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Detection of ExoT Gene in Local Isolates of *Pseudomonas auroginosa* in a Sample of Burn Infection

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Abstract

Background *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic microorganism that requires damaged mucus membranes and epithelial tissues to cause acute infections. It had been stated that *P. aeruginosa* alters mammalian cytokinesis in a type III secretion system and exotoxin T (ExoT)-related way.

Objective To identify exoT gene local isolates of *P. aeruginosa* isolated from burn infections.

Methods Forty bacterial isolates of *P. aeruginosa* (isolated from burn infection) were identified by standard laboratory methods and polymerase chain reaction (PCR) technique was applied for the detection of the gene encoding for ExoT.

Results The results showed that PCR amplification of exo T gene occurred in 24 (60 %) isolates out of the enrolled 40 isolates of *P. aeruginosa* while 16 (40 %) of the isolates showed negative amplification reactions.

Conclusion It appeared that exoT can be a significant virulence factor expressed by 60 % of *P. aeruginosa* isolates as indicated by positive PCR-amplification results.

Keywords Burn infections, Exotoxin T, type III secretion system, PCR, *P. aeruginosa*

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List of abbreviations: PCR = Polymerase chain reaction, *P. aeruginosa = Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic bacteria that has the ability to infect virtually all kinds of tissues, affect immunosuppressed patients and can cause nosocomial infections [(1)]. Burn affected individuals, patients with assisted ventilation, and cystic fibrosis (CF) victims are specifically susceptible to be infected with *P. aeruginosa*. It has been documented that these bacteria are the leading cause of increased morbidity and mortality in persons with cystic fibrosis [(2)]. *P. aeruginosa* is responsible for an elevating percentage of infections acquired in the modern hospitals, especially in intensive care units (ICU) and in patients with urological disorders and has held almost unchanged position in the rank order of pathogens causing ICU infections during the last 4 decades [(3)].

P. aeruginosa utilizes a wide variety of virulence factors, which either help the pathogen to adhere to target cells or act as toxins. These toxins are either released by passive transport from the cells or actively secreted via one of the three secretion systems

namely, type I secretion system (T1SS), type II secretion system (T2SS) or the type III secretion system (T3SS) [4].

The complex III secretion system is an essential and newly recognized virulence factor of *P. aeruginosa* responsible for injecting certain toxin molecules into the target mammalian cells. The chromosome of *P. aeruginosa* harbors the genes encoding for type III secretion system in an evenly distributed manner [5].

P. aeruginosa has been shown to have the so-called type III secretion system (T3SS), along with a group of effector molecules (ExoT, ExoS, ExoY and ExoU). These protein factors can be directly delivered to the host cells; and once transferred, can elicit various host responses, facilitating successful dissemination and infections [6].

ExoT and ExoS, the first enzymes in this group are known. ExoT produced during the release and escape of pathogens. In addition, it has ADP-ribosil transferase activity, which acts similar pathology cholera toxin [7]. ExoT attacks host kinases mainly responsible for focal adhesion and eventual phagocytosis and has been linked with dissemination of infection from the lung to the liver in mice model and induction of apoptosis in HeLa cells [8].

The present study aimed to identify exoT gene local isolates of *P. aeruginosa* isolated from burn infections.

Methods

Sampling

Burn wound swabs were obtained from forty patients. Specimen collection started from January to April 2012 from the laboratories of Al-Imamein Al-Kadhimein Medical City, Baghdad. Identification of the obtained isolates was performed according to previous work [9].

DNA Extraction

Wizard genomic DNA purification kits (Promega®, USA) was used for the extraction of bacterial DNA as indicated by the manufacturer's instructions. Agrose gel (1%) electrophoresis on 1 % agarose was applied to confirm the results of DNA extraction [10].

Primers

Primers were synthesized by Bioneer ® (South Korea). Polymerase chain reaction (PCR) product size and melting temperature were 1000 bp and 45 °C, respectively (Table 1).

Table 1. Primers sequences and molecular weight of the relevant PCR products of exoT

Gene	Forward primer	Reverse primer	Product size (bp)
*exo*T	5′TCACTGCAGTTCCGCGTGCTCCGACG 3′	5′TCAGGTACCTGCTGGTACTCGCCGTT -3′	1000

Polymerase chain reaction (PCR)

Polymerase chain reaction was performed by adding 3 µl of the bacterial DNA to the preloaded master mix eppendorff tubes (AccuPower PCR premix-® (South Korea)) then 2.5 µl (10 pmol\µl) of the specific primers was also added, the final volume of 20 µl was attained by adding distilled water. Table 2 describes the running conditions for the amplification of exoT (Table 2).

