was *Staphylococcus aureus* [113 (75.3%)] and 37(24.7%) were Coagulase negative *Staphylococcus* (CoNS). The age wise distribution of the isolated *Staphylococcal* species has been shown in [Table/Fig-1].

Out of the 113 *Staphylococcus aureus* isolates, 67(59.3%) were MSSA and 46 (40.7%) were MRSA. Among the 37 Coagulase negative Staphylococcus Isolates (CoNS), 23 (62.2%) were found to be Methicillin-Sensitive (MSCoNS) and 14 (37.8%) were found to be Methicillin-Resistant (MRCoNS). [Table/Fig-2] shows the distribution of the Staphylococcal isolates with respective to the methicillin sensitivity.

Among the CoNS isolates, 27(18.0%) were *Staphylococcus epidermidis,* followed by *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* [3 (2.0%)] each and *Staphylococcus hominis* and *Staphylococcus simulans* [2(1.3%) each].

Among the 150 strains of the *Staphylococcus* species, 5(3.3%) were resistant to mupirocin and the remaining strains were sensitive to it. Out of these five; three were *Staphylococcus aureus* (2 MSSA, 1MRSA) and two were MRCoNS. The distribution of the high level (200 µg) and the low level (5µg) Mupirocin resistance which were found in the *Staphylococcus* species has been shown in [Table/Fig-3].

The Minimum Inhibitory Concentration (MIC) for the low level mupirocin (5µg) and the high level mupirocin (200µg) resistances were 256mg/L and ≥512mg/L respectively. The difference in the proportion of the mupirocin resistance between MRSA and MSSA was not statistically significant. Similarly, the difference in the mupirocin resistance between MRCoNS and MSCoNS was not statistically significant. The difference in the proportions of

the resistance between the different classifications was tested for its statistical significance by using the Chi-square test and the Student's t test (unpaired).

DISCUSSION

Mupirocin is an effective topical antibacterial agent that is used for the management of skin infections and for the colonization with MRSA in both patients and health care workers. The first report on *Staphylococcus aureus* which was resistant to mupirocin began to emerge shortly after the introduction of mupirocin into the clinical practice [4]. But still, Mupirocin is considered as an effective therapy for the elimination of the *Staphylococcal* species.

In the present study, 150 *Staphylococcal* isolates were speciated and the prevalence of the methicillin-resistant *Staphylococcal* species and the mupirocin resistance was analyzed. Among the 150 *Staphylococcal* isolates, hundred and thirteen were *Staphylococcus aureus* and the remaining 37 were Coagulase negative *Staphylococci.* Among the 113 *Staphylococcus aureus* isolates, 46(30.7%) were MRSA and 67(44.7%) were MSSA. The percentage of MRCoNS and MSCoNS were 14(9.3%) and 23 (15.3%) respectively. A similar study which was done at a tertiary care hospital in south India [18] had almost the same percentage of MRSA (29.0%), whereas the isolates such as MSSA, MSCoNS and MRCoNS were 50(29.9%), 30(17.9%) and 39(23.3%) respectively.

Age	MSSA	MRSA	MSCoNS	MRCoNS
Below 40	45(69.2%)	20(30.8%)	15(60.0%)	10(40.0%)
Above 41	22(45.8%)	26(54.2%)	8(66.6%)	4(33.3%)
Total	67(59.3%)	46(40.7%)	23(40.0%)	14(40.0%)

[Table/Fig-1]: Age wise distribution of isolates *Staphylococcal* species

Organism isolated	Methicillin sensitivity	No. of isolates	Percentage (%)
Staphylococcus aureus	a) MSSA b) MRSA Total	67 46 113	59.3 40.7 100
CoNS	a)MSCoNS b)MRCoNS Total	23 14 37	62.2 37.8 100

[Table/Fig-2]: Distribution of *Staphylococcal aureus* and CoNS with respective to methicillin sensitivity

Methicillin Sensitivity	Mupirocin resistant (%)	Low level mupirocin resistant (MuL)	High level mupirocin resistant (MuH)
MRSA	1(2.2)	0	1
MSSA	2(2.9)	1	1
MRCONS	2(14.3)	1	1
MSCONS	0	0	0

[Table/Fig-3]: Distribution of MuL and MuH resistant isolates of *Staphylococcus* species

Among the 46 MRSA isolates, 26 were found to be from patients who were above 41 years of age and 20 isolates were from patients who were below 40 years of age. The proportion of MRSA in patients who were above 41 years of age was 54.2% (26/48) and it was 30.8% (20/65) in patients who were below 40 years. The difference was statistically significant (P< 0.05). In support to our findings, a study which was done in a Saudi hospital [19] reported to have isolated more MRSA in the elderly age groups (above 60 years).

