**Original article**

**Methicillin and Clindamycin resistance in biofilm producing Staphylococcus aureus isolated from clinical specimens**

Pankaj A. Joshi, Dhruv K. Mamtora, Neeta P. Jangale, Meena N. Ramteerthakar, Vidya P. Arjunwadkar, Vishakha V. Shikhare

Department of Microbiology, Govt. Medical College Miraj, Maharashtra, India
Corresponding author: Dr Pankaj Joshi

**Abstract**

**Introduction:** Antimicrobial resistance in *S. aureus* is a growing concern of all microbiologists and clinicians. It has acquired resistance to almost all classes of antimicrobials, by various mechanisms including beta lactamase production, altered receptors, ribosome modification and active efflux. Biofilm formation by *S. aureus* is one of its virulence markers which, in addition to providing a survival advantage by firm adherence to underlying surface, also protects the organism from the antimicrobials.

**Material & Methods:** In present study, a total of 148 isolates were studied for biofilm production by Congo Red Agar (CRA) method and Christensen’s tube method. The methicillin resistance and clindamycin resistance in *S. aureus* was detected by disc diffusion method using cefoxitin and clindamycin. The clindamycin disc was placed adjacent to erythromycin disc to detect the inducible clindamycin resistance (D test).

**Results:** Out of 148 *S. aureus* isolates, 89 were biofilm producers and 59 were non-biofilm producers. Amongst biofilm producing *S. aureus*, 41 were Methicillin Sensitive *Staphylococcus aureus* (MSSA) & 48 were Methicillin Resistant *Staphylococcus aureus* (MRSA) as against 54 MSSA & 5 MRSA in non-biofilm producers. Amongst biofilm producing MRSA, clindamycin resistance was seen in 81.25% (Inducible-43.75%, Constitutive-37.5%) of the isolates. In non-biofilm producing MRSA, clindamycin resistance was seen in 60% (Inducible-20%, Constitutive-40%) of the isolates. In biofilm producing MSSA isolates 73.16% showed clindamycin resistance (Inducible-58.53%, Constitutive-14.63%) and in non-biofilm producing MSSA isolates, 27.77% showed clindamycin resistance (Inducible-20.37%, Constitutive-7.40%).

**Conclusion:** It can be observed that biofilm production was more frequently associated with resistance to antimicrobials (both methicillin resistance as well as clindamycin resistance)

**Key words:** Biofilm, MRSA, MLSBi.
penicillin binding proteins. Macrolides, Lincosamides and Streptogramin B (MLS\textsubscript{B}) group of antibiotics are used as an alternative in penicillin-allergic patients. However, \textit{S.aureus} may develop resistance to clindamycin (lincosamide) during therapy particularly in isolates with erythromycin resistance. Therefore, it becomes clinically important to identify isolates with biofilm production and inducible resistance in order to prevent treatment failures.

We conducted a study to know the rates of methicillin and clindamycin resistance amongst clinical isolates of biofilm producing \textit{S.aureus}.\textsuperscript{1,2,3,4,5}

**Methodology**

A total of 148 isolates of \textit{Staphylococcus aureus} were collected during one year period. All isolates were identified as per standard protocol and an antimicrobial susceptibility testing (AST), using Kirby Bauer’s disc diffusion method, was performed for all the isolates as per CLSI guidelines\textsuperscript{6}. In addition the D-test, as suggested by Jenssen, was performed for all the isolates for detection of Inducible clindamycin resistance\textsuperscript{7}. All isolates were tested for biofilm production by Congo red agar method\textsuperscript{8} and Christensen’s tube method\textsuperscript{9}.

For D test the discs of erythromycin (15µg) and clindamycin (2µg) were placed at the distance of 20 mm and the flattening of zone of inhibition adjacent to erythromycin disc (referred to as D test) was considered as inducible clindamycin resistance\textsuperscript{10}.

**Observations & Results**

The observed results amongst the study isolates are depicted in the table.

