Original Research Article

Biofilm: Comparison between the *Staphylococcus aureus* and coagulase negative staphylococcus species isolated from a rural medical college hospital in North Kerala, India

Ramakrishna.Pai. Jakribettu, Syed Ahamed Mustaq*, V.C. Ashthami, M.M. Anju and M.I.V. Safeera

Department of Clinical Microbiology, M.E.S. Medical College, Perinthalmanna, Kerala, India

*Corresponding author

**A B S T R A C T**

Biofilm is a serious threat for the patient with infected with the biofilm producing microbes, as it may cause therapeutic failure with regular antibacterial therapy. The Gram positive bacteria are also known to produce biofilm. This study conducted to know the biofilm producer among the *Staphylococcus* species isolated from the patients attending a medical college in North Kerala. The clinical isolates of *Staphylococcus* species were subjected to speciation and antibacterial susceptibility test by standard methods, then for biofilm producing by tissue culture plate method. Fifty isolates of *Staphylococcus aureus* and Coagulase Negative (CoN) *Staphylococcus* were included in the study. The antimicrobial susceptibility pattern showed that both *Staphylococcus aureus* and CoNS*Staphylococcus* species are resistant to Ampicillin, Penicillin and Erythromycin. The rate of MRSA and MRCoN isolation was 6% and 4% respectively. CoN*Staphylococcus* (12%) were more biofilm producer than compared to *Staphylococcus aureus* (4%). Coagulase Negative *Staphylococcus* species have a higher capability of biofilm production and thus a therapeutic failure in infections. Hence CoN*Staphylococcus* cannot be neglected if isolated from the nosocomial infection sample.

**Keywords**

Nosocomial infections; *Staphylococcus aureus*; Coagulase Negative *Staphylococcus*; Biofilm.

**Introduction**

Biofilm are a group of microbes which are encased in an exo-polysaccharide matrix on both biotic and abiotic surfaces. Various changes occur during their transition from a planktonic to a surface attached community. This causes a number of persistent infections which respond poorly to conventional antibiotic therapy. After adherence to a surface, organisms adapt to the environment of the biofilm by increasing the secretion of exo-polysaccharide. This helps the microorganisms to escape their killing by antibiotics. Biofilm formation is commonly regulated by inter and intraspecies quorum sensing mechanisms. Availability of nutrients, chemotaxis towards the surface, motility of bacteria,
surface adhesion and the presence of surfactants influence biofilm formation in microorganisms (Seema et al., 2011).

Microscopy of biofilm formation in-vitro suggests that two steps involved:(1) the attachment of the bacterial cells to the surface, which may occur rapidly; and (2) the growth dependent accumulation to form multilayered cell clusters surrounded by a slimy matrix (Friedrich et al., 2002). Biofilm may be formed by one or several types of microorganism’s. The cultural morphology of biofilm forming bacteria is usually different from those strains which do not form biofilms. It has been observed that biofilm forming bacteria attach themselves to solid surfaces by using their sticky appendages and employing a rolling motion, which results in their continuous attachment and detachment to the surface and the formation of micro aggregation. First they detach from the top of the microorganisms where apparently the shearing force overcomes the attachment forces; then the micro aggregations roll slightly and attach some place downstream (Gerald et al., 2009).

The two important species which producing biofilm are Staphylococcus aureus and Coagulase Negative Staphylococci e.g., Staphylococcus epidermidis, Staphylococcus saprophyticus. Staphylococcus aureus is a ubiquitous pathogen. Its role in a variety of clinical conditions has been recognized to include nosocomial infections, septicemia, wound sepsis, septic abortion, osteomyelitis, septic arthritis, post-surgical infections and toxic shock syndrome. Staphylococcus aureus infection is one of the most common bacterial infections in AIDS patients (Shittu et al., 2006).

Staphylococcus aureus has emerged as one of the pathogen, and has over the past numerous decades, been a leading foundation of hospital acquired infections. Staphylococcus aureus is well characterized and known to have a diverse arsenal of virulence factors that cause a prominent inflammatory response. Biofilm formation does not only occur with foreign body infections. If one closely examine native tissues removed from patients with recurrent Staphylococcus aureus infection the cells are organized with a confluent colonies (Longauerova et al., 2006).

Coagulase negative Staphylococci are among the bacteria routinely isolated at various microbiological departments. There were presently 41 taxon, designated Coagulase Negative Staphylococci (Longauerova, 2006). Coagulase negative Staphylococci can be divided into two groups depending on whether they resistant to or susceptible to novobiocin. Novobiocin susceptible species are Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus chleiferi, as well as novobiocin resistant species Staphylococcus saprophyticus (Christof, 2002).

Staphylococcus species including MRSA can cause painful skin infection resembling a pimple, insect bites, and furuncle and have pus or drainage. Pus is formed by white blood cells that rush in to kill bacteria and enlarge blood vessels—a way for the body to purify infections. The resistance of biofilms to antibiotics is increased compared with what is normally seen with planktonic cells. Infact, when cells exist in a biofilm, they can become 10-1000 times more resistant to the effects of antimicrobial agents (Heikens et al., 2005).

Regarding too many infections due to Staphylococcus aureus and coagulase
negative *Staphylococcus* species and increasingly resistant to antibiotics agents, the present study was conducted to identify frequency of *Staphylococcus aureus* and coagulase negative *Staphylococci* isolated from pyogenic infections and determination of biofilm productions and susceptibility pattern of these species to antibacterial agents. Aims and Objectives: To know the antibiogram and biofilm production of the staphylococcus species isolated from the pyogenic infection.

**Materials and Methods**

This was prospective study was conducted at the MES Medical College, Perinthalmanna for a period of 3 month from April 2013 to June 2013. The pus sample was collected from patients attending the rural medical college Hospital. The sample was inoculated on 5% Sheep Blood agar (SBA) and Mac Conkey agar, incubated at 37°C for 24-48 hours. The β hemolytic colonies on SBA were subjected to Gram’s staining. The gram positive cocci in clusters, catalase positive were identified as *Staphylococcus* species. The tube coagulase test was done to differentiate between *Staphylococcus aureus* and Coagulase Negative *Staphylococcus* species.

The isolates identified as Coagulase negative Staphylococci and Coagulase positive Staphylococci were subjected to test for biofilm production described by (Christensen et al., 1985) by Tissue Culture Plateand antibiotic susceptibility according to CLSI guidelines (Wilker et al., 2009).

**Results and Discussion**

A total of 100 isolates of *Staphylococcus* species were isolated during the study period, of which 50 isolates of *Staphylococcus aureus* and 50 Coagulase negative Staphylococci were included in the study.

The antimicrobial susceptibility pattern showed that both *Staphylococcus aureus* and CoN *Staphylococcus* species are resistant to Ampicillin, Penicillin and Erythromycin. It was observed that the CoN *Staphylococcus* Species were resistant to Cotrimoxazole (24%) compared to *Staphylococcus aureus* (4%). These are the common drugs used in the community for most of the infectious disease. Of the 100 *Staphylococcus* species isolated, 6% strains were Meticillin Resistant *Staphylococcus aureus* (MRSA) and 4% strains were Meticillin Resistant Coagulase Negative *Staphylococcus* (MRCoNS). No isolates were resistant to Vancomycin, Linezolid and Teicoplanin. The above figure shows that the CoN *Staphylococcus* species were biofilm producer than the *Staphylococcus aureus*. Higher percentage of CoN *Staphylococcus* (12%) were highly biofilm producer than *Staphylococcus aureus*(4%).

*Staphylococcus* species are known to produce biofilm in device related infections. These biofilms prevent the entry and the action of the antibacterial administered and rendering the pathogen drug resistant.

In modified TCP method, extended incubation for 24 hour could lead to a better discrimination between moderate and non-biofilm producing Staphylococci and biofilm forming formation was observed in 62% isolates. This observation suggested a strong dependence between growth condition and biofilm formation in *Staphylococci*.
Fig 1. Age-wise Distribution Of Patient In Whom S. aureus And Coagulase Negative Staphylococcus Isolated

<table>
<thead>
<tr>
<th>Age Group</th>
<th>S. aureus</th>
<th>CONS</th>
</tr>
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<tbody>
<tr>
<td>0-15</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>16-30</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>31-45</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>46-60</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>&gt;60</td>
<td>11</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig 2. Antibioticogram of Isolates Studied

- Staphylococcus aureus n=50
- Coagulase Negative Staphylococcus n=50

Fig 3. Comparison of Biofilm production among the Staphylococcus aureus and Coagulase Negative Staphylococcus Isolated

<table>
<thead>
<tr>
<th>Biofilm Production</th>
<th>S. aureus</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non</td>
<td>62%</td>
<td>16%</td>
</tr>
<tr>
<td>Moderately</td>
<td>72%</td>
<td>34%</td>
</tr>
<tr>
<td>Highly</td>
<td>4%</td>
<td>12%</td>
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</table>
This study was similar to that of study done by (Christensen et al., 1985) shows that 53.2% were producing biofilm.

