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ORIGINAL ARTICLE

Phenotypic and Molecular Characteristics of *Pseudomonas Aeruginosa* Isolated from Burn Unit

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ABSTRACT

Background: *The biggest concern for a burn team is a nosocomial infection in burn patients, which is a significant health issue. Pseudomonas aeruginosa is an extremely troublesome drug-resistant bacterium in the world today. We are now faced with rising P. aeruginosa pan-drug-resistant clones in hospital settings.* ***Objectives:*** *To evaluate the distribution of different virulence factors generated by P. aeruginosa isolated from burn wound infections, together with its antimicrobial susceptibility.* ***Methodology:*** *The isolates reported as P. aeruginosa were further tested for the presence of various phenotypic and genotypic virulence factors including (Biofilm formation, lipase, protease, gelatinase, DNase, bile esculin hydrolysis & hemolysin). Also, genes encoding (nan 1 and Exo A) were investigated by PCR using specific primers. All the isolates were tested for their antimicrobial susceptibility patterns.* ***Results:*** *The study reported that toxins and enzymes were expressed by the tested strains in varying proportions; (92.0%) were producing β-hemolysin, lipase (86%), and protease (86%). The formation of biofilm was observed in 84%. Exo A (70%) was the main virulence gene found in the tested strains. Nan 1 gene was identified in 30% of the samples. 82% of MDRPA isolates were found. There is indeed a relationship between biofilm production and drug resistance, as well as the presence of virulence genes (nan 1 and Exo A) were associated with certain patients and burn wounds characteristics as burn size, burn wound depth, length of hospital stays, and socioeconomic status.* ***Conclusions:*** *Correlation of Pseudomonas aeruginosa virulence profiles with burn wounds and patient-related data can be useful in establishing of an appropriate preventive protocol for hospitalized patients with P. aeruginosa burn serious infections. The targeting of these bacterial virulence arsenals is also a promising approach to developing alternative drugs, which act by attenuating the aggressiveness of the pathogen and reducing its potential to cause vigorous infection.*

INTRODUCTION

Burn is characterized as a serious injury to the skin or other biological tissue that is assumed to be caused by heat or exposure to electrical discharge, friction, chemicals, and radiation[1].

Patients that have burned wound are easily subjected to microorganism infection because of the weakened immune system resulting from skin loss and burning injuries[2].

Pseudomonas aeruginosa is a gram-negative microorganism that is considered as the third nosocomial pathogen after *Staphylococcus aureus* and *Escherichia coli* [3]. It is also the main cause of hospital infections, especially in patients with cystic fibrosis (CF), burns, or immunodeficiency and intubation[4].

P. aeruginosa can produce an amazing array of virulence factors e.g. Flagellum, Lipopolysaccharide, Type IV Pili, Type III Secretion System, Proteases, Exotoxin A, alginate, Quorum Sensing, Biofilm formation, Type VI secretion systems, and oxidant production in the airspace. These virulence factors act in different ways in the immune system[5].

Sialidases also referred to as neuraminidases, are hydrolytic enzymes involved in the invasion of the host by bacteria and the spread within the host. The enzyme activity forms part of the first step in the degradation of sialic-acid bonds[6].

P. aeruginosa generates neuraminidase that virulence gene (nan), releases residues of terminal sialic acids from glycoproteins, glycolipids, and gangliosides expressed on host cells. This enzyme plays a pivotal role in bacterial attachment and subsequent invasion of host cells, particularly epithelial cells[7].

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Also, exotoxin A encoded by the Exo A gene is a protein toxin that inhibits polypeptide synthesis by ADP elongation factor 2 ribosylation, as does diphtheria toxin that contributes to cell death[8].

About virulence factors, the biofilm formation of *P. aeruginosa* plays a role in biofilm-associated antimicrobial tolerance as it contains bacterial subpopulations characterized by a wide distribution of metabolic activity. The sub-populations in the biofilm's periphery display high physiological activities, and the sub-populations in the inner sections show low metabolic response[9].

