Incidence of Biofilm Producing \textit{Staphylococcus epidermidis} isolated from Clinical Samples and Skin of Healthy Individuals: A Correlative Study


\textbf{Abstract}

\textbf{Purpose:} Biofilm formation impairs wound healing and also protects the organisms from the action of antibiotics thereby associated with various hospital acquired infections. Currently \textit{S.epidermidis} has been increasingly recognised as an important nosocomial pathogen due to its capability in forming biofilm. Hence this study is aimed to screen the incidence of biofilm producing clinical and skin isolates of \textit{S.epidermidis} in order to determine its capacity to cause infections in health care setting.

\textbf{Materials and Methods:} A total of 100 \textit{S.epidermidis} isolates, 50 from clinical samples and 50 from skin of healthy volunteers were collected during Nov 2014- Jan 2015. All the isolates were subjected for biofilm production by tube and tissue culture plate method.

\textbf{Result:} Out of 50 clinical isolates 50% were from blood sample. 52% of clinical isolates and 26% of skin isolates were found to be a strong biofilm producer by tube method where as tissue culture plate (TCP) method showed 60% of clinical and 34% skin isolates as strong biofilm producer. 20% of strong biofilm producer were from blood sample which may be because of various intravenous catheter related septicaemia infection during hospitalisation. TCP method was considered as more sensitive.

\textbf{Conclusion:} Our study reveals that there was no difference between clinical and skin isolates in producing biofilm, as skin isolates were equally more virulent as that of the clinical isolate. Hence proper screening and management may possibly reduce the risk of hospital acquired \textit{S.epidermidis} infection.

\textbf{Keywords:} Biofilm, Clinical isolate, \textit{S.epidermidis}, Skin isolate, Tissue culture plate method and Tube method.

1. \textbf{Introduction}

Coagulase negative Staphylococci (CoNS) are gram positive, coagulase negative, immobile, prevailing non-encapsulated spherical cocci found as a normal skin and mucosal microflora\(^1\). The organism remains non-pathogenic in healthy people. But immunocompromised patients are at high risk for developing an infection \(^2\). Isolation of CoNS frequently from samples such as blood, other normally sterile body fluids, intravenous catheters, peritoneal dialysates and the various indwelling devices becomes a challenge to both clinical microbiologist and clinicians. But in recent years \textit{S.epidermidis} has emerged as a common cause of nosocomial infections in immunocompromised patients \(^3\). The factors associating the CoNS as etiological agents include (i) the isolation of strains in pure culture from infected sites or body fluids and (ii) the repeated isolation of the same strain(s) over the course of an infection \(^4\).

Biofilm formation is the major pathogenic factor of \textit{S.epidermidis} mediated by a capsular polysaccharide adhesin (PSA) and Polysaccharide Intercellular Adhesin (PIA). PSA helps in initial adherence and formation of slimy-biofilm, whereas PIA mediates accumulation of cells thereby actual formation of biofilm occurs \(^5\). PIA production in \textit{S.epidermidis} is encoded by the ica operon consisting of icaADBC genes and is regulated by icaR gene \(^6\). Septicaemia caused by \textit{S.epidermidis} is mainly associated with the use of catheters and indwelling medical devices. Various strains of \textit{S.epidermidis} are capable of forming biofilms that remains a major concern for patients with catheters, heart valves and other implants. It has also been demonstrated that biofilm formation by Staphylococcus impairs wound healing and protects CoNS against the action of antibiotics administered for treatment of infection \(^7\). One critical factor for transmission of CoNS from a person (patient or health care worker) to the environment and then to another person is the ability of these agents to survive on environmental surfaces \(^8\). Hence the present study is aimed to screen and compare the significance of biofilm producing \textit{S.epidermidis} from patients who are admitted in our hospital and also from the skin of healthy individuals.
individuals, in order to determine its capacity to cause infection especially in the hospital setting, because these organisms are considered as a major alarm for catheter related infections.

Materials and Methods:
Collection of Samples:
A total of 100 S.epidermidis isolates were collected in SSSMC&RI. Of which 50 isolates were collected from clinical specimens such as blood, urine, surgical wounds, intravascular catheters, DTT and the remaining 50 isolates were collected from skin of healthy volunteers such as forehead, anterior nares, and arm joint using a moistened swab. The study was conducted over a period of 3 months (from Nov 2014- Jan 2015) after obtaining informed consent from patients and healthy volunteers.

Isolation of S.epidermidis:
Isolates from the samples were identified and characterized by standard microbiological techniques including Gram stain, catalase, mannitol fermentation, coagulase production and susceptibility to bacitracin and novobiocin. Isolates were then subcultured on Mannitol Salt Agar (MSA), in which S.epidermidis produced pink colonies on MSA. Cultures were maintained on Tryptic Soy Broth (TSB) as per standard protocol [9].