In our study among the *Staphylococcus aureus* isolates studied 62% were non biofilm producers, 34% were moderate biofilm producers and 4% strong biofilm producers, and in CoN *Staphylococci* 16% were non biofilm producers, 72% were moderate biofilm producers and 12% highly biofilm producers. It was found that CoN *Staphylococci* isolates were strong biofilm producer. In a study done in Western India, it was observed similar percentage of the Staphylococcal isolates were biofilm producer, 15.64 % were highly biofilm producers, 38.55% were moderate biofilm producers and 45.81% were non biofilm producers. (Bose S et al., 2009).

In this study antibiotic susceptibility pattern of various biofilm producers and non-producer *Staphylococci* spp. isolated from clinical materials were obtained. The significant and clinically relevant observation was that the high resistance shown by biofilm producers to conventional antibiotics than non-biofilm producers. These observations supported the study by (Gürkan Mert et al., 2011). All the strains were sensitive to linezolid and vancomycin.

The Susceptibility pattern of *Staphylococcus aureus* against antibacterial agents in this study showed that the majority of the resistance of Staphylococcus was belonging to Ampicillin (31%), Penicillin (28%), Cephalexin (24%) and Erythromycin (12%) and there is no resistance towards Vancomycin, Teicoplanin, and Linezolid. This study was supported by Asma Bashir et al (2007) that most effective antibiotic for *Staphylococcus aureus* is vancomycin showing 80.5% efficacy, then methicillin with 68.0% efficacy, erythromycin with 55.6% efficacy (Asma Bashira et al., 2007).

*Staphylococcus aureus* is highly versatile and adaptable pathogen capable of causing a diverse array of infections in hospitals and community settings. *Staphylococcus aureus* has been reported to be the cause of most wound infections.

In this study prevalence of MRSA was 6%. The MRSA also showed resistance to cotrimoxazole, erythromycin, and gentamicin. This study was supported by (Murugan et al., 2008), as expected all the strains were resistant to ampicillin and most of them to penicillin. But significant and clinical relevant observation of their study is moderate resistance shown by MRSA to other conventional antibiotics.

Coagulase negative *Staphylococci* have emerged in recent years as pathogens in a growing number of nosocomial infections. Production of an exopolysaccharide, allowing adherence and subsequent formation of a multilayered biofilm, appears to be essential for the pathogenesis of Coagulase negative *Staphylococcus* species. Because many isolates are multi-drug resistant, their infections are difficult to treat and can even fatal. A detailed characterization of isolates of CoNS through speciation, genetics and antibiotic susceptibility may be necessary to distinguish infecting from contaminating isolates and to plan suitable therapy. In this study, the CoNS isolates were resistant to the common antibacterials used for wound infections; hence an accurate sensitivity pattern has to be determined before starting of the antibacterial therapy to prevent therapeutic
failure. Hence, CoNS cannot be neglected as nonpathogenic as infections with these can be life-threatening and the biofilm producing isolates may be multi-drug resistant too. It is better to isolate the patients if they are infected with MRCoN for the better infection control measures.

The Methicillin Resistant Staphylococcal isolates, ie both MRSA and MR CoN were strong biofilm producers. The similar findings were found by (Rachna et al., 2010); there is a significantly decreased penetration of antibacterial drugs. This results indicate that either a high density growth phase or its metabolic state which is related to a biofilm growth mode rather than biofilm producing ability contribute significantly to the resistance of CoNS biofilm to antibiotics. Thus, we conclude that the biofilm producing CoN Staphylococcus species are difficult to be eradicated from the site of infection using antibacterial drugs. These biofilm producing CoN Staphylococcus even though considered contaminant in the clinical specimens sometimes can cause serious infections in patient with reduced immunity. They can be multidrug resistant as biofilm restrict the entry of the drug to the point of action. Hence, when CoN Staphylococcus is isolated the biofilm producing capacity has to evaluate before treating the patients with the antibacterial.

References


Longauerova, A., 2006. Coagulase


