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MUPIROCIN RESISTANCE IN METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Mupirocin is the topical antimicrobial agent which is used for the treatment of methicillin resistant Staphylococcus aureus (MRSA). However the prevalence of mupirocin resistance in MRSA has increased with the extensive and widespread use of this agent in hospital settings. The present study was attempted to evaluate the low level and high level resistance to mupirocin in MRSA isolates by different phenotypic and genotypic methods. Isolates of MRSA were collected from different clinical specimens obtained from SRM hospital and were identified by routine biochemical reaction. Resistance to mupirocin was determined by Kirby Bauer disc diffusion method (using 5µg mupirocin) as per CLSI guidelines. Low level and high level resistance to mupirocin was confirmed by minimum inhibitory test (MIC) using agar dilution method and comb test. The presence of *mupA* gene in resistant strains of MRSA was confirmed by PCR method. Among 75 MRSA isolates, 16(21.3%) were mupirocin resistant. Based on MIC (comb test) 9 (12%) showed low level resistance and 7(9.3%) showed high level resistance to mupirocin.*Mup-A* gene was present in all the 16 strains.

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INTRODUCTION

MRSA is emerging as a common human pathogen causing several nosocomial infections and is generally recognised as the most significant pathogen because of the burden of serious diseases it causes and its multi drug resistance nature[1]. Mupirocin (pseudomonic acid A) is a topical antibiotic that has been extensively used for treating MRSA skin and soft-tissue infections, decreasing certain types of surgical site infections and eliminating nasal colonisation of MRSA among patients and medical staff[2,3,6]. However, the massive use of this agent has led to the rapid emergence of resistance worldwide. Mupirocin specifically binds to bacterial isoleucyl-tRNA synthetase (IRS) and inhibits protein synthesis [2]. Two mupirocin resistance phenotypes namely low level (MuL) and high level (MuH) mupirocin resistance are defined in *Staphylococci*.MuL (with MICs of 8-256 µg/ml) is frequently related to site mutations in the chromosomal isoleucyl-tRNA synthetase gene, where as MuH (with MIC ≥512 µg/ml) is generally due to acquisition of a plasmid hosting the mupA gene encoding an additional novel isoleucyl tRNA synthetase whose function is not inhibited by mupirocin [4]. High level mupirocin resistance is mediated by *mup A* gene that was previously reported exclusively on mupirocin resistance plasmids. However *mup A* gene has also been reported in the genomic DNA of a few MRSA isolates expressing low level mupirocin resistance, suggesting that the *mup A* gene may also be located in the chromosome. The study was carried out to primarily determine the rates of high level and low level mupirocin resistance in MRSA by disk diffusion and MIC methods and also to investigate whether *mup A* gene conferred mupirocin resistance in low and high level mupirocin resistant MRSA isolates [5].

MATERIALS AND METHODS

Bacterial isolates

Isolates of MRSA were collected from various clinical specimens: pus(59),blood(5),urine(6),tracheal aspirate(1),wounds swab(2),surgical pus(1),synovial fluid(1) in SRM hospital. *MRSA* was identified by routine microbiological procedures including gram staining, catalase test and coagulase test.

Antimicrobial susceptibility testing

Susceptibility to antimicrobial agent was done by *Kirby Bauer* disk diffusion method using cefoxitin(30µg/ml),cefuroxime(30µg/ml),cephalothin(3oµg/ml),clindamycin(2µg/ml),cotromoxazole(1.25/23.75µg/ml),erythromycin(15µg/ml), penicillin(10units),chloramphenicol(30µg/ml),linezolid(30µg/ml),vancomycin(30µg/ml) and mupirocin(5 µg) as per CLSI guidelines. *Staphylococcus aureus* strains resistant to cefoxitin were considered as MRSA. Mupirocin resistance in MRSA strains were determined by disk diffusion method. It was confirmed by MIC agar dilution method with mupirocin concentration varying from 0.016 to 1024 µg/ml. *Staphylococci* requiring concentrations of mupirocin of ≤4 µg/ml for growth inhibition were considered as "susceptible", those requiring concentrations of 8-256 µg/ml for inhibition were considered as "low level resistance", and those requiring concentrations of ≥ 512 µg/ml were considered as "high level resistance". The MIC of mupirocin was further confirmed by using comb test (Hi Media MD034) ranging from 0.01-240 µg/ml according to manufacturer's instructions.

Mup-A gene detection using PCR.

Genomic DNA extraction was done by using (Pure Fast Bacterial genomic DNA purification kit) from all strains of *MRSA* which showed resistance to mupirocin by both disk diffusion and MIC (comb test and agar dilution method).

Primers used

Mup-A (500bp) -F TATATTATGCGATGGAAGGTTGG
R AATAAAATCAGCTGGAAAGTGTTG

Amplifications conditions were, initial denaturation at 94^0C for 3min ,30 cycles of 94^0C for 1 min,55^0C for 1 min,72^0C for 1 min and final extension at 72^0C for 5 min.

RESULTS

Total 130 isolates of *Staphylococcus aureus* were collected from different clinical samples. Among 130 isolates *Staphylococcus aureus*,75(57.69%) were resistant to cefoxitin hence considered as MRSA.The percentage of resistance in MRSA to different antibiotics were,cefuroxime(40%),cephalothin(44%),clindamycin(13%),erythromycin(30%),cotrimoxazole(56%),penicillin(88%),chloramphenicol(17%), vancomycin(0%),linezolid(7%) and mupirocin(20%). Among 75 MRSA isolates 16(21.3%) were resistant to mupirocin by disk diffusion method. Based on MIC agar dilution and comb test (figure-1), out of 16 mupirocin resistant strains by disk diffusion method, 9 (12%) show low level resistant and 7(9.3%) show high level resistance to mupirocin . All the 16 strains (both high level and low level mupirocin resistant) were positive for *Mup-A* gene by PCR (Figure-2).

Figure-1 ***MIC by Comb test***

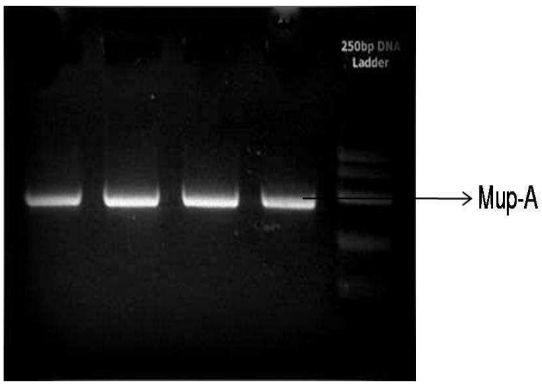

Figure-2 ***Positive mup-A gene of 500bp size***

DISCUSSION

The emergence of mupirocin resistance among MRSA isolates has been a well-defined phenomenon in many parts of the world[8].Confluence of several current clinical and epidemiologic trends contribute to increase mupirocin use in both community and health care settings and could trigger an increase in mupirocin resistance. Widespread use of mupirocin ointment has been considered as a major cause to develop and propagate mupirocin-resistant organisms. According to one study the high level and low level mupirocin resistance in MRSA isolates are 26.1% and 44.1% respectively [7], similarly in our study we found less high level (9.3%) and more low level (12%) mupirocin resistance. Genotypic method such as PCR can be used as the final confirmatory test for detection of mupirocin resistance in *MRSA* isolates.

CONCLUSION

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 even in facilities where mupirocin is

not administered. This will facilitate the early detection of resistance and assist in the control and spread of mupirocin-resistant MRSA.

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