Prevalence of biofilm and betalactamase-producing staphylococcus in nasal and throat isolates from healthy volunteers: A medical alert

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ABSTRACT
Objective: To study the prevalence of virulence factors such as Biofilm and Beta-lactamase in Staphylococcus isolates residing in nasal and throat mucosa in healthy volunteers.

Materials and Methods: Nasal and throat swabs were taken from 100 healthy volunteers at Gulf Medical University and Gulf Medical College Hospital, Ajman, UAE, and cultured for Staphylococcus isolates on appropriate culture media. The isolate were classified as Staphylococcus aureus or Coagulase Negative Staphylococcus (CoNS) based on the growth characteristics on Mannitol Salt Agar and standard tube coagulase test. They were further tested for Biofilm production by Christensen’s tissue culture plate and Congo red agar methods. The positive samples were identified for beta-lactamase by iodometric tube method.

Results: Of the 100 Staphylococcus isolates, 41 were Staphylococcus aureus of which 25 (61%) were positive for biofilm production whereas 19 (46.3%) were Beta-lactamase positive. Of the 16 (39%) biofilm negative Staphylococcus aureus isolates, 13 (31.7%) were Beta-lactamase positive. Among the 59 CoNS isolates, 38 (64.4%) were positive for biofilm production and 18 (30.5%) were Beta-lactamase positive. Twenty one CoNS samples (35.5%) were negative for both biofilm and Beta-lactamase production. Biofilm production in Staphylococcus aureus and CoNS did not show any significant difference (61% and 64.4%). Predominance of Staphylococcal isolation was in males between the age group of <20 years, mostly from the nasal site.

Conclusion: Biofilm-producing Staphylococcus appear to inhabit the normal flora of the nasal and throat mucosa of healthy individuals. Beta-lactamase production was found to be higher in Staphylococcus aureus positive for biofilm producers as compared to CoNS. Transmission of these biofilm producers with drug resistance factors from the healthy individuals to those at risk, like patients on long term catheterization or with indwelling devices need to be considered.

Key words: Staphylococcus aureus, CoNS, Biofilm, Beta-lactamase

INTRODUCTION
Staphylococcus epidermidis and Staphylococcus aureus are the predominant and persistent inhabitants in the anterior nares and the throat as part of the normal flora1−3. The nasal site serves as a breeding ground for the Staphylococcus species to grow and multiply remaining as non-pathogens, until they disseminate through the blood stream or breached epithelial surface to other sites4. There the growth and up regulation of adherence factors occur5,6. The virulence of the coagulase negative Staphylococcus species is related to its capacity to produce biofilms. Such biofilm-related infections are extremely difficult to treat and have to be diagnosed early. The major components of the extracellular polymeric substance (EPS) of the Staphylococcal biofilm consist of poly-N-acetyl glucosamine (PNAG). Certain strains lack PNA Gand among these strains the extracellular teichoic acid was noticed as a new component of Staphylococcal biofilm7. Formation of biofilm in Staphylococcus is considered a four step process, consisting of adherence,
accumulation, maturation and later dispersal.

Biofilm is an important colonization factor as well as a virulence factor in bacterial adherence. Colonization occurs in the principal implants like central venous catheters, heart valves, ventricular assist devices, coronary stents, neurosurgical ventricular shunts, implantable neurological stimulators, fracture-fixation devices, arthro-prostheses, inflatible penile implants, breast implants, cochlear implants, intra ocular lenses and dental implants. The objective of this study was to determine the presence of biofilm formation and beta-lactamase production in Staphylococcus aureus and Coagulase negative Staphylococcus inhabiting the normal flora in the anterior nares and throat of normal healthy individuals belonging to a Medical University.

