Molecular detection of *mecA* gene from Methicillin Resistant Coagulase Negative *Staphylococci*

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DOI: 10.24896/jmbr.2017723

ABSTRACT

In the present research, a total of 27 Wound swab samples were collected for the isolation of Methicillin Resistant-Coagulase Negative *Staphylococci*. Out of these, 3.7% of the samples have been shown the presence of Methicillin Resistant-Coagulase Negative *Staphylococci* (MR-CoNS). The preliminary coagulase activity has been identified by Coagulate slide and tube method. The HiCrome MeReSa agar medium has been used for the preliminary identification of Methicillin resistance. During the isolation, the other strains of Coagulase Negative *Staphylococci* (29.6%), Methicillin Sensitive *Staphylococcus aureus* (44.4%) have been also isolated. 6 samples (22.2%) were sterile. The genomic DNA was isolated from Methicillin resistant Coagulase Negative *Staphylococci* and other species of *Staphylococci* through Modified DNA extraction method. The isolated DNA was amplified through the thermal cycler using suitable forward and reverse oligonucleotide primers, for the amplification of 527bp of *mecA* gene. The amplified DNA product has been identified by agarose gel electrophoresis using suitable 100bp ladder marker.

**Keywords**: Methicillin Resistance, Coagulase Negative *Staphylococci*, *mecA* gene, Polymerase Chain Reaction

INTRODUCTION

*Staphylococci* were first observed in human phylogenetic lesions by Von Recklinghausen in 1871. These are gram positive cocci that occur in grape-like clusters and causes of localized superlative lesions in human beings. The genus *Staphylococcus* contains 32 species, 16 of which are found in humans. Coagulase-Negative *Staphylococci* are a heterogeneous group of organisms and the infections caused by Coagulase-negative *Staphylococci* in addition to the well recognized species includes *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* [1]. These are major nosocomial pathogens rending today among the five most frequently isolated organisms in nosocomial sepsis and infections related to foreign biomaterials, like intravascular and peritoneal dialysis catheters, cerebrospinal fluid shunts, prosthetic joints, vascular grafts, cardiac pacemakers and intraocular lenses [2]. These are associated with the normal skin flora and mucous membranes and it may cause bacteremia, endocarditis, catheter related infections, central nervous system shunt infections, urinary tract infections and infections of prosthetic joints [3]. The coagulase production test is performed to separate *Staphylococcus aureus* from other species that are collectively referred to as Coagulate – Negative *Staphylococci* and Coagulate – Positive *Staphylococci*. The enzyme coagulase produced by *Staphylococcus aureus* binds plasma Fibrinogen and activates a cascade of reactions that cause plasma to clot. An organism can produce two types of coagulase,
referred to as bound and free. It is still one of the major problems of drug resistance and it should be a frequent and important human pathogen both in community and in hospital [4].

Methicillin was introduced in 1959 to treat infections caused by penicillin resistant Staphylococci. In 1961, there were reports of artificial induction of Methicillin resistance in Staphylococci and by 1963, there appeared the first infections with methicillin resistant Staphylococcus aureus. The first three infections were reported by Harding in 1963 in United Kingdom but soon these infections were documented from other European countries, Japan, Australia and the USA in 1968[5]. The gene responsible for methicillin resistance in Staphylococcus aureus is mecA with mecDNA. This is possible through the introduction of exogenous DNA into its genome and this region has been bound to be mobile as known as Staphylococcal chromosomal cassette mec (SSC mec). A mutation in the mecA gene of Staphylococcus will provide resistance to all beta-lactams. This gene codes for penicillin binding protein- a mutation in the gene prevents penicillin from effectively binding foreign particles [6]. Resistance to methicillin in Coagulase-Negative Staphylococci has also been shown to be heterogeneous and in vitro testing has entailed the use of osmotic support in the medium incubation at 30°C and prolonged incubation. The detection of methicillin resistance may be affected by the medium used for both Staphylococcus aureus and Coagulase-Negative Staphylococci [7].

During the 1980’s, the National Nosocomial Infection Surveillance Surveys demonstrated the increasing significance of gram positive organisms in hospital acquired infections recent report have confirmed that Coagulase-Negative Staphylococci remain an important cause of bacteremia in hospitalized patients [6]. The detection of Coagulase Negative Staphylococci has been very difficult and therefore detection of the mecA gene has been recommended as the gold standard. Fast methods for use on samples drawn from blood cultures positive for Staphylococci are needed in order to speed up response time which for conventional methods in 24-48hrs[9]. In this present study, Methicillin Resistant Coagulase Negative Staphylococci have been isolated from wound swab samples and the mecA gene from the specific strain should be amplified and identified.

