Production of Slime Layer by Staphylococcus Epidermidis Isolated from Hip and Knee Joint Infections during Revision Arthroplasty of Patients in Abn- Sina Hospital in Baghdad

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Abstract

This study was carried out to determine the ability of Staphylococcus epidermidis strains to produce slime layer and biofilm formation during a period of study which extended from 1 October, 2016 till 1 October, 2017. Analytic Profile Index (API) system for Staph (Bio-Merieux, France) was used to identify of bacteria to species level. According to Kirby-Bauer (disk diffusion) technique, the antimicrobial susceptibility test was done. Frequency of Staphylococcus epidermidis strains isolated from hip and knee joint infections was higher in male than female patients, 12(85.7%) vs. 2 (14.3%) (P-value ≤ 0.05). From antimicrobial susceptibility test, the most effective antibiotic was Vancomycin which showed 100% susceptibility rate and the highest resistance rate was to Benzylpenicillin (85.7 %). followed by Erythromycin and Ampicillin (78.6%). Staphylococcus epidermidis strains were revealed different degrees of resistance towards remaining antibiotics. Out of 14 Staphylococcus epidermidis strains under study, 11(78.6%) of Staphylococcus epidermidis strains were slime layer production, while only 3(21.4%) of Staphylococcus epidermidis strains were non slime layer production (P-value ≤ 0.05) this indicate that Staphylococcus epidermidis strains from Hip and knee infections have the ability to format biofilm.

Key words: Staphylococcus epidermidis, Hip and knee infections, Osteomyelitis, Slime layer production and Biofilm formation.

Introduction

The infection of hip and knee joint can occur in different ways, (I) via injection or during joint operation through direct colonization, (II) by direct contact with a neighboring infected site, or (III) by haematogenous or lymphogenous seed of the pathogen. Among joint infections, the hip is the second most frequent localization after the knee joint [1, 2].

The most serious complication of hip and knee arthroplasty, is infection of the joint, which occurs in <1% of patients. Risk factors for infection are related to both patient and surgical factors [3].Gram-positive (GP) bacteria, especially Staphylococcus, classically cause this disease [4, 5]. Staphylococcus epidermidis (S. epidermidis) is a Gram-positive bacterium, and one of over 40 species belonging to the genus Staphylococcus [6].

It is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora [7].

S. epidermidis is a particular concern for people with catheters or other surgical implants because it is known to form biofilms that grow on these devices [8]. In osteomyelitis following inoculation, bacteria adhere to bone extracellular matrix and surgical implants. Staphylococci possess a large variety of adhesive proteins and glycoproteins which mediate binding to bone via receptors to fibronectin and other structural proteins [9, 10].

They cause osteolysis by interaction of bacterial surface components with immune system cells leading to cytokine production. Bacteria elude antibiotics and host defenses by lowering their metabolic rates, formation of a glycocalyx coat and “hiding” intracellular
The ability to form biofilms on plastic devices is a major virulence factor for S. epidermidis.

It produces an extracellular material known as polysaccharide intercellular adhesin (PIA), which is made up of sulfated polysaccharides (slime layer) [8]. A bio film is defined as bacterial aggregates in a matrix (slime layer), attached to various biotic and abiotic surfaces which interact with each other to adapt themselves to environmental stressors compared to plank tonic existence [12].

Bio films have great importance for public health because of their role in certain infectious diseases and importance in a variety of device-related infections.

Microbes within bio film communities become much less susceptible to antibiotics, even if highly susceptible as individual cells [13]. This study was aimed to determine the ability of S. epidermidis strains isolated from hip and knee aspiration specimens cultures to slime layer production and biofilm formation.

**Methods**

**Collection of Specimens**

A total of 38 aspiration specimens were collected from hip and knee joint during revision arthroplasty of patients with osteomyelitis infections in Abn- Sina hospital in Baghdad during the period extended from 1 October, 2016 till 1 October, 2017.

**Isolation and Identification of Bacterial Isolates**

Fourteen *Staphylococcus epidermidis* strains were isolated from hip and knee aspiration specimen’s cultures.

These isolates were isolated according to standard microbiology methods [14], and then bacteria were identified at species level by using API Staph (Analytic Profile Index) system (Bio-Merieux, France), for the identification of the *Staphylococcus* spp. according to the manufacturer’s instructions.