A rapid increase in multidrug-resistant isolates of *P. aeruginosa* (MDRPA) in clinical settings around the world has resulted in increased mortality rates. Several factors are proposed in *P. aeruginosa* antimicrobial resistance such as β-lactamase production, target mutation, overexpression of efflux systems, decreased membrane permeability, and chromosomal resistance to gene encoding[10].

The aim of this research is to test the in vitro behaviour of different virulence determinants and to examine the susceptibility pattern of isolates of *P. aeruginosa* burn wounds.

METHODOLOGY

This cross-sectional research was done in Clinical and Chemical Pathology Laboratory, Benha University Hospital between June 2017 and July 2019. A total of 50 *P. aeruginosa* strains were collected from patients suffering from moderate to serious burn wounds with clinical signs and symptoms of burn-infection at the burn unit, the Benha Teaching Hospital. Patient's median age was 5 years (age range 1 − 55 years). They were 46% males and 54% females.

Identification of isolated *P. aeruginosa*

The specimens were cultivated on the following agar plates: MacConkey, and 5% Blood agar, incubated at 37 ° C for 24 hours under aerobic conditions. Well isolated colonies were identified as *P. aeruginosa* by 42ºC growth, positive oxidase reaction, pigment production and other biochemical characteristics[11].

Phenotypic characterization of the virulence determinants

Biofilm formation:

It was detected using microtiter plate assay as previously described by O'Toole[12]. In trypticase soy broth with 0.25% glucose, all tested species isolated from fresh agar media were inoculated and findings were interpreted according to the O'Toole microtiter plate assay.

Protease activity[13]:

All bacterial isolates, were tested for their ability to produce protease enzymes (casein hydrolysis) using skim milk agar. On the skimmed agar, the isolates were streaked and incubated for 24 hr at 37ºC; plates were inspected for halo-regions around the streaks. A clearance zone around the streaks has been confirmed to be positive for protease development (Figure 1).

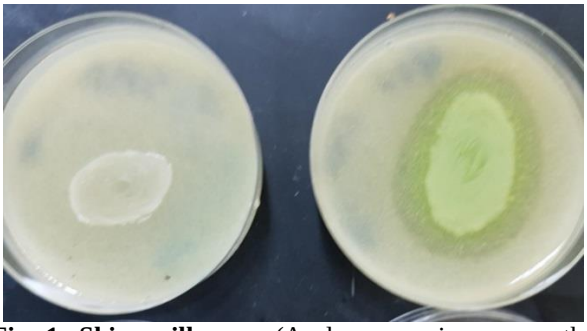

Fig. 1: Skim milk agar (A clear zone is seen on the right Petri dish around the spotted inoculum indicating positive protease activity while the left one is negative)

Lipase activity[14]:

Each tested isolate was inoculated on Tween esterase agar plates and incubated at 37ºC. The presence of opaque zone surrounding the inoculum was checked on the plates after 24-48hrs and this finding suggest a positive lipolytic activity (Figure 2).

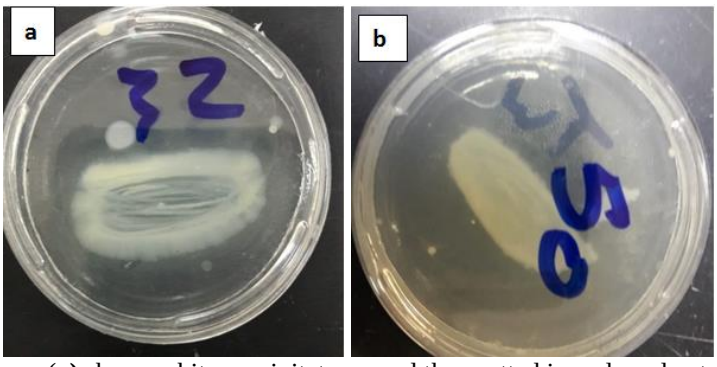

Fig. 2: Tween esterase agar (**a)** shows white precipitate around the spotted inoculum due to precipitation of Ca salts around the released fatty acids indicating that it is positive for lipase production, while (**b)** plate: is negative for lipase

Gelatinase activity*[15]*:

Gelatinase production was tested via straight-line stab inoculation of tubes containing nutrient gelatine medium. The tubes were incubated at 37ºC for 24-48hr. Uninoculated tubes were run beside the inoculated ones as a negative control. At the end of the incubation period, the liquefaction of the culture medium was observed after placing the culture at 4ºC for 2 hours.