Note: Running conditions were adopted after several trials depending upon other's work [11].

Agarose Gel Electrophoresis

Agarose at 1 gm, 1.5 gm was dissolved in 100 ml of 1X tris-borate EDTA buffer for genomic DNA and PCR products, respectively. These mixtures were then solubilized by heating, then they were left to cool at 40°C and poured into the taped plate [10].

Table 2. Cycling conditions for the PCR-amplification of exoT gene of *P. aeruginosa*

Step	Temperature (°C)	Time (minutes)	No. of Cycles
Initial denaturation	94	3	1
First loop:			
Denaturation	94	30 seconds	
Annealing	50	30 seconds	40
Extension	72	1	
Final extension	72	5	

Results

The results of the present work revealed that all the enrolled bacterial isolates were primarily identified as *P. aeruginosa* because they looked as gram negative, oxidase positive rods, and able to grow at 42 °C, the growth of the colonies characterized by sweet musty odor. Oxidation/fermentation test was also applied for the confirmation of the identity of the isolates.

Figure 1 shows the genomic DNA of the bacterial species enrolled in the current work.

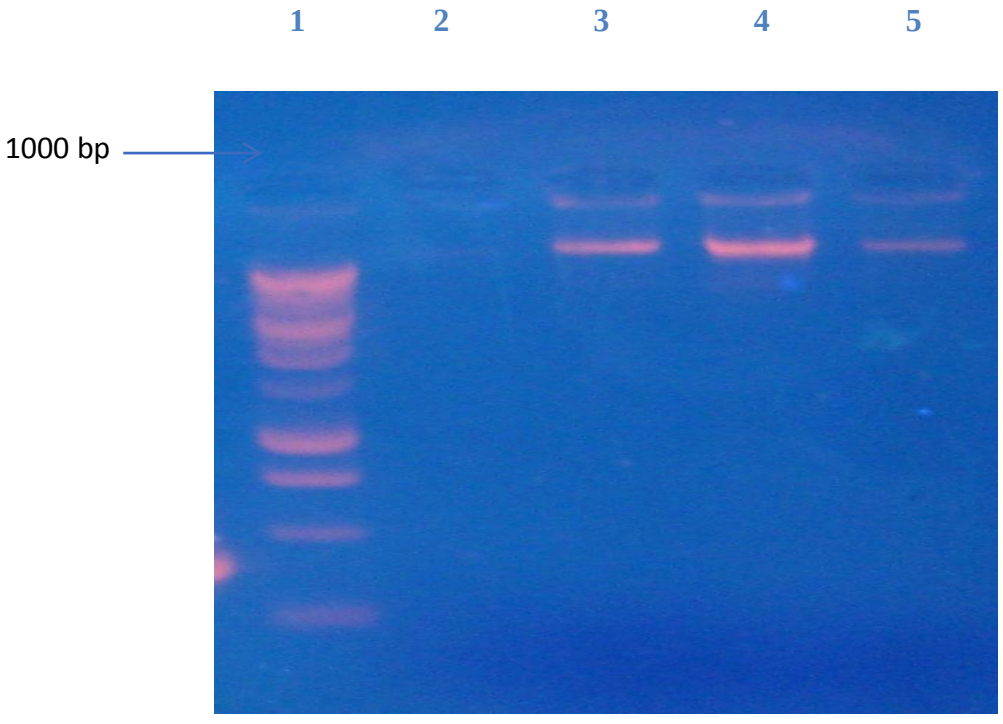

Figure 1. Bacterial chromosomal DNA. Lane 1: 1000 bp molecular marker, lane 3, 4, and 5. Bands run on 1% agarose at 4 V/cm for 60 minutes

PCR experiment indicated that exoT gene was successfully amplified in twenty-four (60 %) out of the forty isolates of *P. aeruginosa*, while only sixteen (40 %) isolates were shown to be negative (Figure 2).

