A Study of Inducible Clindamycin Resistance among *Staphylococcus aureus* Skin and Soft Tissue Infections in A Tertiary Care Hospital

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ABSTRACT

Objectives: Drug resistant phenotypes like MRSA are difficult to treat requiring higher group of antibiotics. Topical agents like clindamycin can be used for the therapy of MRSA. The knowledge of prevalence of inducible clindamycin resistance phenotype is essential to prevent treatment failure.

Methods: A total of 204 staphylococcal isolates obtained from skin and soft tissue infections and MRSA was detected by Cefoxitin disc diffusion method and detection of *Mec A* gene by Polymerase chain reaction (PCR). Antibiotic susceptibility testing was performed by Kirby Baeur disc diffusion method. The Erythromycin resistant isolates were tested for D test. The differences in antibiotic susceptibility pattern between MRSA and MSSA was compared by Chi Square test using Graph pad Quick Calcs software and p value less than 0.05 was considered as significant

Results: Out of the 204 Staphylococcus aureus isolates, 48 (23.5%) were identified as MRSA by Cefoxitin disc diffusion method. All these 48 (23.5%) isolates were also positive for *Mec A* gene by PCR. Inducible clindamycin resistance (iMLSB resistance phenotype) was observed among 24 (11.7%) of the isolates. MRSA showed comparatively lesser susceptibility than MSSA (p ≤0.05). Among the MRSA inducible clindamycin resistance was seen among 11 (22.9%).

Conclusions: Emergence of drug resistance warrants antibiotic susceptibility testing for all the isolates in the laboratory. Cefoxitin disc diffusion method can be used in resource constraint laboratory where PCR facilities are not available. Inducible clindamycin resistance phenotype (iMLS phenotype) must be checked for all isolates showing erythromycin resistance to prevent treatment failure. *J Microbiol Infect Dis 2019; 9(3):125-128.*

Keywords: *Methicillin resistant Staphylococcus aureus (MRSA), iMLSB resistance phenotype*

INTRODUCTION

Infections caused by Staphylococcus aureus is a growing problem worldwide with Methicillin resistant Staphylococcus aureus (MRSA) posing a therapeutic challenge. The various risk factors associated with MRSA infections are previous hospitalizations [1]. Erythromycin and clindamycin are used as topical therapeutic agents for treatment of skin and soft tissue infections associated with Staphylococcal infections [2].

However wide spread use of the Macrolide, Lincosamide, Streptogramin group of drugs have led to emergence of resistance. The *erm* gene is responsible for clindamycin resistance. The knowledge of prevalence of inducible clindamycin resistance phenotype is essential to prevent treatment failure.

The present study aims to isolate and identify the MRSA by Cefoxitin disc diffusion method and Polymerase chain reaction for detection of *Mec A* gene and to detect inducible clindamycin resistance by D test. A positive D-test indicates the presence of an *erm* gene resulting in clinical failure [3].We also aim at ascertaining the difference in D test among MRSA and Methicillin Sensitive *Staphylococcus aureus* (MSSA).

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METHODS

The present study was conducted from September 2016 to June 2017 at the department of microbiology and approved by the institutional ethical committee. A total of 204 Staphylococcus aureus isolates were obtained from skin and soft tissue infections and included in the study. The isolates were identified as *Staphylococcus aureus* by Standard Microbiological techniques.

Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method using Amoxicillin (30 µg), Erythromycin (15 µg), Clindamycin (2 µg), Gentamicin (30 µg), Cotrimoxazole (25 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Vancomycin (30 µg) and linezolid (30 µg).

Methicillin resistance was detected by Cefoxitin disc diffusion method and results interpreted as per CLSI guidelines [4].

The isolates showing Erythromycin resistance were subjected to D test. The results were interpreted as per CLSI guidelines [4].

The erythromycin disc (15 µg) disc was placed at a distance of 15 mm edge to edge and incubated at 37^0 C. The results were interpreted as per CLSI guidelines. Flattening of zone (iMLSB resistance phenotype, D shape near the clindamycin disc) indicates a positive test for inducible clindamycin resistance. Two other phenotypes was also be interpreted [5].MS phenotype- circular zone of inhibition around clindamycin disc and resistance to erythromycin, cMLS$_B$ phenotype- resistance to both clindamycin and erythromycin.

Polymerase chain reaction for detection of *Mec A* gene

HiPurATM Genomic purification kit (HIMEDIA) was used for DNA isolation and the steps were followed as per the manufacturer's instruction. MRSA Detection Kit (Uniplex) was utilized for amplification of *Mec A* gene using specific primers and controls. The following PCR program was followed. Initial denaturation of 94 ^{0}C for 10 minutes following by denaturation at 94^0 C for 1 minute, Annealing at 60 ^{0}C for 1 minute followed by extension at 72 ^{0}C for 1 minute (30 cycles) and final extension at 72 ^{0}C for 10 minutes. After amplification the products were subjected to Agarose gel electrophoresis [6].

Agarose gel electrophoresis

The amplicons were loaded along with 6 X gel loading dye on to 1.5% agarose gel incorporated with Ethidium bromide .DNA ladder (100 bp) was also loaded for confirming the size of the amplicon and observed under UV trans illuminator. For the isolates positive for *Mec A* gene bands were observed at (293 bp) [6].

Statistical Analysis

The differences in antibiotic susceptibility pattern between MRSA and MSSA was compared by Chi Square test using Graph pad Quick Calcs software and p value less than 0.05 was considered as significant.

