Comparison the presence of exotoxin A, T, S isolated from clinical and environmental sample Pseudomonas aeruginosa in hospitals of Qom city

Ali Javadi, Yaser Sharifi, Rouollah Khodadadian, Farzaneh Fakharian, Abbas Morovvat, Azadeh Abedzadeh, Fariba dastjani Farahani, Mehdi Rostami Rad

ABSTRACT

One of the most important bacterial pathogen is Pseudomonas aeruginosa causing nosocomial infections. The presence of several virulence factors such as exotoxins of the bacteria causing a serious threat to the patient. The purpose of this study was to investigate the presence of three exotoxin A, T, S, genes in the isolation and characterization of Pseudomonas aeruginosa isolated were confirmed by standard biochemical tests. Bacterial DNA was extracted using the SinaGlon Extraction kit. After the primers design and evaluation of Primer BLAST software on NCBI site, PCR reactions were performed to evaluate each gene. After electrophoresis, the bands obtains results from the reaction of PCR, was observed in gel doc device. The frequency of bacteria was obtained in the urine samples %22.8, in skin samples %28.94, in blood samples %19.29, in lungs %15.78 and the humid environments, samples %13.15. The Presence frequency of exotoxin T, S was obtained in clinical samples %37.9, %39.1 and in environmental samples %27.8, %24.3 respectively. Exotoxin A was present in all samples. Electrophoresis of PCR reaction producing results for all genes exotoxin was showed the expected bands, S fragment 318 bp, exotoxin T 471 bp, exotoxin A piece 664 bp. The results showed that the bacterial strains isolated from the skin samples are more than of other isolated. As well as the presence of Pseudomonas aeruginosa exotoxin genes in clinical samples is more than environmental samples. This shows exotoxins of the bacteria are a serious threat to patients. Therefore, as regards Pseudomonas aeruginosa bacteria are an opportunist pathogen in hospitals, It is important for isolation and identification of clinical and environmental samples. 

KEY WORDS: Pseudomonas aeruginosa, Exotoxin, clinical samples, environmental samples

INTRODUCTION

Pseudomonas bacteria are gram-negative aerobic and Pseudomonadaceae families. Members of this family have a variety of metabolic and ability to live in different environments (Japoni, 2009). Pseudomonas aeruginosa is an Associate in opportunist microorganism related to a variety of nosocomial infections (Shahcheragi, 2010). Tend to grow in humid environments, leading to the success of ecology and its importance as a major virulence of nosocomial infections (Stone, 2002). Pseudomonas aeruginosa is commonly found in soil, water, wet environments as well as in humans and animals (World Health Organization, 2000; Goldmann, 1996). This bacterium is an opportunistic pathogen and is acquired frequency of resistance to antibiotics, which make to abundant participation in nosocomial infections, While this negative bacteria to enter the blood causing to be one of the most lethal septicemia (Foca, 2000; Stone, 2002). One of the most important factors of the type III secretion system of the bacteria that cause direct injection of a series of proteins into the cytosol of the target cell (Strateva, 2010). Several researches have shown that five major enzymes involved Exo T, Exo U, Exo S, Y and Exo A in this system are transferred to the target cell. The three Exo A, Exo T, Exo S was investigated. Exo T and Exo S, the first enzyme in this group are known. Exo S is a cytotoxin which plays
an important role in the colonization of bacteria. Exo T produced during the release and escape of pathogens. In addition, both exoenzymes are ADP-ribosyl transferase activity which acts similar pathology cholera toxin (Bradbury, 2010). Briefly, exoenzymes S and T is the activity of ADP-Ribose transfers that decrease the activity of macrophages and phagocytosis. Exo Y increases the activity of adenylate cyclase and its effect on cell morphology. Exo U is a cytotoxin affecting epithelial cells and causing lung injury and also has a toxic effect on macrophages, while its mechanism of action not specified (Bush, 1995). Exo A is toxic to eukaryotic cells and inhibit protein inside the cell and Damage to lung tissue and the brain. Also the toxin is the activity of ADP-ribosil transferase and induced the production of interleukin-1 (Luzzaro, 2001). The presence of several virulence factors such as exotoxins of the bacteria which the most important pathogenic factors causing a serious threat to the patient. With regard to the Pseudomonas aeruginosa bacteria an opportunistic pathogen in hospitals, its important Isolation and identification of clinical and environmental samples. The aim of this study is the first, isolate Pseudomonas aeruginosa of various clinical and environmental samples, then investigate the presence of three exotoxin A, T, S, genes in the isolation and characterization of pollution samples Qom hospitals in different parts of this bacteria and comparison of the presence of exotoxin in clinical and environmental samples.

**Methodology:**

**Isolating and Identification bacteria:**

The study on the total sample suspected of Pseudomonas aeruginosa (170 samples) were collected from various hospitals in Qom. Isolated has been collected from urine, blood, skin, lung and humid environments hospitals. Of the 170 samples, 144 strains of Pseudomonas aeruginosa were confirmed by standard biochemical. For Identification, The first, gram-negative samples was inoculated in TSB medium and incubation for 24 hours 37 °c. Then were cultured in cetrimide agar medium by streaking method incubation for 24 hours 42 °c. Then bacteria were transferred to the TSI medium. The isolates that are alkaline reaction(non-fermentable sugars) and was produced without H2S gas, were cultured on Cetrimide agar medium and incubation for 24 hours in c°37. After growth, Single colonies transferred to OF medium containing glucose. The strains that were able to grow in aerobic OF medium But did not grow in anaerobic OF medium were identified as oxidizing glucose (non-fermented) or aerobic. All strains of Pseudomonas aeruginosa were stored at -70 °c.

**Extraction of bacterial genomic**

The Bacterial isolated were cultured in Brain Heart Infusion broth (Merck Co.) medium and incubated at room temperature for 24 hours in 37 °c. Bacterial DNA was extracted using the SinaClon Extraction kit.

**Sequence selection and specific primer design of PCR amplification:**

In Primer design process, first of were reviewed relevant articles. After have been studied the characteristics different of genes, the three major genes were selected and synthesized for exoenzymes T, S, A genes by the SinaClon Co. The gene sequence was designed to detect, S fragment 317 bp, T fragment 471 bp and exotoxin piece A 664 bp. For evaluation of specificity primer design, were evaluated by Primer BLAST software on the NCBI site (Figure 1).

<table>
<thead>
<tr>
<th>Fragment size</th>
<th>Melting temperature</th>
<th>Sequence primer</th>
<th>Primer name</th>
</tr>
</thead>
<tbody>
<tr>
<td>664bp</td>
<td>60.8</td>
<td>CAGGTGATCCGCCAACGCCCC</td>
<td>tox A F:</td>
</tr>
<tr>
<td></td>
<td>60.7</td>
<td>TCAGCCGTTCGAACCCTGCC</td>
<td>tox A R:</td>
</tr>
<tr>
<td>471bp</td>
<td>60.2</td>
<td>GCCTGCTCTCCGCTGCCGG</td>
<td>Exo TF</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>TTTCCGCCAGTGCTCCGG</td>
<td>Exo TR</td>
</tr>
<tr>
<td>318bp</td>
<td>60.5</td>
<td>GATGCGGAAAAGTACCTGGGC</td>
<td>Exo SF</td>
</tr>
<tr>
<td></td>
<td>60.9</td>
<td>CTCTCGCGACACCCGGG</td>
<td>Exo SR</td>
</tr>
</tbody>
</table>

**Fig. 1:** Primer sequences used in this study

**Polymerase chain reaction:**

PCR reaction was performed to examine each of the genes with Condition 25 microliter in a standard size. The reaction volume of 1.5 micro molar of Mg2+, 0.2 micro molar of dNTPs, 100 nano gram of DNA extracted from the bacteria, forward and Reverse primer concentration of 0.5 micro molar and Taq DNA Polymerase enzyme 1 unit of enzyme were used. The PCR reaction started with the initial melting temperature of 95 °c with 30 cycles for 2 minute, and continued in 94 °c for 30 Second, Annealing temperature of 60 °c for 30 Second, amplification temperature of 72 °c for 40 Second and finished at 72 °c for 5 minutes eventually. Microtubes containing deionized water were used as a negative control. Then was mixed the 7 microliter of the PCR product with 1 microliter of 6X Loading dye Buffer (Sinaclon Co.) and electrophoresis was performed by 1% agarose gel containing ethidium bromide at a 100 volts of voltage for 40 minutes. The reaction primers were designed so that it is possible to perform the reaction at the same time (Figure 6).
Result:

The level of bacteria was obtained in the urine samples %22.8, in skin samples %28.94, in blood samples %19.29, in lungs %15.78 and the wet environments, samples %13.15(Figure 3). The bacterial strains isolated from the skin samples are more than of other isolated. Figure 2, shows Distribution of Pseudomonas aeruginosa strains isolated of clinical and environmental samples from Qom hospitals.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Beheshti hospital</th>
<th>Nekouei hospital</th>
<th>Kamkar hospital</th>
<th>Total</th>
<th>(Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>9</td>
<td>10</td>
<td>7</td>
<td>26</td>
<td>%22.8</td>
</tr>
<tr>
<td>Skin</td>
<td>10</td>
<td>13</td>
<td>10</td>
<td>33</td>
<td>%28.94</td>
</tr>
<tr>
<td>Blood</td>
<td>7</td>
<td>9</td>
<td>6</td>
<td>22</td>
<td>%19.29</td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>18</td>
<td>%15.78</td>
</tr>
<tr>
<td>Humid environment</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>15</td>
<td>%13.15</td>
</tr>
<tr>
<td>(Hospital waste,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bathroom...)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>44</td>
<td>32</td>
<td>114</td>
<td>%100</td>
</tr>
</tbody>
</table>

n=170

Fig. 2: Distribution of Pseudomonas aeruginosa strains isolated of clinical and environmental samples.

The Presence of exotoxin T, S frequency was obtained in clinical samples %37.9, %39.1 and in environmental samples %27.8, %24.3 respectively. Exotoxin A was present in all samples (Figure 4, 5).

Figure 6, Electrophoresis of PCR reaction producing results for all exotoxin genes were shown the expected bands, S fragment 318 bp, exotoxin T 471 bp, exotoxin A piece 664 bp. On the one hand, the result of electrophoresis of PCR products, did not show any proliferative in the negative control sample which confirms the authenticity of the PCR reaction.

Fig. 3: Distribution of Pseudomonas aeruginosa strains isolated of clinical and environmental samples

Fig. 4: Abundance the presence of exotoxin genes in clinical samples.
Fig. 5: Abundance the presence of exotoxin genes in environmental samples.

Discussion:
In this study, examined 144 clinical and environmental isolates of Pseudomonas aeruginosa exotoxin genes and the presence of three exotoxin A, S, T genes were evaluated as described previously. The bacteria exotoxins have an important role in the pathogenesis of nosocomial infections. Some of these toxins, several studies have been conducted as follows in Iran and other countries. But the abundance had a different frequency of each exotoxin A, S, T genes. Accordingly, among the 144 isolates, were positive all isolates for the A exotoxin gene. (39.1%) isolates were positive for the production Exo S, Also (37.9%) isolates were positive for the production Exo T and the samples were humid hospital isolates of 24.3 and 27.8 percent, respectively, for the production of Exo S and Exo T. Also (37.9%) isolates were positive for the production Exo T. Khan et al in 1994 studied of Pseudomonas aeruginosa A exotoxin Gene in clinical and environmental samples And established that it can be useful for epidemiological studies and The samples were detected by PCR, to identify P. aeruginosa genes (Khan, 1994). Kamali et al in 2010 isolated P. aeruginosa of burn hospitalized patients and confirmed the presence of exotoxin A. To provide the recombinant proteins, the genes were isolated and cloned into the appropriate vector (Bayat, 2010). Wolska et al in 2009 examined the genetically characterized clinical isolates of Pseudomonas aeruginosa in the presence of 6 virulence genes. In their study, only exoS and exoA gene was determined by the 49 isolates that only 46.15 percent of their genes were exoS and 76.9 percent exoA (Wolska, 2009). In a study of Pollack et al in 1997 isolated the 75 samples of P. aeruginosa from the skin patients and
evaluated in the presence of exotoxin in vitro, which the 87 percent of them had the ability to produce exotoxins (Pollack, 1977). Berthelot et al in 2003 the first, isolated P. aeruginosa of bacteremia patients and evaluated of Exo S and Exo U by Real-time PCR. These two genes were examined among 16 serotypes, only stereotypes 4, both genes had (Berthelot, 2003). In this study, the most isolated are related to skin and urine samples of 28.94% and 22.8%, respectively, which was indicative of this matter that urinary tract infections and skin infections in patients still are among the most common hospital infections.

**Conclusion:**

The results showed that the bacterial strains isolated from the skin samples are more than of other isolated. As well as the presence of Pseudomonas aeruginosa exotoxin genes in clinical samples is more than of environmental samples. This matter shows exotoxins of the bacteria is a serious threat to patients. Therefore, as regards Pseudomonas aeruginosa bacteria are an opportunistic pathogen in hospitals, it’s important for isolation and identification of clinical and environmental samples.

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**REFERENCES**


