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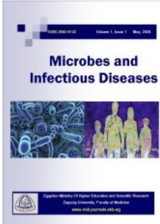

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Original article

Type III secretion system effector proteins genes, drug resistance profiles and biofilm formation in *Pseudomonas aeruginosa* isolated from clinical specimens

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ABSTRACT

Background: *Pseudomonas aeruginosa (P.aeruginosa*) is one of the most clinically important bacteria. It can cause serious infections because it has many virulence factors which are the main reasons for emergence of antibiotic resistance. The most important one is type III secretion system (T3SS) that plays a main role in bacterial invasion and poor clinical outcome. **Objectives:** This study aimed to detect some phenotypic virulence factors and the antimicrobial susceptibility patterns among clinical isolates of *P. aeruginosa,* and to evaluate the prevalence of T3SS virulence genes among these isolates. **Methods:** The study included one hundred strains of *P.aeruginosa* isolated from different clinical specimens from patients in Menoufia University Hospitals. The isolates were identified by microbiological methods and tested for biofilm formation, bile esculin hydrolysis, hemolysin and DNase production. Antimicrobial susceptibility of the isolates was determined by disc diffusion method. Multiplex PCR technique was used for targeting the virulence genes. **Results:** virulence factors were recorded as follows: hemolysin (72%), biofilm formation (77%) and bile esculin hydrolysis (40%). The most frequent genes were *exoT* and *exoY* genes (90% and 77%) respectively, while the least detected one was *exoU* (27%). High antibiotic resistance was detected to most of the used antibiotics. The *exoT*, *exoY* and *exoS* genes were significantly associated with high level of antibiotic resistance (p<0.001). **Conclusions:** This study highlights the prevalence of T3SS virulence genes among clinical isolates of *P.aeruginosa* obtained fom different clinical specimens, Moreover, there was association between *exoT*, *exoY* and *exoS genes* and antimicrobial resistance in these isolates.

Introduction

Pseudomonas aeruginosa is a Gram-negative, rod shaped, oxidase positive, and motile bacterium. It is an opportunistic pathogen causes serious health care associated infections (HAIs), including endocarditis, septicemia, urinary tract infections, cystitis, pneumonia, and infections of surgical wounds [1]. It is the leading cause of burn infections and the most prevalent colonizer of medical devices. Multidrug-resistant *P. aeruginosa* has become a significant public health concern among hospitalized patients. *Pseudomonas aeruginosa* infections can be fatal and managing

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them is difficult and frequently associated with high morbidity and mortality rates [2].

Pseudomonas aeruginosa's ability to colonize, invade, and spread is tied to the release of a number of virulence elements. These elements include biofilm, mucoid exopolysaccharides, pili, lipopolysaccharides, exotoxin A, pigments, lipases, proteases, hemolysins, histamine, exoenzymes, leukocidin, and rhamnolipids**.** These elements impair host's immune system and create an antibiotic resistance barrier [3].

Biofilms of *P. aeruginosa* can cause serious infections especially in imunocompromised patients. These infections are challenging to eradicate due to the fact that extraceullar matrix of biofilms is one of the leading factors that can reduce the penetration of antibiotics and contribute to the evolution of resistance [4].

The type III secretion system (T3SS) is important for tissue lysis, bacterial invasion, and unfavorable clinical outcomes [5]. This virulence factor plays an important role in the pathogenesis of *P. aeruginosa* infection. This system is unique in that it transports and secretes four different exotoxins(ExoS, ExoT, ExoU, and ExoY) from bacteria to their host cells through a needle-shaped complex.The secreted effector proteins are injected directly into cytosol of host cells to begin their damaging effects [6]. ExoU is an exoenzyme with phospholipase and cytolytic activity that causes severe tissue damage [7]. ExoS is an exoenzyme with ADP- ribosyl transferase activity which inhibits protein synthesis and causing apoptosis [5]. ExoY is an exoenzyme with adenylate cyclase activity [8]. ExoT shows a lower ADP-ribosyl transferase activity in comparison to ExoS [9]. These exoenzymes help *P. aeruginosa* evade the host immune response and stay alive by altering the inflammatory response.Moreover, it may disrupt physiological barriers including the actin cytoskeleton and endothelial cell membrane. This makes *P. aeruginosa* infection worse and cause significant tissue damage [9].

The emergence of *P. aeruginosa* as a major health threat is alarming because of its ability to release many virulence factors and its resistance to many antibiotic classes [1]. Therefore, it is important to learn more about the virulence and resistance characteristics of this bacteria. This should contribute to better clinical management of the infected patients. Our study aimed to detect some phenotypic virulence factors and the antimicrobial susceptibility patterns among clinical isolates of *P. aeruginosa,* and to evaluate the prevalence of T3SS effector proteins genes (*exoS*, *exoT*, *exoU* and *exoY*) among these isolates.

Material and Methods

This research was performed at Microbiology Laboratory of Clinical Pathology Department, Menoufia University Hospitals, Egypt, during the period from June 2021 to June 2022. The study included 100 non-repetitive strains of *P. aeruginosa*. Burn, wound, blood, urine, sputum, central venous catheter, endotracheal aspirate and ascitic fluid were the used samples to isolate the organism. The samples were obtained from patients in Menoufia University Hospitals by using standard microbiological sample collection methods after obtaining written consents from all participants.

Sample-size estimation

Based on a review of past literature [5], the least sample size calculated using statistics and sample size program version 6 is 94 participants and increased to be 100 to avoid 10% dropout. The power of the study is 80% and the confidence level is 95%.

Ethical approval

Menoufia Faculty of Medicine Ethical Review Board authorized this research. The ethical approval number was 19719 CPATH 41, in compliance with the Declaration of Helsinki's Principles of Ethical Conduct.

Isolation and identification of *P. aeruginosa*

Different samples were cultured on a variety of media including nutrient, blood and MacConkey's agar plates (Oxoid, UK), they had a 37°C incubation for a period of 24 to 48 hours. Colony morphology, Gram staining, growth at 42 degrees Celsius, exopigment production and different biochemical reactions including oxidase test, Lysine iron agar (LIA) test, Motility Indole Ornithine (MIO) test, and Citrate utilization test (Oxoid, UK) were used to identify *P. aeruginosa* colonies [10]. The identification was confirmed by VITEK -2 compact system (BioMerieux). Isolates of *P. aeruginosa* were stored in tryptic soy broth (TSB) (Oxoid, UK) containing 20% glycerol at - 80°C until further steps. *P. aeruginosa* (ATCC 27853) was used as reference strain.

Phenotypic detection of *P. aeruginosa* virulence factors

Hemolysin production

On blood agar plates, the tested isolates were cultured. After a 24-hour period of incubation at 37°C, hemolysis surrounding the colonies on the plates were inspected. Greenish zones indicated α-hemolysis, whereas clear zones indicated β-hemolysis, and no zone of hemolysis indicated γ-hemolysis [11].

Esculin hydrolysis

Isolates were inoculated into bile esculin medium (Oxoid, UK) containing Fe^{3+} citrate then incubated for 24h at 37° C. A brown precipitate around colonies indicated positive esculinase activity [12].

DNase activity

Colonies were inoculated into DNase agar plates (Oxoid, UK) and were incubated at 37° C for 24-48 h. Clear zone around the inoculum was considered positive test [13].

Biofilm formation

Congo Red Agar method (CRA)

Biofilm production was performed using a qualitative method described by **Freeman et al.** [14]. CRA was prepared as follows :Brain heart infusion (BHI) broth (37 g/L), agar (10 g/L), (5%) sucrose and Congo red stain (0.8 g/L) (Oxoid, UK). Separate from the other components of the medium, CR is autoclaved at 121 degrees Celsius for 20 minutes. Then, the autoclaved agar with sucrose was added at 55°C. Specimens were plated then incubated aerobically at 37 degrees Celsius for 24 to 48 hours. Colonies that changed colour from pink to black with a dry crystalline consistency were biofilm producers.

Microtiter plate assay (MTP)

The biofilm quantification assay was performed using MTP. Adjusting *P. aeruginosa* culture after an overnight incubation to a turbidity of 0.5 McFarland standard was done . Tryptic soy broth (TSB) containing 1% glucose was used to dilute suspensions to 1:100 concentration. 200µl of the dilution were inoculated into sterile flat bottomed 96 well of polystyrene tissue culture plates (Sigma Aldrich, USA) .For the next 24 hours, the plates were kept at 37 degrees Celsius. Distilled water was used three times to completely rinse the wells and get rid of any remaining germs. (0.1%) crystal violet was used to stain bacterial biofilm for 15 minutes at room temperature. To get rid of the excess discoloration, we used deionized water and let the plates dry at room temperature. Ethanol was gently added and left at room temperature for 30mins [15].

All strains were examined in triplicate using the biofilm-producing strain *P. aeruginosa* (PAO-1) as positive control and sterile uninoculated BHI broth as negative control. Using an Enzyme-linked Immunoassay reader, the optical density (OD) of each well was determined at 570 nm (Tecan.Sunrise remote Austria). The ODc value was determined statistically as three SDs above the mean OD of the negative control [15].

Antimicrobial susceptibility testing

Tests for antimicrobial susceptibility met Clinical and Laboratory Standards Institute guidelines (CLSI 2020) [16]. It was done by modified Kirby Bauer's disc-diffusion method on Mueller-Hinton agar (Oxoid, UK) plates. The following discs were used: meropenem (10 µg), imipenem (10 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), cefepime (30 µg), piperacillin - tazobactam (100/10 µg), tetracycline (30 µg), and ceftazidime - avibactam (50 µg) (Oxoid, UK). MDR was defined previously as resistant to at least one agent across three or more classes of antimicrobials [6].

Molecular detection of T3SS effector proteins genes

DNA extraction of *P. aeruginosa*

Using a Gene JET^{TM} Genomic DNA Purification Kit (Thermo Fisher Scientific, UK), bacterial DNA was extracted and purified from overnight cultures of *P. aeruginosa*. We followed the manufacturer's guidelines for the extraction procedure. After DNA extraction, its concentration and purity were evaluated spectrophotometrically using the Nanodrop (Implen, Germany). Until amplification, the isolated DNA was stored at -20 degrees Celsius.

Detection of *exoS, exoT, exoU and exoY* genes

Multiplex PCR assay was done to detect T3SS virulence genes (*exoS*, *exoT*, *exoU* and *exoY*) by Thermal Cycler (Biometra, Germany) using the primers listed in **table (1)**. The total volume PCR reaction mixture was 25µl. It was done as follows: 12.5µl Taq Green PCR Master Mix (Thermo Fisher Scientific, UK), 5µl of template DNA, 0.25µl from each primer, and 5.5µl of nuclease free water. The protocol used to amplify the DNA was as follows: initial denaturation at 95°C for 3 min, then 36 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 40 s, and extension at 72°C for1 min, with final extension at 72°C for 10 min [17].

Pseudomonas aeruginosa ATCC 27853 was used as positive control, while sterile distilled water was used as negative control. Electrophoresis on ethidium bromide-stained 2% agarose gel allowed us to examine the amplified products. A 50-pb DNA ladder (Life Technologies) was used. DNA bands were visualized using UV transilluminator and photographed. Genes were determined by the size of amplified products in comparison with known ladder bands, *exoS* (118bp), *exoT* (152 bp), *exoU* (134 bp) and *exoY* (289bp) [5] **(Figure 1)**

Statistical analysis

The information that was entered into the computer was analysed using IBM SPSS version 20.0 (Armonk, NY: IBM Corp)**.** Qualitative data were described using number and percentages. Quantitative data were described using mean, median, range and standard deviation. Chi-square test $\chi 2$, Fisher's Exact test and Monte Carlo correction were used. The significance of the obtained results was judged at the 5% level. In all tests, *p* value of less than 0.05 was considered statistically significant.

Results

One hundred *P. aeruginosa* isolates were obtained and identified from non-repetitive patients' specimens, including burn (n=28), blood (n = 16), wound (n = 21), urine (n = 14), sputum (n = 8), endotracheal aspirate (n=1), bed ulcer (n=6), ascitic fluid (n=2) and central venous catheter (n=4). Of these 100 *P. aeruginosa* isolates, 54 (54%) were recovered from males and 46 (46%) from females with a mean age of 36.99±23.84 years old, ranging from 3 month to 86 years old.

Antibiotic susceptibility test results demonstrated that *P. aeruginosa* isolates showed the highest resistance against ceftazidime (82%), followed by gentamycin (74%), amikacin (66%), piperacillin-tazobactam, cefepime, levofloxacin (63% for each), ciprofloxacin (59%), meropenem (56%) and imipenem (54%). In the present study, the highest frequency of resistance was observed with ceftazidime while the highest frequency of sensitivity was observed with imipenem and meropenem. 64 (64%) of the 100 *P. aeruginosa* isolates were multidrug resistant. The complete antibiotic susceptibility profile data were summarized in **table (2)**.

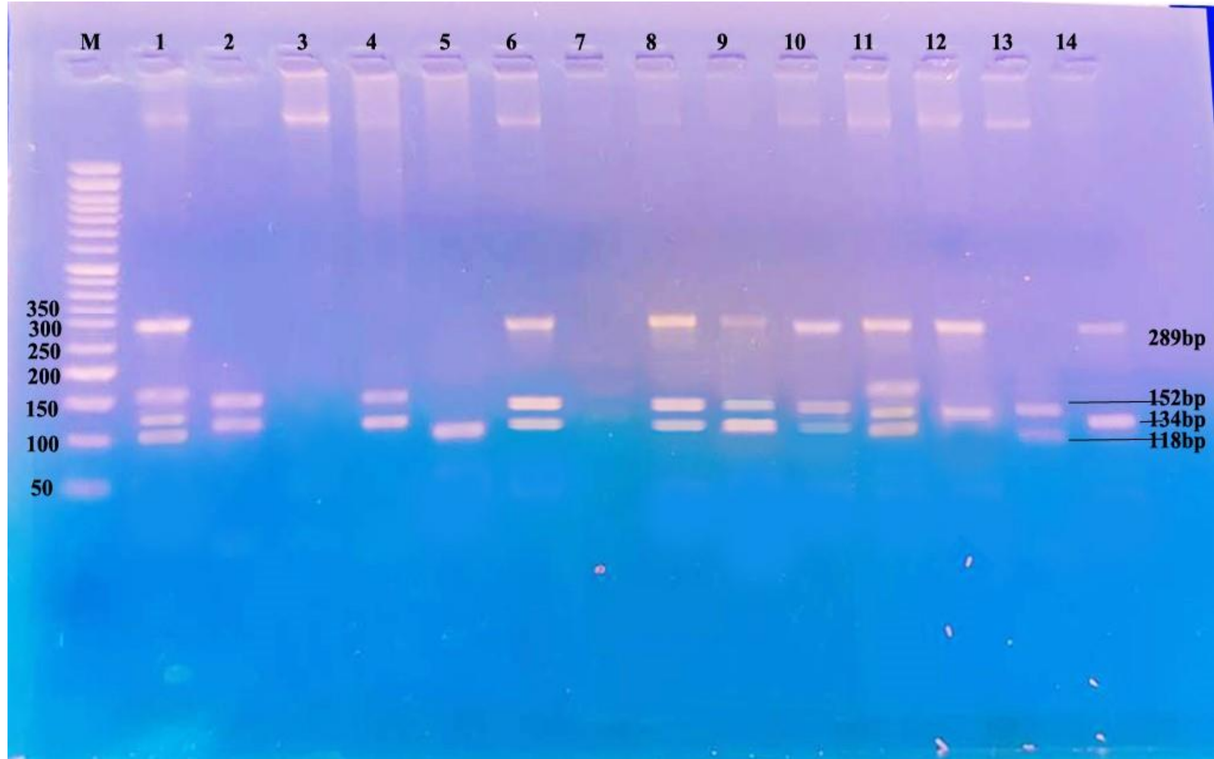
This study demonstrated that the frequency of virulence factors by phenotypic methods was as follows: the highest percentage was detected in biofilm formation by MTP (77%), followed by hemolysin production (72%), biofilm formation by CRA (56%), bile esculin hydrolysis (40%). The least detected virulence factor among *P. aeruginosa* isolates was DNase (2%), as shown in **figure (2)**.

Multiplex PCR analysis was used for detection of T3SS virulence genes, the results revealed that *exoT* and *exoY* were the most prevalent genes among *P. aeruginosa* strains (90% and 77%) respectively, followed by *exoS* (73%), while the least detected gene was *exoU* (27%), as demonstrated in **table (3)** and **figure (1)**.

In this study, the frequency of virulence genes among isolates from different clinical specimens was as follows: *exoU* was higher among burn isolates (29.6%) followed by urine isolates (18.5%), while *exoS* showed the highest prevalence among burn isolates (26%) followed by wound isolates (20.5%). The *exoT* was higher among burn and wound isolates (22% for each) than other clinical isolates. The *exoY* gene was higher among burn isolates followed by blood isolates (24.75 and 19.5%) respectively, as shown in **table (4)**.

There was a statistically significant association between *exoT, exoY* and *exoS* genes and increased antibiotics resistance and MDR rate (*p* value <0.001), while *exoU* gene showed no significant association (*p* value= 0.735). The results also showed significant differences between MDR and non-MDR isolates regarding the total number of T3SS virulence genes detected in the tested isolates (*p* value <0.001), as illustrated in **table (5).**

Figure 1. Agarose gel electrophoresis for PCR amplified products of *P. aeruginosa* virulence genes *exoS, exoT*, *exoU* and *exoY*.



Lane (M) 50bp DNA ladder. Lane (1) is a positive control. Lane (3) is a negative control
Lane (2) and (4) showed *exoU* and *exoT genes*.
Lane (5) showed *exoS*. Lane (6), (8), (9) and (10) showed *exoT*, *exoY* and *exoU*.
Lane (7) is negative for genes. Lane (11) showed *exoT*, *exoY*, *exoS* and *exoU*.
Lane (12) showed *exoY* and *exoT*. Lane (13) showed *exoT* and *exoS*. Lane (14) showed *exoU* and *exoY*.

Figure 2. Distribution of phenotypic virulence factors among isolated *P.aeruginosa*.

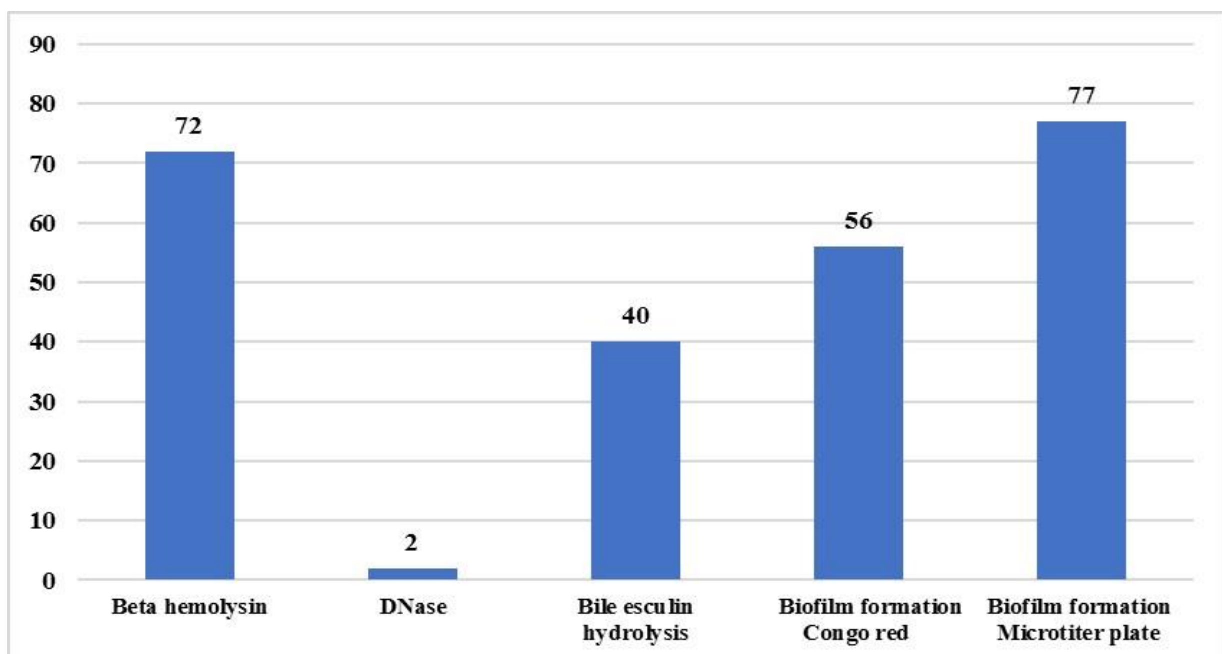


Table 1. The sequences of the used primers and the size of PCR products (pb).

Target gene		Primer sequence (5′-3′)	(bp)	Reference
exoS	Forward	5′-GCGAGGTCAGCAGAGTATCG-3′	118bp	[5]
	Reverse	5′-TTCGGCGTCACTGTGGATGC-3′		
exoT	Forward	5′-AATCGCCGTCCAACTGCATGCG-3′	152 bp	
	Reverse	5′-TGTTCGCCGAGGTACTGCTC-3′		
exoU	Forward	5′-CCGTTGTGGTGCCGTTGAAG-3′	134bp	
	Reverse	5′-CCAGATGTTCACCGACTCGC-3′		
exoY	Forward	5′-CGGATTCTATGGCAGGGAGG-3′	289bp	
	Reverse	5′-GCCCTTGATGCACTCGACCA-3′		

Table 2. Antimicrobial susceptibility pattern of *P. aeruginosa* isolates.

Antimicrobial agent	**Antibiotic disc concentration (µg)**	***P. aeruginosa* isolates (n=100)**	
		Sensitive (S)	**Resistant (R)**
		No. (%)	**No. (%)**
β-lactam combination agents			
Piperacillin-tazobactam (TZP)	100/10	37(37%)	63(63%)
Ceftazidime-avibactam (CZA)	30/20	40(40%)	60(60%)
Cephalosporins			
Ceftazidime (CAZ)	30	18(18%)	82(82%)
Cefepime (CEF)	30	37(37%)	63(63%)
Carbapenems			
Imipenem (IPM)	10	46(46%)	54(54%)
Meropenem (MEM)	10	44(44%)	56(56%)
Aminoglycosides			
Gentamicin (GEN)	10	26(26%)	74(74%)
Tobramycin (TOB)	10	36(36%)	64(64%)
Amikacin (AK)	30	34(34%)	66(66%)
Fluoroquinolones			
Ciprofloxacin (CIP)	5	41(41%)	59(59%)
Levofloxacin (LEV)	5	37(37%)	63(63%)
Norfloxacin (NOR)	10	44(44%)	56(56%)
Ofloxacin (OF)	5	38(38%)	62(62%)
Gatifloxacin (GAT)	5	38(38%)	62(62%)

Table 3. The prevalence of type III secretion system virulence genes among the studied isolates.

Virulence genes	***P. aeruginosa* isolates (n=100)**
	No. (%)
exoT	90(90%)
exoY	77(77%)
exoS	73(73%)
exoU	27(27%)

Table 4. Distribution of exoenzymes virulence genes among different clinical samples.

Sample	***exoT* (n=90)**		***exoY* (n=77)**		***exoS* (n=73)**		***exoU* (n=27)**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Burn (n=28)	20	22.2	19	24.7	19	26.0	8	29.6
Wound (n=21)	20	22.2	14	18.2	15	20.5	4	14.8
Blood (n=16)	16	17.8	15	19.5	13	17.8	4	14.8
Urine (n=14)	14	15.6	12	15.6	12	16.4	5	18.5
Sputum (n=8)	8	8.9	7	9.1	4	5.5	3	11.1
Bed ulcer (n=6)	5	5.6	5	6.5	4	5.5	1	3.7
CVC (n=4)	4	4.4	2	2.6	3	4.1	2	7.4
Ascitic fluid (n=2)	2	2.2	2	2.6	2	2.7	0	0.0
ET aspirate (n=1)	1	1.1	1	1.3	1	1.4	0	0.0

Table 5. Frequency of T3SS virulence genes among MDR and non-MDR *P. aeruginosa* isolates.

Virulence genes	**MDR (n= 64)**		**Non- MDR (n = 36)**		**Test of significance**	***p*.value**
	No.	**%**	**No.**	**%**	χ^2	
exoT	64	100	26	72.2	19.753	$^{FE}p<0.001^*$
exoY	56	87.5	21	58.3	11.067	$p<0.001^*$
exoS	62	96.9	11	30.6	51.414	$p<0.001^*$
exoU	18	28.1	9	25	0.114	$p=0.735$
Total number of virulence genes						
0	0	0	4	11.1	54.996	$^{MC}p<0.001^*$
1	0	0	9	25.0		
2	1	1.6	12	33.3		
3	54	84.4	10	27.8		
4	9	14.1	1	2.8		

χ^2: chi square test MC: Monte Carlo FE: Fisher Exact. MDR: multiple drug resistant Non- MDR: non multiple drug resistant

Discussion

Pseudomonas aeruginosa is considered one of the most significant organisms causing opportunistic hospital acquired infections because it has the ability to invade tissue through a variety of virulence factors including exotoxin, exoenzymes and biofilm. Understanding the pathogenesis of this organism and creating efficient policies against *P. aeruginosa* infections depend on identification of these virulence factors [1,3]. A key determinant of *P. aeruginosa* is its remarkable resistance to antibiotics especially in immunocompromised patients and many of the isolates are multiple drug resistant [15].

The primary objective of this research was to assess the prevalence of type III secretion system effector proteins genes (*exoS*, *exoT*, *exoU* and *exoY*) among clinical isolates of *P. aeruginosa* and to detect the antimicrobial susceptibility patterns of these isolates.

In the current study, 100 *P. aeruginosa* isolates were obtained and identified from different patients' specimens. The rate of *P. aeruginosa* isolation was higher among burn specimens than respiratory and urinary tract specimens. Another research by **Saleh et al.** also noted this finding, as 55% of their isolates were from burn samples [18].

Sixty-four percent of the isolates were classified as MDR, indicating that they exhibited a high level of antibiotic resistance.The resistance was recorded as follows: ceftazidime (82%), gentamycin (74%), amikacin (66%), tobramycin (64%), piperacillin-tazobactam, cefepime, levofloxacin (63% for each), ceftazidime-avibactam (60%) and ciprofloxacin (59%). Consistent with our result; **Ismail et al.** [13] and **Kunwar et al.** [19] showed high resistance rate observed for ceftazidime as it was (70%), gentamycin (82%), tobramycin (74%) and ciprofloxacin (68%). This may be related to *P. aeruginosa* biofilm and its exogenous DNA which increase their tolerance to antimicrobial peptide and to aminoglycoside antibiotics [13]. However, **Khosravi et al.** [20] and **Nasirmoghadas et al.** [21] observed higher antimicrobial resistance rates (>80%) against all tested antibiotics.

In our findings, the prevalence of carbapenems susceptible *P. aeruginosa* was 46% for imipenem and 44% for meropenem. This was in accordance with the finding of others studies [6,20], while higher carbapenem resistance was documented by **Nasirmoghadas et al.** [21]. On the contrary **Wang et al.** [22] reported that 100% *P. aeruginosa* strains were susceptible to imipenem and meropenem, while a survey conducted in Nigeria showed imipenem resistance of (9.6%) [23]. Possible explanations for this discrepancy between studies' findings on *P. aeruginosa*'s susceptibility to various antibiotics lie in regional variations in drug use, the prevalence of antibiotic resistance in different parts of the world, and the different mechanisms of resistance that have been described for the bacterium [24].

In the current study the percentage of *P. aeruginosa* virulence factors was as follows: hemolysin (72%), bile esculin hydrolysis (40%) and DNase (2%). **Ismail et al**. [25] demonstrated higher percentage in hemolysin production (92%), while **Iseppi et al.** [1] demonstrated lower percentage (35%). Beta-hemolysin production by *P. aeruginosa* plays an important role in spreading the infection and the invasion of the organism into eukaryotic cells, protecting the bacterium from the host's defense mechanisms and the antimicrobial treatment [26].

In this study 40% of the isolates were positive for esculin hydrolysis. This went with **Gautam et al.** [27] where 50% of isolates had bile esculin activity, and this was slightly higher than those reported by **Georgescu et al.** [12]. In our study only two isolates gave positive DNase test in sputum samples. This matched with another study [1]. DNases are useful for bacteria in certain conditions, as for escaping viscous secretions and neutrophil extracellular traps (NETs). It is unusual to find thick mucus and NETs within the burn, wound area and within urinary tract and this may explain the lack of DNase production in burn, wound and urine *P. aeruginosa* isolates [25].

Biofilms inhibit antibiotic penetration, shield microbes from the human immune system, and confer antimicrobial resistance via induction of persistence [4, 25]. Due to inadequate aseptic practises, biofilms often develop on indwelling and implanted medical devices used in immunocompromised patients in clinical settings. Our results showed low positivity in biofilm production by CRA method regarding quantitative MTP (56% and 77%) respectively, indicating that the quantitative technique was more efficient than the qualitative technique. This was in agreement with **Bakir and Ali** [28], while **Pramodhini et al.**

[29] disagreed with our results and reported only 34% were biofilm producers. This may be due to variable conditions like site of infection and presence of device. Several studies were in agreement with our results as they reported higher percentage of biofilm production 76% [25,30] and 80% [31].

Studying whether *P. aeruginosa* isolates have genes for T3SS was the primary objective of our research. In this study, the prevalence of *exoT* and *exoY* genes was (90% and 77%) respectively. Both genes were the most frequent genes among *P. aeruginosa* strains, this was in agreement with earlier studies [3,5,6]. However, this result was different from the results obtained by **Elbargisy et al.** [7] who demonstrated a lower prevalence rate of *exoT* (36.3%).

The results showed that, the prevalence of *exoS* gene was higher than *exoU* (73% vs 27%), this was in agreement with results of previous studies [5,12]. However, higher prevalence rates of *exoU* than exo*S* was reported in other studies by **Bazghandi et al.** and **Rodulfo et al.** (48% vs 43.5%) and (56% vs 51.2%), respectively [6,26].

In this study, prevalence of *exoU* was higher among burn isolates (29.6%) followed by urine (18.5%), while *exoS* showed highest prevalence among burn (26%) followed by wound isolates (20.5%). The *exoT* was higher among burn and wound (22.2% for each) than other clinical isolates. The *exoY* was higher among burn followed by blood isolates (24.75 and 19.5%), respectively. This was in agreement with similar findings shown in other studies [17,32,33].

Pseudmonas aeruginosa utilizes the T3SS to deliver effector toxins (exoS, exoU, exoY, and exoT) directly into host cells, which can cause rapid cell necrosis or can modulate the actin cytoskeleton, allowing the pathogen to invade the host cells and evade phagocytosis depending on the disease site or patient background [6].

The exoT, *exoY* and *exoS* genes had statistically significant association with higher antibiotics resistance and MDR rate (p<0.001). Similar results were found by **Bazghandi et al.** who also found a strong correlation between the expression of *exoT* and *exoS* genes and resistance [6]. This was unlike with results obtained by previous study [26]. Our study demonstrated no significance association between *exoU* positivity and MDR (p value=0.735) which was matched with other studies [6,32]. The results also showed significant differences between multidrug resistant (MDR) and non-MDR isolates regarding total number of virulence genes detected in tested isolates (*p*<0.001), as all MDR isolates had at least two or more virulence genes with maximum four virulence genes which were detected in 9 MDR isolates. On the other hand, 11.1% of non-MDR isolates had not any effector proteins genes and 25% of those isolates had one virulence gene. This coincided with other studies [3,32].

Conclusions

This study highlights the prevalence of T3SS effector proteins genes (*exoT*, *exoY*, *exoS* and *exoU*) among isolates of *P. aeruginosa* obtained fom different clinical samples, Moreover there was association between *exoT*, *exoY* and *exoS* genes and antimicrobial resistance in these isolates. Biofilm formation by *P. aeruginosa* is frequent and is used to overcome the host immunity and increase antimicrobial resistance. Further studies are required to determine the role of each virulence factor and for the development of novel anti-virulence therapeutic strategies.

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Conflicts of interest: None.

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