Detection of mecA genes of Methicillin-Resistant Staphylococcus aureus by Polymerase Chain Reaction

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Background: Methicillin-Resistant Staphylococcus aureus (MRSA) infections have become a global health problem particularly in hospital setup causing simple skin infections to life-threatening infections. In many developing countries, including India, the situation appears gloomy due to inadequate or poor implementation of policy on infection control, lack of political will, inadequate resources including shortage of skilled manpower, poor motivation of health care workers and researchers.

Objective: The present study describes a rapid and accurate PCR for detection of clinically relevant antibiotic resistance gene of Staphylococcus aureus.

Materials and Methods: A total of 586 Staphylococcus positive clinical samples were collected under aseptic precautions from patients attending various departments in a tertiary care hospital in south India. Staphylococcus aureus isolates were identified based on cultural characteristics, biochemical reactions and positive tube coagulase test. Methicillin resistance was determined by Kirby-Bauer’s disc diffusion method. The PCR was used for mecA gene detection from MRSA strains.

Results: Of the total 586 Staphylococcal aureus isolated, 236 (40.2%) strains were MRSA. Thirty-five MSRA strains were randomly selected for PCR assay. Thirty three MRSA strains (94%) were mecA gene positive and two MRSA strains were mecA negative visualised on 2% agarose gel electrophoresis.

Conclusion: The PCR assay was found to be rapid and accurate procedure for the detection of MRSA strains as compared to the conventional methods since the reporting time is less and can help efficiently in infection management.

Keywords: MRSA, PCR, mecA genes, Staphylococcal aureus

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INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an important bacterial pathogen causing nosocomial and community onset infections. The prevalence of MSRA has increased in many parts of the world causing serious infections in hospitals that pose a serious burden in terms of medical and socio-economic costs and cause significant morbidity and mortality.

The resistance in MRSA is due to the expression of Penicillin binding protein (PBP2a) encoded by *mec* genes, which is located on the Staphylococcal cassette chromosome (SCC). Different types of SCC *mec* cassettes were extensively studied by PCR techniques. The resistance mechanism involves changes or defects brought about by mutation on *mec* gene which results in the organism’s resistance to antibiotics. In addition, other antibiotic resistance genes may also be present in the cassette rendering resistance to multiple antibiotics.

Detection of *mec* gene by Polymerase chain reaction is considered as the gold standard for methicillin resistance as these genes are highly conserved among Staphylococcal species. In the present study, the PCR method was used for the detection of *mec* genes among the MRSA strains. The study aimed to set up a rapid and accurate detection procedure for methicillin resistance among Staphylococcal isolates through the amplification of specific gene determinants by PCR in order to treat clinical condition and in eradication of the pathogen.

MATERIALS AND METHODS

Clinical Isolates

A total of 586 clinical samples of Staphylococci were isolated from 2645 patients. Hospital ethical clearance (R.M.M.C.H ethical committee) was obtained prior to the start of the study. After obtaining an informed consent clinical sample which includes pyogenic materials, aspirates and wound secretions from patients who attended various departments at Rajah Muthiah Medical College and Hospital, Chidambaram (India) were collected. The specimens were collected under sterile aseptic conditions using sterile test tubes and swabs and transported immediately to the Microbiology laboratory for processing.

Culture

Direct smear examination by Gram’s stain followed by culture on nutrient agar, blood agar, Mac Conkey’s agar and chocolate agar at 37°C overnight incubation. The isolates were identified by characteristic colony morphology of *Staphylococcus* colonies in clusters was identified as staphylococci. Yellow-colored colonies of *Staphylococcus aureus* were obtained on Mannitol salt agar. The identification of *Staphylococcus aureus* was further confirmed by biochemical and tube coagulase test according to the methods described by Mackie and MacCartney.

Antibiotic Susceptibility Test

The Kirby-Bauer’s disc diffusion method using oxacillin 1 µg disc on 5% Muller-Hinton agar incubated overnight at 35°C was done for the detection of MRSA according to the guidelines recommended by the Clinical Laboratory Standards Institute (CLSI).