Detection of esculinase activity*[16]*:

The strains were plated on a bile esculin agar plate. After incubation at 37 °C for 18-24 h, blackening of the medium occurs which indicate positive esculinase activity.

Detection of DNase activity*[17]*:

DNase agar plates test an organism's ability to generate an exoenzyme called deoxyribonuclease that cleaves DNA and releases free nucleotides and phosphate into the media. As a positive response, a clear zone around the inoculum was registered.

Haemolysin production*[18]*:

The bacterial isolates have been tested using blood agar media for their ability to generate hemolysin. At 37°C for 24 hours, plates were incubated and then tested for hemolysis around the colonies. The results were recorded as follows: α-haemolysis (greenish zones), β-hemolysis (clear zone), or γ-hemolysis (no hemolysis).

Antibiotic susceptibility testing:

The drug susceptibility test was carried out by the Kirby-Bauer agar disk diffusion method for all isolates, and the inhibition zones were measured per the recommendations of CLSI, 2018 [19]. The susceptibility profiles were determined for 10 antibiotic discs including ceftazidime (CAZ, 30 µg/disk), aztreonam (ATM, 30 µg/disk), amikacin (AK, 30 µg/disk), gentamicin (CN, 10 µg/disk), tobramycin (TOB, 10 µg/disk), piperacillin-tazobactam (TZP, 100/10µg/disk), norfloxacin (NOR) and ciprofloxacin (CIP) each (5µg/disk), imipenem and meropenems each (10 µg/disk), (all Oxoid disks, UK).

Index of Multiple antibiotic resistance (MAR):

MAR index was determined for each isolate in this analysis by dividing the number of the resistant antibiotics over the total number of tested antibiotics (10 antibiotic discs).

Detection of Exo A and nan 1 virulence genes by Polymerase Chain Reaction (PCR):

A particular set of primers listed in (table 1) was used to perform PCR. DNA from *P. aeruginosa* isolates was extracted and purified using Thermo Scientific Gene JET Genomic DNA Purification Kit (Cat. No. #K0721, Thermo Fisher, California).

The Exo A and nan 1 genes were amplified in a 50 µL reaction mixture containing 200 ng DNA template, 1 µL of each primer, and 25 µL Master Mix. Ultra-pure water was then added to make up a final volume to 50 µL.

The amplification for Exo A gene had been achieved in these steps: one cycle of denaturation at 94˚C for 2 min, followed by 30 denaturation cycles (94˚C for 30 sec), annealing (68˚C for 1 min) and extension (72˚C for 1 min), then a single final extension of 7 min at 72˚C. The nan 1 gene was amplified as follows: initial denaturation at 94˚C for 2 min, 30 cycles of denaturation (94˚C for 30 sec), annealing (55˚C for 30 sec), extension (72˚C for 90 sec) and a single final extension at 72˚C for 5 min.

Amplified products were separated at 130 V, stained with ethidium bromide (0.5µg / ml) and assessed by UV transillumination in 1.6 % agarose gel for 30 min (Figure 3,4).

Table (1): Primers sequence

Target gene		Primer sequence (5′–3′)	Size (bp)	Reference
Exo A	Forward	5′GACAACGCCCTCAGCATCACCAGC3′	396 bp	**19**
	Reverse	5′CGCTGGCCCATTCGCTCCAGCGCT3′		
nan 1	Forward	5′ATG AAT ACT TAT TTT GAT AT3′	1316 bp	**20**
	Reverse	5′CTA AAT CCA TGC TCT GAC CC3′		

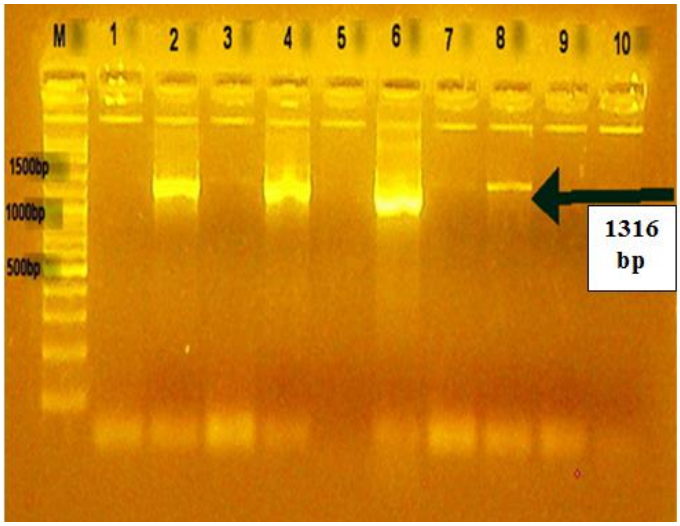

Fig. 3: Agarose gel electrophoresis for the detection of (nan 1) gene

- Lane M: molecular marker.
- Lanes 2,4,6 and 8: positive (+ve) cases (1316 bp).
- Lanes 1,3,5,7,9,10: negative (–ve) cases

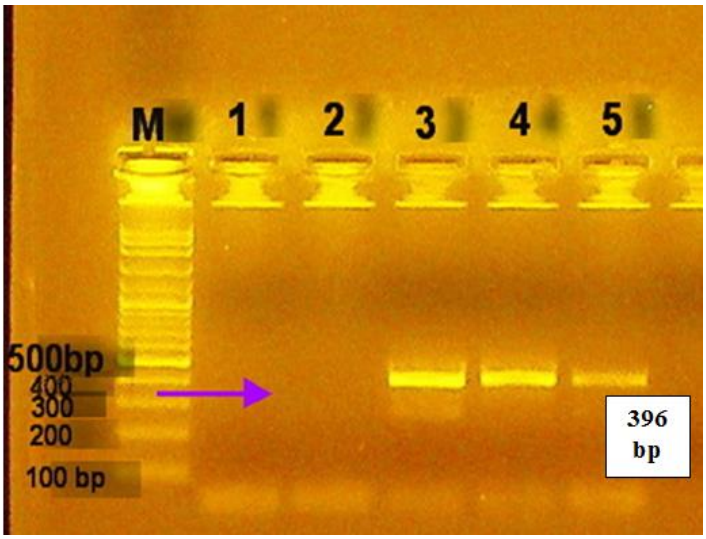

Figure 4: Agarose gel electrophoresis for the detection of (Exo A) gene

- Lane M: molecular marker.
- Lanes 3, 4 and 5: Positive (+ve) cases (396bp)
- Lanes 1, 2: Negative (–ve) cases

Statistical Methods

Statistical packages (IBM Corp. Released 2017. IBM SPSS, Version 25.0. Armonk, NY: IBM Corp.) were used to analyse the collected data. P-value <0.05 is significant at confidence interval 95%. Descriptive statistics: median, minimum, and maximum for numerical data. Frequency and percentage of non-numerical data. Analytical statistics: Mann Whitney Test (U test), Chi-Square test (X^2), Fisher's exact test (FET), and logistic regression analysis.

RESULTS

A total of fifty *P. aeruginosa* isolates have been recovered from burn infected patients with hot fluids (scald) causing 50% of injuries and flames causing the

other 50%. Burn injury occurred in 66% of patient's due to indoors related accident, and 34% of patient's due to outdoors related accident, with median hospitalization period of 14.5 days. Only few patients (22%) had surgery in the form of skin grafts. The median total burn area (TBSA) was 15%, varying from 8% to 45%. TSBA was 11-20 percent in most cases (46%). More than half of cases had 1st degree burn (58%), while 42% of cases had 2nd and 3rd grade burn.

The recovered isolates were tested for virulence factors; haemolysin production, protease, DNase, lipase, gelatinase, esculinase enzymes and biofilm together with the Exo A and nan 1 genes.

As shown in (table 2), the spread of the pathogenicity factors in clinical *P. aeruginosa* isolates showed that β-hemolysin (92%) was the most frequently detected phenotypic virulence factor, followed by protease and lipase (86% each), then biofilm formation (84%) and finally gelatinase and esculine hydrolysis (34% each). In any of the analyzed isolates, DNase activity was not observed. The virulence genes (Exo A and nan 1) detected among the bacterial isolates were 72% for Exo A gene (36 isolates), and 30% for nan 1 gene (15 isolates).

Table 2: Frequency of studied virulence factors in *P. aeruginosa* burn wound isolates.

		Cases (N=50)	
β-hemolysin	N, %	46	92%
Gelatinase	N, %	17	34%
Protease	N, %	43	86%
DNAse	N, %	0	0%
Esculin hydrolysis	N, %	17	34%
Lipase	N, %	43	86%
Biofilm formation	N, %	42	84%
Exo A	N, %	36	72%
nan 1	N, %	15	30%

The existence of different virulence factors displayed by each of the *P. aeruginosa* isolates tends to influence the synthesis of the Exo A and nan 1 genes, in which 50% of the positive Exo A genotypes were contained in isolates expressing 6 virulence factors ($P<0.05$) and 40% of the positive strains of the nan 1 gene were also found in isolates expressing 6 virulence factors ($P < 0.001$) (table 3).

There was an important correlation of positive Exo A isolates with positive β-hemolysin ($P<0.05$) and protease ($P= 0.001$), but no substantial association was observed with gelatinase, esculine hydrolysis and biofilm formation (table 4).

Table 3: Number of virulence factors according to nan 1 and Exo A in studied cases.

			Negative nan 1 N=35		**Positive nan 1 N=15**		***Test***	***p***	**Negative Exo A N=14**		**Positive Exo A N=36**		***test***	***p***
Number of virulence factors	**Median (min-max)**		**5**	**1-7**	**6**	**4-8**	**U=3.213**	**0.001**	**4**	**1-6**	**6**	**3-8**	**U= 4.192**	**<0.001**
	1	**N, %**	**2**	**5.7%**	**0**	**0%**	**FET= 12.306**	**0.023**	**2**	**14.3%**	**0**	**0%**	**FET= 19.393**	**<0.001**
	2	**N, %**	**0**	**0%**	**0**	**0%**			**0**	**0%**	**0**	**0%**		
	3	**N, %**	**5**	**14.3%**	**0**	**0%**			**4**	**28.6%**	**1**	**2.8%**		
	4	**N, %**	**3**	**8.6%**	**1**	**6.7%**			**3**	**21.4%**	**1**	**2.8%**		
	5	**N, %**	**10**	**28.6%**	**2**	**13.3%**			**3**	**21.4%**	**9**	**25.0%**		
	6	**N, %**	**14**	**40.0%**	**6**	**40.0%**			**2**	**14.3%**	**18**	**50.0%**		
	7	**N, %**	**1**	**2.9%**	**5**	**33.3%**			**0**	**0.0%**	**6**	**16.7%**		
	8	**N, %**	**0**	**0%**	**1**	**6.7%**			**0**	**0.0%**	**1**	**2.8%**		

Table 4: Frequency of different phenotypic virulence factors with genotypic virulence factors in all studied isolates.

	Negative nan 1 N=35			**Positive nan 1 N=15**		***test***	***p***	**Negative Exo A N=14**		**Positive Exo A N=36**		***test***	***p***
β-hemolysin	**N, %**	32	91.4%	14	93.3%	FET= 0.052	0.820	11	78.6%	35	97.2%	**FET= 4.764**	**0.029**
Gelatinase	**N, %**	13	37.1%	4	26.7%	X^2= 0.514	0.474	5	35.7%	12	33.3%	FET= 0.025	0.873
Protease	**N, %**	29	82.9%	14	93.3%	FET= 0.957	0.659	8	57.1%	35	97.2%	**FET= 13.449**	**0.001**
Esculin hydrolysis	**N, %**	11	31.4%	6	40.0%	X^2= 0.344	0.558	3	71.4%	14	38.9%	FET= 1.369	0.327
Lipase	**N, %**	30	85.7%	13	86.7%	FET= 0.008	0.929	10	78.6%	33	91.7%	FET= 3.429	0.085
Biofilm formation	**N, %**	28	80.0%	14	93.3%	FET= 1.389	0.407	11	78.6%	31	86.1%	FET= 0.426	0.670

The study of different burn wound characteristics showed that positive nan 1 (P<0.05) was significantly correlated with only the duration of hospital stay and low socioeconomic status. Exo A production was significantly related to total burn surface area, burn wound depth and burn wound infections affecting the trunk.

In this study, an analysis of the antibiotic susceptibility pattern of *P. aeruginosa* isolates to 10 antibiotics showed an increased rate of resistance to antibiotics. The highest incidence level was observed for ciprofloxacin (82%) and gentamycin (76%), while imipenem and meropenem (26%) were the most effective antibiotics. The *P. aeruginosa* isolates' total multiple antibiotic resistance (MAR) index was (0.9).

Evaluation of the expression of Exo A and nan 1 genes in relation to the pattern of drug susceptibility showed that positive Exo A was strongly associated with resistance to Amikacin (AK) (P<0.05). Positive nan 1 was significantly correlated with imipenem (IPM) and meropenem (MEM) resistance (P<0.05).

Patients with MDR *P. aeruginosa* isolates (P <0.05) were significantly affiliated with a longer hospital stay.

MDR isolates have also been significantly related to higher biofilm - forming isolates (P < 0.05), where 90.2% of MDR isolates were positive for biofilm formation. No significant differences were detected regarding β- hemolysin, gelatinase, protease, esculine hydrolysis, lipase, Exo A and nan 1 (table 5).

A logistic regression analysis was performed to predict MDRPA using age, gender, burn type, TBSA, burn depth, place of occurrence, biofilm formation, β-hemolysin, gelatinase, protease, esculine hydrolysis, lipase, Exo A, nan 1 and MAR index as covariates. Higher MAR index and biofilm formation were assumed to be independent variables for MDR *P. aeruginosa* isolates in uni- and multivariable study (table 6).

Table 5: Comparison between MDR and non-MDR isolates regarding the frequency of different phenotypic virulence factors.

		No MDR N=9		**MDR N=41**		***test***	***p***
Beta hemolysin	**N, %**	7	77.8%	39	95.1%	FT=3.016	0.144
Gelatinase	**N, %**	5	55.6%	12	29.3%	FT=2.273	0.242
Protease	**N, %**	8	88.9%	35	85.4%	FT=0.076	0.783
DNAse	**N, %**	0	0%	0	0%	-	-
Esculin hydrolysis	**N, %**	4	44.4%	13	31.7%	FT=0.534	0.467
Lipase	**N, %**	6	66.7%	37	90.2%	FT=3.407	0.100
Biofilm formation	**N, %**	5	55.6%	37	90.2%	**FT=6.607**	**0.026**

Table 6: Regression analysis for prediction of MDR occurrence in pseudomonas isolates.

	Univariable				**Multivariable**			
	p	**OR**	**95% CI**		***p***	**OR**	**95% CI**	
Age	0.221	931	0.911	1.131				
Gender	0.436	1.347	0.234	4.387				
Type of burn	0.636	1.387	0.423	3.109				
TBSA	0.080	1.064	0.993	1.140				
Depth of burn	0.108	1.799	0.879	3.683				
Place	0.934	1.432	0.487	3.109				
Biofilm formation	**0.021**	3.254	1.199	8.830	**0.032**	1.536	1.153	4.100
Beta hemolysin	0.123	2.793	0.757	10.300				
Gelatinase	0.142	0.534	0.231	1.233				
Protease	0.432	0.309	0.129	2.762				
Esculin hydrolysis	0.353	0.786	0.412	1.385				
Lipase	0.090	2.466	0.868	7.003				
Exo A	0.866	1.164	0.683	1.937				
nan 1	0.572	0.871	0.579	1.375				
MAR index	**<0.001**	5.948	2.242	12.653	**0.011**	2.324	2.121	10.546

OR, odds ratio; CI, confidence interval

DISCUSSION

Pseudomonas aeruginosa, which is a gram-negative, aerobic, non-spore-forming rod, is capable of causing a number of infections among both immunocompetent and debilitated populations. It is an extremely difficult organism to treat in modern medicine because of its intent to inflict illnesses among immunocompromised hosts, severe versatility, a broad range of complex defenses and antibiotic resistance [20].

