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Research Article

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

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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 strians (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* ATTC 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)

Target gene	Primer Sequence (5´- 3´)	PCR Denaturation	Annealing	Extension	Cycles	Amlicon size
tox A gene	GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTCGCTCCAGCGCT	94°C 1 min	68°C 1min	72°C 1min	35	396 bp

Electrophoresis was used to confirm PCR reaction products in agarose gel1% 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).

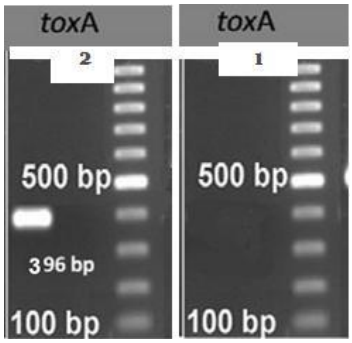

Figure 1: PCR products electrophoresis on agarose gel 1%. (1) Band is not observed-(2) Band is observed

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

Bonomo RA and Szabo D (2006). Mechanisms of multidrug resistance in Acinetobacter species and Pseudomonas aeruginosa. *Clinical Infectious Diseases* **43**(suppl 2) 49–56.

Chopp DL, Kirisits MJ, Morgan B and Parsek MR (2003). The Dependence of Quorum Sensing on the Depth of a Growing Biofilm. *Bulletin of Mathematical Biology* **65** 1053-1079.

De Kievit, Teresa R., Richard Gillis, Steve Marx, Chris Brown and Barbara H (2001). Iglewski, Quorum-Sensing Genes in *Pseudomonas aeruginosa* Biofilms: Their Role and Expression Patterns. *Applied and Environmental Microbiology* **67** 1865-1873.

Donlan Rodney M and William Costerton J (2002). Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. *Clinical Microbiology Reviews* **15** 167-193.

Feltman H, Schulert G, Khan S, Jain M, Peterson L and Hauser A (2001). Prevalence of type III secretion genes in clinical and environmental isolates of Pseudomonas aeruginosa. *Microbiology* **147** 2659-69.

Hentzer Morten, Kathrin Riedel, Thomas B Rasmussen, Arne Heydorn, Jens Bo Andersen, Matthew R Parsek, Scott A Rice, Leo Eberl, SorenMolin, NielsHoiby, StaffanKjelleberg and Michael Givskov (2002). Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furane compound. *Microbiology* **148** 87-102.

http://www.tjclarkminerals.com/bacterial_diseases/pseudomonas_aeruginosa.htm. Accessed 2004 October 26.

Jaffe R, Lane JD and Bates CW (2001). Real-time identification of *Pseudomonas aeruginosa* direct from clinical samples using a rapid extraction method and polymerase chain reaction (PCR). *Journal of Clinical Laboratory Analysis* **15**(3) 131-7.

Khan AA and Cerniglia CE (1994). Detection of *Pseudomonas aeruginosa* from clinical and environmental samples by amplification of the exotoxin A gene using PCR. *Applied and Environmental Microbiology* **60**(10) 3739-45.

Lanotte Ph, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, Goudeau A and Quentin R (2004). Genetic features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients compared with those of isolates from other origins. *Journal of Medical Microbiology* **53** 73–81.

Lavenir R, Jocktane D, Laurent F, Nazaret S and Cournoyer B (2007). Improved reliability of Pseudomonas aeruginosaPCR detection by the use of the specific ecfx gene target. *Journal of Microbiological Methods* **70**(1) 20-9.

Pai LH, Wittes R, Setser A, Willingham MC and Pastan I (1996). Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to Pseudomonas exotoxin. *Nature Medicine* **2**(3) 350-3.

Qin X, Emerson J, Stapp J, Stapp L, Abe P and Burns JL (2003). Use of real-time PCR with multiple targets to identify Pseudomonas aeruginosaand other nonfermenting gram-negative bacilli from patients with cystic fibrosis. *Journal of Clinical Microbiology* **41**(9) 4312-7.

Schulert GS, Fltman H, Rabin SDP, Martin CG, Battle SE and Rello J *et al.*, (2003). Secretion of the toxin ExoU is a marker for highly virulent *Pseudomonas aeruginosa* isolates obtained from patients with hospital-acquired pneumonia. *JID* **188** 1695-706.

Shih Pei-Ching and Ching-Tsan Huang (2002). Effects of quorum-sensing deficiency on *Pseudomonas aeruginosa* biofilm formation and antibiotic resistance. *Journal of Antimicrobial Chemotherapy* **49** 309-314

Soong G, Muir A, Gomez MI, Waks J, Reddy B and Planet P *et al.*, (2006). Bacterial neuraminidase facilitates mucosal infection by participating in biofilm production. *Journal of Clinical Investigation* **116**(8) 2297-305.

Theilacker C, Coleman FT, Mueschenborn S, Losa N, Grout M and Pier GB (2003). Construction and characterization of a Pseudomonas aeruginosamucoidexo-polysaccharide-alginate conjugate vaccine. *Infection and Immunity* **71** 3875-3884.

Wendelboe A and Baumbach J (2007). Outbreak of *Pseudomonas aeruginosa* infections caused by a contaminated cystoscope. *New Mexico Epidemiology Report* **6** 1-4.

Whiteley Marvin and Greenberg EP (2001). Promoter Specificity Elements in *Pseudomonas aeruginosa* Quorum-Sensing-Conrolled Genes. *Journal of Bacteriology* **183** 5529-5534.

Winstanley C, Kaye SB, Neal TJ, Miksch S and Hart CA (2005). Genotypic and phenotypic characteristics of *Pseudomonas aeruginosa* isolates associated with ulcerative keratitis. *Journal of Medical Microbiology* **54**(Pt 6) 519-526.