

# Characterization of Uropathogenic *Pseudomonas aeruginosa*: Serotypes, Resistance Phenotypes, and Virulence Genotypes

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## Abstract

*Pseudomonas aeruginosa* is a major cause of urinary tract infections. This organism has extended resistance to antimicrobials along with multiple virulence factors, making it difficult to treat. In this study, 49 isolates from urine samples were identified as *P. aeruginosa* and serotyped by the slide agglutination method. The sensitivity of isolates against 10 antipseudomonal drugs was determined. Phenotypically, lipase, protease, hemolysin, and biofilm production were detected. Genes for the type III secretion system, elastase B, and exotoxin A were detected by PCR. Serotype O11 was the most predominant serotype among test isolates. High levels of resistance were observed against ceftazidime, cefepime, piperacillin, and piperacillin/tazobactam while 10.2% of isolates were resistant to amikacin. MDR was detected in 20.4% of the isolates and was significantly associated with strong biofilm producers. About 95.9% and 63.3% of *P. aeruginosa* isolates had proteolytic and lipolytic activity, respectively. Among the genes detected, the *exoY* gene was the most prevalent gene (79.6%), while the *exoU* gene was the least frequent one (10.2%). *toxA* and *lasB* genes were amplified in 63.27% and 75.5% of the isolates, respectively. In addition, the *exoU* gene was significantly associated with MDR isolates. The high incidence of *exoS*, *exoT*, *exoY*, *lasB*, and *toxA* genes in uropathogenic *P. aeruginosa* implies that these genes can be considered markers for virulent isolates. Furthermore, the coexistence of *exoU* and *exoS* genes, even in 6% of isolates, poses a significant treatment challenge because those isolates possess both the invasive and cytotoxic properties of both effector proteins.

**Keywords:** Serotypes, Type III secretion system, antimicrobial resistance, *P. aeruginosa*, Urinary tract infections

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## INTRODUCTION

Urinary tract infections (UTIs) represent a great health problem that affects people of all ages.<sup>1</sup> These infections may be hospital-acquired. Catheterization of the urinary tract is an important predisposing factor to this type of infection by introducing opportunistic pathogens into the urinary tract.<sup>2</sup> *P. aeruginosa* is commonly isolated from complicated UTIs. This bacterium is widespread in healthcare facilities and can get accessed through tap water, food, disinfectant, medical supplies, and healthcare staff resulting in severe nosocomial infections, especially among immunocompromised patients.<sup>1,3</sup>

Treatment of infections caused by *P. aeruginosa* is a great challenge facing physicians all over the world. The bacterium has both intrinsic and acquired resistance to many varieties of antimicrobials, which limits the therapeutic options available. Besides, multiple virulence factors, either cell-associated (e.g., lipopolysaccharides, flagellum, and adhesins) or cell-free (e.g., protease, elastase, lipase, hemolysins, exotoxin A) have been linked to *P. aeruginosa* pathogenicity, allowing it to persist in a wide range of settings. Some of these virulence factors are needed for colonization, while others help the invasion process.<sup>4,5</sup>

The most potent virulence toxin is exotoxin A (encoded by the *toxA* gene). The majority of clinical isolates of *P. aeruginosa* produce this toxin. It has destructive effects on host cells including suppression of protein synthesis, apoptosis of host cells, and impairment of the host cellular immune response.<sup>6,7</sup>

The second important virulence factor is the type III secretion system (TTSS) which plays a significant role in serious infections caused by *P. aeruginosa*. This system transports the effector proteins, ExoS, ExoT, ExoU, and ExoY, to the cytosol of host cells to start their harmful effects. Both ExoS and ExoT can disrupt actin cytoskeleton and cause host cell death in addition to inhibition of phagocytosis.<sup>8</sup> ExoU is an exoenzyme with phospholipase activity. It has rapid and potent cytotoxic activity that damages the host cells including macrophages. Besides, exoU increases the expression of inflammation genes. ExoU-mediated cell death is characterized by a fast loss of plasma membrane integrity, which is associated

with necrosis.<sup>9,10</sup> ExoY possesses adenylate cyclase activity, which disrupts the actin cytoskeleton and promotes the synthesis of the second messengers cGMP and cUMP in host cells.<sup>11</sup> Another virulence factor secreted by *P. aeruginosa* isolates is LasB elastase, which is encoded by the *lasB* gene. This enzyme targets host proteins such as collagen and elastin causing degradation of host tissues which then facilitates the invasion process.<sup>12</sup>

The capacity of *P. aeruginosa* to induce an infection has been linked to its potential to form biofilms. Biofilms can escape the host defense mechanisms as well as resist antimicrobial therapy.<sup>13</sup> *P. aeruginosa* has a high tendency for sticking to surfaces forming biofilms. Thus, long-term bladder catheterization predisposes to adhesion and biofilm formation by *P. aeruginosa* leading to recurrent UTIs.<sup>14</sup>

Based on the O-antigen portion of their lipopolysaccharide molecule, *P. aeruginosa* isolates were divided into 20 serotypes by the international antigenic system (IATS). Several investigations have found a link between various virulence factors of *P. aeruginosa* isolates and their serotypes.<sup>15-17</sup>

Characterization of uropathogenic *P. aeruginosa* will help in the selection of the proper therapeutic strategy and improve the treatment outcome. Therefore, the present study aimed to investigate the antimicrobial resistance, serotypes, biofilm formation, and TTSS genotypes of *P. aeruginosa* isolated from urinary tract infections in Egypt. The correlations between the studied virulence factors, antimicrobial resistance, and recorded serotypes were also investigated.

