Biofilm Formation and Presence of Esp and cylA Genes Enterococcus faecalis Isolated from Hospital Infection

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Abstract

Background and purpose: Enterococcus faecalis normal intestinal flora of humans and one of the menagaditise the ability to form biofilm on surfaces such as catheters, venous catheters artificial heart valves and ocular lenses, the ESP and cylA virulanse factors in E. faecalis. The purpose of this study was to evaluate the ability of the bacteria in the biofilm formation and detection of virulence factors in clinical isolates of enterococci surface protein and cytolysin.

Method and Materials: A total of 54 clinical E. faecalis isolates was collected from hospitals Ability of biofilm formation was measured by Microtiter plate assay. All isolates were then examined for presence of the Esp and cylA genes by PCR.

Results: The microtiter plate assay results showed that attachment abilities in 4 (7%) strains were strong, 10 (26%) strains were moderate, and in 14 (56%) strains were weak and 5 (11%) strains didn't form biofilm. The prevalence of the ESP and cylA genes identified by PCR among clinical isolated strains, and the results were 83% and 70%, respectively.

Conclusion: Our results suggest different biofilm formation ability in clinical isolated strains of E. faecalis. Biofilm formation on medical devices such as intravascular catheters, artificial heart valves and ocular lenses can increase antibiotic resistance and cause many health problems.

Introduction

Enterococcus faecalis Gram-positive coci and natural inhabitant of the human is the most common bacterial pathogens worldwide [1]. The third most common cause of nosocomial bacteria is then Staphylococcus aureus and Escherichia coli [2]. Of the causes endocarditis, meningitis, urinary tract infections- endophthalmitis and antibiotic resistance is extensive [3]. The emergence of antibiotic resistance threatens the successful treatment of various infections [2]. It is the third leading cause of infection after abdominal surgery and trauma caused by the removal of the epithelial layer continuity and colon cancer [4]. Biofilms are complex microbial cell surface polysaccharide matrix that binds irreversibly causing is bacteria survive in unfavorable conditions [5]. The biofilm formation in bacteria is a social behavior that it passes over three decades of research Enterococcus infections in the host's ability to form biofilms, biofilms cause survival stability and continuity in medical devices such as catheters - prosthetics - implants and trauma treatment and medical cost increases. In addition to the tolerance of the host immune system, such as phagocytosis cause genetic exchange between cells forming the biofilm is very fast [6]. With increasing drug resistance in enterococci studying virulence factors associated with colonization and pathogenesis of this bacteria is essential Expression of specific genes and environmental conditions with close ties to the biofilm formation of E. bonding on surfaces of medical tools such as catheters - catheter and ocular lenses and the production of biofilm reported [7]. Enterococcus faecalis virulence factor of several hydrolytic enzymes, Surface proteins and toxins [8]. ESP: This gene was first identified in MMH594 strains with1873 amino acid and 202 kDa, 13 sequence conservation (744-1665) which N-terminal from (50-743), which is essential in the action against the host. The c terminal (1666-1873), which is located in the hydrophobic membrane [9] (Figure 1).

The presence of this gene is associated with urinary tract infections, bacteremia [10]. Tendolkar and preeti in research found the presence of this factor in the ability to form biofilms and biofilm thickness is proved. Removal of N-terminal region of the
virulence factor in reducing the ability of bacteria to form biofilms was impressive [11].

cylA: Part of an eight-subunit operon is the gene for the removal of the N-terminal amino acid subunits and activation is essential L-S subunit [4] (Figure 2).

Virulence factor in causing the uncontrolled release of inflammatory mediators from damaged tissue and cells are phagocytic. It is the production of extracellular enzymes and toxins. Cytolysin moved on Mobile genetic elements such as plasmids and exacerbated destruction of blood cells. Further access pathogen to food and increased infection [12].

Biofilm Formation Assay

A modified microtiter plate method was followed as previously described [13]. Briefly, the wells of microtiter plate were filled with 180 μl of trypticase soy broth (TSB) supplemented with 5% glucose. Then, a 20 μl quantity of previously prepared bacterial suspensions with turbidity equal to 0.5 Macfarland standards was added to each well. The negative control wells contained 200 μl of TSB supplemented with 5% glucose. Incubation was carried out at 37°C for 24 h before removal of the cultures. Then, the cells were decanted, and each well was washed 3-times with sterile phosphate buffered saline, fixed by methanol for 20 min, dried at room temperature and finally strained with 0.1% safranin. The safranin dye bound to the adherent cells was dissolved with 1 mL of 95% ethanol per well, and the plates were read at 490 nm (A490) using ELISA reader. Optical density cut-off (ODc) was determined. It is defined as average OD of negative control + 3× standard deviation (SD) of negative control. Formation of biofilm by isolates was analyzed and categorized relying on the absorbance of the safranin-stained attached cells (Table 1).

PCR screening for virulence-related genes: Genomic DNA was extracted from pure cultures using a Bacterial Genomic DNA Extraction Kit (cinapureTM DNAKIT, iran) and PCR was used to detect the presence of the virulence determinants esp and cylA. The primer sequences were blast NCBI site and synthesized by pishgam biotechnology company and PCR procedures were set based related references (Table 2).

