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Detection of high and low level mupirocin resistance among clinical isolates of *Staphylococcus aureus*

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Abstract

Introduction*: Staphylococcus aureus* infections have become more difficult to treat due to multidrug resistance. Therefore removal of nasal carriage of Staphylococci plays a major role in infection control. Mupirocin is an effective topical agent for nasal decolonization. Existence of high level mupirocin resistance (HLMR) excludes its use and low level resistance (LLMR) need higher dosage recommendations. Hence screening of HLMR and LLMR in a hospital is essential.

Materials and Methods: A total of 100 isolates obtained from skin and soft tissue infections and blood were used and the study was carried out at Chettinad hospital and Research Institute, Kelambakkam, Chennai, India. HLMR and LLMR were detected using 200 µg and 5µg mupirocin discs respectively. The MIC for mupirocin resistant isolates was determined by agar dilution method.

Results: Among the 100 strains tested 79 were MSSA (Methicillin sensitive) and 21 were MRSA (Methicillin Resistant). Out of 100 strains 7 (7%) were mupirocin resistant. Out of the seven resistant isolates, one isolate (MSSA) was resistant to 5µg mupirocin and six (2 MRSA and 4 MSSA) isolates were resistant to 200 µg mupirocin. MIC for LLMR and HLMR isolates was 8µg/ml and ≥1024µg/ml respectively.

Conclusion: Emergence of LLMR and HLMR isolates has become more common because of its repeated use. Hence continual monitoring for its prevalence is very much needed and also can guide the clinician to select appropriate empirical therapy.

Keywords: Staphylococcus aureus; Low level Mupirocin resistance; High level Mupirocin resistance; Methicillin Resistance.

Introduction

Staphylococcus aureus is a pathogenic Gram positive coccus that can also occur as normal microbial colonizers in the external nares. These colonizers can be introduced into sterile sites of the body during trauma resulting in life threatening infections both in the community as well as in nosocomial settings. Burden of treating these infections have become more complicated due to the emergence of Methicillin resistant and Vancomycin Intermediate Resistant Staphylococcus aureus (MRSA and VISA) which result in treatment failures[1,2,3]. So decolonization of these isolates from exogenous sources especially from external nares can help in control of MRSA and VISA infections particularly in hospitals[4,5]. Decolonization is usually done by intranasal application of Mupirocin[6,7]. Low level mupirocin resistance is due to point mutations that occur in the gene coding for tRNA synthetase, the native gene *ileS-1* and High level mupirocin resistance (HLMR) is due to a gene encoded in a plasmid called Mup A gene also called as *ileS- 2*, which encodes an additional modified isoleucyl t-RNA synthetase. HLMR strains have a MIC range ≥ 512 µg/ml[8]. Since use of mupirocin for nasal decolonization plays a major role in infection control, its repeated use for nasal decolonization and as topical agent for treatment has resulted in the emergence of mupirocin resistant isolates[9,10].

Materials and Methods

Collection of Bacterial strains: A total of 100 non-duplicate S.aureus strains were obtained from various clinical specimens. About 97 isolates were from skin and soft tissue infections, two isolates were obtained from blood stream infection and one isolate was from the sputum of a patient with respiratory tract infection. All these strains were obtained during a period of 4 months, from May to August 2014. These isolates were stocked in Brain Heart Infusion broth with 20% glycerol and stored until used for the study. The present study was carried out in the Department of Microbiology at Chettinad Hospital and Research Institute, Kelambakkam.

Identification of S.aureus isolates: Staphylococcus aureus isolates were identified by standard microbiological procedures. Identification protocol includes growth on blood agar, gram staining (Gram positive cocci in clusters), positive reaction for catalase, tube coagulase and mannitol fermentation[11,12].

Detection of MRSA isolates: Detection of Methicillin resistance was carried out according to CLSI guidelines 2014. Kirby Bauer disc diffusion technique was carried out by using 30µg Cefoxitin disc, a surrogate marker for mecA mediated resistance. A standard bacterial suspension of 0.5 MacFarland standards was inoculated into Muller Hinton agar plates as a lawn inoculum. The plates were incubated at 37^0C overnight and zone of inhibition for the isolates were measured. A zone diameter of ≥ 22mm was considered as sensitive and ≤ 21mm was considered as resistant[13].

Detection of Mupirocin resistance: Detection of Mupirocin resistance was carried out for all the 100 S.aureus strains which included both Methicillin sensitive and resistant isolates. This was performed as per the CLSI guidelines 2014, by the Kirby Bauer disc diffusion technique. 5µg mupirocin disc was used for the detection of Low level Mupirocin resistance and 200µg mupirocin disc was used for the detection of High level Mupirocin resistance. A zone diameter of ≤ 13mm was considered as resistant and ≥ 14mm was considered as sensitive. S.aureus ATCC 25923 strain was used as a quality control strain[13].

Detection of Minimum inhibitory concentration: For the mupirocin resistant isolates, Minimum inhibitory concentration was determined by agar dilution method as per the CLSI guidelines. Mupirocin powder was obtained from Himedia Laboratories. Agar dilution method was performed in Muller Hinton agar plates with varying dilutions of the antibiotic prepared from stock solution. The final mupirocin concentration ranged from 0.016 to 1024µg/ml. Strains that required mupirocin concentration of ≤4µg/ml for its inhibition were considered as sensitive strains, those strains showing an MIC between 8 and 256µg/ml were considered as low level resistance strains and finally those strains that required ≥ 512µg/ml for inhibition were considered as high level resistance strains. ATCC 25923 was used as a quality control strain and the acceptable range usually falls between 0.016-0.5µg/ml[13,14].