**Antimicrobial Susceptibility Test**

Antibiotic susceptibility test towards different groups of antibiotics (Oxacillin, Benzylpenicillin, Tetracycline, Ampicillin, Erythromycin, Levofloxacin, Vancomycin, Aoxicillin/Clavulanic acid, Aztreonam, Clindamycin, Cefazolin, Ciprofloxacin, Gentamicin and Trimethoprim-Sulphamethoxazole). Was performed according to Kirby-Bauer (disk diffusion) technique [15], using Muller-Hinton agar. Inhibition zones developed around the discs were measured by millimeter (mm) using a metric ruler according to Clinical Laboratories Standards Institute (CLSI, 2011).

Results were read according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS) [16].

**Detection of Bio film Formation**

All *Staphylococcus epidermidis* strains were subjected to biofilm production. The method used for testing biofilm production in isolated bacteria include qualitative Congo red agar plate test (CRA test).

**Congo Red Agar (CRA) Method**

This method is based on the characteristic cultural morphology of biofilm-forming bacteria on Congo red medium. Qualitative evaluation of biofilm producers using the Congo red agar method to detect slime production was performed as follows: The medium is comprised of brain heart infusion broth (Hi media /India) 37 g/l, sucrose (BDH / England) 50 g/l, Congo red (Fluka) 0.8 g/l and agar (Bio life /Italy) 10 g/l. Inoculated plates were incubated at 37°C for 24 hr.

Slime producing strains presented black colonies while non-producing strains developed red colonies. The scale of colony color evaluation was assessed as follows: very black and black colonies were biofilm producing strains; almost black colonies were weak biofilm producers while very red to Bordeaux colonies were considered as non bio film producing [15].

**Statistical Analysis**

The Chi-square (χ²) test was employed for comparison among groups. P value ≤ 0.05 was considered statistically significant.

**Results**

**Study Patients**

Aspiration specimens were collected from 38 patients with hip and knee joint infections during revision arthroplasty in Abn- Sina hospital...
hospital in Baghdad along a period extended from 1 October, 2016 till 1 October, 2017.

Isolated Bacteria
Out of 38 aspiration specimens of hip and knee joint infections, 14(36.8%) *Staphylococcus epidermidis* strains were isolated. Isolated bacteria were from patients within different ages, but according to gender, frequency of *S. epidermidis* strains isolation from hip and knee joint infections was higher in male than female patients, 12(85.7%) vs. 2 (14.3%) (P-value ≤ 0.05) as shown in Figure-1.

![Figure 1: The numbers of male and female infected with *Staphylococcus epidermidis* strains of hip and knee joint infection](image)

Antimicrobial Susceptibility Test
Susceptibility of 14 *Staphylococcus epidermidis* strains towards different antibiotics under study was summarized in table-1. From this table, we notice the most effective antibiotic was Vancomycin which showed 100% susceptibility rate. This study also showed a highest resistance to Benzylpenicillin (85.7 %) followed by Erythromycin and Ampicillin (78.6%). On the other hand, the bacterial isolates were revealed different degrees of resistance towards remaining antibiotics.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S (%)</th>
<th>R (%)</th>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>3 (21.4%)</td>
<td>11 (78.6%)</td>
</tr>
<tr>
<td>Aoxicillin/Clavulanic acid</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>7 (50%)</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>2 (14.3%)</td>
<td>12 (85.7%)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>6 (42.9%)</td>
<td>8 (57.1%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6 (42.9%)</td>
<td>8 (57.1%)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>3 (21.4%)</td>
<td>11 (78.6%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>6 (42.9%)</td>
<td>8 (57.1%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>4 (28.6%)</td>
<td>10 (71.4%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
</tr>
<tr>
<td>Trimethoprim-Sulphamethoxazole</td>
<td>6 (42.9%)</td>
<td>8 (57.1%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>14 (100%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Detection of Bio film Formation
In the Congo red agar method, slime production was investigated. Out of 14 *Staphylococcus epidermidis* strains, 11 (78.6%) of *Staphylococcus epidermidis* strains were slime production, while only 3(21.4%) of *Staphylococcus epidermidis* strains were non slime production (P-value ≤ 0.05) (Figure-2).
Discussion

Osteomyelitis is defined as an infection of the bone, and can be caused by a variety of bacteria or fungi. However, Staphylococci provide the majority of cases [18]. Bacteria can be introduced through a hematogenous route or direct inoculation during surgery, trauma or an overlying infection [19]. Staphylococci can produce a multilayered biofilm embedded within a glycocalyx or slime layer with heterogeneous protein expression throughout [20]. In this study, Out of 14 Staphylococcus epidermidis strains, 11(78.6%) of Staphylococcus epidermidis strains were slime production and only 3(21.4%) strains were non slime production. This gave an evidence that the most Staphylococcus epidermidis strains under study were slime layer produced and have the ability to format biofilm.

References