## METHODS

### Isolation and identification of *P. aeruginosa*

The research ethics committee of Faculty of Pharmacy, Mansoura University authorized the experiments carried out in this work (Code number: 2021-236). Forty-nine isolates of *P. aeruginosa* were isolated from urine samples from urinary tract infected patients admitted to the Urology and Nephrology center, Mansoura University, Mansoura, Egypt. The identification of *P. aeruginosa* isolates was based on their gram reaction, growth on cetrимide agar, oxidase production, and pyocyanin pigment production.

### Serotyping of *P. aeruginosa* isolates

Isolates of *P. aeruginosa* were serotyped by the slide agglutination method using 4 polyvalent and 16 monovalent antisera (Bio-Rad®, France), and the test was done following the manufacturer's instructions. The serotype groups were determined as described in the International Antigen Typing Scheme (IATS).<sup>18</sup> The isolates that did not agglutinate with any antisera were described as non-typeable.

### Antimicrobial susceptibility testing

The antimicrobial activity of ten antipseudomonal drugs was determined using the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute guidelines.<sup>19</sup> The used antimicrobial discs were ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), piperacillin (PRL, 100µg), imipenem (IPM, 10µg), meropenem (MEM, 10 µg), levofloxacin (LEV, 5µg), ciprofloxacin (CIP, 5µg), amikacin (AK, 30µg), gentamicin (CN, 10µg), and piperacillin/tazobactam (TPZ, 100 µg /10 µg) (Bioanalyse, Turkey). Resistance to 3 or more classes of antimicrobials was considered multidrug resistance (MDR).

### Phenotypic detection of some virulence factors of *P. aeruginosa* isolates

#### • Qualitative detection of Lipase and protease enzymes

Lipase enzyme production was detected on nutrient agar plates supplemented with Tween 80. Following incubation, lipase-producing colonies showed a zone of precipitation surrounding them.<sup>20</sup> Skimmed milk/ brain heart infusion agar

was used to detect the proteolytic activity of the tested isolates. After 24-48 h of incubation, protease producers showed a zone of clearance surrounding growth.<sup>21</sup>

#### • Quantitative detection of Hemolysin

Test isolates with optical density  $0.257 \pm 0.002$  at  $\lambda_{600}$  nm were inoculated in nutrient broth and incubated for 48 h at 28°C with shaking at 120 rpm. The hemolytic activity was assessed using human erythrocytes suspension in hemolysin assay buffer (2% v/v). For each isolate, the supernatant was collected by centrifugation, mixed with an equal volume of erythrocyte suspension, and incubated at 37°C for 2 h. The mixture was then centrifuged, and the optical density of the supernatant at  $\lambda_{540}$  nm was determined. Control experiments for spontaneous lysis (negative control) and complete lysis (positive control, 0.2% sodium dodecyl sulfate) were carried out. The percentage (%) of lysed erythrocytes was calculated.<sup>22</sup>

$$\% \text{ of lysed erythrocytes} = [(X-B) / (T-B)] \times 100$$

B is the absorbance of the negative control, T is the absorbance of the positive control, and X is the absorbance of the test sample.

#### • Quantitative detection of biofilm

Colonies of overnight cultures were suspended in tryptic soy broth to yield 1 McFarland turbidity. 100 µl of each bacterial suspension was inoculated in triplicate into the wells of a microtiter plate and incubated for 24 h at 37°C. The content of wells was aspirated. The wells

**Table 1.** Primer sequences used for the detection of target genes and their amplicon sizes

Target gene	Primer sequence (5' - 3')	Amplicon size (bp)	Annealing Temp.	Ref.
<i>lasB</i>	F TCATCACCGTCGACATGAAC	490		[22]
	R TGCCCTTCTTGATGTCGTAG			
<i>exoT</i>	F CAATCATCTCAGCAGAACCC	1159		[25]
	R TGTCGTAGAGGATCTCCTG			
<i>exoY</i>	F TGCCATAGAATCCGCCTC	145	60 ° C	[26]
	R GATGACCGCCGATTATGAC			
<i>exoS</i>	F AGGCATTGCCATGACCTTG	372		
	R ATACTCTGCTGACCTCGCTC			
<i>exoU</i>	F CTAGAAGAGAAAGGCATGCTCG	274		[22]
	R CTATGCGTGGGAGTACATTGAG			
<i>toxA</i>	F GACAACGCCCTCAGCATCAACAGC	390	65 ° C	
	R CGCTGGCCCATTCGCTCCAGC			

were then washed three times with phosphate-buffered saline. Absolute methanol was added for 15 min to fix adherent cells, then the plates were emptied and left to dry. Crystal violet (1%) was added for 20 min and excess stain was washed away. After air drying of the plates, glacial acetic acid [33% (V/V)] was added. Finally, a microtiter plate reader was used to measure the optical density of each well at  $\lambda_{492}$  nm. Negative control wells were included. Consequently, *P. aeruginosa* isolates were classified as strong/moderate/weak slime producers or as non-adherent.<sup>20</sup>