Detection of Mec A Genes by PCR

Thirty-five MRSA isolates were further subjected for the detection of *mec* genes using PCR at Sir Dorabji Tata Centre for Research in Tropical Diseases, Indian Institute of Science Campus, Bangalore (India).

Bacterial Genomic DNA Extraction

An overnight culture in brain heart infusion broth was collected by centrifugation and processed according to the procedure of Arakere *et al* (2005). The isolated DNA was stored at -20°C till further use.

PCR Protocol

A three step PCR method reported by Oliveira *et al* was carried out using XP thermal cycler. The already published primers (Bangalore genei) used for amplification was...
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The forward primer sequence (TCCAGATTACAACCTTCCACCGG) and reverse primer sequence (CAATTCATACTTGTAAACG) were used for amplification at 162 bp region and the forward primer sequence (ATCGATGGTAAAGGTTGGC) and reverse primer sequence (AGTTCTGCAGTACCGGATTTGC) for amplification at 530 bp region.

The conditions of PCR were as described by Murakami et al. which includes an initial denaturation at 94°C for 4 minutes followed by 35 cycles of an initial denaturation at 94°C for 30 seconds, annealing at 53°C for 30 seconds and extension at 72°C for 1 minute, final extension at 72°C for 4 minutes. The PCR products were then electrophoresed on 2% agarose gel, and amplified bands were analyzed in UVI tech gel documentation system (Bio-Rad Laboratories, Inc, USA).

RESULTS

Of the total 586 Staphylococcal aureus clinical isolates studied, 236 (40.2%) isolates were MRSA (inhibition zone of 10mm or less by Kirby-Bauer’s disc diffusion method using 1µg oxacillin disc on 5% Mueller-Hinton agar).

Detection of mec A Genes of MRSA by PCR

PCR results indicated that thirty three (94%) out of 35 MRSA isolates were positive for mec A genes as indicated by 162 and 530 base pairs regions whereas two isolates (6%) were mec A gene negative (absence of the corresponding band) (Fig - 1).

DISCUSSION

Early and accurate diagnosis of MRSA is crucial in effective management and control of spread of MRSA infections. PCR-based assays are considered as the gold standard for the detection of MRSA, due to the heterogeneous resistance by various phenotypic detection methods displayed by many clinical isolates. Genotypic methods are more accurate in detecting methicillin resistant Staphylococci as compared to conventional susceptibility methods.

In addition, several culture conditions can also influence methicillin resistance such as the temperature, pH and concentration of NaCl in the medium. Strains with low resistance level are usually complicated by these factors and impair the process of detection. The greatest disadvantage of phenotypic methods is that it is time-consuming and sometimes difficult to detect some strains. PCR can produce results within 24 hours as compared to the conventional methods which requires at least 48 hours. This would help the treating physician to quickly arrive at the diagnosis and to initiate therapy promptly. Hence, the molecular detection methods are more preferred, favorable and accurate than the phenotypic method.

In the present study it was interesting to note that about 236 strains (40.2%) out of 586 Staph. aureus isolates were MRSA by conventional Oxacillin (1µg) disc diffusion method, which is slightly lower than earlier studies, but almost similar to those reported by Hanumanthappa et al.

The study results indicate that 94% of the 35 MRSA samples were mec A gene positive.
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whereas 6% samples as mec A negative by PCR method. Despite of being mecA positive only 94% of the samples were genotypically mecA positive. However, the reason for the 6% of the mecA negativity can be attributed to one of the following

1. Differing levels of mecA gene expression of methicillin resistance, occurring every 10^4 or 10^6 cells 20
2. Absence of penicillinase plasmid, which otherwise plays an important role in the stability and phenotypic expression of the mec A gene 21

CONCLUSION
To conclude, PCR assay was found to be a rapid and accurate procedure for the detection of MRSA infection compared to conventional methods, since the time taken for PCR assay is much less, prompt treatment can be initiated in view of medical and socio-economic costs.

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CONFLICTS OF INTEREST
None declared

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