MATERIALS AND METHODS

Clinical Specimens and Screening
A total of 27 Wound swab samples were collected (Using sterile Hi-Media Cotton Swabs) from in and around Salem District through various hospitals and laboratories. The collected samples were immediately transferred to the laboratory and processed. The collected samples were plated on Nutrient agar medium, Mannitol salt agar medium, Blood agar medium, MacConkey agar medium, DNase Agar medium and HiCrome MeReSa agar medium and incubated at 37°C for 12-24 hours. The incubated agar media plates were studied for Morphological characteristics, Staining reaction, Biochemical characteristics, Confirmation of Methicillin Resistance Coagulase negative Staphylococcus aureus by the amplification of mecA gene through Polymerase Chain Reaction.

Confirmation of Coagulase Negative Staphylococci

Coagulase Test
Slide test
A loopful of plasma was placed on glass slides and emulsifies a colony of the isolated organism in the plasma to make two thick suspensions and mix gently, Look for clumping of the organisms within 10 seconds.

Tube Test
0.5 ml of diluted citrated plasma in a small tube was incubated with heavy saline suspension of the organisms and was incubated at 37°C for 1-4 hours. It was examined for every 15 minutes for the formation of the coagulum compared with control. Observed results were noted.

Growth on HiCrome MeReSa Agar
A Colony from Nutrient agar medium was streaked on the HiCrome MeReSa Agarmedium and incubated for 18-24 hours at 37°C. After the incubation, the results should be observed for bluish-green color colonies on the agar medium.

Genomic DNA Isolation from Coagulase-Negative Methicillin Resistant Staphylococci
The genomic DNA were isolated from Methicillin resistant Coagulase Negative Staphylococci through Modified DNA extraction for rapid PCR detection of Staphylococci and confirmed through Agarose gel electrophoresis. The Methicillin resistant Coagulase Negative Staphylococci isolates were grown in Luria BertaniBroth for 18 – 24 hours at 37°C. The grown isolates were
centrifuged at 8000 rpm for 10 minutes at 4°C and the supernatant was discarded. The pellet was resuspended with 5ml Phosphate Buffer Saline, shaken for 30 minutes and then centrifuged at 8000 rpm for 10 minutes and the supernatant was discarded. Again the pellet was resuspended with 0.5ml of Phosphate Buffer Saline and the washed bacterial cells were transferred into 1.5ml microfuge tubes and 10μl of Lysozyme was added and incubated at 55°C for 30 minutes. After the incubation, the tubes were added with 200μl of 10% Sodium Dodecyl Sulphate (SDS) and 5μl of (10mg/ml) Proteinase K and the tubes were incubated in water bath at 33°C for 60 minutes. The tubes containing the suspension was extracted twice with Phenol: Chloroform (1:1) and once with Chloroform (equal volume). Each step of the extraction was performed using sterile microfuge tubes and centrifuged at 12,000 rpm for 5 minutes. The Staphylococcal DNA containing fraction was recovered from supernatant and precipitated with Isopropanol for overnight at -20°C freezer. The precipitated DNA was washed with 70% ethanol to separate the protein contamination and then resuspended with 40μl of TE buffer [10].

**Amplification of mecA gene**

The isolated DNA were amplified through the thermal cycler (TECHNE, UK) using suitable Forward (5'GGGATCATAGCGTCATTATTC_3) and reverse (5'AACGATTGTGACAGATAGCC_3) Oligonucleotide primers (First Base, Singapore), for the amplification of 527bp of mecA gene. The total volume of the PCR reaction mixture was 50μl and included as 33μl of sterile deionized water, 10μl of 5X buffer, 1.0μl of dNTP mix, 1.0μl of primer I, 1.0μl of primer II, 0.5μl of Taq DNA Polymerase, 1.5μl of MgCl2 and 2μl of template DNA. The cyclic parameters were included as the initial denaturation for 2 minutes at 94°C, annealing for one minute at 49°C, extension for one minute at 72°C, repeat the step for 35 cycles, final extension for 7 minutes at 72°C and finally the tubes were stored at 4°C. The amplified gene was identified through Agarose gel electrophoresis with suitable 100bp ladder marker [11].

**RESULTS**

**Bacterial Strains**

A total of 27 Wound swab samples were collected and cultured, out of these, 12 samples (44.4%) yielded Methicillin-Sensitive *Staphylococcus aureus* (MSSA), 8 samples (29.6%) with Coagulase-Negative *Staphylococci* (CNS), 1 sample (3.7%) yielded Methicillin-Resistant Coagulase-Negative *Staphylococcus aureus* (MR-CoNS) and 6 samples (22.2%) were sterile. The results have been shown in Table 1.

**Confirmation of MR-CoNS**

The confirmation of Methicillin Resistant Coagulase Negative Staphylococci on the basis of Colony morphology with different media includes, Nutrient agar medium, Mannitol salt agar medium, MacConkey agar medium, Blood agar medium, DNAse agar medium and HiCrome MeReSa agar medium, and biochemical analysis have been shown in Table 2.

**Isolations of Genomic DNA**

Agarose gel electrophoresis was done and the purified genomic DNA was shown under UV illuminator as seen as clear area. The results have been shown in Fig 1.