#### Molecular detection of the type III secretion system, exotoxin A and elastase B

##### • Extraction of the tested isolates' genomic DNA

A single colony from overnight grown culture was picked up and suspended in 0.1 ml of DNase / RNase-free water. The bacterial cell suspensions were held in a thermocycler at 95°C for 10 min, cooled on ice, and centrifuged. The supernatants were kept at -20°C in aliquots of 5  $\mu$ l until their use as template DNA in PCR.<sup>23</sup>

##### • Polymerase chain reaction for the detection of virulence genes

The virulence genes (*exoS*, *exoT*, *exoU*, *exoY*, *toxA*, and *lasB*) were amplified by PCR

as described previously.<sup>24</sup> Positive control and negative control reactions were performed simultaneously. Primer sequences, annealing temperature, and expected amplicon sizes are presented in Table 1.

For the 49 isolates of *P. aeruginosa*, all data obtained in the previous tests were scored using a binary code system. Then, analysis was done by the Past 4.03 application through the unweighted pair group method with arithmetic mean (UPGMA) and Dice coefficient. A dendrogram was then constructed based on the analyzed data.

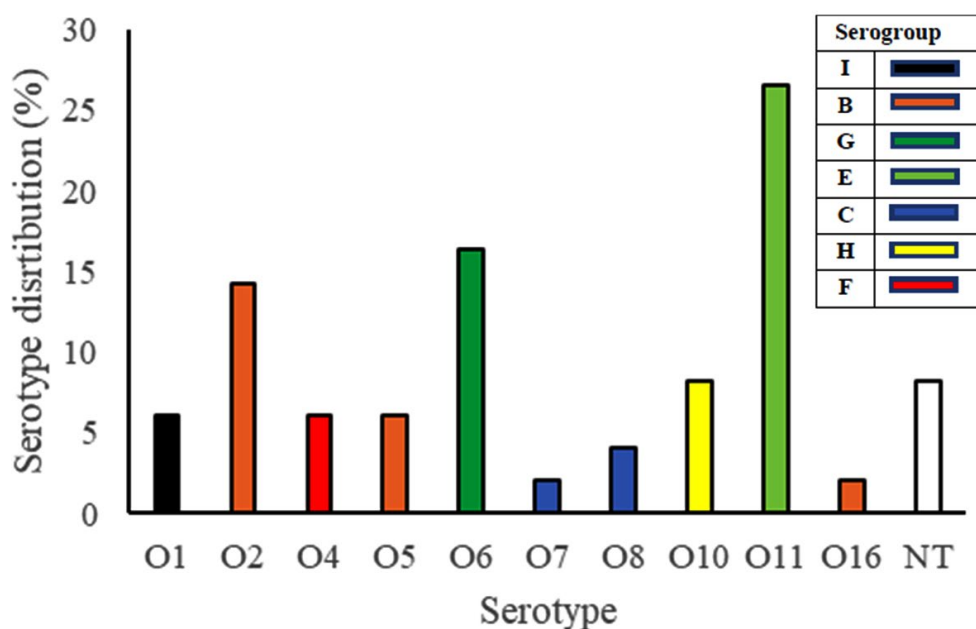
#### Statistical analysis of data

The Spearman rank correlation coefficient test was used to determine whether there is a significant association between the virulence factors, biotypes, and serotypes studied using SPSS software (version 13; SPSS Inc.). At a P-value of  $\leq 0.05$ , the results were statistically considered significant.

## RESULTS

### Serotyping of *P. aeruginosa* isolates

Ten serotypes (O1, O2, O4 - O8, O10, O11, and O16) with 7 serogroups (B, C, E, F, G, H, and I) were detected in *P. aeruginosa* isolates. Serotype



**Fig. 1.** Distribution of serotypes among urine isolates of *P. aeruginosa*.

NT: Non-typeable

O11 was the most prevalent (26%) followed by serotype O6 (16.3%) and serotype O2 (14%). The least frequent serotypes were O7 and O16 (one isolate each, 2%). Four isolates of *P. aeruginosa* (8%) were non-typeable, Fig. 1.

**Antimicrobial susceptibility testing**

Resistance to ceftazidime and cefepime was demonstrated by all tested *P. aeruginosa* isolates. A high resistance rate was also recorded for piperacillin (95.9%) and piperacillin/tazobactam (91.8%). The resistance rate to meropenem, imipenem, and gentamicin was relatively low (16.3%, 20.4%, and 24.5%, respectively). About 34.7% of isolates were resistant to both ciprofloxacin and levofloxacin. Most *P. aeruginosa* isolates (89.8%) were susceptible to amikacin. The antibiogram of *P. aeruginosa* isolates is shown in

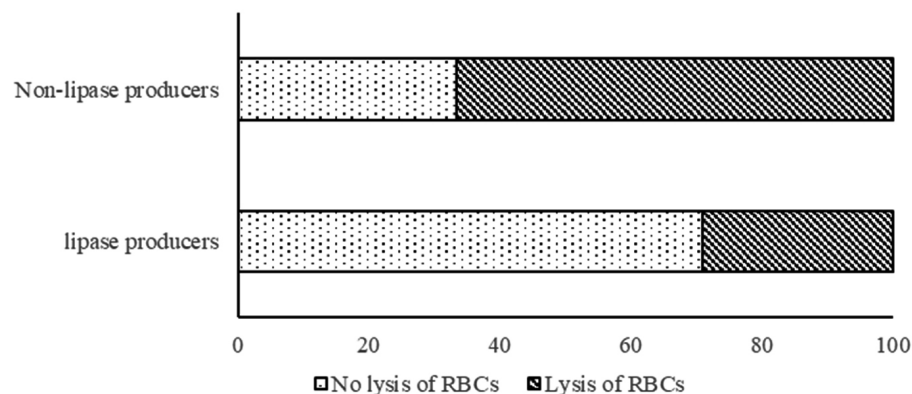
Table 2. Twelve antimicrobial resistance patterns were detected (A1: A12). Resistance pattern A3 was the most frequent pattern among the tested isolates (46.9%). Multidrug resistance was detected in 10 isolates (20.4%, patterns A5, A9, A10, A11, and A12) and three of them were resistant to all tested antimicrobials.

**Correlation between serotypes of *P. aeruginosa* isolates and their antibiogram**

Among the most frequent serotypes (O2, O6, O10, and O11), the isolates of serotype O11 were the most sensitive to all tested antimicrobials. A positive significant correlation between quinolone resistance and serotypes O6 (P=0.008) and O10 (P=0.004) was observed. In contrast, quinolone resistance was negatively associated with serotype O11 (P=0.017). In

**Table 2.** Distribution of antimicrobial resistance patterns among *P. aeruginosa* isolates

Code	Resistance pattern	No. of resistant isolates (%)
A1	FEP/CAZ	2 (4.08)
A2	FEP/PRL/CAZ	1 (2.04)
A3	FEP/PRL/TPZ/CAZ	23 (46.9)
A4	IPM/FEP/PRL/TPZ/CAZ	1 (2.04)
A5	CIP/LEV/ CN/FEP/PRL/CAZ	1 (2.04)
A6	CIP/LEV/FEP/PRL/TPZ/CAZ	7 (14.29)
A7	IPM/MEM/FEP/PRL/TPZ/CAZ	3 (6.12)
A8	IPM/MEM/CN/FEP/PRL/TPZ/CAZ	2 (4.08)
A9	CIP/LEV/ CN/FEP/PRL/TPZ/CAZ	4 (8.16)
A10	CIP/LEV/ CN/AK/FEP/PRL/TPZ/CAZ	1 (2.04)
A11	IPM/CIP/LEV/ CN/AK/FEP/PRL/TPZ/CAZ	1 (2.04)
A12	IPM/MEM/CIP/LEV/ CN/AK/FEP/PRL/TPZ/CAZ	3 (6.12)



**Fig. 2.** The correlation between lipase and hemolysin production in *P. aeruginosa* isolates.

**Table 3.** Correlation between antimicrobial resistance and the commonly detected serotypes

Serotype	Antimicrobials, No. of resistant isolates (%)								
	CIP	LEV	AK	CN	IPM	MEM	PRL	TPZ	MDR
O2	1(14.3)	1(14.3)	1(14.3)	2(28.6)	1(14.3)	1(14.3)	7 (100)	7 (100)	1(14.3)
Correlation Coefficient	-0.175	-0.175	0.055	0.039	-0.062	-0.023	0.084	0.122	-0.062
Significance	0.229	0.229	0.707	0.792	0.672	0.878	0.565	0.405	0.672
O6	6 (75)	6 (75)	2 (25)	3(37.5)	3(37.5)	1(12.5)	8 (100)	8 (100)	3(37.5)
Correlation Coefficient	0.374	0.374	0.216	0.134	0.187	-0.046	0.091	0.132	0.187
Significance	0.008**	0.008**	0.136	0.360	0.197	0.755	0.533	0.367	0.197
O10	4(100)	4(100)	1 (25)	2 (50)	1(25)	1(25)	4 (100)	3 (75)	2(50)
Correlation Coefficient	0.409	0.409	0.146	0.177	0.034	0.070	0.062	-0.183	0.219
Significance	0.004**	0.004**	0.318	0.224	0.817	0.633	0.675	0.207	0.131
O11	1 (7.7)	1 (7.7)	0	1 (7.7)	2(15.4)	2(15.4)	11(84.6)	10(76.9)	1(7.7)
Correlation Coefficient	-0.341	-0.341	-0.203	-0.235	-0.075	-0.015	-0.343	-0.327	-0.190
Significance	0.017*	0.017*	0.163	0.104	0.609	0.917	0.016*	0.022*	0.192

\*\* Significant at 0.01 level; \* Significant at 0.05 level.

addition, 37.5% and 50% of serotypes O6 and O10, respectively showed multidrug resistance, Table 3.

