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Research Article

DETECTION OF MRSA USING THREE DIFFERENT METHODS SUCH AS OXACILLIN AGAR SCREENING METHOD, CEFOXITIN DISC DIFFUSION METHOD AND MECA GENE BY PCR AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS FROM TERTIARY CARE HOSPITAL IN KANCHEEPURAM DIST

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A B S T R A C T

Cefoxitin is a good marker of the *mecA* regulatory system. It is being recommended for detection of methicillin resistance in *Staphylococcus aureus* (MRSA) using disk diffusion testing. The aim of our study was to evaluate the efficacy of cefoxitin disc diffusion test to characterize MRSA and compare it with oxacillin agar screening and detection of *mecA* gene by PCR. We have observed 100% sensitivity and specificity in cefoxitin disc diffusion method over detecting *mecA* gene by PCR. Results of cefoxitin disc diffusion test is in concordance with the PCR for *mecA* gene. Thus, the test can be an alternative to PCR for detection of MRSA in resource constraint settings.

INTRODUCTION

Staphylococcus aureus is an everyday bacterial pathogen causing infection among humans. It causes skin infections, osteoarthritis and respiratory tract infections in the community, as well as postoperative and catheter-related infections in hospitals [1]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a chief bacterial pathogen causing nosocomial and community onset infections [2-3]. It's prevalence has increased in various parts of the world causing serious infections in hospitals that poses a serious challenge in terms of medical and socio-economic costs and cause significant morbidity and mortality [4].

The increase in colonization rates of *S. aureus* haslead to an increase of infection rates among communities and in hospitals. The consequences faced by the health care systems are longer hospital stays and greater costs of treatment, which approximately doubles the expenditure per patient and this is ultimately a huge burden on patients with low socio-economic status [5]. A patient's risks include significantly higher mortality and morbidity rates with invasive MRSA infection. Health care workers may carry MRSA on their hands or clothes following their contact either with asymptomatic carriers or patients who have clinical infection [6].

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Resistance of Staphylcocci to methicillin and all the beta-lactam antibiotics is linked with the low affinity of a penicillin binding protein PBP2a, which is not present in susceptible staphylococci [7-8]. This protein is encoded by the *mecA* gene, which is located in the mec region and is DNA of foreign origin [9].

MATERIALS AND METHODS

Bacterial isolates

A total of 20 clinical isolates of *S.aureus* were collected from different clinical specimens of patients attending Saveetha Medical Collage and Hospital. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy medium and stored at 4ºC until further use.

Antibiotic susceptibility test

Antibiotic susceptibility testing was determined for these isolates to the following antibiotics such as penicillin, erythromycin, clindamycin, ciprofloxacin, tetracycline, cotrimoxazole and linezolid. These antibiotics were procured from Himedia, Mumbai. This was performed by Kirby-bauer disc diffusion method as per CLSI guidelines [10].

Detection of MRSA

Oxacillin agar screening method

Mueller-Hinton agar (MHA) plates containing 4% NaCl and 6 µg/ml of oxacillin were prepared. Plates were inoculated with 10 µL of 0.5 McFarland suspension of the isolate by streaking in one quadrant and incubated at 35 ^{0} C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant [11].

Cefoxitin disc diffusion method

All the isolates were subjected to cefoxitin disc diffusion test using a 30µg disc. A 0.5 McFarland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37 ^{0} C for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 19 mm was reported as oxacillin resistant and ≥20 mm was considered as oxacillin sensitive [12].

Detection of mecA gene by PCR

S. aureus isolates were detected for the presence of *mecA*gene by PCR analysis. Detection of the gene was carried out using primer as depicted in table 1. Bacterial DNA was extracted by boiling lysis method. 1 µL of DNA extract was used as template for PCR reaction. A 50 µl PCR reaction consisted of 45 µl of master mix containing PCR buffer (1X), dNTP mix (0.2 mM of each), primer (0.5 µM), Taq DNA polymerase (0.25 U), and MgCl2 (1.5 mM) with 5 µL of template DNA. Cycling conditions were started at 94°C for 3 minutes followed by 30 cycles of denaturation at 94°C for 55 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute and final extension step at 72°C for 2 minutes. PCR products were visualized on 1.5% agarose gel with ethidium bromide dye under UV trans illuminator. A 100bp ladder was including in all the gel analysis.[13]

Table 1 Gene sequence of *mecA* gene

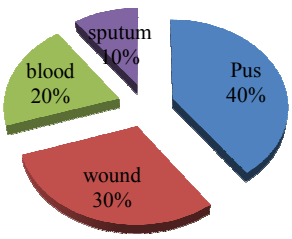
Primer	Primer sequence	Product size
mecA	5'-GTA GAAATG ACT GAA CGT CCG ATA A-3 5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'	310 bp

RESULTS

Sample wise distribution of clinical isolates of S. aureus

Of 20 clinical isolates of *S. aureus*, 8/20 (40%) were obtained from pus, 6/20 (30%) were from wound, 4/20 (20%) and 2/20 (10%) were from blood and sputum respectively (Figure 1).

Figure 1: pie chart showing the sample wise distribution of S. aureus



Antibiotic susceptibility pattern

We have observed a varied pattern of sensitivity among one *S. aureus*isolates. There was complete resistance observed for penicillin (100%), 9/20(45%) isolates were shown to the resistant to erythromycin, 6/20(30%) were to cotrimoxazole, 4/20(20%) were to linezolid followed by 3/20(15%) were resistant to ciprofloxacin and clindamycin respectively (Table 1) (Figure 2).