Detection Of Biofilm Production:
Biofilm production was detected by two methods

Tube Method:
Tube method was performed as per previous protocol. 10ml of TSB with 1% of glucose was inoculated with a loopful of over night cultured organism from nutrient agar. Broths were incubated at 37º C for 24 hours. The cultures were decanted and tubes were washed with phosphate buffer saline (pH 7.3). The tubes were dried and stained with 0.1% crystal violet. Excess stain was washed with deionized water. Then tubes were dried in inverted position and observed for biofilm formation. Biofilm production was considered positive, when a visible film lined the wall and bottom of the tube. Ring formation was scored as 0-absent, 1-weak, 2-moderate, 3-strong (10).

Quantitative Detection of Biofilm Production:
Quantitative detection of biofilm production was performed using tissue culture plate assay (TCP) as reported earlier. 96-well tissue culture plates (TCP) were used. In each well 200 µl of sterile TSB was added. Into which 2 µl of each sample was added. The plate was incubated aerobically for 24 hours at 37 ºC. After 24 hours, the contents of each well were gently removed by tapping the plate. Then each well was washed 4 times with 200 µl 1X PBS. After that, 100 µl of 0.1% crystal violet was added to each well to stain the biofilm. The tissue culture plate was incubated at room temperature for 15 minutes. Each well was then washed repeatedly with double distilled water to remove excess stain. The tissue culture plate was allowed to dry and then read at 570nm using ELISA plate reader. The assay has been repeated with double distilled water to remove excess stain. The tissue culture plate was allowed to dry and then read at 570nm using ELISA plate reader. The assay has been performed in triplicate for each sample. Mean OD Value were considered as strong biofilm producers. Values between 0.06-0.12 was considered as moderate to weak biofilm producers and less than 0.06 values considered as non-biofilm producers. The results were validated by using a compound microscope (11).

Results:
A total of 100 S.epidermidis were studied, among which 50 were from various clinical samples such as blood, urine, surgical wounds, intravascular catheters and DTT. 50% of clinical isolate of S.epidermidis were from blood sample followed by which the next predominant number of isolates were from urine with 30% (TABLE: 1) and the remaining 50 isolates were from skin of healthy subjects. Among 100 isolates tested for the production of biofilm by Tube method, 26 isolates were from clinical specimen and 13 isolates were from skin, showed 52% and 26% of strong biofilm production which was indicated with score 3. Followed by that 7 and 4 isolates from clinical and skin samples showed 14% and 8% of moderate biofilm production with score 2, where as the remaining 17 clinical and 33 skin isolates showed 34% and 66% of weak/ non biofilm production with score 0-1. (TABLE: 2)(FIGURE: 1).

In tissue culture plate method, among 50 clinical isolates of S.epidermidis, 30 isolates were found to be strong biofilm former accounting for 60%, 10 isolates were moderate biofilm producer with 20% and the remaining 10 isolates were weak/ non biofilm producers with 20%. Among skin isolates, 17 were strong biofilm former with 34%, followed by 10 and 23 isolates were detected as moderate and weak or non biofilm biofilm producer which showed 20% and 46%. (FIGURE: 2) (TABLE: 2).

In our study, from all the 50 clinical isolates tested, highest number of strong biofilm producer were from blood with 10 isolates, followed by 9 from intravenous catheters, 5 from DTT, 5 from Urine and 1 from wound. From the above mentioned samples, blood was the maximum and the predominant isolate accounting for 20% of strong biofilm production. This may be due to intravenous catheter related septicaemia infections during hospitalization. Simultaneously the least producer was from wound sample with 2%.

Table: 1 S.epidermidis isolates from various clinical samples

<table>
<thead>
<tr>
<th>Clinical samples</th>
<th>No. Of S.epidermidis (n=50)</th>
<th>Percentage (%)</th>
</tr>
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<tbody>
<tr>
<td>Blood</td>
<td>25</td>
<td>50%</td>
</tr>
<tr>
<td>Urine</td>
<td>15</td>
<td>30%</td>
</tr>
<tr>
<td>Surgical wound</td>
<td>3</td>
<td>6%</td>
</tr>
<tr>
<td>DTT</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>Intravascular catheters</td>
<td>5</td>
<td>10%</td>
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Table: 2 Score of biofilm producing clinical and skin isolate of S.epidermidis by tube method