#### Phenotypic detection of protease, lipase, hemolysin, and biofilm

Most *P. aeruginosa* isolates (95.9%) were proteolytic on skimmed milk-BHI agar (Isolates 1 and 4 were negative), while 63.3% of isolates were positive lipase producers. For hemolysin production, 28 isolates (57.14%) were non-hemolytic while 16 isolates (32.7%) caused lysis of less than 10% of RBCs in the suspension. The highest % of lysed RBCs were observed with isolates No. 20 and 33 (75.49 % and 79.83 %, respectively).

Biofilm production was assessed using a microtiter plate assay. *P. aeruginosa* isolates were classified into 3 categories: strongly adherent (10 isolates, 20.4%), moderately adherent (30 isolates, 61.2%), and weakly adherent (9 isolates, 18.4%).

#### Correlation between the studied virulence factors, antimicrobial susceptibility, and serotypes of *P. aeruginosa* isolates

The association between lipase and hemolysin production was studied. Fig. 2 shows that hemolysin production had a significant negative relationship with lipase production,  $P=0.01$ .

The correlations between strong and weak biofilm producers and antimicrobial resistance, lipase, and hemolysin production were also investigated, Table 4. There were significant correlations between each of the following pairs: strong biofilm production and gentamicin resistance ( $P=0.036$ ), strong biofilm production and MDR ( $P=0.009$ ), weak biofilm production, and quinolone resistance ( $P=0.026$ ). In addition, all weak biofilm producers were lipase producers ( $P=0.011$ ) while one isolate only caused lysis of RBCs ( $P=0.034$ ).

Moreover, the correlation between the tested virulence factors and the most frequently encountered serotypes was analyzed. Hemolysin production was more prevalent in serotype O2 isolates (71.4%) while lipase production was detected more in serotype O10 isolates. In addition, a high percentage of isolates of serotypes O2, O6, and O11 isolates (71.4%, 75%, and 92.3%, respectively) were strong to moderate biofilm producers. Statistically, none of these observations proved significant.

#### PCR-based detection of genes encoding TTSS, exotoxin A, and elastase B

For TTSS genes, *exoY* was the most

prevalent gene (39 isolates, 79.6%) followed by *exoS* (33 isolates, 67.3%) and *exoT* (31 isolates, 63.3%) while the *exoU* gene was the least frequent (5 isolates, 10.2%). The *toxA* and *lasB* genes were amplified in 31 isolates (63.27%) and 37 isolates (75.5%), respectively. Three isolates (6.12%) did not amplify any of the tested genes. Table 5 illustrates the coexistence profiles of the tested genes. Twenty-one profiles were recorded and 11 of them were presented by 2 or more isolates

with the highest predominance for profile P20 (8 isolates, 16.33%).

Correlation studies detected a positive significant correlation between the *lasB* and *exoY* genes ( $P=0.003$ ) and the *exoS* and *exoT* genes ( $P=0.05$ ). In addition, the *lasB* gene was significantly detected in isolates sensitive to quinolones ( $P=0.049$ ). *exoU* gene was significantly associated with MDR isolates ( $P=0.000$ ) in contrast to the *exoT* gene that had a negative association with MDR isolates ( $P=0.014$ ).

**Table 4.** Correlation between biofilm production and antimicrobial resistance, hemolysin, and lipase production

Biofilm category	Antimicrobial agents Resistant isolates (%)									Lipase	Hemolysin
	CIP	LEV	AK	CN	IPM	MEM	PRL	TPZ	MDR		
Strong	5(50)	5(50)	2(20)	5(50)	3(30)	2(20)	10(100)	10(100)	5(50)	4(40)	6(60)
Correlation Coefficient	0.163	0.163	0.164	0.300	0.121	0.050	0.104	0.151	0.372	-0.244	0.175
Significance	0.264	0.264	0.261	0.036*	0.409	0.731	0.475	0.300	0.009**	0.091	0.228
Weak	7(77.8)	7(77.8)	1(11.1)	3(33.3)	0	0	9(100)	8(88.9)	3(33.3)	9(100)	1(11.1)
Correlation Coefficient	0.319	0.319	0.014	0.098	-0.240	-0.210	0.098	-0.051	0.152	0.361	-0.304
Significance	0.026*	0.026*	0.923	0.505	0.096	0.148	0.504	0.727	0.297	0.011*	0.034*

\*\* Significant at 0.01 level; \* Significant at 0.05 level.

