MOLECULAR EVALUATION OF PSEUDOMONAS AERUGINOSA
ISOLATED FROM PATIENTS IN BURN WARD, ICU, CCU AND ITU IN A
NUMBER OF HOSPITALS IN KERMAN PROVINCE

*Habibollahi Mohammad Hossein1, Roshdi Maleki Mehdi2, Abbasi Masoumeh2, AbediGholamreza3
and Dabighi Mohammad Masoud4

1Department of Microbiology, College of Sciences, Karaj Branch, Islamic Azad University, Alborz, Iran
2Department of Microbiology, Malekan Branch, Islamic Azad University, Maleka, Iran
3Department of Biology, Tabriz Branch, Payame Noor University, Tabriz, Iran
4Department of Microbiology, Hamedan Branch, Islamic Azad University, Hamedan, Iran

ABSTRACT
Pseudomonas aeruginosa has appeared as one of the most problematic Gram-negative nosocomial pathogens. It can cause a wide array of hospital-associated diseases, including respiratory tract infections, urinary tract infections, wound infections and bacteraemia. P. aeruginosa is an opportunistic pathogen that rarely causes disease in healthy individuals. Most infections are able to take hold by the loss of the integrity of a physical barrier to infection (eg, skin, mucous membranes) or the presence of immune deficiency. Two extracellular toxins, Exoenzyme S and exotoxin A, are important for the pathogenic activity of P. aeruginosa. Exotoxin A causes ADP-ribosylation of eukaryotic elongation factor 2, which results in inhibited protein synthesis, which has the same mechanism of action as the diphtheria toxin. Purified exotoxin A is highly lethal to animals, including primates and humans. In this study, 102 isolated strains (95/33%) of all strains had toxA gene, which suggests that exotoxin A is a major virulence factor of P. aeruginosa in hospitalized patients in burn ward, ICU, CCU and ITU.

Keywords: Pseudomonas aeruginosa, Exotoxin A, toxAgene, Nosocomial Infections

INTRODUCTION
Pseudomonas aeruginosa is one of the most pathogenic bacteria found in the human body. It is an opportunistic pathogen. Typically, an opportunistic pathogen will not cause disease in healthy individuals. However, given the opportunity, P. aeruginosa will multiply and release toxins within immune compromised body tissues. It is from the family Pseudomonadaceae. Members of this genus are known for their ability to live in both plant and animal tissues. P. aeruginosa is a gram negative bacterium most commonly found in soil, water, or any decomposing organic matter. This bacterium may usually be found in slimy layers of rocks in water or free living in soil particles (Donlan and William, 2002; Jaffe et al., 2001).

Though P. aeruginosa is commonly found in nature, it is also naturally occurring and lives symbiotically in many regions of the human body. For example, P. aeruginosa may be found in the intestinal tract, contact lenses, teeth or even the skin (Wendelboe and Baumbach, 2007). The only requirements for the survival of the bacterium are moisture and a carbon source, where as both may be readily obtained in the human body. All of these well adaptive characteristics combine to allow P. aeruginosa to realistically live almost anywhere. The most common targets of a P. aeruginosa infection are persons with cystic fibrosis, burn sites, and AIDS. This bacterium is responsible for death rates as high as 50%, 60%, and 50%, respectively. Other cases of infection involve medical devices such as catheters, joint replacements and heart valves. P. aeruginosa is the most common pathogen in all nosocomial infections (Donlan and William, 2002; Hentzer et al., 2002). According to the T.J. Clark website, “P. aeruginosa is responsible for 16% of nosocomial pneumonia cases, 12% of hospital-acquired urinary tract infections, 8% of surgical wound infections, and 10% of bloodstream infections [5]. P. aeruginosa may quickly become problematic once introduced to a hospital because of its virulence factors and antibiotic resistance (Feltman et al., 2001; Schulert et al., 2003). P. aeruginosa produces two
extracellular protein toxins, exotoxin A and exoenzyme S. Exotoxin A is an extracellular enzyme that is produced by most clinical strains of *P. aeruginosa*. It is a single-chain polypeptide with A and B fragments that mediate enzymatic and cell-binding functions, respectively. Exotoxin A catalyzes the transfer of the adenosine diphosphate-ribosyl moiety from nicotinamide-adenine dinucleotide to elongation factor 2, which results in the inactivation of the latter and the inhibition of protein biosynthesis (Feltman *et al.*, 2001; Khan and Cerniglia, 1994; Soong *et al.*, 2006; Winstanley *et al.*, 2005).

The purpose of this research is to recognize *P. aeruginosa* containing toxA gene and to study the relationship of this gene with virulence.

**MATERIALS AND METHODS**

**The Collected Samples and Biochemical Detection**

This study was conducted to cross-sectional. In this research the specimens were collected from patients in burn ward, ICU, CCU and ITU in a number of hospitals in Kerman province.

To isolate *P. aeruginosa* bacteria, the different samples were sent to the laboratory and cultured in MacConkeyagar, Chocolate agar, Blood agar and EMB at 37°C for one day, then the colony formation were examined by biochemical tests including Oxidase, Catalase, Arginine dihydrolase, Simmon's citrate medium, Characteristic pigments, Growth at 42°C, L-lysine decarboxylase, L-ornithine decarboxylase, SIM, MR-VP and TSI.

**DNA Extraction**

DNA strains were extracted with phenol-chloroform method. Accordingly, the bacterial suspension in lysis buffer (TrisHCl, EDTA, NaCl), SDS 25% and proteinase K was incubated for an hour at 60°C. Then the mixture of phenol-chloroform-Isoamyl alcohol was added according to precipitate and separate proteins. The obtained bacteria DNA was precipitated with cold ethanol and finally dissolved in TE buffer contain RNase (TrisHCl, EDTA, RNase).

**PCR and Electrophoresis**

PCR test for check in the presence of toxA gene which was performed by using the temperatures and primers listed in Table 1.

Also strains of *P. aeruginosa* ATCC 27853 as a positive control and Echerichia coli ATTC 25922 as a negative control were used. For PCR amplification, we used a mixture containing 0.5μl of DNA extracted to the PCR mastr mix with final volume25μl (2/5μl PCR 10X buffer, 1/5μl MgCl$_2$ 25mM, 0/6μl dNTP 10Mm, 0/5μl of each primer 10pmol and 0/1μl Taq polymerase 5U/μl).

**Table 1: The sequence of the primers and the temperatures used in the PCR test (Winstanley *et al.*, 2005)**

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer Sequence (5′-3′)</th>
<th>PCR</th>
<th>Amlicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>tox A gene</td>
<td>GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTGCTGCCAGCGCT</td>
<td>94°C 68°C 72°C 1 min 1min 1min</td>
<td>35</td>
</tr>
</tbody>
</table>

© Copyright 2014 | Centre for Info Bio Technology (CIBTech)
Electrophoresis was used to confirm PCR reaction products in agarose gel 1% at a voltage of 90V in 50 Minute, and the results were analyzed after staining with ethidium bromide. Size marker 100bp DNA Ladder was used to determine the size of the PCR products (Figure 1).

![Figure 1: PCR products electrophoresis on agarose gel 1%. (1) Band is not observed-(2) Band is observed](image)

**RESULTS AND DISCUSSION**

**Results**

107 strains of *P. aeruginosa* were recognized from the taken samples by using biochemical diagnostic tests. After PCR on 107 isolated *P. aeruginosa*, 102 (95.33 %) of them had tox A gene. ANOVA test was used to evaluate the differences between the frequencies of toxA gene which was isolated from wounds, burns, urine and blood. The results of the test isolates burn significantly more than the other isolates (p<0.05).

**Conclusion**

The results of this study showed that 102 strains (95.33%) out of 107 strains had toxA gene and also it showed the presence of this gene, especially in isolated bacteria from burn patients. In research conducted by Qin et al., (2003) of the 63 strains of *P. aeruginosa* isolated from patients with Cystic fibrosis (CF), 93.7% had toxA gene (Qin et al., 2003) and in research Lavenir et al., (2007) of 59 strains of *P. aeruginosa* isolated, 55 strains (93.22%) had tox A gene (Lavenir et al., 2007).

Khan and Cerniglia (1994) isolated 130 strains of *P. aeruginosa* from various sources (clinical and environmental samples) and Showed that 96% of them had toxA gene (Khan and Cerniglia, 1994). In the study by Lanotte et al., (2004) all specimens of *P. aeruginosa* (100 %) had tox A gene (Theilacker et al., 2003). So this demonstrator is the relationship between tox A gene with pathogenic and It seems likely that exotoxin A has a direct role in the pathogenesis with tissue damage and decreased phagocytic activity, especially in patients who suffer burn. Therefore exotoxin A was recommended to produce vaccine For people who are at risk of infection such as patients in burn, cystic fibrosis, etc. (Pai et al., 1996) and used exotoxin A for other purposes Such as the fight against cancer cells (Bonomo and Szabo, 2006).

**REFERENCES**


Research Article