Table 2 Results of antibiotic susceptibility pattern of *S.aureus*

Antibiotics	Sensitive(%)	Intermediate(%)	Resistant(%)
Penicillin	0	0	20(100)
Erythromycin	14(70)	4(20)	2(10)
Clindamycin	15(75)	2(10)	3(15)
Ciprofloxacin	9(45)	8(40)	3(15)
Tetracyclin	14(70)	4(20)	2(10)
Cotrimoxazole	10(50)	4(20)	6(30)
Linezolid	10(50)	6(30)	4(20)

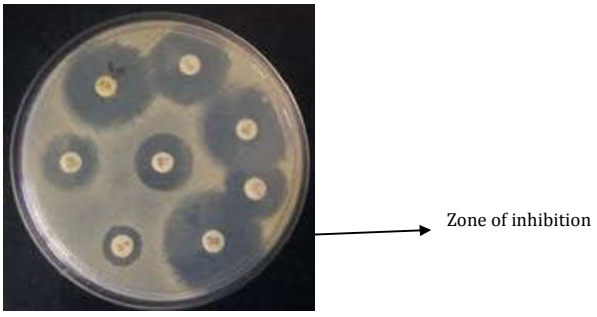


Figure 2 Representative picture showing antibiotic sensitivity pattern of S.aureus

MRSA detection by cefoxitin disc diffusion and oxacillin agar screening methods

Using cefoxitin distribution method 15/20(75%) were found to be MRSA isolates. Whereas, oxacillin agar screening method yielded 2/20(10%) of MRSA isolates (Figure 3 and 4).

Figure 3 Representative picture showing MRSA by Cefoxitin disc diffusion method

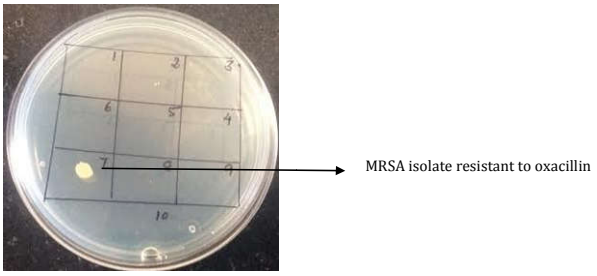


Figure 4 Representative picture showing MRSA by Oxacillin agar screening method

Result of*mecA* gene in *Staphylococcus aureus* by PCR:
Out of 20 clinical isolates of *S. aureus*, 15/20 (75%) of isolates were found to be positive for *mecA* gene.

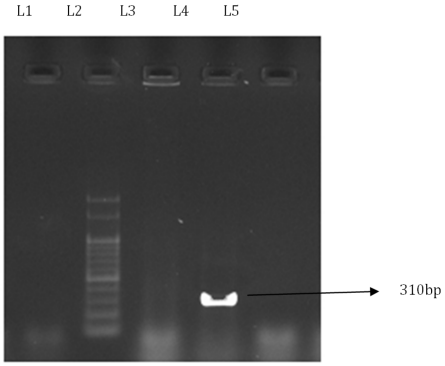


Figure 5 Representative gel picture showing *mecA* gene positive
L2 – 100bp ladder, L4 – *mecA* gene positive

Table 3: Results of MRSA detection using 3 different methods

***S.aureus*isolates**	**Cefoxitin disc diffusion method**	**Oxacillin agar screening method**	***mecA* by PCR**
20(100%)	15(75%)	2(10%)	15(75%)

DISCUSSION

Detection of MRSA is important for fine patient care and appropriate employment of infection control resources. Methicillin-resistant *S. aureus* (MRSA) is a substantial pathogen that has emerged over the last four decades, causing both nosocomial and community-acquired infections. Rapid and accurate detection of methicillin resistance in *S. aureus* is important for the use of appropriate antimicrobial therapy and for the control of nosocomial spread of MRSA strains [11].

Detection of *mecA* gene or its product, penicillin binding proteins (PBP2a) by PCR, has always been the gold standard for MRSA detection [14]. However recent studies have documented that disc diffusion testing using cefoxitin disc is more superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion and oxacillin screen agar testing due to its feasibility, and simplicity and is now an accepted method for the detection of MRSA by many reference groups including CLSI [15]. The prompt and early detection of methicillin resistance is crucial in the prognosis of infections caused by *S. aureus*. In this study, we have attempted to evaluate different methods for detecting MRSA.
During the last many years, the CLSI has attempted to improve the accuracy of detecting *mecA* strains among *S. aureus*. In our study, the observations using the CLSI disc diffusion criteria to define the resistance (cefoxitin zone diameters of ≤19 mm for resistance and ≥20 mm for sensitivity), sensitivity and specificity were 100% in the 20 strains tested in the study whereas the results of oxacillin screen agar were not as precise. Results of cefoxitin disc diffusion test is in concordance with the PCR for *mecA* gene, and thus the cefoxitin disk diffusion method is very suitable for detection of MRSA and the test can be an alternative to PCR for detection of MRSA in resource constraint settings.

CONCLUSION

Although the number of isolates used in this study are less, this study provides substantial evidence that cefoxitin can be used as an accurate marker in routine susceptibility testing. In addition, the results have shown 100% sensitivity and specificity as compared to *mecA* gene detection by PCR. Hence, it can be used as an alternative to the technically demanding PCR.

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