**Table 5.** Virulence genotypes of *P. aeruginosa* isolates

Code	Gene profile	No. of isolates
P1	No genes	3
P2	<i>toxA</i>	1
P3	<i>toxA, exoS</i>	1
P4	<i>lasB, exoY</i>	1
P5	<i>toxA, lasB, exoT</i>	1
P6	<i>toxA, lasB, exoY</i>	2
P7	<i>lasB, exoU, exoY</i>	1
P8	<i>lasB, exoT, exoY</i>	2
P9	<i>exoS, exoT, exoY</i>	3
P10	<i>lasB, exoS, exoY</i>	1
P11	<i>toxA, exoS, exoT</i>	1
P12	<i>toxA, exoU, exoY</i>	1
P13	<i>lasB, exoS, exoT</i>	1
P14	<i>toxA, lasB, exoS, exoY</i>	6
P15	<i>toxA, lasB, exoS, exoT</i>	2
P16	<i>lasB, exoS, exoT, exoY</i>	5
P17	<i>toxA, exoS, exoT, exoY</i>	2
P18	<i>lasB, exoS, exoU, exoY</i>	1
P19	<i>toxA, lasB, exoT, exoY</i>	4
P20	<i>toxA, lasB, exoS, exoT, exoY</i>	8
P21	<i>toxA, lasB, exoS, exoT, exoU, exoY</i>	2

Cluster analysis of the constructed dendrogram divided thirty-eight isolates of *P. aeruginosa* into 10 clusters with a similarity of 70%. Eleven isolates with a lower similarity percentage (< 70%) were not included in these clusters. The largest cluster had 12 isolates that were moderate biofilm producers of A3 and A8 biotypes. The second large cluster had 5 isolates that were also moderate biofilm producers of the A3 biotype. Only two isolates (Nos. 5 and 36) had 100% similarity with respect to the analyzed data, Fig. 3.

## DISCUSSION

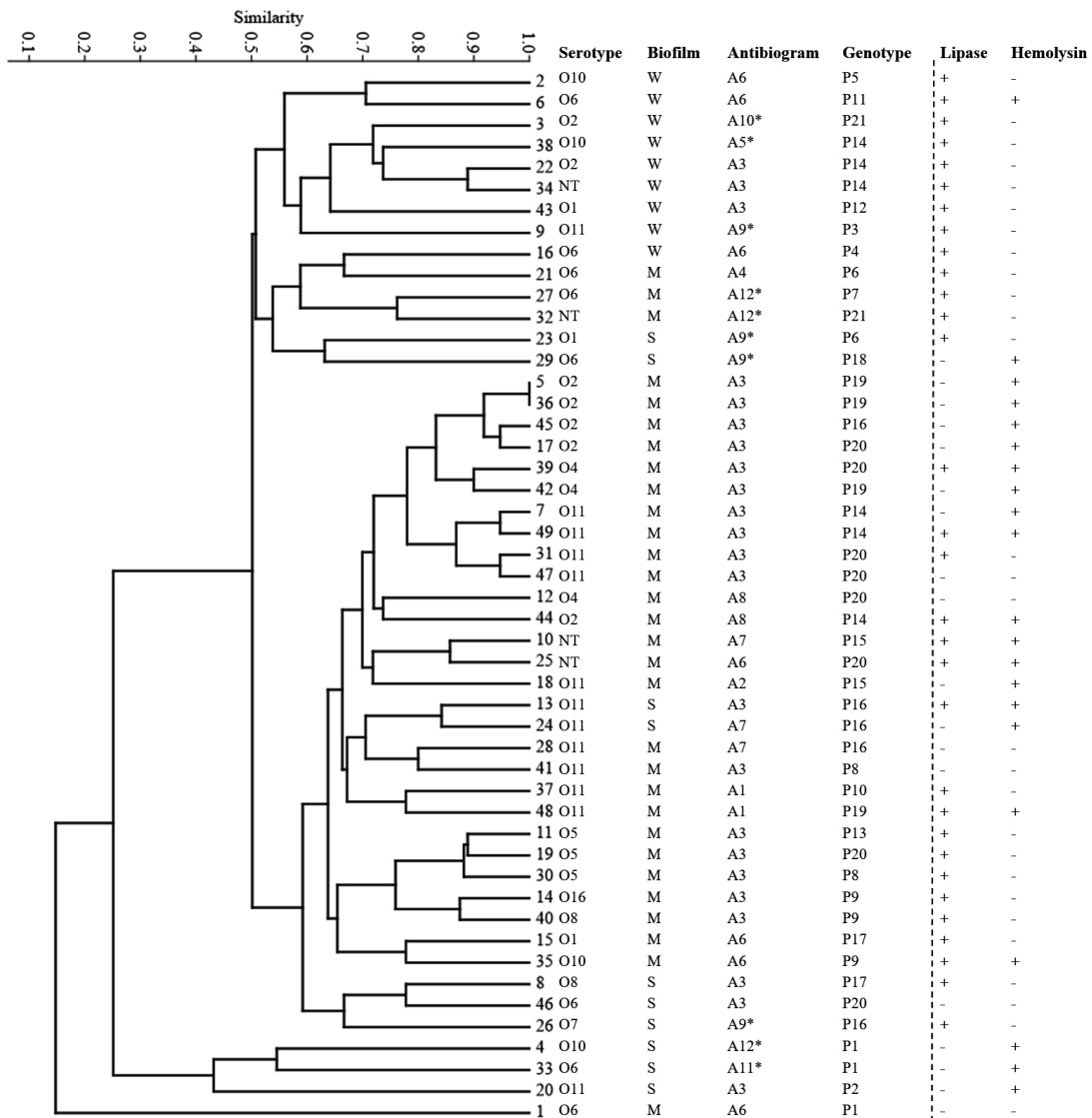
*P. aeruginosa* is well-known as a major cause of UTIs especially those associated with catheterization. In addition, this bacterium contributes significantly to the morbidity and death rates associated with nosocomial infections. In the current study, 49 uropathogenic isolates were identified as *P. aeruginosa*. Although the study population was different from those involved



in previous studies, the high prevalence of O11 and O6 serotypes was also recorded.<sup>15,17,27-29</sup> Lu et al.<sup>17</sup> reported the failure of the standard technique to serotype about 8% of their isolates which is similar to this study where four isolates (8%) were non-typeable. Other studies recorded non-typeable isolates in higher proportions.<sup>30-32</sup>

The antibiogram of the infecting isolates is very helpful for physicians to select the appropriate antimicrobial to shorten the treatment

period and improve the outcome. In UTIs, improper antimicrobial will delay the treatment process and increase the risk for urosepsis.<sup>33</sup> In this study, the antimicrobial susceptibility testing demonstrated high levels of resistance (91.8%-100%) for ceftazidime, cefepime, piperacillin, and piperacillin/tazobactam. Previous studies conducted in Egypt also revealed a significant rate of resistance to these antimicrobials.<sup>34-40</sup> However, other investigations in Egypt reported a



**Fig. 3.** Dendrogram of 49 *P. aeruginosa* isolates based on antibiogram, serotyping, virulence genotype, virulence phenotype (lipase, hemolysin, and biofilm production). \*MDR isolates, NT: non-typeable, W, M, S: Weak, moderate, and strong biofilm producers.



lower rate of resistance (29%-43%).<sup>41,42</sup> The same discrepancies in resistance rate were observed in different countries, where some studies recorded a high resistance rate,<sup>43-45</sup> while others recorded a relatively low resistance rate with respect to the used antimicrobials.<sup>46-48</sup> Isolates of the current study showed low to moderate resistance (20.4%-34.7%) to gentamicin, ciprofloxacin, levofloxacin, imipenem, and meropenem. Although some reports coincide with current data,<sup>34,37,49</sup> several research groups reported high resistance levels to quinolones, gentamicin, and/or carbapenems.<sup>36,40,50</sup> In agreement with previously published data,<sup>34,41,51-53</sup> amikacin showed the highest efficacy against tested isolates.

Multidrug resistance of *P. aeruginosa* is alarming everywhere, but the problem is escalating in Egypt and other developing countries because there are no regulations that limit the prescription of antibiotics even the broad-spectrum ones. Antibiotics are also sold without a doctor's prescription as over-the-counter medications. In the current study, 20.4% of the isolates were MDR compared to 48.8% -100% of the MDR demonstrated by many research groups in Egypt.<sup>34,35,37-39,42</sup> In addition, 37.5% and 50% of isolates belonging to serotypes O6 and O10 isolates were MDR and a significant association was found between these two serotypes and quinolone resistance. These results agree partially with Abdel-Rhman and Rizk who found a significant association between MDR and O6 and O10 serotypes.<sup>39</sup> In contrast to many earlier studies,<sup>54,55</sup> *P. aeruginosa* isolates of serotype O11 were the most sensitive to all tested antimicrobials, and a negative significant correlation was detected between these isolates and quinolone resistance.

The pathogenesis of *P. aeruginosa* in different illnesses has been linked to its ability to express multiple virulence factors. Previous studies had shown that among the multiple virulence factors produced by *P. aeruginosa*, individual virulence factors may control and contribute to the outcome of various infections. Like other infections, the establishment of UTIs requires colonization of host tissues, which is facilitated by many virulence factors of the bacterium. In this study, most isolates were proteolytic on skimmed milk-BHI agar while 63.3% of isolates were positive lipase producers. Proteases, according to Gupta

et al., play an important role in the pathogenesis of *P. aeruginosa* in UTIs because they enhance bacterial colonization of renal tissues by degrading a variety of substrates that aid tissue invasion and dissemination.<sup>56</sup> Lipases also destroy host tissues.<sup>57</sup> The case was different for hemolysin production, where 21 isolates (42.86%) were hemolytic and 16 of them caused lysis of less than 10% of the RBCs and there was a significant negative association between lipase and hemolysin production. Gupta et al. observed that mutant strain producing hemolysin but not producing proteases and elastase was incapable of colonizing the renal tissues of the used mice model. This may suggest that hemolysin has a minimal role in UTIs compared to proteases and elastases.<sup>56</sup>

The ability of *P. aeruginosa* to cause infections has been linked to its tendency to form biofilms. Its capacity to adhere to catheter surfaces makes catheterized patients more likely to get UTIs.<sup>58,59</sup> In addition, urea, the major solute in urine may encourage the biofilm development of *P. aeruginosa* through alterations in the cell membrane or production of additional extracellular matrix.<sup>60</sup> In this study, all isolates had biofilm-forming capacity, where 20.4%, 61.2%, and 18.4% of them were strong, moderate, and weak biofilm producers, respectively. The same finding was recorded in two previous studies conducted in Egyptian hospitals where 100% of the isolates were biofilm producers.<sup>38,61</sup> Also, a lower percentage of biofilm production by *P. aeruginosa* was reported by other studies.<sup>37,62-65</sup>

In contrast to studies that found no relationship between biofilm and MDR,<sup>38,62,63</sup> this research concurred with other reports that found a significant link between biofilm production and MDR.<sup>37,65,66</sup> In addition, weak biofilm production was associated with quinolone resistance which agrees with previous investigations.<sup>63,67,68</sup> Most weak biofilm producers were nonhemolytic but all of them had lipolytic activity. This also may reflect the importance of lipase production in the pathogenesis of *P. aeruginosa*. Besides, the current work found no significant correlation between the serotypes predominant in the isolates and any of the investigated virulence factors, contradicting the findings of Mittal et al.<sup>69</sup> and Visca et al.<sup>70</sup>

Some virulence factors are recognized as major in acute infections caused by *P. aeruginosa*,

among which is TTSS. The distribution of TTSS genotypes among *P. aeruginosa* clinical isolates can help in understanding the epidemiology of such infections. The distribution frequency of TTSS genes varied between studies. In this study, the *exoS* gene was prevalent in 67.3% of isolates, which was consistent with previous reports.<sup>38,71-73</sup> Also, the *exoU* gene was detected in only 10.2% of isolates; however, higher prevalence rates were reported in Egypt and other countries.<sup>38,72,74,75</sup> While the *exoT* gene was amplified in 63.3% of isolates and it was significantly associated with *exoS* gene, a recent study in Egypt recorded *exoT* gene in only 6.7% of isolates.<sup>38</sup> Other research studies showed high prevalence rates of the *exoT* gene.<sup>10,74</sup> The *exoY* gene was the most predominant gene (79.6%), which is consistent with earlier studies.<sup>38,74</sup> Statistical analysis revealed a significant association between MDR and *exoU* gene, which coincides with previous reports.<sup>72,73</sup> Few isolates (6%) harbored both *exoS* and *exoU* genes which agrees with prior studies.<sup>72,73,76,77</sup> These isolates will possess the invasive and cytotoxic properties of both toxins. Although the *exoU* gene was found in only a few isolates, earlier studies correlated its existence with high mortality rates.<sup>77-79</sup> In the current investigation, the elastase encoding gene *lasB* was detected in 75.5% of isolates and it was statistically associated with *exoY* gene. Similar results were reported in Egypt<sup>38,80</sup>; however, higher prevalence was observed in studies conducted by Benie et al. (89.2%),<sup>81</sup> and Babour et al. (100%).<sup>82</sup> About 63.3% of the *P. aeruginosa* isolates amplified the *toxA* gene which is regarded as an important virulence factor in catheter-associated UTIs.<sup>83</sup> A higher frequency of this gene was reported in Iran (95.7%)<sup>9</sup> but recently in Egypt, a lower frequency (45.6%) was recorded.<sup>38</sup>

Based on biotypes, serotypes, virulence factor production, and PCR results, the dendrogram grouped 38 isolates into 10 clusters (70% similarity), while 11 isolates had similarities lower than 70%. The 17 isolates that represented the two largest clusters were moderate biofilm producers and of definite biotypes but were of different serotypes. This confirms that serotyping is still a more useful tool to discriminate between *P. aeruginosa* isolates.<sup>84</sup> Two isolates showed

100% similarity, which may indicate cross-infection among hospitalized patients.<sup>39</sup>

## CONCLUSION

In this study, isolates of serotype O11 were the most sensitive to all antimicrobials tested compared to isolates of other commonly encountered serotypes. Amikacin was the most effective antimicrobial against test isolates. Although MDR was detected in 20.4% of isolates, it was significantly associated with strong biofilm production, making treatment more difficult. Most of the isolates had proteolytic and lipolytic but nonhemolytic activity, which may reflect the importance of lipase and protease enzymes in the pathogenesis of *P. aeruginosa* in UTIs. The current work found no significant correlation between the serotypes predominant in the test isolates and any of the investigated virulence factors. In addition, the high prevalence of *exoS*, *exoT*, *exoY*, *lasB*, and *toxA* genes in uropathogenic *P. aeruginosa* isolates suggests identifying these genes as a maker of virulent strains. Although *exoU* gene was detected in only 10.2% of isolates, it is commonly associated with a high mortality rate. Furthermore, the coexistence of *exoU* and *exoS* genes even in a small % of isolates constitutes a great challenge in treatment as those isolates will have both the invasive and cytotoxic properties of the two effector proteins, especially that *exoU* gene was found significantly in MRD isolates. Isolates of different serotypes were distributed in all clusters in the dendrogram, emphasizing that serotyping is still a more useful tool to discriminate between *P. aeruginosa* isolates.

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None.

## DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Faculty of Pharmacy, Mansoura University, Egypt with reference number:2021-236.

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