<table>
<thead>
<tr>
<th>Score of biofilm formation</th>
<th>No. of clinical S.epidermidis (%)</th>
<th>No. of skin isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1(non/weak biofilm)</td>
<td>17(34%)</td>
<td>33(66%)</td>
</tr>
<tr>
<td>2 (moderate biofilm)</td>
<td>7(14%)</td>
<td>4(8%)</td>
</tr>
<tr>
<td>3 (strong biofilm)</td>
<td>26(52%)</td>
<td>13(26%)</td>
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Table: 3 OD value of biofilm producing clinical and skin isolate of S.epidermidis by tissue culture plate method

<table>
<thead>
<tr>
<th>Mean OD Value</th>
<th>No. Of Clinical S.epidermidis (%)</th>
<th>No. Of Skin isolates (%)</th>
</tr>
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<tbody>
<tr>
<td>&lt;0.06</td>
<td>10(20%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>0.06-0.12</td>
<td>10(20%)</td>
<td>10(20%)</td>
</tr>
<tr>
<td>&gt;0.12</td>
<td>30(60%)</td>
<td>17(34%)</td>
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Discussion:
Coagulase-negative staphylococci (CONS) are the most commonly isolated organisms, colonizes skin and mucous membrane of humans and animals. Initially, these organisms were considered as contaminants of clinical specimens from humans. But, during the last few decades, CONS have emerged as important nosocomial pathogens. Among all CONS, *S. epidermidis* strains represent highly significant nosocomial pathogen and the most common agents of infections with implanted devices (e.g., catheters and prostheses). It has also been implicated as the etiological agent in infections of wound, urogenital tract, respiratory tract, meninges, conjunctiva and skin. Based on these previous reports, our study was conducted and correlated significance of *S. epidermidis* in causing infection. *S. epidermidis* is one of the very few bacteria that can cope with the extremes of salt concentration, pH, and other factors that contribute to the harsh environment on human skin. This exceptional ability is mainly due to the biofilm forming virulence properties associated with *S. epidermidis*. Epidemiological studies have demonstrated that healthy people carry between 10 and 24 different strains of *S. epidermidis* at any one time. Similarly we have proved 50% of isolation of *S. epidermidis* from skin of healthy volunteers.

In our paper, 50% of isolates were detected from blood sample likewise Shubhra Singh et al (19) reported higher number of blood isolates 72 among 150 strains of CONS with 60% and followed by another study from Iran also proved the higher prevalence of *S. epidermidis* in blood sample with 40%.

Many reports have demonstrated biofilm formation by *S. epidermidis*, isolated from device associated infection followed by IV catheter associated septicaemias, prosthetic cardiac valves, CSF shunts, orthopedic appliances and other devices. In our study, blood isolates were detected to produce strong biofilm formation which may be due to various intravenous catheter related septicaemia infections as mentioned in other studies.

Many studies showed Biofilm production was considered to be less in case of samples collected from healthy skin when compared to the patients associated with infection and implants. A study reported that 44% of the samples collected from patients were strong biofilm producers when compared to 0% of the samples that were collected from healthy volunteers. But in 2013 a study from Chennai detected the prevalence of biofilm producing *S. epidermidis* from skin healthy volunteers; they reported 91.4% of isolates as biofilm producers. Similar to this report, our study also revealed 34% of skin isolates and 60% of clinical isolates as a strong biofilm producer by Tissue culture plate method. This shows that there is no difference in the capability of *S. epidermidis* in producing biofilm even though it is from symptomatic patients or healthy volunteers.

Overall 39% of *S. epidermidis* isolates were found to be a strong biofilm producer by tube method. Whereas, 47% of isolates were detected as strong biofilm producers by tissue culture plate method. This shows that there was an association between detection of strong biofilm producers by both the methods but those isolates that were considered as moderate to weak biofilm producer in tube method were picked up as strong biofilm producer by tissue culture plate method.

This is mainly due to the difficulty in differentiating moderate, weak and non biofilm producers due to change in the ability of different observer in detecting the result of tube method. Hence in correlation with the previous studies we are strongly recommending the tissue culture plate as more sensitive and accurate method for detecting biofilm production.

Conclusion:
Our study has established the high incidence of biofilm-producing *S. epidermidis* both from healthy skin and patient’s sample. Clinical isolates from blood revealed high range of biofilm production which may be due to various catheter associated septicaemia infection. It has also demonstrated the similarity of skin isolates in producing biofilm as that of the clinical isolates which is of utmost importance in causing nosocomial outbreak, as medical devices gets easily contaminated with these bacteria from skin of hospital staffs and visitors during hospitalisation. Hence early screening of biofilm production with highly sensitive method might be helpful in proper management and control of disease.

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Reference: