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Molecular detection of different virulence factors genes harbor *pslA*, *pelA*, *exoS*, *toxA* and *algD* among biofilm-forming clinical isolates of *Pseudomonas aeruginosa*

Rehab E. Farhan, Samar M Solyman, Amro M Hanora*, Marwa M Azab

Department of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University Pharmacy, Ismailia, 41522, Egypt

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ABSTRACT

Pseudomonas aeruginosa (P. aeruginosa) is considered as the foremost cause of hospital-acquired infections due to its innate and plasmid-mediated resistance to multiple antibiotics making it a multi-drug resistant (MDR) pathogen. This study aimed to determine the biofilm formation ability and the presence of different virulence factors genes (*pslA, pelA, exoS, toxA* and *algD*) among biofilm-forming strains of *P. aeruginosa* clinical isolates from burn units in Ismailia Hospitals, Egypt. In our cross-sectional study, one hundred and twenty-six (126) non-duplicate clinical *P. aeruginosa* isolates were recovered from 450 clinical specimens from burn units in Ismailia Hospitals. The antibiotic sensitivity of strong and moderate biofilm producer isolates was investigated using the disc diffusion method. The isolated bacteria were tested for their ability to form biofilm using a microtiter plate assay. The expression of (*pslA, pelA, exoS, toxA* and *algD*) genes in biofilm producers isolates was detected using PCR. The MPA detected 80% (95 /126) isolates as biofilm producers, 18% (22/126) were strong biofilm producers, 34% (43/126) were moderate biofilm producers, 28% (35/126) were weak biofilm producers and 20% (31/126) non-biofilm producers. Susceptibility pattern analysis of biofilm-forming *P. aeruginosa* isolates (95) detected that 60% (68/ 95) were multi-drug resistant isolates (MDR). Resistance to all used antibiotics and multidrug resistance was higher among biofilm-producing than non-biofilm-producing strains, but the difference was statistically non-significant. Investigation of virulence factors associated genes revealed that 96%, 94%, 86.4%, 80.0% and 74% of the biofilm producers isolates were harboring *algD, pslA, pel A, toxA* and *exoS* gene, respectively. The present study confirmed that antimicrobial resistance and virulence genes were more prominent in biofilm-producing *P. aeruginosa* than in non-biofilm-producers.

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Introduction

Pseudomonas aeruginosa is an opportunistic, Gram-negative, non-fermenting bacterium that is a common cause of human infections (1). This pathogen causes a wide range of infections, including urinary tract infections, respiratory infections, dermatitis, soft tissue infection, bacteremia, and a variety of systemic infections, especially in hospitalized patients and immunocompromised individuals. Patients with severe burns are particularly susceptible to *P. aeruginosa* infection during hospitalization, often resulting in significant morbidity and mortality (1). The high mortality rate of *P. aeruginosa* infection is due to the ability of the bacterium to easily adapt to environmental conditions, to rapidly develop resistance to antimicrobials and to produce a variety of virulence factors (2, 3). In addition to the low permeability of the *P. aeruginosa* cell wall to anti-pseudomonal agents, this bacterium has a high genetic capacity to quickly acquire drug resistance (4, 5). Multidrug-resistant (MDR) *P. aeruginosa* isolates can cause life-threatening and, in some cases, untreatable infections and are considered to be a major problem in infection control in recent years (6, 7). *P. aeruginosa* also has a large number of cell-associated and extracellular virulence factors. Exotoxin A, a major virulence factor of *P. aeruginosa* encoded by the *toxA* gene, inhibits protein synthesis. Exoenzyme S, encoded by the *exoS* gene, is a major virulence factor involved in burn infections. This cytotoxic effect changes the function of the cytoskeleton of the host cell, resulting in bacterial colonization, invasion and dissemination during infection (8). In the biofilm matrix, diverse biomolecules, including polysaccharides and proteins, protect bacteria from the host's immune response and from antimicrobials. Alginate, encoded by the *algD* gene, is a common type of polysaccharide and is found in the biofilm structure. In addition, the *pslA* gene encodes a neutral-charge exopolysaccharide providing structural support during the primary stage of biofilm formation and facilitating cell-to-cell and cell-to-substrate attachment (7). Because of this, infections related to biofilm-forming strains are difficult to treat and can create serious problems in burn hospitals (9).ost of the previous studies focused on the presence or absence of genes of biofilm in biofilm-producing bacteria. This study aimed to determine the biofilm formation ability and the presence of different virulence factors genes (*pslA, pelA, exoS, toxA* and *algD*) among biofilm-forming strains of *P. aeruginosa* clinical isolates from burn units in Ismailia Hospitals, Egypt.

* Corresponding author. Email: a.hanora@pharm.suez.edu.eg

Materials and Methods

Experimental materials

All antibiotic disks used in this study (Piperacillin, (PRL), Ceftazidime, (CAZ), Cefoxitin (FOX), Ceftriaxone (CRO), piperacillin/tazobactam (TZP), Imipenem (IPM), Meropenem (MEM) Aztreonam, (ATM), Cefepime (FEP), Cephradine (CE), Amox/Clav (AMC), Amikacin (AK), Sulpha/Trimethoprim (STX), Ciprofloxacin, (CIP), Cefuroxime, (CXO), Ampicillin/sulbactam (SAM) and Ertapenem (ETP) were purchased from (Oxoid Ltd., Basingstoke, and Hampshire, England). The 96-well flat-bottomed polystyrene plate and Mueller-Hinton broth were purchased from (Sigma-Aldrich, Poland), glacial acetic acid was purchased from (Zorka Pharma, Šabac, Serbia) and Crystal violet used for Gram staining was purchased from (Merck, Germany).

Specimens collection

In our cross-sectional study, 126 non-duplicate clinical *P. aeruginosa* isolates recovered from 450 clinical specimens, were collected over 14 months (November 2015 until April 2017). Samples were taken from clinically diagnosed infected burns, wounds sepsis and septicemia at inpatients and outpatients from the burns unit and different departments in Suez Canal University Hospital and General Ismailia Hospital (Table 1 and Figure 1).

Specimens samples culture

All samples were cultured on Cetrimide agar media then the isolated organisms were identified by standard microbiological techniques as colony morphology (pale yellow colonies on MacConkey agar and blue-green colonies on Nutrient agar and Cetrimide agar), Gram staining (Gram-negative bacilli) and biochemical reactions (oxidase positivity, catalase positivity and oxidative-fermentative (OF) tests according to (10).

Biofilm formation assay

Biofilm formation was quantified using a microtiter plate test method described by (11). Briefly, standard overnight cultures (1.5×108CFU/mL) were diluted 100-fold in brain–heart infusion broth. Bacterial suspension made of strong and moderate biofilm producer isolates. From each culture dilution, 200 μL [180 μL of Mueller-Hinton broth (MHB) and 20 μL of bacteria (5×105 CFU/mL)] were transferred into individual wells of a 96-well flat-bottomed polystyrene plate and incubated at 37 ◦C for 48 h. Negative control wells contained broth only. The plates were incubated aerobically for 24 h at 35◦C. Thereafter, the content of each well was aspirated and the wells were washed three times with 300 µl of sterile physiological saline. Biofilm was fixed with 200 µl of methanol per well, and after 20 min the plates were emptied and left to air dry. The plates were stained with 150 µl per well of Crystal violet used for Gram staining) for 5 min. After the plates were air dried, the dye bound to the adherent cells was resolubilized with 150 µl of 33% glacial acetic acid per well. The optical density of each well was measured at 570 nm by using an automated Multiscan EX reader (Lab Systems, Helsinki, Finland). Based on the optical densities of bacterial biofilms, all strains were classified into the following categories: no biofilm producers (0), weak (+), moderate (++), or strong (+++) biofilm producers, as previously described (11). (Table 2).

Antimicrobial susceptibility testing

Susceptibility pattern analysis of strong and moderate biofilm forming of 65 *P. aeruginosa* isolates was carried out according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014) against 17 different antimicrobial agents including PRL (100 µg), CAZ (30 µg),

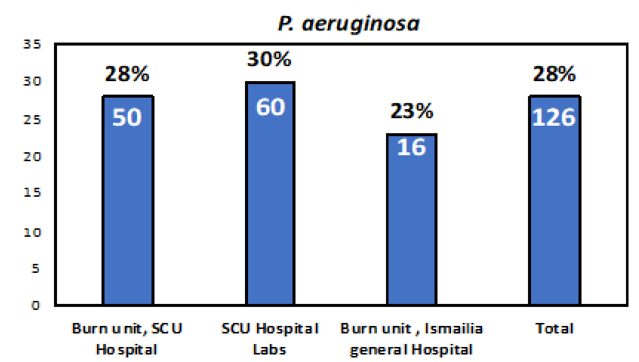

Figure 1. Bar chart presenting the prevalence of *P. aeruginosa* from different study sites.

Table 1. The different sources, numbers, and percentages of *P. aeruginosa* isolates.

Source	Number of clinical samples	*P.aeruginosa* isolates (n%)		Chi-square Sign
		No	Yes	
Burn unit at Suez Canal University Hospital	180	130 (62%)	50 (28%)	<0.001***
Suez Canal University Hospital Labs	200	140 (70%)	60 (30%)	<0.001***
Burn unit at Ismailia General Hospital	70	54 (67%)	16 (23%)	<0.001***
Total	450	126 (28%)	126 (28%)	<0.001***
Chi-square test	<0.001***			

*, **, *** significant at p<0.05, <0.01, <0.001, ns, non-significant at p>0.05

Table 2. Biofilm production assay using microtiter plate method.

Biofilm activity	Strong	Moderate	Weak	Non-Biofilm Producer	Total
Number	22	43	35	26	126
Percentage	17.5%	34.1%	27.8%	20.6%	100%
hi-square	Chi= 8.41; sign. = 0.038*				

*, **, *** significant at p<0.05, <0.01, <0.001, ns, non-significant at p>0.05

FOX (30 µg), CRO (30 µg), TZP (100/10µg), IPM (10 µg), MEM (10 µg), ATM (30 µg), FEP (30 µg), CE (30µg), AMC (30 µg), AK (30µg), STX (25ug), CIP (5µg), CXO (30ug), SAM (10/10ug) and ETP (10ug). *P. aeruginosa* ATCC 27853 reference strain was used as a control. The turbidity of the suspension was matched to the turbidity of 0.5 McFarland standards. The isolates with resistance to at least 3 additional antibiotic classes were selected as MDR *P. aeruginosa*, as already explained (12).

Molecular detection for virulence genes of *P. aeruginosa*

Genomic DNA was extracted from the overnight TSB cultures of *P. aeruginosa* isolates using the boiling method as previously described (13, 14). Conventional PCR analysis was carried out using primer pairs used to identify five virulence genes are shown in Table 3. Extracted nucleic acid was used as template DNA for PCR. Each gene was amplified separately. The reaction mixture consisted of 5 µl 1× PCR buffer, 2 µl of each primer, 1 µl MgCl2, 0.8 µl each of the dNTPs, 0.6 µl Taq DNA polymerase, and a 2 µl DNA each of the isolates. PCR amplification was performed in 50 µl reaction volume using Taq DNA polymerase. The thermal cycler programmer consisted of an initial denaturation at 95 ◦C for 5 min; 30 cycles of denaturation at 95 ◦C for 60 sec, annealing at 55 ◦C for 45 sec and extension at 72 ◦C for 75 sec; followed by a final extension step at 72 ◦C for 10 min. PCR products were detected by electrophoresis on a 1% agarose gel. Finally, the sizes of the PCR products were determined by comparing them with the migration of the 3000-bp DNA ladder (Fermentas, Germany). Finally, the amplicon was visualized and photographed using a Gel Documentation System (Syngene, England).

The 16s sample no. 8, 3, 6, 23, 24, 21, 20, 19, 14, 18, 15, 17, 12, 9, 10, 2, 7, 5, 4 and 13 were provided with accession no. Accession numbers; MG584716, MG571640, MG571638, MG571616, MG571615, MG571613, MG571612, MG571611, MG571608, MG571610, MG571609, MG571606, MG571605, MG571598, MG571579, MG571577, MG571569, MG571568, MG571567, MG571566, and MG571607; respectively. Moreover, the virulence genes tested were OM567543 (pslA1), OM567544 (pslA2), OM567545 (pslA3), OM567546 (exoA1), OM567547 (exoA2), and OM567548 (exoA3)

Statistical Analysis

Each experiment was carried out at least in triplicate, and all data were presented as Mean ± SD. Data were checked for normality using Kolmogorov-Smirnov to check whether variables are parametric or nonparametric. Differences between two independent groups of nonparametric data were performed using Mann-Whitney U. Analysis of statistical significance was performed by one-way ANOVA and the post-hoc Tukey Test ($p < 0.05$). All analysis was conducted in SAS 9.4 for Windows x64 from SAS Institute (Cary, NC) and graphical outputs were generated by GraphPad Prism software (Version 8, GraphPad Software Inc.) and SPSS version 28.0 for Mac OS.

Results

Biofilm formation

Resistance to antimicrobials, biofilm production and the frequency of various virulence-associated genes in clinical isolates of *P. aeruginosa* were investigated. A number of 126 isolates of *P. aeruginosa* were tested for their ability to form biofilm using a microtiter plate test method. Our study results found that the phenotypic detection of biofilm formation revealed that 80% (100/126) of clinical isolates were positive biofilm producers; 18% (22/126) were strong biofilm producers, 34% (43/126) were moderate biofilm producers, 28% (35/126) were weak biofilm producers and 20% (31/126) non-biofilm producers the results are shown in Table 4. Results of virulence gene PCRs performed upon biofilm-forming isolates versus non-biofilm-forming isolates showed a highly significant difference in algD and *pslA*, genes, and non-significantly in *pela*, *toxA*, *exoS* as revealed by Mann- Whitney U for independent samples (Table 4 and Figures 2 and 3).

Table 3. Primer selection sequences for conventional PCR.

Gene	Primer	Primer sequence	Amplicon size	(bp)
algD	algD-F algD-R	**5′-ATGCGAATCAGCATCTTTGGT-3′** **5′-CTACCAGCAGATGCCCTCGGC-3′**	1310	(12)
pslA	pslA-F pslA-R	**5′-CACTGGACGTCTACTCC GACGATAT-3’** **5′-GTTTCTTGATCTTGTGCAGGGTGTC-3’**	1119	(11)
toxA	toxA-F toxA-R	**5′-GGTAACCAGCTCAGCCACAT-3’** **5′-TGATGTCCAGGTCATGCTTC-3’**	325	(12)
exoS	exoS-F exoS-R	**5′-CTTGAAGGGACTCGACAAGG-3′** **5′-TTCAGGTCCGCGTAGTGAAT-3′**	504	(12)

Table 4. Results of virulence gene PCRs performed upon biofilm-forming isolates of *P. aeruginosa*.

Gene	+VE biofilm-forming isolates		+VE Non-biofilm-forming isolates		Mann-Whitney U
	N (95)	%	N (31)	%	
algD	91	95.8%	24	77.4%	0.002**
pslA	89	93.7%	23	74.2%	0.003**
pelA	82	86.3%	27	87.1%	0.912ns
toxA	76	80.0%	25	80.6%	0.938ns
exoS	70	73.7%	23	74.2%	0.956ns

*, **, *** significant at p<0.05, <0.01, <0.001, ns, non-significant at p>0.05.

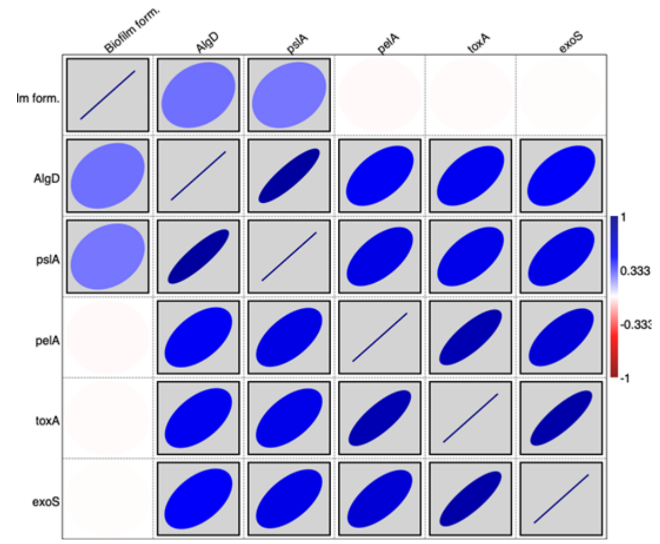

Figure 2. Heatmap presenting the interrelationship between virulence gene PCRs performed upon biofilm-forming isolates of *P. aeruginosa*.

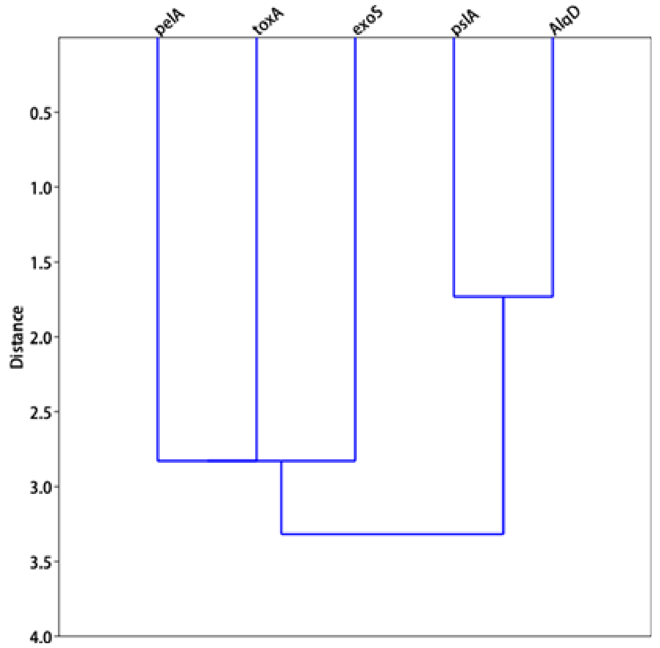

Figure 3. Clustering showing the virulence gene PCRs performed upon biofilm-forming isolates of *P. aeruginosa*, Cluster constructed using PAST version 4.04.

Antibiotic Susceptibility testing

In this technique, the concentration of antibiotics used is aimed at inhibiting the planktonic cell, which differs from cells in the biofilm state. The bacterial biofilm is 10-1,000 times more resistant to antimicrobial agents than the planktonic cell. Therefore, the conventional antibiotic susceptibility test cannot predict the bacteria involved in biofilm production. This can be one explanation as to why there is a higher failure rate in the eradication of biofilm-related infections. Antibiotic Susceptibility testing for the biofilm-forming *P. aeruginosa* isolates (100) under the standard CLSI guidelines for different antimicrobial agents showed that 68% (68/ 100) were multi-drug resistant isolates (MDR) Pattern. The results of the susceptibility testing were categorized as sensitive, intermediate and resistant as shown in Table 5 and Figures 4 and 5.

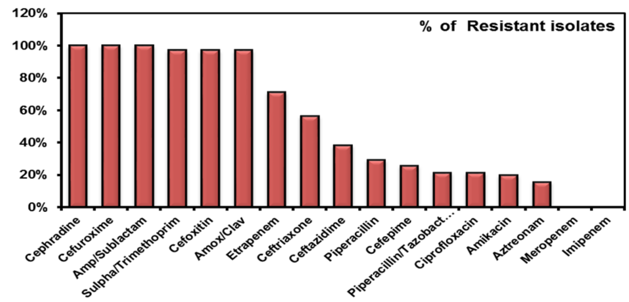

Figure 4. Histogram showing resistance of *P. aeruginosa* to tested antimicrobials.

Multidrug resistance (MDR) (resistant to three or more antimicrobial classes) was higher among biofilm-producing than non-biofilm-producing strains but the difference between the two groups was not statistically significant. Furthermore, the results showed that all isolates were susceptible to Meropenem and Imipenem, sensitivity was absolute (100%) and the highest resistance rate was observed against (Cefuroxime), (Cefoxitin, Amox/Clav, Sulpha/Trimethoprim) and Ceftriaxone showed resistance rates of

Table 5. Percentage of resistance of biofilm-forming *P. aeruginosa* to 68 tested antibiotics samples.

Antimicrobial Agent(s)	Conc. (μg)	Resistant		Intermediate		Sensitive		Chi-square sign.
		NO	%	NO	%	NO	%	
Cephradine	30	68	100.0	0	0.0	0	0.0	>0.999ns
Ampicillin/Sulbactam	10/10	68	100.0	0	0.0	0	0.0	>0.999ns
Cefuroxime	30	68	100.0	0	0.0	0	0.0	>0.999ns
Sulpha/Trimethoprim	19:1	64	94.1	1	1.5	1	1.5	<.001
Amoxycillin/clavulanic acid	30	64	94.1	2	2.9	0	0.0	<.001
Cefoxitin	30	64	94.1	2	2.9	0	0.0	<.001
Ertapenem	10	47	69.1	12	17.6	7	10.3	<.001
Ceftriaxone	30	34	50.0	29	42.6	0	0.0	0.225
Ceftazidime	30	25	36.8	10	14.7	31	45.6	0.004
Piperacillin	100	19	27.9	0	0.0	47	69.1	<.001
Cefepime	30	17	25.0	3	4.4	46	67.6	<.001
Piperacillin/Tazobactam	100/10	14	20.6	6	8.8	46	67.6	<.001
Ciprofloxacin	5	14	20.6	1	1.5	51	75.0	<.001
Aztreonam	30	13	19.1	14	20.6	39	57.4	<.001
Amikacin	30	10	14.7	2	2.9	54	79.4	<.001
Meropenem	10	0	0.0	0	0.0	68	100.0	>0.999ns
Imipenem	10	0	0.0	0	0.0	68	100.0	>0.999ns

*, **, *** significant at p<0.05, <0.01, <0.001, ns, non-significant at p>0.05.

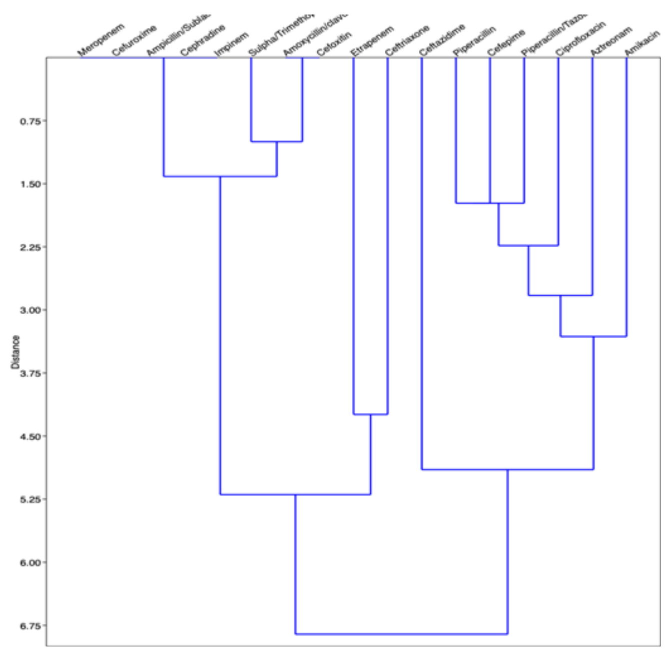

Figure 5. Clustering showing the resistance of *P. aeruginosa* to tested antimicrobials, Cluster constructed using PAST version 4.04.

100%, 97% and 71% and 56% respectively. Whereas, the lowest resistance rate was to amikacin at 15.2% and moderate resistance rate was observed against Ceftazidime, Piperacillin, Cefepime, levofloxacin showing resistance rates of 38%, 29%, 25.5% and 21%, respectively.

Molecular detection for virulence factors associated genes of *P. aeruginosa.*

The occurrence of virulence genes upon strong and moderate biofilm-forming isolates of *P. aeruginosa* was evaluated by using

conventional PCR, where it was detected that 96%, 94%, 86.4%, 80% and 74% of the biofilm producers isolates were harboring *algD, pslA, pelA, toxA* and *exoS* gene respectively, the results are shown in Figures 6 and 7.

The frequency of *algD* and *pslA* in biofilm-forming strains were 96% to 94% respectively, while the frequency of *algD* and *pslA* in biofilm-forming strains were 77% to 78% respectively, whereas 91/95 isolates (96%) of biofilm-producing strains have expressed the *algD* gene, while 24/31 isolates (78%) of non- biofilm-producing strains have expressed *pslA* gene. Furthermore, 89/95 isolates (94%) of biofilm-producing strains have expressed the *pslA* gene, while 23/31 isolates (77%) of non-biofilm-producing strains have expressed *pslA* gene (Figure 6).

The frequency of *pelA* in biofilm-forming strains was 86.4%, where 82/ 95 isolates (86.4%) of biofilm-producing strains have expressed the *pel A* gene, while 27/31 isolates (87%) of non - biofilm-producing strains have expressed *pel A* gene (Figure 7).

The frequency of *toxA* and *exoS* in biofilm-forming strains was 80% and 74% respectively. A number of 76/ 95 isolates (80%) of biofilm-producing strains have expressed the *toxA* gene, while 25/31 isolates (82%) of non -biofilm-producing strains have expressed *toxA* gene, meanwhile, 70/ 95 isolates (74%) of biofilm producing strains have expressed the *exoS* gene, while 23/31 isolates (77%) of non -biofilm-producing strains have expressed this gene (Figures 8-10).

Discussion

Antimicrobial resistance is one of the major problems

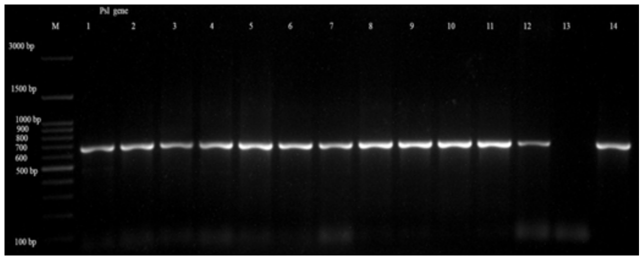

Figure 6. PCR amplification of *pslA gene* in *P. aeruginosa* isolates Lane M: 100 bp DNA size marker; Lane 1-13 PCR product of *pslA* gene (656bp); lane 13: negative sample.

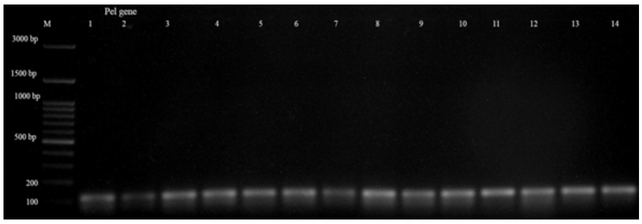

Figure 7. PCR amplification of *pelA* gene in *P. aeruginosa* isolates Lane M: 100 bp DNA size marker; Lane 1-13 PCR product of *pelA* gene (118bp).

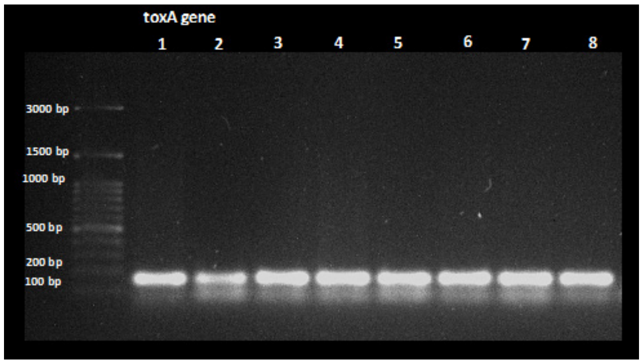

Figure 8. PCR amplification of *toxA* gene in *P. aeruginosa* isolates Lane M: 100 bp DNA size marker; Lane 1-8 PCR product of *toxA* gene (188bp).

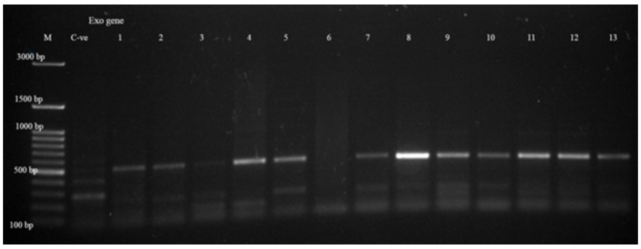

Figure 9. PCR amplification of *exoS* gene in *P. aeruginosa* isolates Lane M: 100 bp DNA size marker; Lane 1-13 PCR product of *exoS* gene (500 bp).

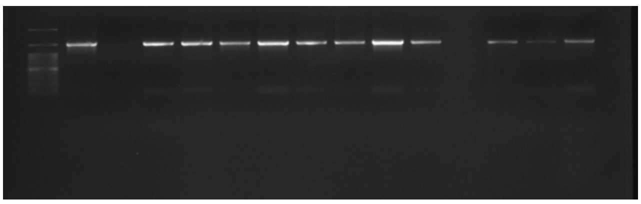

Figure 10. PCR amplification of 16S rRNA gene in *P. aeruginosa* isolates (1500 bp). Lane M:100 bp DNA size marker.

in the treatment of infectious diseases worldwide. *P. aeruginosa* is inherently resistant to multiple antimicrobials owing to the low permeability of the outer membrane, constant expression of several efflux pumps and the production of various antimicrobial-inactivating enzymes. It also has a high biofilm production capacity that makes antimicrobial penetration and access to the bacteria difficult. Several previous studies reported different rates of biofilm production by *P. aeruginosa* isolates. A previous study in Egypt on biofilm production reported that 27% (27/100) of clinical isolates were positive biofilm producers; 14% (14/100) produced strong biofilm, 7% (7/100) produced moderate biofilm and 6% (6/100) produced weak biofilm (15). Another study in Egypt also reported that biofilm formation was detected in 32/35 (91.4%) *P. aeruginosa* isolates; 25.7%, 40%, 25.7% and 8.6% of isolates were strong, moderate, weak and non-biofilm producers, respectively (13). Maita and Boonbumrung (16) reported that 60% (82/136) of *P. aeruginosa* isolates obtained from different clinical samples were strong biofilm producers, 11% (14/136) were moderate biofilm producers and 7% (9/136) were weak biofilm producers (16).

Our results are in accordance with Harika, Shenoy (17) reported that 78.2% (72/92) of clinical isolates were positive biofilm producers; 69.5% (64 /92) produced strong biofilm, 8.7% (8/92) produced moderate biofilm and 21.7% (20/92) produced weak biofilm (17). The variability in results between different studies may be attributed to many factors such as the difference in type and number of samples collected in each study and differences in isolates capacity to form a biofilm. A better understanding of the route of biofilm development and its control may constitute a platform for the design of strategies that are used to combat and eradicate the infection.

Similar previous results of antibiotic Susceptibility testing were obtained by Ijaz, Siddique (18), who reported that 58.6% were multi-drug resistant (MDR) for the biofilm-forming *P.aeruginosa* isolates (18). In addition, Maita and Boonbumrung (16) reported results that 51% of MDR were multi-drug resistant (MDR) strains of *P.aeruginosa* (16). Furthermore, our results are nearly similar to the previous studies who's reported that the resistance pattern against the carbapenem group i.e., meropenem and imipenem was only 6.67% which correlates with other studies in India, Nepal, Spain and Italy (19-22). All of those studies suggested meropenem and imipenem as the most effective anti-pseudomonal drugs. Asma and Noura (23) showed sensitivity to meropenem (91.6%), imipenem (90.2%) and piperacillin/tazobactam (81.3%). Raja and Singh (24) showed sensitivity to imipenem (90.1%) and piperacillin/tazobactam (90.6%) (24). However, several reports indicated increasing resistance towards this antibiotic group day by day (25, 26). In agreement with our study, El Kholy, Baseem (27) further reported the highest resistance rate against ampicillin and chloramphenicol (100%) and the lowest against ceftazidime (38%) (27).

In addition, a previous study in Bangladesh reported 89.5% resistance against Ampicillin and 89.3% resistance against Amoxiclav (28). Our results are nearly similar to Abdelraheem, Abdelkader (29) that reported a lower incidence of amikacin resistance of 13.2% (18/136) (29). Another study in Egypt reported nearly similar results of lower resistance to amikacin (12%) (30).

In addition to, Kannan, Nallasamy (31) from Pakistan showed that 30% of *P. aeruginosa* strains were MDR with the highest resistance rate against cefuroxime and cefixime (each with 100%) and the lowest resistance rate against amikacin (10%). In contrast to our study, an Indian study reported that imipenem and meropenem presented with resistance rates of 13.5%, and 21.6% respectively (31). Also, Our results were dissimilar to the results of the Hakemi et al. (32), which shows that resistance of *P. aeruginosa* isolates to tested antibiotics in antibiogram test were 100% to cefpodoxime, 82.98% to ceftriaxone, 78.73% to imipenem, 75% to meropenem, 72.72% to gentamicin, 69.23% to ciprofloxacin and aztreonam, 67.57% to cefepime, 65.95% to ceftazidime, and 61.53% to piperacillin.

Furthermore, a study in Egypt reported dissimilar results, where 12/35 (34.3%) strains were resistant to ceftazidime, 9/35(25.7%) strains were resistant to levofloxacin and 7/35(20%) strains were resistant to imipenem but lower resistance 28.6% of *P. aeruginosa* isolates were resistant to amikacin (13). The European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2015 reported an increasing trend for resistance against piperacillin/tazobactam during 2011–2015, with the highest resistance related to piperacillin/tazobactam (36.1%) and levofloxacin (36.6%), and the lowest (1%) was against colistin in European hospitals (33). Similarly, resistance to piperacillin/tazobactam, levofloxacin, and colistin was reported as 27.1%, 29.5%, and 1.1%, respectively in the U.S. hospitals (34). The variation in the level of resistance between different studies may be attributed to the difference in geographical distribution, type and number of samples collected in each study and the difference in antibiotic policies implemented in each country.

However, we detected the molecular detection for virulence factors associated with genes of *P. aeruginosa* revealed that 96%, 94%, 86.4%, 80.% and 74% of the biofilm producers isolates harboring *algD*, *pslA*, *pel A*, *toxA* and *exoS* gene, respectively. There- fore, these virulent genes may have a significant role in biofilm formation as these genes were heavily expressed in biofilm-producing strains of *P. aeruginosa*. Very similar results were previously obtained by Maita and Boonbumrung (16), who reported that the prevalence of *pslA* gene was 94% in biofilm-forming *P. aeruginosa* strains (16). In addition, Abootaleb et al. (2020) in Iran showed 100% presence of *pslA* gene in biofilm-forming *P. aeruginosa* (35). Ghadaksaz, Sekhavatjou (36) was nearly similar to our result, reporting that *algD* and *pslA* genes were positive in 100% and 86.9% of the isolates, respectively (36). The percentage of biofilm-former strains is in accordance with the obtained results of Wang, Schmidt (37), who reported 70% biofilm capability in burn isolates, but the frequency of the *pslA* gene was higher in the present study (37). Previous findings reported that *pslA* gene expression had proven itself a good marker of biofilm formation in *Pseudomonas aeruginosa* isolates, owing to the fact that *pslA* plays an essential role in initial biofilm formation (38). However, another study by Heydari and Eftekhar, 2015, reported that *pslA* gene was also detected in non-biofilm-producing isolates (39). Sharma and Choudhury (40) showed in previous studies, observed that *pel A* gene was expressed heavily (80%) among biofilm-producing strains (40). Also, AL-Sheikhly et al. reported that *pel A* gene is present in all biofilm-producing *P. aeruginosa* isolates (41). In the study conducted by Ghadaksaz et al. the prevalence of the *pslA* and *pelA* genes was 83.7% and 45.2%, respectively (42). Also, Pournajaf et al. reported that *pel A* gene is present only in 57.3% of the isolates (43). The frequency of the *toxA* and *exoS* genes in the present study was similar to the results reported by Amirmozafari, Fallah (44) and Bogiel, Depka (45), in wound isolates of *P. aeruginosa*. The present study has some limitations. So, we recommend further studies with more strains of *P. aeruginosa* to prove the potential relationship between biofilm formation and expression of different resistance and virulence genes. Continuous monitoring and identification of these resistant organisms is essential for the selection of appropriate infection control strategies and proper treatment strategies for regarding the role of virulence genes in the significant increase in the pathogenicity of *P. aeruginosa* (46).

In conclusion, the results of this study indicate a high percentage of virulence-associated genes in burn infection isolates of *P. aeruginosa* in Egypt. In particular, the increasing rate of resistance to β-lactam antimicrobials is considerable, limiting choices for suitable treatment of patients with severe burn infections. Regarding the role of virulence genes in the significant increase in the pathogenicity of *P. aeruginosa*, continuous monitoring and identification of these resistant organisms is essential for the selection of appropriate infection control strategies and proper treatment strategies.

Interest conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publications

The author read and proved the final manuscript for publication

Availability of data and material

All data generated during this study are included in this published article

Ethics approval and consent to participate

No human or animals were used in the present research. The study protocol was approved by the Ethics Committee of Suez
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