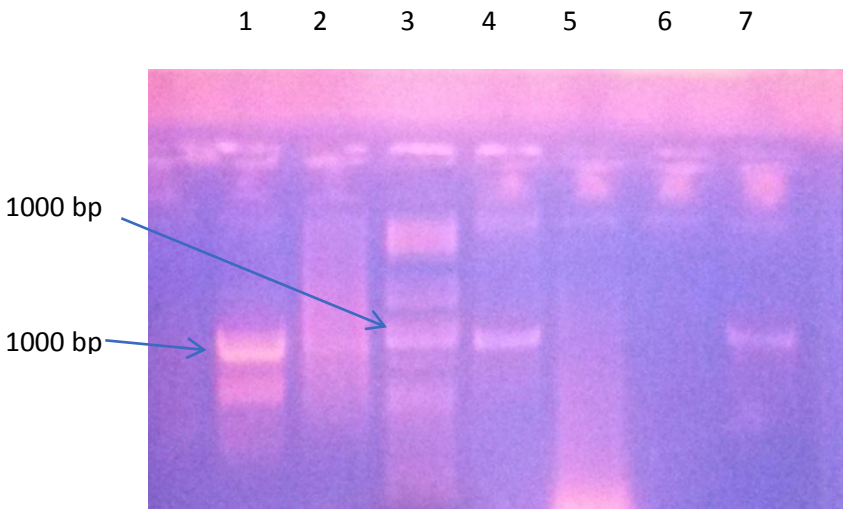

Figure 2. Electrophoresis of PCR products of exoT of *P. aeruginosa*. From left to right: Lane 3: 1000 bp ladder, lane: 1, 2, 4 and 7 amplification products of exoT gene (1000 bp) Lane 5 and 6 negative results. Electrophoresis conditions were: 5V/cm for one hour, the concentration of agarose gel was 1.3%

Discussion

The type III secretion system (T3SS) is a needle-like nanomachine that delivers virulence proteins (exotoxins) directly into target cells to initiate infection. These exotoxins trigger and maintain infection by altering target cell functions, such as signaling pathways, secretory trafficking, constant movement of the cytoskeleton, and cellular reaction to inflammatory stimuli. T3SS is fundamental for survival and pathogenesis of several Gram-negative organisms including *Pseudomonas, Salmonella, Escherichia, Shigella, Yersinia, and Chlamydia spp*. [12]. This complex cellular machinery acts in a very well-organized manner and can modify the target cell in many variable ways. The opportunistic nature of *P. aeruginosa* in humans is well defined, therefore, it is unlikely that type III secretion system has evolved as the result of survival pressure within host cells. The usual target of the *P. aeruginosa* T3SS is unknown. Probably, this system may have been established to combat nearby predators (e.g. amoebae that inhabit the soil and water), and broad conservation of targeted materials across eukaryotic creatures culminating in a system that is effective against human cells as well [13].

Effector proteins delivered by type III secretion system have been shown to have important contribution to the virulence of *P. aeruginosa* in a variety of in vivo animal studies [14-16]. Moreover, analysis of *P. aeruginosa* clinical isolates has also revealed a prominent correlation between production of T3SS effectors and enhanced severity of the disease with an elevated mortality rate [17]. Interestingly, the significance of T3SS in dictating clinical consequences and enhancing pathogenic process in animal studies is well documented, nevertheless, clinical isolates usually do not express T3SS in vitro and can cause illness in a T3SS-independent path [18].

The results of the current work contradicted those of other study which stated that exoT gene was found in 100 % of the enrolled *P. aeruginosa* isolates. The difference between the results might be attributed to the variation of the samples, from which the isolates were obtained and that environmental isolates of P. aeruginosa may show varying expression patterns for the virulence factors [19].

In s study conducted in Iran, over 144 clinical and environmental isolates of *P. aeruginosa*, it was shown that 37.9% of the isolates were positive for the production exoT when clinical

samples were considered while much less figure of 27.8 % was obtained when hospital settings isolates was estimated [20]; both results showed less frequent isolation of *P. aeruginosa* when compared to the result of the current study.

It was postulated that the ExoT gene is not a variable trait since it has been found in all the examined isolates [21,22] and that the existence of this gene in all the studied environmental isolates indicates that there may be selective advantage for this gene in hospital environments [8].

In a separate study, exoT gene prevailed in case of wound samples (95 %); despite higher figure that what is recorded in the present work, both studies support the previous knowledge indicating an important role for ExoT in bacterial dissemination [23]. It is of note to state that the abundance of type III secretion genes in clinical isolates is in line with its critical role in the pathogenicity of *P. aeruginosa* and the understanding of the particular contribution of ExoT to the clinical outcome of the infection may have substantial guiding for the therapeutic approach of patients infected with *P. aeruginosa* [24].

In conclusion, exoT gene plays an important role in the infectious process caused by *P. aeruginosa*.

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Authors Contribution:

Dr. Auda conducted the sampling, isolation, and molecular work. Rana and Dr. Aziz guided and finished writing and editing the study.

Conflict of interest

The authors declare no conflict of interest.

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