Potential of Biofilm Formation by Staphylococci on Polymer Surface and its Correlation with Methicillin Susceptibility

Dear Editor,

*Staphylococcus epidermidis* and *Staphylococcus aureus* are major causes of colonization and bio film formation in intravenous catheters. This frequently leads to catheter associated blood stream infection. Polysaccharide inter cellular adhesin (PIA) plays an important role in pathogenesis as it mediates the contact of bacterial cells with each other, resulting in the accumulation of a multilayered biofilm. PIA production is under the control of the *ica* operon and this operon has been well documented not only in *S. epidermidis* but also in *S. aureus*. It has been documented that the regulation of *ica* operon expression and the resultant biofilm formation may be altered by various environmental factors like anaerobicity, CO₂ levels, glucose and osmotic changes. In recent years, the influence of methicillin susceptibility on biofilm formation and *ica* expression among *S. aureus* isolates have been studied, revealing some important findings; though similar studies have not been done for *S. epidermidis*. Our study has examined the methicillin sensitivity / resistance on biofilm formation among *S. aureus* and *S. epidermidis* isolates from venous catheter tips.

Isolates of Staphylococci from venous catheters have been used. A total of 60 representative strains consisting 15 each of methicillin sensitive *S. aureus* (MSSA), methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. epidermidis* (MSSE) and methicillin resistant *S. epidermidis* (MRSE) were included. Methicillin sensitivity testing was done using oxacillin, 1 µg disks (Hi Media, India) and reconfirmed with cefoxitin, 30 µg disks (Hi Media, India). The antibogram of the *S. aureus* and the *S. epidermidis* strains were studied and only those strains which differed in their susceptibility patterns included in the study. This was done to ensure that the strains were clonally distinct. For quantitative biofilm measurement, the method suggested by Rachid *et al.*, with slight modifications, was used. An 1:100 dilution of overnight broth culture of the organism was done in Brain heart infusion broth (Hi-Media) with or without 4% Sodium chloride or 1% Glucose. This was transferred to 96 well polystyrene flat bottomed ELISA plates (Tarsons, India) and incubated overnight at 37°C, followed by washing, air drying and staining with crystal violet solution. The plates were read at 490 nm and optical density of greater than 0.120 was considered biofilm positive.

Among the MRSA stains, six could produce biofilms without any external influence while four additional strains were able to produce the same under the influence of glucose [Table]. One strain was biofilm positive in the presence of Sodium Chloride as well as glucose. In contrast, none of the MSSA could produce biofilm with or without Glucose, but two could produce it in the presence of Sodium Chloride.

The *S. epidermidis* strain exhibited much less biofilm forming potential with only one strain each of MSSE and MRSE showing this effect independently. Two additional strains of MSSE were biofilm positive in presence of glucose. The single strain of biofilm positive MRSE strain was able to produce it in glucose, but not in the presence of sodium chloride.

Bio film formation among *S. epidermidis* and to some extent *S. aureus* is a well documented phenomenon, particularly with reference to the production of PIA. However, only limited studies have been done in the recent years to find out the effect of methicillin susceptibility / resistance on PIA production. In our study, a greater number of MRSA could produce bio film compared to the other three groups (MSSA, MSSE and MRSE). The latter three groups showed very limited potential to form bio film on smooth surfaces, even in the presence of glucose or sodium chloride.

Bio film formation among MRSA strain was significantly increased under the influence of glucose, although the same could not be seen in presence of sodium chloride. However, among the *S. epidermidis* strains, none of the MRSE strains could produce bio film in the presence of sodium chloride. Glucose was able to induce biofilm formation in two and one additional strain among MSSE and MRSE respectively, while sodium chloride was unable to induce bio film production in any of the MRSE strains.

Sodium chloride is a known activator of *ica* operon transcription and from our study; it is more likely to induce bio film formation among methicillin sensitive isolates. Among MRSA isolates, bio film formation seems to be predominantly glucose induced. There seems to be a complex relationship in terms of bio film formation and its

<table>
<thead>
<tr>
<th>Table: Bio film production by Staphylococci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Without stimulus</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>MSSA (15)</td>
</tr>
<tr>
<td>MRSA (15)</td>
</tr>
<tr>
<td>MSSE (15)</td>
</tr>
<tr>
<td>MRSE (15)</td>
</tr>
</tbody>
</table>

www.ijmm.org
regulation under the influence of sodium chloride or glucose which may be resolved if a larger series is studied.

The other important finding of our study was the variability of adherence of the strains to the polymer surfaces. Although the isolates were all from plastic catheters, they showed varying ability in their adherence potential to polystyrene surface in vitro. It seems likely that there may be strain to strain variation in the expression of genes responsible for PIA production and/or protein adhesins which play an important role in biofilm formation. Another probable explanation is the more complex milieu existing in the inserted catheter under in vivo conditions where various host proteins may form a coating on the catheter surface. The organisms can anchor themselves directly to those proteins and other substances using multiple receptors and surface proteins.

Further studies are needed to elucidate the factors influencing ica expression and the different mechanisms involved in biofilm formation among methicillin susceptible/resistant strains. This will give a more comprehensive picture of Staphylococcus induced catheter related infection and effective therapeutic interventions.

References

* A Chaudhury, M Nagaraja, AG Kumar
Department of Microbiology, SV Institute of Medical Sciences, Tirupati, Andhra Pradesh -517 507, India

*Corresponding author (email: <ach1964@rediffmail.com>)
Received: 13-12-2008
Accepted: 06-03-2009
DOI: 10.4103/0255-0857.55450

Laboratory Microbiology to Clinical Microbiology: Are we Ready for a Transition?

Dear Editor,

We read with interest the guest editorial in the April 2009 issue of IJMM.[1] The author has succinctly brought out the need for bridging the divide between clinical care and laboratory work in microbiology.

We in India appear to be in a state of transition as we attempt to adopt this ‘service delivery model’. The concept and approach, however, is not entirely unfamiliar to us. Through this letter we would like to share our experience in this regard.

The department has undertaken an initiative to build a clinical association with physicians and intensive care experts in our hospital. As part of this effort, postgraduate students along with faculty members attend daily medical and surgical ICU rounds with the respective treating physicians. This approach revealed several benefits in a short span of time. For microbiologists — first hand patient information, clinical correlation, treatment trends and suggestions for additional / alternate tests were a few.

For healthcare providers, direct liaison with laboratory personnel, guidelines on specimen collection, update on new findings (ESBLs / MBLs) and feedback on antibiotic susceptibility patterns proved invaluable. Subsequently, we also extended this service to the pediatric ward, Oncology and Hematology Units.

Marked reduction in MRSA infections in the ICU, return of susceptibility patterns in certain Gram negative pathogens and investigation and source tracking of a nosocomial outbreak of neonatal septicemia were other notable benefits.

Other proactive efforts included a study of the bacteriological profile of air in the ICU / Operating Units, up-to-date antibiotic policy recommendations and liaison with anesthetists, to resolve doubtful cases of ventilator associated pneumonia.

Although the experience has proved to be mutually rewarding and educative, we continue to face certain challenges. Shortage of staff to cover all units and the ability to sustain the effort on a long-term basis are some challenges.