The mupirocin resistances among the 150 *Staphylococcal* isolates were 5 (3.3%) and the remaining strains were sensitive. This showed that a majority of the coagulase positive and the coagulase negative *Staphylococcus* species were sensitive to mupirocin. A marginal increase in this percentage was observed by another author [5], in which, out of 297 clinical isolates, 4.8% *Staphylococcus* species showed resistance to mupirocin. However, the percentage (3.3%) of resistance to mupirocin in our study was analyzed in detail, in terms of high level and low level mupirocin resistances. The high level mupirocin resistance among the 46 MRSA isolates was only one (2.2%). In a study which was done at a Pakistan hospital,[20] among 156 MRSA isolates, one (0.7%) showed a low-level resistance and none of the isolates showed high level resistances. As per the surveillance program reports [8] of 1995 to 1999, the proportions of the MRSA strains with high and low-level mupirocin resistances were 1.6% and 6.4%, respectively, whereas as per those of 2000 to 2004, the resistant rates were 7.0% and 10% respectively, which showed that there was a considerable increase in the percentage of the resistance upon the usage of mupirocin.

In our study, 67 (44.7%) were Methicillin-Sensitive *Staphylococcus Aureus* (MSSA). Out of the 67 MSSA, only 2(3.0%) were mupirocin resistant, in which one showed high-level (200 µg) and another one showed low level (5µg) mupirocin resistance, whereas a study that included Staphylococci from 19 European hospitals [5] showed 0.9% of low-level and high-level resistance in the MSSA, particularly in skin and soft tissue infections. The difference in the proportions of the mupirocin resistance between the MRSA and the MSSA was not statistically significant, but a high level of resistance is very important in the MRSA isolates, as it confers an additional *mupA* gene in a plasmid that can be transferred to other strains by plasmid conjugation [21].

The mupirocin resistance in CoNS was quite less in the present study as compared to that of *Staphylococcus aureus.* Among the 14 methicillin resistance CoNS, two (14.3%) showed mupirocin resistances. Among these, one high and one low level resistance were detected. A much higher percentage (28.8%) of mupirocin resistance was reported among the MRCoNS by a Korean hospital [22]. Moreover, almost double the percentage (14.7%) of the low-level mupirocin resistance and almost a similar percentage (8.3%) of the high-level mupirocin resistance were recorded by them. None of the MSCoNS were resistant to mupirocin in our study.

Studies	MSSA		MRSA		MSCoNS		MRCoNS	
	Low (%)	High (%)	Low (%)	High (%)	Low (%)	High (%)	Low (%)	High (%)
Present study	1 (1.5)	1 (1.5)	_	1 (2.2)	_	_	1 (7.1)	1 (7.1)
Hee-Jeong Yun et al [22]	_	1(0.3)	_	15 (4.7)	4 (2.0)	4 (2.0)	30 (14.7)	17 (8.3)
Franz-Josef Schmitz*et al* [5]	2 (0.9)	2 (0.9)	2 (3.5)	1 (1.8)	1 (9.1)	2 (18.2)	3 (25.0)	1 (8.3)
Oommen *et al* [18]	_	_	_	1 (2.08)	_	_	_	11 (28.2)

[Table/Fig-4]: Distribution of low and high level mupirocin resistant *Staphylococcal* isolates in various studies

Among the three mupirocin resistance strains of *Staphylococcus aureus*; one each from MSSA and MRSA showed MICs of 1024 mg/L, thus confirming their high-level resistances by the disc diffusion method and one another methicillin-sensitive *Staphylococcus aureus* showed an MIC of 256mg/L, thus confirming its low-level resistance. The comparison charts of various studies showed the low and high level mupirocin resistances in MRSA, MSSA, MSCoNS and MRCoNS, as has been shown in [Table/Fig-4].

Varying percentages of MRCoNS and MSCoNS were reported by different studies, which ranged from 7.1%-25.0% for the low-level mupirocin resistance among the MRCoNS and which ranged from 7.1%-28.2% for the high-level mupirocin resistance among the MRCoNS. Those studies which reported the mupirocin resistance among the MSCoNS have a range of 2% to 9% in low-level and 2%-18.2% in high-level mupirocin [5].

In the hospitals where the mupirocin use was common, the prevalence of the mupirocin resistance among the MRSA was higher as compared to the hospitals where the mupirocin use was infrequent. As compared to other studies, a considerably lower percentage (2.2%) of high-level mupirocin resistance among *Staphylococcus aureus* was noted in this study, which suggested that in spite of the usage of mupirocin, the resistance was low in our hospital setup.

As has been cited in the literature, [21] the mupirocin resistances are transferred from the mupirocin resistant strains of *Staphylococcus aureus* to the mupirocin susceptible *Staphylococcus aureus* strains via a conjugative plasmid. Furthermore, this plasmid was transferred between *Staphylococcus aureus* and CoNS, mainly to *Staphylococcus epidermidis*. This result indicates the possibility of a horizontal transfer of the conjugative plasmid among the *Staphylococcus* species and it also suggests that *Staphylococcus epidermidis* could be a reservoir of this plasmid. In our study, we isolated mupirocin resistant CoNS which were identified as *Staphylococcus epidermidis and Staphylococcus saprophyticus.*

Hence, we conclude that the screening for mupirocin resistance, in terms of high-level and low-level resistances among the *Staphylococcus* species from patients with skin and soft tissue infections is warranted and that it is important for the clinicians in selecting the appropriate empirical antimicrobial therapy, once the prevalences of these resistant pathogens are known in their own hospital setup.

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