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**Table 1: Total Number of Staphylococcal Isolates**

<table>
<thead>
<tr>
<th>Number of Wound Swab Specimens</th>
<th>Total Number of Staphylococcal Isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 (100%)</td>
<td>Methicillin Sensitive <em>Staphylococcus aureus</em> (MSSA)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Coagulase Negative <em>Staphylococcus aureus</em> (CoNSA)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Methicillin Resistant Coagulase Negative <em>Staphylococcus aureus</em> (MR-CoNSA)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No Growth Identified</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 2: Morphological and Biochemical Characterization of Coagulase Negative Staphylococci

<table>
<thead>
<tr>
<th>S.No</th>
<th>Tests Performed</th>
<th>Result Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram Staining</td>
<td>Gram Positive Cocci</td>
</tr>
<tr>
<td>2</td>
<td>Catalase Test</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Oxidase Test</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Coagulase Test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slide Test</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Tube Test</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Indole Test</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Methyl Red Test</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Voges-Proskauer Test</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrate Fermentation Test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>Acid Production only</td>
</tr>
<tr>
<td></td>
<td>Mannitol</td>
<td>No Acid and Gas Production</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>Acid Production only</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>Acid Production only</td>
</tr>
<tr>
<td>9</td>
<td>Citrate Utilization Test</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Growth on HiChrome MedReSa Agar Media</td>
<td>Bluish Green Color Colonies</td>
</tr>
</tbody>
</table>

Fig. 1: Agarose Gel Electrophoresis of Isolated MR-CoNS and MRSA

Fig. 2: Amplification of mecA gene from MR-CoNS

Detection of mecA Gene by PCR

The oligonucleotide primers used in the PCR procedure were derived from the published sequence of the mecA gene and designed to give an amplification product of 527bp. In a previous study, we have shown that this amplified product is specific for mecA gene, because it does not hybridize with DNA of methicillin-susceptible strains and hybridizes with DNA of methicillin resistant strains. The amplification reaction was first performed with crude cell lysate corresponding to 10^8 bacteria and 1μg of purified DNA by using the PCR protocol. After 15, 20, 30 and 35 cycles the Samples were taken from each reaction tube and were submitted to agarose gel electrophoresis. The results have been shown in Fig 2.
DISCUSSION

Most of the countries have been reported an increase in colonization and infection in hospitalized patient by Coagulase-Negative Staphylococci, which are resistant to methicillin. This present study was designed to determine the species distribution of Staphylococcus aureus and the pathogenicity of the hospital strain compared to PCR for mec A gene [12]. The amplification reaction was carried out with crude cell lysate as a source of target DNA [13]. The amplification reaction was carried out with crude cell lysate as a source of target DNA [13]. The amplification reaction was carried out with crude cell lysate as a source of target DNA [13]. The amplification reaction was carried out with crude cell lysate as a source of target DNA [13]. The amplification reaction was carried out with crude cell lysate as a source of target DNA [13]. The amplification reaction was carried out with crude cell lysate as a source of target DNA [13]. The amplification reaction was carried out with crude cell lysate as a source of target DNA [13].

Previously, the detection of Penicillin Binding Protein 2a by the MRSA screen latex agglutination test with 2001 clinical Coagulase-negative Staphylococci had an initial sensitivity of 98% and a high degree of specificity for Staphylococcus epidermidis strain compared to PCR for mec A gene [13]. The amplification reaction was carried out with crude cell lysate as a source of target DNA and seventy four isolates of Coagulase negative Staphylococci were tested by Polymerase Chain Reaction [14]. It is related to amplification process of isolated Methicillin-Resistant Coagulase-Negative Staphylococcus epidermidis. The mecA gene that mediates methicillin resistance and its accompanying mec locus DNA, in Staphylococcus aureus to investigate whether there is a similar relationship between mec A in Coagulase-Negative Staphylococci [15]. However, since the size of the small fragments containing mec A varied from 73 to 527kb, the distance between the two genes were determined and it is more similar to our product.

CONCLUSION

It is concluded that the detection of mecA gene in Methicillin Resistant-Coagulase Negative Staphylococci through polymerase chain reaction may be a beneficial alternative to the standard antibiotic susceptibility testing. This molecular method is very much useful for the identification of intrinsic resistance in a timely and reliable manner. Although, the application of PCR related diagnosis represents the increasing of equipment and chemical costs, but their reliability, as well as considerations of the time and labor, these molecular methods have been recommendable for early detection of methicillin resistance in Coagulase Negative Staphylococcal infections.

Acknowledgement

Authors wish to thank the Muthayammal Educational and charitable Trust, Rasipuram, Tamil Nadu, India, for providing the laboratory facilities.

Authors contribution

All authors have an equal contribution in this research work.

Conflict of interest

We declare that we have no conflict of interest.

REFERENCES


