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GENOTYPIC INVESTIGATION OF TYPEIII EXOTOXINS AMONG CLINICAL AND ENVIRONMENTAL PSEUDOMONAS SPP. ISOLATES IN HILLA-CITY, IRAQ

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ABSTRACT

Pseudomonas spp. is an opportunistic pathogens that can cause an arrays of diseases for human especially wound infections. It is success can be attributed to many virulence factors which leads to adaptation and withstand for different inconvenient niches. It is also wildly spread in environment and isolated from different samples. This present study intended to display the difference in the most important virulence traits (TypeIII exotoxins) of Pseudomonas spp. among clinical and environmental isolates. Thirty eight Pseudomonas spp. isolates were used in This study. Eighteen isolates were recovered from wound infections (clinical isolates) and eighteen isolates were recovered from sewage and soil (environmental isolates) during a period of 3 months. All isolates inoculated on Pseudomonas chromogenic agar for primary screening of Pseudomonas spp. and then confirmed by PCR using specific primer for 16S rDNA gene of Pseudomonas spp. The results revealed that 17 (73.7%) and 8(42.1%) of isolates have exoS gene in clinical and environmental isolates respectively while 17 (89.5%) of clinical and 17 (89.5%) of environmental Pseudomonas spp. isolates have exoT gene. For exoU gene the results showed it is presence in all (19(100%) clinical isolates while present in only 18(94.7%) of environmental isolates. Only 12(63.2%) and 8(42.1%) of clinical and environmental isolates respectively have exoY. Our study not settle a significant differences between the virulence of clinical and environmental Pseudomonas spp. isolates and this is may indicate that the source of many environmental Pseudomonas spp. isolates may be from the medical waste or due to leakage from untreated municipal waste water.

Keywords: ExoS, ExoT, ExoU, ExoY, PCR

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INTRODUCTION

Pseudomonas aeruginosa has a battery of virulence traits that augment its potential for disease including pigments ; pyocyanin, fluorescein, pyoverdin and pyorubin; toxins and lytic enzymes and for this reasons it can be recovered from clinical, environmental and hydrocarbon polluted pools [1-5]. As a nosocomial pathogen, P. aeruginosa has a notable capacity to colonize catheters and wounds and thus it can be nourished among different hospital sectors like burn unite, urology unites and intensive care units [6-8]. Respiratory system infection, urinary tract infections, wound and burn with blue green pus, eye infection and ear infection were common infections caused by this pathogen [9,10]. Settlement of infection and avoidance of immune response can be attributed to set of virulence characters. Pseudomonal toxin delivered directly to the eukaryotic host cell via T3SS to start infection [11,12]. The four effector exotoxins (also called Exoenzymes) includes: ExoS, ExoT, ExoU, and ExoY. ExoS, is a GTPase-triggering protein domain, as well as ADP ribosyl transferase (ADPRT) activity. It can prompt a cytotoxic effect and is linked with the capability to cause lung impairment and depraved consequence from P. aeruginosa infection. ADP ribosyl transferase (ADPRT) domain of ExoS accountable for aptitude to hinder DNA synthesis in cultured cells the [13]. ExoT is a GTPase-triggering protein (GAP) for Rho family. It was established to be in charge for preclude of wound repair in vitro [14,15].

Exotoxin U(ExoU) revealed to carry phospholipase activity which play a role in hydrolyzing the ester bond of cell membrane phospholipids leading to membrane disruption, fatty acid elaboration and may be signal [16,17]. Exotoxin Y (ExoY) is adenylate cyclase which when injected to mammalian cells leads to raising of intracellular 3',5'- cyclic adenosine monophosphate (cAMP) focus and finally disturbance of the actin cytoskeleton, enhanced endothelial penetrability and hindrance of bacterial uptake host immune cells [18,19]. The immune effector cells seem designate the crucial goal of ExoY which enable the endurance of the pathogenic bacteria in the host (Stevens et al., 2014). Actually, the environmental bacterial isolates less pathogenic, and therefore handling of them may be less risky compared to clinical strains. The study's aim is to scrutinize the risk degree of clinical isolates and environmental isolates of Pseudomonas spp. isolates via comparing presence of their type-III secretion system exoenzymes.

Materials and Methods

Bacterial Isolates

Thirty-eight Pseudomonas spp. isolates were used in This study. Eighteen isolates were recovered from wound infections (clinical isolates) and eighteen isolates were recovered from sewage and soil (environmental

isolates) during a period of 3 months. All isolates inoculated on Pseudomonas chromogenic agar for primary screening of Pseudomonas spp. and then confirmed by PCR using specific primer for 16S rDNA gene of Pseudomonas spp.

Genomic DNA Extraction

All isolates were inoculated to Lauria Bretani broth (LB broth) as a first step of genomic DNA extraction. After incubation (37 °C, 18 hrs) cells harvesting by centrifugation at 14000 rpm for 5 min were performed. The supernatant was disposed and decontaminated by alcohol and the pellet of bacterial cells were resuspended in phosphate buffer saline for washing, centrifugation and this step can be repeated three times. The rest of steps were performed according to the manufacturer company (Favorgen/Tiwan).

Primer Pair Preparation

The primer pairs for 16S rDNA, exoS, exoT, exoU and exoY were purchased from Realgene (Realgene/China). Stock primer of 100pmole/µl were prepared and then working primer of 10pmole/µl used directly in PCR. The reaction mix of 25µl were prepared using 12.5µ of master mix (New England Biolabs/UK), 2µl of forward working primer, 2µl of reverse working primer, 4µl of DNA and 4.5µl of nuclease free water (New England Biolabs/UK). The sequence of primer pair, PCR products were stated (table 1).

Table 1. Primer Sequences and Amplicon Size

Primer Name	Primer's sequence (5´-3´)	Amplicon (bp)	Reference
pa16S-F	GGGGGATCTTCGGACCTCA	618	[21]
pa16S-R	TCCTTAGAGTGCCCACCCG		
exoS-F	GCGAGGTCAGCAGAGTATCG	118	[22]
exoS-R	TTCGGCGTCACTGTGGATGC		
exoT-F	AATCGCCGTCCAACTGCATGCG	152	[22]
exoT-R	TGTTCGCCGAGGTACTGCTC		
exoU-F	CCGTTGTGGTGCCGTTGAAG	134	[22]
exoU-R	CCAGATGTTCACCGACTCGC		
exoY-F	CGGATTCTATGGCAGGGAGG	289	[22]
exoY-R	GCCCTTGATGCACTCGACCA		

Biosafety and Hazard Material Disposing

Biosafety aspects followed during the work include disposing of all swabs, petri dishes and all contaminated supplies by autoclaving and then incineration. All benches cleaned with alcohol before and after the work. Simplysafe were used instead of ethidium bromide.

PCR Conditions

The PCR tubes with complete mix were place in thermocycler (Techno/UK) and optimization were performed in This study to get the best conditions. The conditions for each primer were mentioned in the table (2).

Table 2. Thermocycler Conditions

Gene name		Init. Denat.	Denat.	Anneal.	Exten.	Final Exten.	Reference
16S rDNA	Temp. (°C) Time	95 2min.	95 30sec.	56 30sec.	72 70sec.	72 5min.	This study
	No. of cycle	1	30			1	
exoS	Temp. (°C) Time	95 2min.	95 30sec.	61 30sec.	72 20sec.	72 5min.	This study
	No. of cycle	1	30			1	
exoT	Temp. (°C) Time	95 2min.	95 30sec.	62 30sec.	72 20sec.	72 5min.	This study
	No. of cycle	1	30			1	
exoU	Temp. (°C) Time	95 2min.	95 30sec.	61 30sec.	72 20sec.	72 5min.	This study
	No. of cycle	1	30			1	
exoY	Temp. (°C) Time	95 2min.	95 30sec.	61 30sec.	72 30sec.	72 5min.	This study
	No. of cycle	1	30			1	

1). Our results were in covenant with Deschaght et. al., (2009) [23] and AL-Shimmary et. al., (2016) [24] who set up that, no alteration in sensitivity could be settled up for Pseudomonas aeruginosa diagnosis between culture and PCR.

Results and Discussion

The results of Pseudomonas spp. isolation reveal no difference between results of identification by Pseudomonas chromogenic agar and results of amplification of 16S rDNA of Pseudomonas spp. (figure

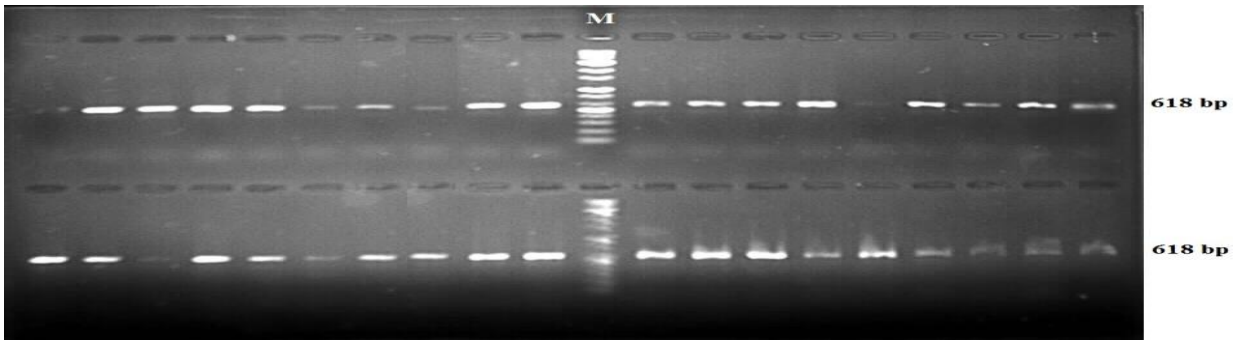

Fig. 1 Electrophoresis. (1.5% Agarose) of PCR products (618 bp) 16S rDNA gene of Pseudomonas spp. M lane "represent 100 bp DNA Marker" while the rest lanes represent samples.

18(94.7%) of environmental isolates. Only 12(63.2%) and 8(42.1%) of clinical and environmental isolates respectively have exoY (figure 2,3,4,5). The results reveal no differences in presence of exoT and exoU among both clinical and environmental Pseudomonas spp. isolates

Results of exotoxins distribution as mentioned in table (3) revealed that 17 (73.7%) and 8(42.1%) of isolates have exoS gene in clinical and environmental isolates respectively while 17 (89.5%) of clinical and 17 (89.5%) of environmental Pseudomonas spp. isolates have exoT gene. For exoU gene the results showed it is presence in all (19(100%) clinical isolates while present in only

Table 3 Distribution of Exotoxins among Clinical and Environmental *Pseudomonas* spp.

Exoenzymes	Clinical isolates (*n*=19)		Environmental Isolates (*n*=19)	
	No.	%	No.	%
ExoS	14	73.7	8	42.1
ExoT	**17**	**89.5**	**17**	**89.5**
ExoU	**19**	**100**	**18**	**94.7**
ExoY	12	63.2	8	42.1

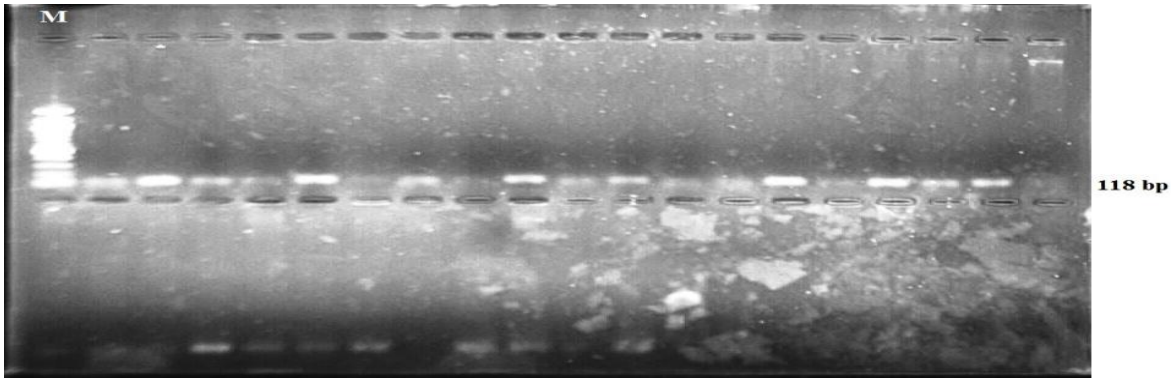

Fig. 2 Electrophoresis. (1.5% Agarose) of PCR products (118 bp) exoS gene of Pseudomonas spp. M lane "represent 100 bp DNA Marker" while the rest lanes represent samples.

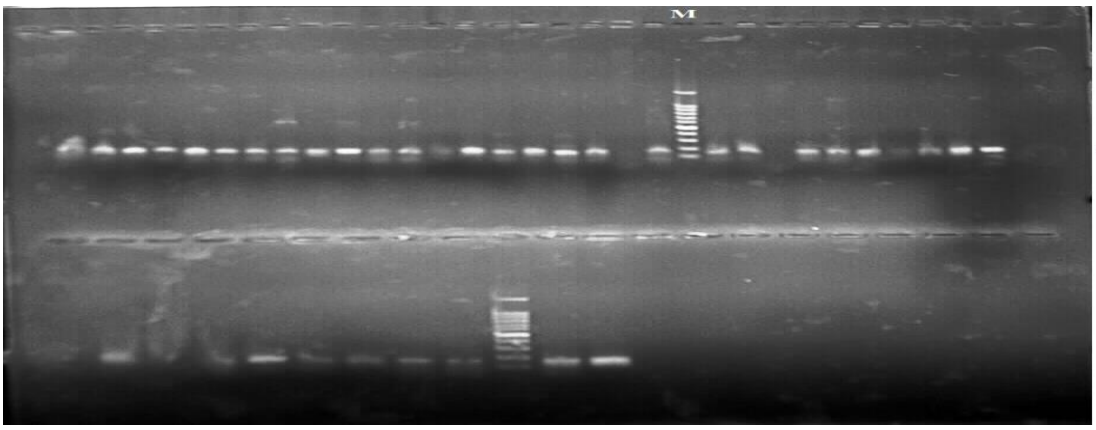

Fig. 3 Electrophoresis. (1.5% Agarose) of PCR products (152 bp) of exoT gene of Pseudomonas spp. M lane" represent 100 bp DNA Marker" while the rest lanes represent samples.

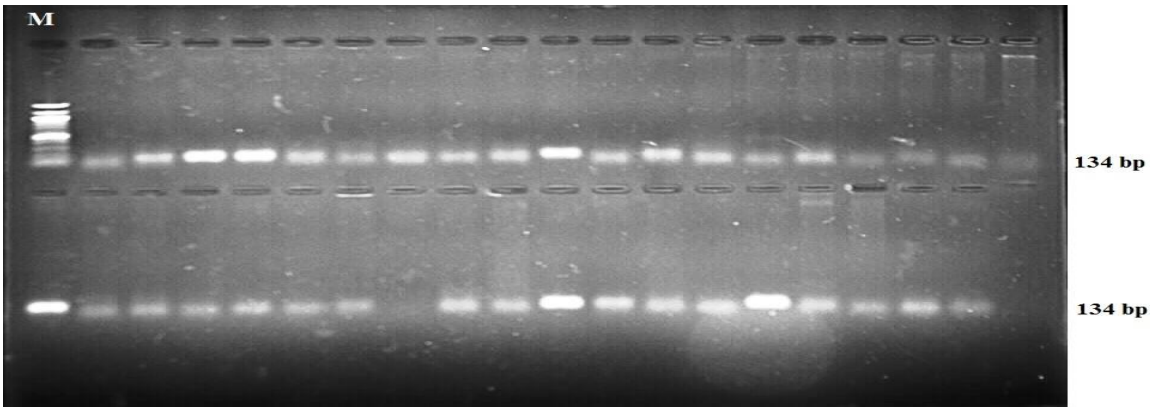

Fig. 4 Electrophoresis. (1.5% Agarose) of PCR products (134 bp) of exoU gene of Pseudomonas spp. M lane "represent 100 bp DNA Marker" while the rest lanes represent samples".

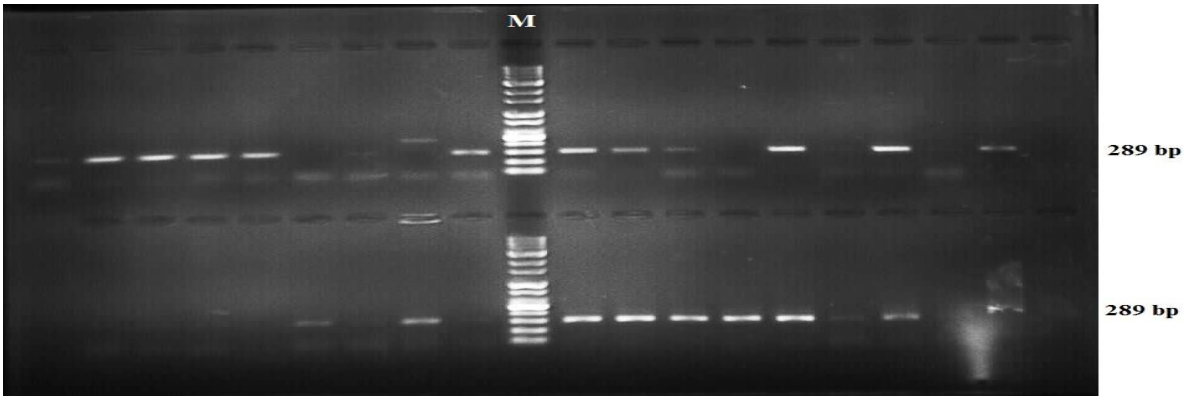

Fig. 5 Electrophoresis. (1.5% Agarose) of PCR products (289 bp) of exoY gene of Pseudomonas spp. M lane "represent 100 bp DNA Marker" while the rest lanes represent samples.

Current study show existence of both exoS, exoU genes in many clinical and environmental isolates and this displayed that no variation in virulence and pathogenicity between many clinical and environmental Pseudomonas isolates. Presence of ExoS and ExoU exoenzymes can secure both mode of infection (cytotoxicity and invasiveness) for isolate that have both of them [25-27].

Presence of all exotoxins (ExoS/ExoT/ExoU/ExoY.) in many clinical, environmental isolates of Pseudomonas spp. increase the possibility of infection to occur and worseness of infection when it is found. Inhibition of immune system, disruption many types of cell and delaying the healing of wounds resulted from presence of one or more of the four exotoxins [28,29].

Current study conclude that, no significant differences between the virulence of clinical and environmental Pseudomonas spp. isolates and this is may indicate that the source of many environmental Pseudomonas spp. isolates may be from the medical waste or due to leakage from untreated municipal waste water.

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