Glucose & sodium chloride induced biofilm production & ica operon in clinical isolates of staphylococci

Asthag Agarwal & Amita Jain

Department of Microbiology, King George’s Medical University, Lucknow, India

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Background & objectives: All colonizing and invasive staphylococcal isolates may not produce biofilm but may turn biofilm producers in certain situations due to change in environmental factors. This study was done to test the hypothesis that non biofilm producing clinical staphylococci isolates turn biofilm producers in presence of sodium chloride (isotonic) and high concentration of glucose, irrespective of presence or absence of ica operon.

Methods: Clinical isolates of 100 invasive, 50 colonizing and 50 commensal staphylococci were tested for biofilm production by microtiter plate method in different culture media (trypticase soy broth alone or supplemented with 0.9% NaCl/ 5 or 10% glucose). All isolates were tested for the presence of ica ADBC genes by PCR.

Results: Biofilm production significantly increased in the presence of glucose and saline, most, when both glucose and saline were used together. All the ica positive staphylococcal isolates and some ica negative isolates turned biofilm producer in at least one of the tested culture conditions. Those remained biofilm negative in different culture conditions were all ica negative.

Interpretation & conclusions: The present results showed that the use of glucose or NaCl or combination of both enhanced biofilm producing capacity of staphylococcal isolates irrespective of presence or absence of ica operon.

Key words Biofilm production - glucose - ica operon - NaCl - staphylococci

Staphylococci are common cause of hospital acquired infections and biofilm is one of its important virulence factors. Its production is dependent on polysaccharide intracellular adhesin (PIA) synthesis. Regulation of PIA synthesis and biofilm production by microbes is a complicated process and is influenced by many factors, e.g. constitutional microbial factors, environmental factors and in clinical situations host proteins, etc. The enzymes involved in PIA synthesis are encoded by the ica operon comprising icaA, icaD, icaB, and icaC genes. Expression of the ica operon and formation of biofilm are highly variable and ica negative biofilm positive strains of staphylococci are also known to have alternative regulatory mechanism of biofilm formation. All colonizing and invasive staphylococcal isolates may not produce biofilm in some situations but may turn biofilm producers in other situations due to change in environmental factors. This study...
was planned to test the hypothesis that non biofilm producer clinical isolates of staphylococci turn biofilm producers in presence of sodium chloride (isotonic) and high concentration of glucose, irrespective of presence or absence of ica operon.

Material & Methods

Isolation and identification of staphylococci was done as reported in our previous study. The isolates were grouped in three categories: (i) Invasive isolates: Isolates obtained from two consecutive blood cultures of same patient. (ii) Colonizing isolates: Isolates from peripheral intravenous device (IVD) of the patients whose blood culture was negative for Staphylococcus. (iii) Commensal isolates: Staphylococcal isolates from skin and or nasal swab of patients, whose blood culture and peripheral IVD culture were negative for Staphylococcus.

Detection of biofilm: Isolates of S. aureus and coagulase negative staphylococci (CNS) were studied for biofilm producing capacity by microtiter plate method at 37° C, aerobically in the following culture media; A= Trypticase soy broth (TSB) alone, B= TSB+5 per cent glucose, C= TSB+10 per cent glucose, D= TSB+0.9 per cent NaCl, E= TSB+5 per cent glucose+0.9 per cent NaCl, F= TSB+10 per cent glucose+0.9 per cent NaCl.

Detection of ica ABDC genes: DNA from four to five colonies of each isolate was extracted and amplification of ica ADBC gene was done by using the protocol of Zeibhur et al.

Statistical analysis: Statistical analysis was done using SPSS (Statistical Package for Social Scientists) software of 15.0 version, USA. Chi square test was used for comparison of proportion.

Results & Discussion

Of the 200 isolates, 139 were S. aureus and 61 were CNS. Of the 100 invasive isolates, 84 were S. aureus and 16 were CNS (3 S. epidermidis, 13 S. haemolyticus); of the 50 colonizing isolates, 30 were S. aureus and 20 were CNS (17 S. epidermidis, 3 S. xylosus), and of the 50 commensal isolates, 25 were S. aureus and 25 were CNS (23 S. epidermidis, 2 S. saprophyticus).