Results

Of the total 100 non – duplicate S.aureus isolates the majority of the strains (97%) was isolated from pus and wound swabs. Out of the 100 S.aureus isolates 65 were from male patients and 35 isolates were from female patients.

Among the 100 strains 79 were found to be MSSA and 21 were found to be MRSA. Of the 21 MRSA isolates 20 isolates were associated with skin and soft tissue infection. Only one isolate was found to be associated with S.aureus bacteremia. Most of the MRSA strains (18) were from male individuals and only 3 from female patients.

Out of 100 strains of S.aureus 7 (7%) were found to be Mupirocin resistant (Table 1). Of the 79 MSSA isolates, only one isolate demonstrated low level Mupirocin resistance (1.26%) and 4 isolates demonstrated HLMR. Of the 21 MRSA, 2 (9.5%) showed HLMR and no isolates displayed low level mupirocin resistance. Out of the seven mupirocin resistant isolates, one isolate was resistant to 5µg Mupirocin and six (2 MRSA and 4 MSSA) isolates were resistant to 200 µg Mupirocin (Table 2). All the Mupirocin resistant isolates were associated with skin and soft tissue infection (Table 3).

Minimum inhibitory concentration for Low and high level mupirocin resistant isolates was found to be 8µg/ml and ≥1024µg/ml respectively. For the other Mupirocin sensitive isolates MIC was found to be ≤ 4µg/ml.

Table 1: Prevalence of Mupirocin resistance among MSSA and MRSA isolates

	No. of isolates (%)	Mupirocin resistant isolates (%)
MSSA isolates	79	6.3
MRSA isolates	21	9.5

Table 2: Distribution of low and high level Mupirocin resistance among MSSA and MRSA isolates

	High level Mupirocin Resistance (%)	Low level Mupirocin resistance (%)
MSSA isolates (n =79)	5.06	1.26
MRSA isolates (n = 21)	9.52	0

Table 3: Distribution of MRSA, HLMR, LLMR strains based of clinical source

	Skin and soft tissue infection (n = 97)	Blood stream infection (n = 2)	Respiratory tract infection (n = 1)
MRSA isolates	20 (20.61 %)	1 (50.0%)	0
Low level Mupirocin resistant isolates	1 (1.03 %)	0	0
High level Mupirocin resistant isolates	6 (6.18%)	0	0

Discussion

Mupirocin acts as an effective topical antibacterial agent for treating *S.aureus* infection and also for nasal decolonization[15]. Frequent use of mupirocin creates selective pressure which will result in development of its resistance. In spite of its emerging resistance mupirocin prevails as one important topical agent for use[16].

Among the 100 isolates 5 MSSA isolates and 2 MRSA isolates were found to possess Mupirocin resistance. Among the MSSA, 6.32% were found to be Mupirocin resistant and among the MRSA 9.52% were found to be Mupirocin resistant. A similar study from south India by Jayakumar et al., gives a prevalence of Mupirocin resistance among MRSA and MSSA isolates as 2% and 3% respectively[17]. The findings of this study were not consistent with our study report on Mupirocin resistance.

Only one S.aureus isolate (MSSA) was found to have Low level Mupirocin resistance. This report was consistent with another study from south India which also gives a 1% prevalence rate of LLMR strains[17]. In our study MIC value for LLMR strains was found to be 8µg/ml where as another study gave MIC range values of 256µg/ml[17].

High level Mupirocin resistance was found to be 9.52% among MRSA isolates and 6.32 % among MSSA isolates. Another study from Korean hospital give a prevalence rate of HLMR prevalence rate in MRSA and MSSA isolates as 4% and 0.3% respectively[18]. HLMR prevalence rate among MRSA isolates was found to be marginally higher than in MSSA in our study. All the HLMR strains showed MIC values of ≥1024µg/ml. A similar study from Korean hospital gives MIC values of ≥1024µg/ml for all the HLMR strains[18].

High level Mupirocin resistance will eliminate the use of Mupirocin for treatment as well as for nasal decolonization of MRSA. However, low level resistant isolates can still be eliminated with a higher dosage of mupirocin. But this again remains as a possible risk factor for the development of treatment failure with mupirocin.

In the present study out of the 100 isolates 21% of the isolates were found to be MRSA. Two studies from south India by Oommen SK et al., & Jayakumar S et al., report MRSA prevalence rates of (28.7%) and (44.7%) respectively[17,14]. MRSA prevalence rates obtained in our study was found to be low when compared to the other studies from south India. ANSORP report gives a MRSA prevalence rate of 22.6% among hospital acquired infections[19]. MRSA prevalence rates were found to be higher among male patients (18%) than females (3%). This was found to be consistent with another study from Nepal where the MRSA prevalence rate among males was higher when compared to females[20].

Conclusion

Emergence of mupirocin resistance in ***Staphylococcus aureus*** is increasing because of its repeated use for nasal decolonization and as topical application for treatment of wound infections. In our study about 6% of the total S.aureus isolates were found to possess High level Mupirocin resistance. Hence continued monitoring for the prevalence of high and low level mupirocin résistance among S.aureus isolates in a hospital remains very essential. This helps the clinician to select appropriate alternate therapy.

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