RESULTS

A total of 204 Staphylococcus aureus isolates were obtained from skin and soft tissue infection over a period of one year. Among them 48 (23.5%) were identified as MRSA by Cefoxitin disc diffusion method. All these strains were also positive for *Mec A* gene by Polymerase chain reaction. Overall highest sensitivity was observed for Ciprofloxacin 164(80.34%) and Gentamicin 156(76.47%). Least susceptibility was observed for Amoxicillin 66(32.3%).All the isolates were sensitive to Vancomycin and Linezolid.

In the present study MS phenotype (Erythromycin resistance) was seen among 63 (31%) of the Staphylococcal isolates. cMLS$_B$ phenotype was observed among 38(18.6%) and Inducible clindamycin resistance was noted among 24 (11.7%) of the isolates (Figure 1). Among the MRSA 11(22.9%) showed inducible clindamycin resistance phenotype. There was a significant difference in the Antibiotic sensitivity pattern among the MRSA and MSSA (Methicillin sensitive Staphylococcus aureus) as depicted in Table1 1 (p ≤0.05). MRSA isolates showed lesser susceptibility than MSSA for all the antibiotics tested except for amoxicillin where MSSA showed a higher susceptibility. (p=0.543).

Among the 48 isolates of MRSA, 22 were of Community origin (CA- MRSA) and 4 (18.18%) isolates showed inducible clindamycin resistance.

DISCUSSION

Emergence of Multidrug drug resistant organism has made determining the antibiotic susceptibility pattern as essential. In the present study the Prevalence of MRSA was 23.5%.Studies across India has reported prevalence of MRSA ranging from 23 % to as high as 74% [7,8].These differences could be attributed to variations in risk factors for acquisition of resistance and infection prevention practices and antibiotic stewardship programs.

We have compared Cefoxitin disc diffusion method and PCR to detect *Mec A* gene for the identification of MRSA isolates. Both the methods were 100 % in concordance with each other in the present study. All the isolates that were identified as MRSA by Cefoxitin disc diffusion method was also *Mec A* gene positive by PCR similar to other studies[6].Detection of *Mec A* gene is the gold standard method for identification of MRSA. In case of resource constraint setups Cefoxitin a inducer of *Mec A* gene can be used for detecting MRSA.

The lesser susceptibility was observed among the MRSA strains for erythromycin, Clindamycin, Gentamicin, Ciprofloxacin, Cotrimoxazole compared to MSSA as depicted in table 1 which is statistically significant (p≤0.001). This was in concordance with other Studies who also reported that drug susceptibility was comparatively lower among the MRSA [9-14].An exception was Amoxicillin where the susceptibility was higher among the MSSA. Susceptibility of 100% was observed for Vancomycin and Linezolid. Usage of these reserve drugs should be restricted only to MRSA. This will prevent the emergence of glycopeptide resistance. Since therapeutic options for multidrug resistant isolates like MRSA is limited. Many treatment options are available for Skin and soft tissue infections caused by Staphylococcus with clindamycin being used for treatment of both MSSA and MRSA infections. Emergence of inducible clindamycin resistance is on the rise. A study by Aleksandra AD et al in central Serbia has reported iMLSB phenotype as high as 50% among the Staphyloccocal isolates [15]. Shenoy MS et al have reported inducible clindamycin resistance as 15.65% in a study in south India[16].A study by Deotale V, et al have reported iMLSB phenotype resistance percentage in the range of 27.6% among MRSA isolates [5]. In our hospital the overall prevalence of inducible clindamycin resistance among Staphylococcal isolates was 24 (11.7%) similar to Phukan C et al [8] .It was also observed that among MRSA 22.9% of the isolates showed inducible Clindamycin resistance.

Reporting of isolates as susceptible to clindamycin without checking for inducible resistance phenotype (iMLS phenotype) will result in treatment failure. The raising resistance to Clindamycin is of important concern as isolates reported as sensitive without checking for inducible resistance by D test on those isolates showing Erythromycin resistance will result in treatment failure.

Table 1. Clindamycin susceptibility patterns of the *Staphylococcal isolates*

Antibiotics	MRSA, n (%)	MSSA, n (%)	P Value
Erythromycin	15 (31.25%)	126 (80.76%)	<0.001
Clindamycin	16 (33.33%)	132 (84.61%)	<0.001
Gentamicin	16 (33.33%)	140 (89.74%)	<0.001
Ciprofloxacin	26 (54.16%)	138 (88.46%)	<0.001
Cotrimoxazole	18 (37.50%)	116 (74.35%)	<0.001
Amoxicillin	10 (20.83%)	56 (35.89%)	0.0543
Vancomycin	48(100%)	156 (100%)	NA
Linezolid	48 (100%)	156 (100%)	NA

MRSA=Methicillin-Resistant Staphylococcus aureus, MSSA= Methicillin-Sensitive Staphylococcus aureus
Number of MRSA=48, Number of MSSA=156

In conclusion Methicillin resistance should always be routinely identified in the laboratory as shown by higher antibiotic resistance among MRSA. Cefoxitin disc diffusion method should be used as method of detection of MRSA in resource constraint labs where *Mec A* gene detection is not feasible. Effective Infection control practices and surveillance protocols can prevent MRSA associated infections. All erythromycin resistant Staphylococcal isolates should be tested for inducible clindamycin resistance to prevent treatment failure.

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