Of the 84 invasive S. aureus isolates, 67 (79%) showed biofilm producing potential, followed by colonizing 73 per cent (22/30) and commensals 28 per cent (7/25) isolates (P<0.001 among groups). All three (100%) invasive S. epidermidis isolates were biofilm producers followed by colonizing [70.5% (12/17)] and commensal isolates [39.1% (9/23)]. Though all S. haemolyticus isolates were invasive, but only 30.7 per cent (4/13) were able to produce biofilm. S. saprophyticus and S. xylosus both were non biofilm producing.

Staphylococcus aureus:

Invasive isolates - The mean absorbance value in different culture conditions increased from 0.386±.05 (control) to 1.67±.20 (highest in presence of both glucose and NaCl). Of the 67 biofilm positive invasive isolates, 63 were ica positive (53 ica ADBC positive, 10 ica AD positive) (Table I). Of the 17 biofilm negative isolates, 15 turned biofilm positive (10 ica ADBC positive, 3 ica AD positive, and 2 ica negative) in presence of either glucose or sodium chloride or both. Two isolates remained biofilm negative (both ica negative) (Table II).

Colonizing isolates - The mean absorbance value in different culture conditions increased from 0.377±.02 (control) to 1.23±.10 (highest in presence of both glucose and NaCl). All 22 biofilm positive isolates were

| Table 1. ica operon in biofilm positive and negative staphylococcal isolates |
|---------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Groups | S. aureus (n=139) | | CNS (n=61) |  |
| | Biofilm +ve | Biofilm –ve | Biofilm +ve | Biofilm –ve |
| | N | ica +ve | ica –ve | N | ica +ve | ica –ve | N | ica +ve | ica –ve |
| Invasive | 67 | 63 | 17 | 13 | 4 | 7 | 7 | 9 | 7 | 2 |
| Colonizing | 22 | 22 | 0 | 8 | 3 | 5 | 12 | 10 | 2 | 8 | 5 | 3 |
| Commensal | 7 | 4 | 3 | 18 | 2 | 16 | 9 | 6 | 3 | 16 | 2 | 14 |
| Total | 96 | 89 | 7 | 43 | 18 | 25 | 28 | 23 | 5 | 33 | 14 | 19 |

CNS, coagulase negative staphylococci; N, number tested; +ve, positive; -ve, negative
Coagulase negative staphylococci:

Invasive isolates - The mean absorbance value in different culture conditions increased from 0.353±.03 (control) to 1.46±.06 (highest in presence of both glucose and NaCl). All nine (S. haemolyticus) biofilm negative isolates turned biofilm positive (5 ica ADBC positive, 2 ica AD positive and 2 ica negative) in presence of glucose/NaCl/both and three isolates remained biofilm negative (all ica negative) (Table II).

Colonizing isolates - The mean absorbance value in different culture conditions increased from 0.453±.04 (control) to 1.33±.10 (highest in presence of both glucose and NaCl). Of the 12 S. epidermidis biofilm positive isolates, 10 were ica positive (7 ica ADBC positive, 3 ica AD positive) and two were ica negative (Table I). Five of eight biofilm negative isolates turned biofilm positive (one S. xylosus ica ADBC positive, one S. xylosus and 2 S. epidermidis ica AD positive and 1 S. epidermidis ica negative) in presence of glucose/NaCl/both but three isolates remained biofilm negative (Table II).

Commensal isolates - The mean absorbance value in different culture conditions increased from 0.301±.03 (control) to 0.755±.07 (highest in presence of both glucose and NaCl). Of the 9 biofilm positive S. epidermidis isolates, six were ica positive (4 ica ADBC positive, 2 ica AD positive). Of the 16 (12 S. epidermidis, 1 S. saprophyticus) biofilm negative isolates, 13 turned biofilm positive (1 S. epidermidis was ica ADBC positive, 1 S. Saprophyticus and 3 S. epidermidis were ica AD positive and 8 ica negative) in presence of glucose/NaCl/both and three isolates remained biofilm negative (all ica negative) (Table I, II).

The present study showed significant increase in biofilm production by clinical isolates of Staphylococcus when exposed to increasing concentration of glucose and sodium chloride. Increase was higher when NaCl and glucose both were supplemented in the medium. No significant biofilm formation has been reported if bacteria are grown in TSB alone irrespective of the fact that TSB already contains substantial amounts of glucose. Supplementation of 0.2 per cent glucose in TSB is sufficient to induce a visible biofilm formation and a further increase in glucose concentration up to 1 per cent increases the biofilm formation significantly. Rode et al. compared individual effects of glucose and NaCl with the combination of glucose plus NaCl. Strains showed highest biofilm production in the presence of both glucose and NaCl.

**Table II. Biofilm negative S. aureus and CNS isolates turned biofilm positive in different culture conditions**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biofilm -ve</th>
<th>Biofilm -ve isolates turned biofilm +ve</th>
<th>Remain -ve</th>
<th>Biofilm -ve</th>
<th>Biofilm -ve isolates turned biofilm +ve</th>
<th>Remain -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Invasive</td>
<td>17</td>
<td>6</td>
<td>11</td>
<td>14</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>COLONIZING</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Commensal</td>
<td>18</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>11</td>
<td>18</td>
<td>26</td>
<td>32</td>
<td>34</td>
</tr>
</tbody>
</table>

Sh, Staphylococcus haemolyticus; Se, Staphylococcus epidermidis; Sx, Staphylococcus xylosus; Ss, Staphylococcus saprophyticus; TSB, trypticase soy broth; A, TSB alone; B, TSB+5% glucose; C, TSB+10% glucose; D, TSB+0.9% NaCl; E, TSB+5% glucose+0.9% NaCl.
formation in the presence of glucose and NaCl rather than for each compound separately.

Expression of the ica ADBC operon is considered to be essential for the synthesis of polysaccharide intercellular adhesin (PIA), which mediates cell-to-cell adhesion. Some biofilm producing isolates were found to be ica operon negative in our study, as has been reported earlier. Cafiso et al. analyzed the transcriptional activity of ica operon genes in a sample of biofilm positive and biofilm negative staphylococcal isolates and concluded that biofilm production takes place only when ica D was co-expressed with ica A. ica independent mechanism for biofilm formation was first studied by Cucarella et al., who demonstrated that biofilm associated proteins (Bap) were involved in ica independent biofilm formation mechanism. The production of PIA is subjected to on-off switching, and may be involved in phase-variation that might improve bacterial survival and growth under changing environmental conditions in vivo. Environmental regulation may play an important role in biomaterial related disease. icaR is a strong negative regulator of the ica locus, as deletion of icaR augmented PIA production by nearly 10-fold and increased transcription of the ica locus by 100-fold. The expression of this gene alone induces low enzymatic activity and the production of low amounts of polysaccharide. However, the simultaneous expression of icaA and icaD promotes a significant increase in N-acetylg glucosaminyl transferase, with a consequent increase in the amount of polysaccharide, forming oligomers of 10-20 β-1,6-N-acetylg glucosamine residues.

Several ica independent regulatory genes have also been reported. The agr gene has been shown to increase biofilm detachment and mutation of the system, leading to decreased biofilm growth. Staphylococcal accessory regulator (Sar) gene regulates genes of cell wall-associated adherence factors increasing the ica transcription and PIA/poly-N-acetyl glucosamine (PNAG) production. Additional components, such as accumulation-associated protein (Aap), DNA and RNA independently or in cooperation with the ica operon, have also been suggested to be important in CNS biofilms. Bap has been shown to be involved in the initial attachment, intercellular adhesion and biofilm formation of S. aureus. NaCl has been found to significantly induce more biofilm in methicillin susceptible Staphylococcus aureus (MSSA) than in methicillin resistant Staphylococcus aureus (MRSA) isolates. NaCl is a known activator of ica transcription and biofilm development in MSSA is ica ADBC dependent. Biofilm development in other isolates is primarily glucose induced, may be ica independent and involves a protein adhesion.

In conclusion, our findings show that increasing the concentration of glucose and sodium chloride enhances biofilm production capacity of pathogenic as well as non pathogenic staphylococcal isolates irrespective of presence or absence of ica operon.

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**References**


Reprint requests: Prof. Amita Jain, Department of Microbiology, King George’s Medical University, Lucknow 226 003, India
e-mail: amita602002@yahoo.com