MATERIALS AND METHODS
The study was conducted among 100 healthy volunteers in Gulf Medical University (GMU), Ajman, UAE after getting the approval from the institutional Ethics Committee. Written consent was taken from all the volunteers after explaining the objective of the study and the procedure of taking samples. The nasal and throat swabs were aseptically collected and processed for Gram staining and isolation on Blood agar, Mannitol salt agar and MacConkey agar. The Staphylococcus isolates were categorized as Staphylococcus aureus and CoNS based on their cultural characteristics and standard tube coagulase test. The biofilm qualitative detection was done by two methods: Christensen’s method and Congo Red Agar method.

In Christensen’s method, a few colonies of the test organism were inoculated in 200µl tryptcase soy broth with 1% glucose in triplicate into flat bottom polystyrene tissue culture plate wells and incubated for 24 and 48 hours at 37°C aerobically. The contents were gently aspirated and the wells were washed several times with phosphate buffer saline with a pH of 7.2 to remove the free floating bacteria. The biofilm formed in the microwells were stained with 1% neutral red. The biofilm positive bacteria stained pink at the bottom and on the walls of the tissue culture plate wells.

In Congo Red Agar method, as described by Freeman et al. the CRA was prepared with brain heart infusion broth 37g/L, sucrose 50g/L, agar 10g/L, and Congo red indicator 8g/L. Sterile Congo red stain was prepared as a concentrated aqueous solution. It was added to the sterilized brain heart infusion agar with sucrose at 55°C. The CRA plates were inoculated by the streak method with the test organisms and incubated at 37°C for 24 hours and after checking incubation was continued for another 24 hours aerobically. Isolates positive for biofilm production formed black colored dry colonies of a crystalline consistency and biofilm negatives produced pink colored smooth colonies.

Further, these isolates were screened for beta-lactamase by the iodometric tube method (ITM). The beta-lactamase positive isolates were screened for antibiotic susceptibility testing by Kirby Bauer disc diffusion method.

RESULTS
The male to female ratio among the 100 healthy volunteers was 54:46. The gender participation in various age groups is shown in the Figure 1. The age distribution was between 18 and 60 years. The majority of the participants (85%) were below 30 years of age.

Of the 100 nasal swabs collected 63 isolated Staphylococcus species, 24 were Gram positive bacilli (Diphtheroids) and 13 swabs had no growth. Out of 100 throat swabs 37 isolated Staphylococcus species, 2 Gram negative bacilli (Klebsiella pneumonia and Enterobacter sp.), 41 Gram negative cocci in groups (non-pathogenic Neisseria) and 20 swabs showed no growth.

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Staphylococcal isolation was higher in the nasal site (63%) compared to the throat (37%). Isolation rate was higher in the age group below 20 years of age (58%), followed by the 21-30 years (26%). In the remaining age groups, isolation was minimal.

As shown in Table 1, irrespective of age and gender, the CoNS isolates were significantly higher than Staphylococcus aureus in nasal and throat specimen (3:2). The number of Staphylococcus isolates was common among the younger age group (<20 years).

The presence of the biofilm formation was tested by two methods: CRA and TCP methods. Beta-lactamase was tested by the nasal site (63%) compared to the throat (37%).

As shown in Table 2, the distribution of Staphylococcus isolates based on age and gender is significant. The CoNS isolates were significantly higher than Staphylococcus aureus in nasal and throat specimen (3:2). The number of Staphylococcus isolates was common among the younger age group (<20 years).

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**Table 1. Distribution of Staphylococcus isolates based on age and gender**

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>Male n=54</th>
<th>Female n= 46</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>CoNS</td>
<td>S. aureus</td>
<td>CoNS</td>
</tr>
<tr>
<td>No.</td>
<td>No.</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>&lt;20</td>
<td>13</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>21-30</td>
<td>08</td>
<td>06</td>
<td>02</td>
</tr>
<tr>
<td>31-40</td>
<td>02</td>
<td>01</td>
<td>02</td>
</tr>
<tr>
<td>41-50</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>51-60</td>
<td>0</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>&gt;60</td>
<td>01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>%</td>
<td>46.2</td>
<td>53.7</td>
<td>34.7</td>
</tr>
</tbody>
</table>

**Table 2. Biofilm and Beta-lactamase production in Staphylococcus isolates**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Congo Red Agar Method (CRA)</th>
<th>Tissue culture Plate Method (TCP)</th>
<th>Tube Iodometric Method (ITM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biofilm (+)</td>
<td>Biofilm (+)</td>
<td>Beta-lactamase (+)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25</td>
<td>61</td>
<td>25</td>
</tr>
<tr>
<td>CoNS</td>
<td>34</td>
<td>57.6</td>
<td>38</td>
</tr>
</tbody>
</table>
iodometric tube method. Biofilm detection by both the methods were alike in Staphylococcus aureus (60.9%), whereas more positivity (64.4%) was obtained by the TCP method compared to the CRA method (57.6%) in the CoNS. Beta-lactamase was positive for Staphylococcus aureus (78%) and positive for CoNS (37.2%) as shown in the Table 2.

Figure 2 shows the number of positive and the negative results for biofilm production by both the tissue culture plate method and beta-lactamase production by Tube iodometric method among the Staphylococcus aureus and CoNS. Of the 100 Staphylococcus isolates 37 were negative for biofilm production and 46 were negative for beta-lactamase production. The beta-lactamase positive isolates were resistant to Penicillin and Ampicillin and susceptible to Vancomycin, Oxacillin and Rifampicin.

**DISCUSSION**

Biofilm formation in bacteria is now a major problem in the medical field since it is responsible for many recalcitrant infections and also difficult to be eradicated. It contributes to virulence factors like the ability to avoid host immune response, restricted penetration of antimicrobial agents into the biofilm and exhibition of resistance to antibiotics due to various mechanisms including beta-lactamase production.

Staphylococcal isolation was high in the nasal site among the Japanese population, as reported by Tadayukiin. Our results were comparable, with increased Staphylococcus isolation (62%) from the nasal site. Karina et al. conducted a similar study among the medical students in Brazil and observed a percentage of nasal Staphylococcus aureus isolation of 40.8%. In our study the nasal Staphylococcus aureus isolation was 21%. Samie et al. conducted a similar study on biofilm and beta-lactamase detection using similar methods and detected 42% were biofilm producers among which 16% were beta-lactamase positive. In our study, 63% were biofilm producers among which 37% were beta-lactamase positive. Our study detected 63% of biofilm producing Staphylococcus isolates, similar to a study done in Pakistan (54.8%) by Joanna et al. The resemblance in the results may be due to similar culture, living conditions and geographical location. Both the conventional methods for biofilm detection (CRA and TCP) gave similar results in Staphylococcus aureus isolates, whereas the positivity in CoNS was slightly higher by the TCP method, agreeing with the earlier studies. However, Ruzicka et al. had done genetic studies detecting ica operon responsible for the biofilm production and compared with the similar
Colonization of biofilm forming CoNS is the current threat to effective antibiotic therapy because of the increasing difficulty in detection and management of infections, leading to fatal outcomes. All the beta-lactamase producers were resistant for Penicillin and Ampicillin but showed 100% sensitivity to Vancomycin corresponding with the earlier reports. There is a possibility of transmission of these virulence factors from the healthy individuals to those at high risk such as patients on long term catheterization, or having indwelling devices in a medical set up, which may be difficult to treat with the commonly available antimicrobial agents.

CONCLUSION
Biofilm detection is more reliable by the Tissue culture plate method than with the Congo Red Agar method. Also, the virulence factors, biofilm production and beta-lactamase production, seem to be present in the Staphylococcus isolates which normally inhabit the upper respiratory tract.

RECOMMENDATION
Since the biofilm and beta-lactamase virulence factors seem to be present in the normal flora of healthy individuals there is a need to screen for these virulence factors among the healthy individuals who are posted in high risk units where medical device implantation, catheterization etc. are commonly carried out.

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REFERENCES