<table>
<thead>
<tr>
<th>S. aureus n=148</th>
<th>S. aureus n=148</th>
<th>ERM –S, CD – S (%)</th>
<th>MS\textsubscript{B} phenotype (%)</th>
<th>Inducible (D test positive) (%)</th>
<th>ERM –R, CD –R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm Producers (89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA (41)</td>
<td>10 (24.39)</td>
<td>1 (2.43)</td>
<td>24 (58.53)</td>
<td>6 (14.63)</td>
<td></td>
</tr>
<tr>
<td>MRSA (48)</td>
<td>8 (16.66)</td>
<td>1 (2.08)</td>
<td>21 (43.75)</td>
<td>18 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Non Biofilm producers (59)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA (54)</td>
<td>35 (64.81)</td>
<td>4 (7.40)</td>
<td>11 (20.37)</td>
<td>4 (7.40)</td>
<td></td>
</tr>
<tr>
<td>MRSA (5)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>2 (40)</td>
<td></td>
</tr>
</tbody>
</table>

Out of 148 \textit{S.aureus} isolates\textsuperscript{89} were biofilm producers and 59 were non biofilm producers. Amongst biofilm producing \textit{S.aureus}, 41 were MSSA & 48 were MRSA as against 54 MSSA & 5 MRSA in non-biofilm producers. Thus methicillin resistance was more frequently observed in biofilm producing \textit{S.aureus} compared to non-biofilm producing \textit{S.aureus}. Amongst biofilm producing MRSA, clindamycin resistance was seen in 81.25% (Inducible-43.75%, Constitutive-37.5%) of the isolates while in non-biofilm producing MRSA, clindamycin resistance was seen in only 60% (Inducible-20%, Constitutive-40%) of the isolates. It was also observed that 73.16% of biofilm producing...
MSSA isolates showed clindamycin resistance (Inducible-58.53%, Constitutive-14.63%) in contrast to 27.77% in non-biofilm producing MSSA isolates (Inducible-20.37%, Constitutive-7.40%). This reveals that biofilm production was more frequently associated with resistance to antimicrobials (both methicillin resistance as well as clindamycin resistance).

**Discussion**

*S. aureus* has acquired resistance to almost all antimicrobials by various mechanisms. Different virulence markers, including biofilm formation, and ability to acquire antimicrobial resistance make it one of the difficult to treat pathogen. Beta lactams have long been used in the treatment of staphylococcal infections. However, their use has been challenged by development of resistance and hypersensitivity (allergy) to these agents in some individuals. The mechanism of resistance to beta lactam group of antimicrobials in staphylococci is either production of beta lactamase or *mecA* mediated modified penicillin binding protein (PBP). These mechanisms can be detected phenotypically by testing the sensitivity of the isolate to penicillin and cefoxitin respectively.\(^1\)\(^2\)\(^6\)

The Macrolides, Lincosamides and Streptogramin B (MLSB) family of antibiotics serve as an alternative to treat staphylococcal infections in penicillin allergic patients, especially for skin and soft tissue infection. However, widespread use of MLSB antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLSB antibiotics, either constitutively or inducibly, leading to treatment failures. The most common mechanism for such resistance is target site modification mediated by *erm* genes which can be expressed either constitutively (Constitutive MLSB phenotype) or inducibly (Inducible MLSB phenotype). These mechanisms can be detected by placing clindamycin disk adjacent to erythromycin disk (D test) during routine antimicrobial susceptibility testing.\(^12\)\(^13\)\(^14\)\(^15\)

In addition, biofilm production increases the probability of the isolate being resistant to methicillin and clindamycin as has been observed in our study. Incorporating simple tests like growth on Congo red agar for detection of biofilm, in routine staphylococcal identification, may provide useful information to clinicians and may aid in selecting the antimicrobials for therapy. The resistance to methicillin and clindamycin was significantly higher in biofilm producing isolates (p value 0.001) suggesting a correlation between biofilm production and methicillin and/or clindamycin resistance. However, this phenotypic correlation needs to be verified at genetic level. It would be interesting to observe if the isolate exhibits methicillin and clindamycin resistance phenotypically, in biofilm producing strains, in the absence of the genetic mechanisms (genes) for the same.

**Conclusions:**

We feel that biofilm per se can attribute methicillin and clindamycin resistance in the absence of known genetic mechanisms for their resistance. But it needs to be extensively studied. We could not verify the same due to resource constraint. Nevertheless we are inclined to suggest that the simple tests like D test and growth on congo red agar can be performed in routine clinical microbiology laboratory, thereby guiding the clinicians in effective management of staphylococcal infections.
References:


