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Antibiotic Resistance and Type III Exotoxin Encoding Genes of *Pseudomonas aeruginosa* Isolates from Environmental and Clinical Sources in Northern West Bank in Palestine

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Abstract

A total of fifty-seven *Pseudomonas aeruginosa* isolates were collected from different sources of clinical (n=47) and environmental samples (n=10) during 2018, in Northern West Bank, Palestine. Molecular techniques were used to detect type III secretion exotoxin-encoding genes (T3SEEG), and clone identity. The antimicrobial susceptibility was carried out by the disk diffusion method. This study is aimed at comparing the distribution of the T3SEEG and antibiotic resistance between *P. aeruginosa* isolated from both sources. The correlation between T3SEEG and antibiotic resistance in both clinical and environmental isolates collected from a limited geographical area, in parallel, over a short period of time was also determined. In addition, clone identity between the clinical and environmental strains was determined. The results showed that all clinical and environmental *P. aeruginosa* isolates carried T3SEEG. *ExoT* was detected in all of the clinical and environmental isolates. The occurrence rates of *exoY* and *exoS* in *P. aeruginosa* isolates were 80.7 % and 36.8 %, respectively. More than one exotoxin gene was observed in 87 % of the clinical isolates and 70 % of the environmental isolates. The most common combination was *exoT* and *exoY*, with prevalence rates of 47 % and 50 % for the clinical and environmental isolates, respectively. *ExoU* was not detected in any isolate. There was no statistically significant difference between the distribution of T3SEEG according to the isolates' source. There were no significant associations between the carriage of T3SEEG and resistance to some antimicrobials such as ciprofloxacin, norfloxacin, levofloxacin, aztreonam, tetracycline, and kanamycin. The profile of RAPD-PCR typing of 44 *p. aeruginosa* isolates (nine environmental isolates recovered from hospital sinks and thirty-five clinical isolates recovered from different hospitals) was clustered into three groups at a 96 % similarity level. There was no significant difference between the strains isolated from both of the environmental and clinical sources according to antibiotic resistance and the prevalence of T3SEEG. In conclusion, there was high similarity between *P. aeruginosa* strains isolated from both environmental and clinical sources. In addition, no significant differences in antibiotic resistance and the distribution of T3SEEG were observed among the isolates from both sources.

Keywords: *Pseudomonas aeruginosa*, Type III secretion system, Environmental isolates, Clinical isolates, *ExoS*, *ExoT*, *ExoY*, *ExoU*.

1. Introduction

Pseudomonas aeruginosa is a major opportunistic human pathogen. It is an important cause of infections, particularly in immunodeficient patients, burn patients, patients using mechanical ventilation, and patients with cancer or cystic fibrosis (Elsen *et al*., 2014; Azimi *et al*., 2016). *P. aeruginosa* can also cause infections in healthy persons (McCallum *et al*., 2002; Kang *et al*., 2005). In addition, *P. aeruginosa* can cause community-acquired infections as well as hospital-acquired infections (Barbier *et al*., 2013).

P. aeruginosa genomes isolated from environmental and clinical sources are highly conserved. All *P. aeruginosa* isolates are able to produce virulence factors, and are thus considered potential pathogens (Alonso *et al*., 1999; Pirnay *et al*., 2002; Pirnay *et al*., 2009; Grosso-Becerra *et al*., 2014). The intrinsic and acquired resistance of *P. aeruginosa* to many types of antibiotics are attributed to several mechanisms, including reduced cell wall permeability, active efflux systems, expression of various enzymes, plasmid acquisition, and biofilm formation (Allydice-Francis and Brown, 2012).

Secreted exotoxins are either passively or actively secreted from the cell by type I secretion system (T1SS), type II secretion system (T2SS), or type III secretion system (T3SS) (Bradbury *et al*., 2010). The T3SS of *P. aeruginosa* has four known secretion effector toxins; these are ADP-ribosylating enzymes exoenzyme (ExoS), exoenzyme T (ExoT), adenylate cyclase exoenzyme Y (ExoY), and acute cytolytic factor (a phospholipase) exoenzyme U (ExoU). Although the *P. aeruginosa* T3SS is not required for infection, it enhanced disease severity in several animal models (Hauser, 2009).

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The prevalence of *exoS*, *exoT*, *exoU* and *exoY* in clinical and environmental *P. aeruginosa* isolates has been previously reported. All tested clinical and environmental *P. aeruginosa* isolates had *exoT* gene sequences (Feltman *et al*., 2001; Strateva *et al*., 2010; Gawish *et al*., 2013; Adwan, 2017). The prevalence of *exoT* gene sequences in *P. aeruginosa* isolates recovered from both environmental and clinical sources ranged from 5 % to 92 % (Lomholt *et al*., 2001; El-Solh *et al*., 2012; Azimi *et al*., 2016). The prevalence of *exoY* gene sequences among *P. aeruginosa* isolates ranged from 55 % to 97 % (Dacheux *et al*., 2000; Azimi *et al*., 2016; Adwan, 2017). The prevalence of *exoS* gene sequences among *P. aeruginosa* isolates ranged from 0 % to 96 % (Rumbaugh *et al*., 1999; Azimi *et al*., 2016; Adwan, 2017). Finally, the prevalence of *exoU* gene sequences among *P. aeruginosa* isolates ranged from 0 % to 80 % (Fleiszig *et al*., 1997; Azimi *et al*., 2016; Adwan, 2017). According to one report, *exoY* and *exoT* gene sequences were detected in all *P. aeruginosa* isolates recovered from hospital environments, and only 80 % and 25.7 % of the isolates carried *exoS* and *exoU* gene sequences, respectively (Bradbury *et al*., 2010). In another study, *P. aeruginosa* isolates recovered from environmental sources were significantly more likely to have *exoU* gene sequences more than other isolates (Bradbury *et al*., 2010). In contrast, in another study, there were no significant differences in the prevalence of type III secretion exotoxin-encoding genes (T3SEEG) between both nosocomial and environmental *P. aeruginosa* isolates, or between isolates recovered from different clinical infection sites (Gawish *et al*., 2013).

The association between antimicrobial resistance, especially to fluoroquinolones, and the clinical isolates of $T3SS^{+ve}$ *P. aeruginosa* has been reported in different clinical studies (Wong-Beringer *et al*., 2008; Mitov *et al*., 2010; Agnello and Wong-Beringer, 2012; Cho *et al*., 2014).

The present study is aimed at comparing the distribution of the T3SEEG and antibiotic resistance between *P. aeruginosa* strains that were isolated from clinical and environmental sources, and to investigate the presence of an association between them in both clinical and environmental isolates collected from a limited geographical area, in parallel, over a short period of time. In addition, clone identity among the clinical and environmental strains was determined and compared using molecular techniques.

2. Materials and Methods

2.1. Bacterial Isolates' Collection and Identification

A total of fifty-seven isolates of *P. aeruginosa* were collected from different clinical (n=47) and environmental samples (n=10) during 2018 (Table 1). Both clinical and environmental samples were collected from different hospitals in Northern West Bank, Palestine. All clinical isolates were identified using the API 20E system at the respective hospital laboratories, and were then confirmed using conventional methods at a microbiology research laboratory. The environmental isolates were identified using conventional methods at the microbiology research laboratory of An-Najah National University.

Table 1. A sample source of 57 of *P. aeruginosa* isolates collected from different hospitals.

Hospital	Sample source (n)												Total
	wound	Urine	tissue	Blood	Bed sore	nasal	Fluid	skin	sputum	swabs	umbilicus	Environment (sink of hospital)	
N	5	4	1	3	1	0	0	0	0	0	0	0	14
W	0	0	0	0	1	0	0	0	0	0	0	0	1
T	1	1	0	0	0	3	0	0	0	0	0	3	8
J	1	7	0	1	0	0	1	2	4	3	0	7	26
TH	2	1	0	0	0	0	0	0	1	3	1	0	8
Total	9	13	1	4	2	3	1	2	5	6	1	10	57

N: An-Najah National University Hospital; W: Alwatany Hospital; T: Tubas Turk Hospital; J: Jenin Hospital; TH: Thabet Hospital.

2.2. Antibacterial Susceptibility Test

Antimicrobial sensitivity testing was performed according to Clinical and Laboratory Standard Institute (CLSI) guidelines using the disk diffusion method (CLSI, 2016). All *P. aeruginosa* isolates were examined using antimicrobial disks (Oxoid) to determine resistance against, ciprofloxacin (5 µg), kanamycin (30 µg), tetracycline (30 µg), norfloxacin (10 µg), aztreonam (30 µg), and levofloxacin (5 µg). Mueller Hinton agar (MHA) plates were seeded with a 6-8 h old culture of the isolates, followed by placement of the antimicrobial disks. The plates were incubated at 37°C for twenty-four hours. The inhibition zones (if any) were measured, and the isolates were classified as resistant, intermediate, or susceptible, according to CLSI guidelines (CLSI, 2016). The reference strain of *P. aeruginosa* ATCC 27853 was used as a quality control in all of the experiments of antimicrobial susceptibility testing.

2.3. DNA Extraction and PCR Amplification

2.3.1. DNA Extraction

The genome of *P. aeruginosa* was prepared for PCR according to the method described previously (Adwan *et al*., 2013). Briefly, the cells were scraped off an overnight MHA plate, washed with 800 µL of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), centrifuged, and the pellet was resuspended in 400 µL of sterile double distilled H_2O, and boiled for 10-15 minutes. The cells were incubated on ice for ten minutes. The debris were pelleted by centrifugation at 11,500 X *g* for five minutes. The concentration of DNA was determined using a nanodrop spectrophotometer (Genova Nano, Jenway). The DNA samples were stored at -20ºC.

2.3.2. PCR Amplification of Type III Secretion Exotoxin-Encoding Genes

The detection of gene sequences encoding T3SEEG (*exoS*, *exoT*, *exoU* and *exoY* genes) was performed using multiplex PCR using specific oligonucleotide primer sets

as described previously (Ajayi *et al*., 2003). Primer sequences and the size of amplicons are listed in Table 2. Each PCR mix (25 µL) consisted of 10 mM PCR buffer pH 8.3; 2.3 mM $MgCl_2$; 0.3 mM of each dNTP; 0.3 µM of each primer; 1.25U of Taq DNA polymerase, and 3 µL (100-200 ng) of DNA template. A negative control without template DNA and a positive control strain (department collection) possessing a T3SEEG (*exoS*, *exoT*, *exoU* and *exoY* genes) were used during PCR. The cycling conditions were: initial denaturation at 94ºC for three minutes; followed by thirty-six cycles of denaturation at 94ºC for forty seconds, annealing at 56ºC for forty seconds, and extension at 72ºC for one minute; followed by a single final extension step at 72ºC for five minutes. The PCR products were detected by electrophoresis on 1.5 % agarose gels to determine the size of amplified fragment after staining with a final concentration 0.5 µg/mL ethidium bromide.

Table 2. Target genes for PCR amplification, amplicon sizes, and primer sequences used.

Target gene	Primer sequence (5′→3′)	Amplicon size (bp)
exoS	Exo S F: GCG AGG TCA GCA GAG TAT CG	118
	Exo S R: TTC GGC GTC ACT GTG GAT GC	
exoT	Exo T F: AAT CGC CGT CCA ACT GCA TGC G	152
	Exo T R: TGT TCG CCG AGG TAC TGC TC	
exoU	Exo U F: CCG TTG TGG TGC CGT TGA AG	134
	Exo U R: CCA GAT GTT CAC CGA CTC GC	
exoY	Exo Y F: CGG ATT CTA TGG CAG GGA GG	289
	Exo Y R: GCC CTT GAT GCA CTC GAC CA	

2.3.3. Random Amplified Polymorphic DNA (RAPD) PCR

Random amplified polymorphic DNA PCR products were generated from forty-four bacterial genomes. The RAPD-PCR was performed using Primer RAPD 208: 5`-ACG GCC GAC C-3` as described earlier (Mahenthiralingam *et al*., 1996). Each PCR mix (25 µL) consisted of 10 mM PCR buffer pH 8.3; 3 mM $MgCl_2$; 0.4 mM of each dNTP; 0.8 µM primer; 1.5 U of Taq DNA polymerase, and 3 µL (100-200 ng) of DNA template. The DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following protocol: initial denaturation for three minutes at 94ºC; followed by thirty-five cycles of denaturation at 94ºC for fifty seconds, annealing at 45ºC for one minute, and extension at 72ºC for one minute, followed by a final extension step at 72ºC for five minutes. The PCR products were analyzed by electrophoresis on a 1.7 % agarose gel. The gel image was scored using a binary scoring system that recorded the absence and presence of bands as 0 and 1, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistical software version 20 (IBM).

2.4. Statistical Analysis

The results were evaluated by the Fisher exact test using SPSS version 20. A *P* value less than 0.05 was considered significant.

3. Results

3.1. Antimicrobial Susceptibility

A total of fifty-seven isolates (forty-seven clinical isolates and ten environmental isolates) of *P. aeruginosa* were subjected for antimicrobial susceptibility testing. All isolates showed high resistance to tetracycline and kanamycin. The clinical isolates showed low resistance rates against aztreonam, while the environmental isolates showed low resistance rates against norfloxacin. There were no significant differences in the resistance profile between strains isolated from environmental and clinical sources (Fisher exact test; *p* 0.084-1). The antimicrobial susceptibility profile of the isolates is presented in Table 3.

Table 3. Antimicrobial susceptibility profile of study isolates.

Antibiotic	Antimicrobial susceptibility result n (%)								
	Clinical source n=47			Environmental source n=10			Total n=57		
	S	I	R	S	I	R	S	I	R
Ciprofloxacin	27 (57.4 %)	3 (6.3 %)	17 (36.3 %)	3 (30 %)	2 (20 %)	5 (50 %)	30 (52.6 %)	5 (8.7 %)	22 (38.7 %)
Norfloxacin	27 (56 %)	6 (12 %)	14 (32 %)	6 (60 %)	2 (20 %)	2 (20 %)	33 (57 %)	8 (14 %)	16 (29 %)
Levofloxacin	27 (57 %)	1 (2 %)	19 (41 %)	6 (60 %)	1 (10 %)	3 (30 %)	33 (58 %)	2 (3.5 %)	22 (38.5 %)
Aztreonam	25 (53.1 %)	14 (29.7 %)	8 (17.2 %)	3 (30 %)	2 (20 %)	5 (50 %)	28 (49.1 %)	16 (28 %)	13 (22.9 %)
Tetracycline	9 (19.1 %)	6 (12. 7%)	32 (68.2 %)	2 (20 %)	2 (20 %)	6 (60 %)	11 (19.2 %)	8 (14 %)	38 (66.8 %)
Kanamycin	7 (14 %)	12 (24 %)	28 (62 %)	1 (10 %)	1 (10 %)	8 (80 %)	8(14.1 %)	13 (22.8 %)	36 (63.1 %)

n: isolates' count; S: susceptible; I: intermediate; R: resistant

3.2. Detection of Type III Secretion Toxins-Encoding Genes

According to the PCR results, all the isolates carried T3SEEG. *ExoT* was detected among all clinical and environmental isolates. The prevalence of *exoY* and *exoS* in isolates from both sources was 80.7 % and 36.8 %, respectively. Two or more genes were observed among 87 % and 70 % of the clinical and environmental isolates, respectively. The most common combination was *exoT*

and *exoY*, with a prevalence rate of 47 % and 50 % among clinical and environmental isolates, respectively. *ExoU* was not detected among the isolates. The results of multiplex-PCR for T3SEEG are presented in Tables 4 and 5. No significant differences were observed in the prevalence of T3SEEG, among the isolates from the environmental and clinical sources (Fisher exact test; *p* values of 1.00, 0.295, and 0.387 for *exoT*, *exoY* and *exoS*, respectively). In addition, the statistical analysis indicated no significant association between the presence of *exoT*, *exoY*, *exoS* in *P. aeruginosa* recovered from clinical and environmental sources and the resistance to antimicrobials such as ciprofloxacin, norfloxacin, levofloxacin, aztreonam, tetracycline, and kanamycin (Fisher exact test; *p* values 0.113-1). A significant association ($p = 0.046$) was observed between the absence of *exoS* in the isolates and the resistance to levofloxacin.

Table 4. Prevalence of type III secretion toxins-encoding genes in the clinical and environmental isolates of *P. aeruginosa* in combination.

Source of *P. aeruginosa* isolate	Distribution of type III secretion exotoxins-encoding genes in combination n (%)			
	exoT	*exoT* and *exoY*	*exoS* and *exoT*	*exoS*, *exoY*, and *exoT*
Clinical (n=47)	6 (13%)	22 (47%)	2 (4%)	17 (36%)
Environmental (n=10)	3 (30%)	5 (50%)	0 (0%)	2 (20%)

n: number of isolates

Table 5. Prevalence of type III secretion toxins-encoding genes in clinical and environmental isolates of *P. aeruginosa.*

Source of *P. aeruginosa* isolate	Distribution of type III secretion toxins-encoding genesn (%)			
	exoT	*exoS*	*exoY*	*exoU*
Clinical (n=47)	47 (100%)	19 (40.4%)	39 (83%)	0 (0%)
Environmental (n=10)	10 (100%)	2 (20%)	7 (70%)	0 (0%)
Total	57 (100%)	21 (36.8%)	46 (80.7%)	0 (0%)

n: number of isolates

3.3. RAPD-PCR Typing

The RAPD-PCR typing profile of 44 *P. aeruginosa* isolates (nine environmental isolates recovered from the sinks of different hospitals and thirty-five clinical isolates recovered from different hospitals) were grouped into three clusters at a 96 % similarity level. Strains grouped in cluster-1 (CL1), which had an identical RAPD-PCR profile were recovered from different hospitals and belonged to environmental (eight isolates) and clinical (nineteen isolates) sources. Strains grouped in cluster-2 (CL2), which had an identical RAPD-PCR profile were recovered from different hospitals and belonged to environmental (one isolate) and clinical (four isolates) sources. Strains grouped in cluster-3 (CL3), which had an identical RAPD-PCR profile, were only recovered from clinical (twelve isolates) sources. Overall, identical clones within the same cluster have been recovered from different hospitals. The results of RAPD-PCR analyses are presented in Figure 1.

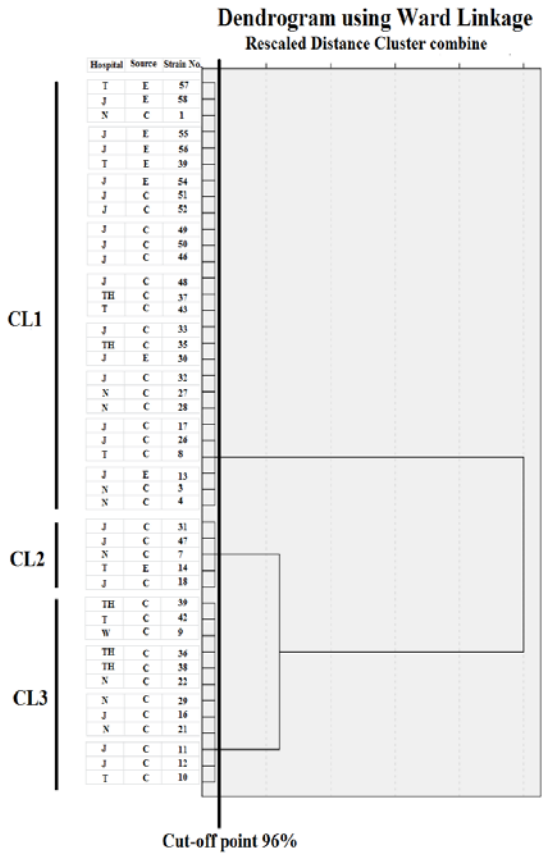

Figure 1. Dendrogram of 44 *P. aeruginosa* isolates recovered from clinical (35 isolates) and environmental (9 isolates) sources based on the UPGMA method derived from analysis of the RAPD-PCR profiles at a 96 % similarity level. CL: Cluster; E: Environmental sample; C: clinical sample; N: An-Najah National University Hospital; W: Alwatany Hospital; T: Tubas Turk Hospital; J: Jenin Hospital; TH: Thabet Hospital.

4. Discussion

Pseudomonas aeruginosa is one of the major causative agents of both nosocomial- and community-acquired infections in humans. This is due to its ability to cause different infections and its high-level resistance to many classes of antimicrobials via several mechanisms (Allydice-Francis and Brown, 2012).

The findings of this study showed that *P. aeruginosa* isolates recovered from both environmental and clinical sources demonstrated high resistance to tetracycline and kanamycin. Results of previous studies carried out in the same country, showed that clinical isolates showed high resistance to different tested antibiotics (Adwan *et al*., 2016a; Adwan, 2017). Results of this study were consistent with previous reports from Jamaica (Allydice-Francis and Brown, 2012) and Iran (Azimi *et al*., 2016), which showed that *P. aeruginosa* isolates from different sources had high resistance to many different classes of antimicrobial agents. The major cause of the high prevalence of resistance among clinical isolates of *P. aeruginosa* in Palestine may be attributed to selective pressure resulting from the uncontrolled, incorrect, and extensive use of antimicrobials inside and outside hospitals. This is exacerbated by the lack of a national antibiotics policy, and due to the over-the-counter antibiotic availability in Palestine (Adwan *et al*., 2016b).

All the isolates recovered from both environmental and clinical sources were *exoT*$^{+ve}$. These results were consistent with previous reports from USA (Feltman *et al*., 2001), Bulgaria (Strateva *et al*., 2010), Egypt (Gawish *et al*., 2013), and Palestine (Adwan, 2017). The prevalence rate of *exoT* was inconsistent with the results of other studies, which showed the prevalence of the gene among environmental and clinical *P. aeruginosa* isolates at a range of 5 % to 92 % (Lomholt *et al*., 2001; El-Solh *et al*., 2012; Azimi *et al*., 2016). In the present study, the prevalence rate of *exoY* among *P. aeruginosa* isolates recovered from clinical and environmental sources was 80.7 %. This is comparable to previously published results from Palestine (Adwan, 2017), which showed that 72.5 % of *P. aeruginosa* recovered from clinical isolates were *exoY*$^{+ve}$. This result is also in agreement with other studies published elsewhere (Strateva *et al*., 2010; Gawish *et al*., 2013), which reported rates ranging from 83.5 % to 85.8 %. The prevalence rate of 80.7 % for *exoY* among the *P. aeruginosa* isolates was lower than that in other studies; 97 % (Dacheux et al., 2000) and 89 % (Finck-Barbancon et al., 1997), and even higher prevalence rates than these rates by other reports such as by Azimi et al., 2016, at 55 %. The prevalence rate of *exoS* among the isolates from both sources was 36.8 %. This is in contrast to a previous report from Palestine (Adwan, 2017), which did not recover the gene among clinical isolates of *P. aeruginosa*. The prevalence rate for this gene among the *P. aeruginosa* isolates by other reports ranged from 0 % to 96 % (Rumbaugh *et al*., 1999; Azimi *et al*., 2016; Adwan, 2017). The product of the *exoS* gene is considered a main cytotoxin involved in the colonization, invasion, and spreading of the infection (Lee *et al*., 2005). Increasing the gene expression results in increased pulmonary damage in cystic fibrosis patients and in animal models, and leads to *in vitro* cytotoxicity, and induction of apoptotic-like cell death (Lee *et al*., 2005; Engel, 2003). In the current study, all the isolates recovered from clinical and environmental sources were *exoU*$^{-ve}$. This result is consistent with a previous report from Palestine (Adwan, 2017), which showed that all the clinical *P. aeruginosa* isolates were *exoU*$^{-ve}$. The prevalence rate of *exoU* gene sequences among *P. aeruginosa* isolates ranged from 0 % to 80 % according to reports published elsewhere (Fleiszig *et al*., 1997; Azimi *et al*., 2016; Adwan, 2017). The product of *exoU* demonstrates specificity to human neutrophils, and is associated with a severe *P. aeruginosa* infection in humans (Finck-Barbancon *et al*., 1997). Strains of *P. aeruginosa* with deleted or mutated *exoU* gene had a lower toxicity during lung infection (Engel, 2003). In addition, an association had been proposed between the product of *exoU* and the invasive *P. aeruginosa* leading to bloodstream infections (Wareham and Curtis, 2007).

In the present study, no association was found between the resistance profile and the *P. aeruginosa* isolates recovered from both clinical and environmental sources that carried T3SEEG. A statistically significant association (p = 0.047) was demonstrated between the absence of the *exoS* gene and resistance to norfloxacin. In a recent study from Palestine (Adwan, 2017), no associations were observed between ciprofloxacin, norfloxacin, meropenem, and imipenem resistance, and the carriage of T3SEEG among clinical *P. aeruginosa* isolates. A significant association between clinical *P. aeruginosa* isolates having the *exoU* gene sequences and fluoroquinolone resistance has been proposed (Wong-Beringer et al., 2008; Cho *et al*., 2014). In another report (Bradbury *et al*., 2010), no significant association was demonstrated between ciprofloxacin resistance and *P. aeruginosa* isolates carrying the *exoU* gene.

RAPD-PCR typing of *P. aeruginosa* genomes of both environmental and clinical isolates showed that the genome is highly conserved (96 % similarity), and that all *P. aeruginosa* isolates, whether from clinical or environmental sources, were able to produce virulence factors, and are thus potential pathogens. Cluster 1 and 2 had identical isolates recovered from both environmental and clinical sources. This indicates that the strains of environmental origin may cause human infections. These results are in accordance with the results published formerly (Hardalo and Edberg, 1997; Pirnay *et al*., 2009). Moreover, others have proposed the absence of significant differences between the environmental and clinical isolates regarding the virulence aspects (Morgan *et al*., 1999; Alonso *et al*., 1999; Pirnay *et al*., 2002). Finally, identical clones have been recovered from different hospitals. This may be attributed to medical referrals and the transportation of patients among different hospitals (Adwan, 2017).

5. Conclusion

According to RAPD-PCR typing, the results of this study revealed a high similarity between the *P. aeruginosa* strains isolated from environmental and clinical sources. In addition, strains from both sources had no significant differences in antimicrobials' resistance profiles and the presence of T3SEEG. These results indicate that the isolates from both sources have the ability to cause clinical infections.

Conflict of Interest

No conflicts of interest have been declared by the authors

References

Adwan G, Shtayah A, Adwan K, Al-Sheboul S and Othman S. 2016a. Prevalence and molecular characterization of *P. aeruginosa* Isolates in the West Bank-Palestine for ESBLs, MBLs and Integrons. *J Appl Life Sci Int*., **8**(2): 1-11.

Adwan G, Abu Hasan N, Sabra I, Sabra D, Al-butmah S, Odeh S, Abd Albake Z and Badran H. 2016b. Detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile. *Int J Med Res Health Sci.,* **5**(5): 75-82.

Adwan G, Adwan K, Jarrar N and Salameh Y. 2013. Prevalence of *seg*, *seh* and *sei* genes among clinical and nasal swab of *Staphylococcus aureus* isolates. *Br Microbiol Res J*., **3**(2):139-149.

Adwan G. 2017. Detection of Type III Secretion Toxins encoding-genes of *Pseudomonas aeruginosa* isolates in the West Bank-Palestine. *J Adv Biol Biotechnol*., **11**(3): 1-10.

Agnello M and Wong-Beringer A. 2012. Differentiation in quinolone resistance by virulence genotype in *Pseudomonas aeruginosa*. *PLoS One*., **7**(8): 1-8.

Ajayi T, Allmond LR, Sawa T and Wiener-Kronish JP. 2003. Single-nucleotide-polymorphism mapping of the *Pseudomonas aeruginosa* type III secretion toxins for development of a diagnostic multiplex PCR system. *J Clin Microbiol*., **41**(8): 3526-3531.

Allydice-Francis K and Brown PD. 2012. Diversity of antimicrobial resistance and virulence determinants in *Pseudomonas aeruginosa* Associated with fresh vegetables. *Int J Microbiol*., **2012**: 1-7.

Alonso A, Rojo F and Martínez JL. 1999. Environmental and clinical isolates of *Pseudomonas aeruginosa* show pathogenic and biodegradative properties irrespective of their origin. *Environ Microbiol.*, **1**(5): 421-430.

Azimi S, Kafil HS, Baghi HB, Shokrian S, Najaf K, Asgharzadeh M, Yousefi M, Shahrivar F and Aghazadeh M. 2016. Presence of *exoY*, *exoS*, *exoU* and *exoT* genes, antibiotic resistance and biofilm production among *Pseudomonas aeruginosa* isolates in Northwest Iran. *GMS Hyg Infect Control*., **11**: 1-6.

Barbier F, Andremont A, Wolff M and Bouadma L. 2013. Hospital-acquired pneumonia and ventilator-associated pneumonia: recent advances in epidemiology and management. Curr Opinion Pulm Med., **19**: 216-228.

Bradbury RS, Roddam LF, Merritt A, Reid DW and Champion AC. 2010. Virulence gene distribution in clinical, nosocomial and environmental isolates of *Pseudomonas aeruginosa*. *J Med Microbiol*., 59(Pt 8):881-890.

Cho HH, Kwon KC, Kim S and Koo SH. 2014. Correlation between virulence genotype and fluoroquinolone resistance in carbapenem-resistant *Pseudomonas aeruginosa*. *Ann Lab Med*., **34**(4):286-292.

Clinical and Laboratory Standards Institute (CLSI). 2016. Performance standards for antimicrobial susceptibility testing. 26th ed. CLSI supplement. M100S. Wayne, PA, USA.

Dacheux D, Toussaint B, Richard M, Brochier G, Croize J and Attree I. 2000. *Pseudomonas aeruginosa* cystic fibrosis isolates induce rapid, type III secretion-dependent, but Exo-U independent, oncosis of macrophages and polymorphonuclear neutrophils. *Infect Immun*., **68**: 2916-2924.

Elsen S, Huber P, Bouillot S, Couté Y, Fournier P, Dubois Y, Timsit JF, Maurin M and Attrée I. 2014. A type III secretion negative clinical strain of *Pseudomonas aeruginosa* employs a two-partner secreted exolysin to induce hemorrhagic pneumonia. *Cell Host Microbe*., **15**(2): 164-176.

El-Solh AA, Hattemer A, Hauser AR, Alhajhusain A and Vora H. 2012. Clinical outcomes of type III *Pseudomonas aeruginosa* bacteremia. *Crit Care Med.*, **40**(4): 1157-1163.

Engel JN. 2003. Molecular pathogenesis of acute *Pseudomonas aeruginosa* infections. *In:* Hauser AR and Dordrecht RJ (Eds.), **Severe Infections caused by *Pseudomonas aeruginosa*.** Kluwer Academic Publishers, pp. 201-229.

Feltman H, Schulert G, Khan S, Jain M, Peterson L and Hauser AR. 2001. Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. *Microbiol*., **147**: 2659-2669.

Finck-Barbancon V, Goranson J, Zhu L, Sawa T, Wiener-Kronish JP, Fleiszig SMJ, Wu C, Mende-Mueller L and Frank DW. 1997. *ExoU* expression by *Pseudomonas aeruginosa* correlates with acute cytotoxicity and epithelial injury. *Mol Microbiol.*, **25**:547-557.

Fleiszig S M J, Wiener-Kronish JP, Miyazaki H, Vallas V, Mostov KE, Kanada D, Sawa T, Yen TSB and Frank DW. 1997. *Pseudomonas aeruginosa*-mediated cytotoxicity and invasion correlate with distinct genotypes at the loci encoding exoenzyme S. *Infect Immun*., **65**: 579-586.

Gawish AA, Mohammed NA, El-Shennawy GA and Mohammed HA. 2013. An investigation of type 3 secretion toxins encoding-genes of *Pseudomonas aeruginosa* isolates in a University Hospital in Egypt. *J Microbiol Infect Dis.*, **3**(3): 116-122.

Grosso-Becerra MV, Santos-Medellín C, González-Valdez A, Méndez JL, Delgado G, Morales-Espinosa R, Servín-González L, Alcaraz LD and Soberón-Chávez G. 2014. *Pseudomonas aeruginosa* clinical and environmental isolates constitute a single population with high phenotypic diversity. *BMC Genomics*., **15**: 318.

Hardalo HC and Edberg SC. 1997. *Pseudomonas aeruginosa:* Assessment of risk from drinking water. *Crit Rev Microbiol*., **23**: 47-75.

Hauser AR. 2009. The type III secretion system of *Pseudomonas aeruginosa:* infection by injection. *Nat Rev Microbiol*., **7**(9): 654-665.

Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, Oh MD and Choe KW. 2005. Clinical features and outcome of patients with community-acquired *Pseudomonas aeruginosa* bacteraemia. *Clin Microbiol Infect*., **11**(5): 415-418.

Lee VT, Smith RS, Tümmler B and Lory S. 2005. Activities of *Pseudomonas aeruginosa* effectors secreted by the type III secretion system in vitro and during infection. *Infect Immun*., **73**: 1695-1705.

Lomholt JA, Poulson K and Kilian M. 2001. Epidemic population structure of *Pseudomonas aeruginosa*: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors. *Infect Immun.*,., **69**: 6284-6295.

Mahenthiralingam E, Campbell ME, Foster J, Lam JS and Speert DP. 1996. Random amplified polymorphic DNA typing of *Pseudomonas aeruginosa* isolates recovered from patients with cystic fibrosis. *J Clin Microbiol*., **34**: 1129-1135.

McCallum SJ, Gallagher MJ, Corkill JE, Hart CA, Ledson MJ and Walshaw MJ. 2002. Spread of an epidemic *Pseudomonas aeruginosa* strain from a patient with cystic fibrosis (CF) to non-CF relatives. *Thorax*., **57**(6): 559-560.

Mitov I, Strateva T and Markova B. 2010. Prevalence of virulence genes among Bulgarian nosocomial and cystic fibrosis isolates of *Pseudomonas aeruginosa*. *Brazilian J Microbiol*., **41**: 588-595.

Morgan JA, Bellingham NF, Winstanley C, Ousley MA, Hart CA and Saunders JR. 1999. Comparison of flagellin genes from clinical and environmental *Pseudomonas aeruginosa* isolates. *Appl Environ Microbiol*., **65**(3): 1175-1179.

Pirnay JP, Bilocq F, Pot B, Cornelis P, Zizi M, Van Eldere J, Deschaght P, Vaneechoutte M, Jennes S, Pitt T and De Vos D. 2009. *Pseudomonas aeruginosa* population structure revisited. *PLoS One*., 4: 1-20.

Pirnay JP, De Vos D, Mossialos D, Vanderkelen A, Cornelis P and Zizi M. 2002. Analysis of the *Pseudomonas aeruginosa oprD* gene from clinical and environmental isolates. *Environ Microbiol*., **4**(12): 872-882.

Rumbaugh KP, Hamood AN and Griswold JA. 1999. Analysis of *Pseudomonas aeruginosa* clinical isolates for possible variations within the virulence genes exotoxin A and exoenzyme S. *J Surg Res*., **82**: 95-105.

Strateva T, Markova B, Ivanova D and Mitov I. 2010. Distribution of the type III effector proteins-encoding genes among nosocomial *Pseudomonas aeruginosa* isolates from Bulgaria. *Ann Microbiol*., **60**: 503-509.

Wareham DW and Curtis MA. 2007. A genotypic and phenotypic comparison of type III secretion profiles of *Pseudomonas aeruginosa* cystic fibrosis and bacteraemia isolates. *Int J Med Microbiol*., **297**: 227-234.

Wong-Beringer R, Wiener-Kronish J, Lynch S and Flanagan J. 2008. Comparison of type III secretion system virulence among fluoroquinolon-susceptible and -resistant clinical isolates of *Pseudomonas aeruginosa*. *Clin Microbiol Infect*., **14**: 330-336.