Cut-off value calculation

<table>
<thead>
<tr>
<th>Mean of OD values</th>
<th>OD &gt; 4×ODc</th>
<th>OD &gt; 2×ODc</th>
<th>ODc &lt; OD ≤ 2×ODc</th>
<th>ODc &lt; OD ≤ 2×ODc</th>
<th>ODc &lt; OD ≤ 2×ODc</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODc &lt; OD ≤ 2×ODc</td>
<td>.054 &lt; OD ≤ .108</td>
<td>.108 &lt; OD ≤ .216</td>
<td>.216 &lt; OD ≤ 4×ODc</td>
<td>OD &gt; 4×ODc</td>
<td>OD &gt; 2×ODc</td>
</tr>
</tbody>
</table>

Table 1: Classification of biofilm formation abilities by Mtp method.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Product Size (bp)</th>
<th>Program PCr</th>
<th>Sequence Target (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[15]</td>
<td>95</td>
<td>60 s 95°C, 60 s 58°C, 60 s 72°C</td>
<td>F-GAACGCCTTGGTATGCTAAC R-CCACTTTATCAGCCTGAACC</td>
</tr>
<tr>
<td>[5]</td>
<td>114</td>
<td>60 s 95°C, 60 s 58°C, 60 s 72°C</td>
<td>F-TTATGCATCAGATCTCTCAA R-CGGAGTGCTTGCACTCAATTGG</td>
</tr>
</tbody>
</table>

Table 2. Primer sequences and program used in PCR.
54 isolates *E. faecalis* were selected for molecular screening for esp and cylA genes using PCR.

PCR amplification was performed with an Ep-pendorf thermal cycler (BIO RAD). Amplification program for ESP consisted of initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 60 sec, annealing at 63°C for 60 sec and extension at 72°C for 60 sec with a final step of 72°C for 10 min. The PCR products were analyzed by electrophoresis in a 2% agarose gel and stained with gel red.

**cylA gene detection**

Amplification program for cylA consisted of initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 60 sec, annealing at 58°C for 60 sec and extension at 72°C for 60 sec with a final step of 72°C for 10 min (Figure 3).

**Clinical bacterial strains**

The microtiter plate assay results showed that attachment abilities in 4 (7%) strains were strong, oundes in 10 (26%) strains were moderate, and in 14 (56%) strains were weak and 5 (11%) strains didn't form biofilm. The prevalence of the ESP and cylA genes identified by PCR among clinical isolated strains, and the results were 83% and 70%, respectively (Figure 4 and Table 3).

**Discussion**

Enterococci are as the natural microflora of the intestinal tract of humans and animals.

The bacteria under certain conditions lead to the emergence of urogenital tract infection, inflammation of bile ducts, endocarditis, meningitis and infections the skin. Several reports suggest an increase in innate and acquired resistance of bacteria and biofilm production is at least 16 the epidemic caused by multi-resistant enterococci have been reported from 1989 to 1998 [11]. Toledo and Arena to study the expression (P<.0001) estimated of the relationship between the presence of Esp gene and biofilm formation on the surface of Polystyrene. Biofilm formation in urine collection bag associated with the presence of the Esp gene [2]. Darini and colleagues in Brazil ESP genes effective in Biofilm formation and increases antibiotic resistance [14]. Shankar et al. stated that the N-terminal region of the gene Esp structural changes in the bacterial will help organism’s ability escape from the host immune response [15]. Chinorose and colleagues stated that the prevalence of ESPgene among strains resistant to highly gentamicin (86%) is associated [9]. Van Dyne D and colleagues in the study stated that the presence cytolisin gene is exacerbated in infection in humans [1]. Coburn and his colleagues in the study stated is related to the expression of this operon cytolisin with Biofilm formation and biofilm formation synergistic factor AS and collaboration with Esp genes are highly interrelated [4]. Chinorose and colleagues in a study of the prevalence of isolates resistant to gentamicin cylA genes, 57 percent indicated this gene is associated with biofilm production [9]. Our research was reported in the gene esp 83% and the prevalence of the gene cylA 70% of the geographical area and genetic characteristics of different strains may be due to differences in prevalence. Enterococcal is second cause bacteremia and endocardit and the third most common cause of urinary tract infection and urinary tract infection and bacteremia is the presence of the esp gene is associated. Given the high prevalence of these genes among the isolates in the study and collection of urine samples of both studies were consistent. Enterococcus effective bonding on surfaces of medical tools such as levels venous catheters - urinary and ophthalmic lenses and the production of biofilm bacteria in biofilms are reported because high concentrations of antibiotics tolerated. Appears at medical centers play an important role in the pathogenesis of antibiotics is the responsibility of Enterococcus. The use of antibiotics in these disease-gene expression and increased levels of inducible factors and pathogenesis of this bacteria increases.

**Conclusion**

Our results suggest different biofilm formation ability in clinical isolated strains of *E. faecalis*. Biofilm formation on medical devices such as intravascular catheters, artificial heart valves and ocular lenses can increase antibiotic resistance and cause many health problems.

We found relationships between biofilm formation and prevalence of virulence genes esp and cylA. 89% of the strains were able to form biofilm. The thickness of the biofilm increased in the presence of these genes.

<table>
<thead>
<tr>
<th>biofilm</th>
<th>esp negative</th>
<th>esp positive</th>
<th>cylA negative</th>
<th>cylA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No biofilm</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Weak biofilm</td>
<td>7</td>
<td>23</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>Moderat biofilm</td>
<td>1</td>
<td>13</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Strong biofilm</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 3.** The relationship between the genes and the level of biofilms.
Suggestions

Due to the presence of Enterococcus faecalis in hospitals, especially in prolonged hospitalization to reduce biofilm formation by bacteria and reduce antibiotic resistance can observe the following:

1. Cleaning and sterilization of medical devices
2. The use of urinary catheters and intravenous disposable
3. Investigation personnel carrier in terms of Enterococcus faecalis
4. Recognized source and possible routes of infection may prevent the formation of biofilms are effective.

Acknowledgment

This article is part of the research work is graduate thesis.

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