One of the reasons that *P. aeruginosa* is considered to be an effective opportunistic pathogen is the emergence of multiple arrays of cell-associated and secreted virulence factors, including structural components, toxins, pigments, and enzymes (elastases, alkaline protease, pyocyanine, and rhamnolipids), aggravates the infection and disease process [21]. At least one or more different virulence variables were carried by all isolates in this research work.

Biofilm production has been calculated as a significant determinant of pathogenicity in *P. aeruginosa* infections [22]. Herein, the majority (84%) were biofilm producers by microtiter plate assay.

A research in Iran with 96 samples, the biofilm development was reported in more than 96% of the isolates collected from burning patients [23], another from Nasirmoghadas, et al.[24] identified that 93% of *P. aeruginosa* isolates extracted from burn patients developed biofilm. But in El Askary study [25] 70.9% of the isolates were biofilm producers by TCP method.

As for the results of lipase development, the vast majority of isolates are said to be lipase positive (86%) and this was slightly lower than those reported by Mohammad [26], who found that 100% of burn wound specimens were positive for lipase. Other studies found that only 42.85% and 37.5% of the tested strains were favourable for lipase secretion respectively [27,28].

The alkaline protease generated by the majority of the strains tested (86%) reflects the tendency of these strains to cause tissue lesions and delay wound healing [29]. This result was consistent with a study by Khalil [30] and his colleagues that recorded 85% protease activity among *P. aeruginosa* isolates tested from different body sites with the highest percentage of protease activity (95%) reported in burn isolates. A study by Shaaban et al [31] investigating the prevalence of lasB virulence gene that code for protease enzyme in *P. aeruginosa* burn wound isolates found that (78.8%) contain the gene.

Another method for assessing protease activity in tested isolates is by detecting gelatinase activity in nutrient gelatine agar media. Studied cases showed that 34% of strains were positive. A research by Pramodhini et al [21] showed a 65% gelatine activity in pus samples, while another analysis by Holban et al [32] the isolated *Pseudomonas aeruginosa* strains did not express gelatinase.

Overall incidence of the neuraminidase virulence gene (nan 1) was (30%) in the study group and this level was close to that of the Elogne et al. [33] study, which recorded 37.1% of neuraminidase 1 genotypic expression in the *P. aeruginosa* clinical strains. While Corehtash et al.[34] reported that the prevalence of nan1 among infected burn wounds was 21.3%.

The frequency of multiple virulence factors with positive nan 1 and positive exo A was significantly high. A similar finding was found in a study by Al-Dahmoshi et al.[35] investigating the existence of exoA, oprL, oprI, lasI, and lasB genes among *P. aeruginosa* isolated from wound infection.

A statistically significant relation was found between Exo A production and burn wound characteristics as total burn surface area, burn wound depth and burn infections affecting the trunk, suggesting its effect in delayed wound healing, a finding that is consistent with a previous study by Badr et al. [36] who reported that all of their 38 toxA+ isolates were associated with delayed wound healing; whereas, their toxA- isolates have been related to a normal wound healing process.

One of the most worrisome characteristics of the tested *P. aeruginosa* was its low antibiotic susceptibility. All tested isolates were almost resistant to all tested antibiotic discs (resistance was over 50%). A study by Khosravi et al. [37] demonstrated that 70.66% of burn wound isolates were multiple drug-resistant. Also, Corehtash et al. [34] found that 93.1% of the isolates were MDR.

The existence of epidemiological and burn related characteristics such as age, sex, site of wound, time to hospital arrival, first aid prior to diagnosis, aetiology of burn injuries, depth, overall burn surface area, degree of burn, existence of comorbidities, residency, hospital stay, season and socio-economic status were examined with regard to MDR and non-MDR isolates. Only patients with a prolonged hospital stay had a higher likelihood of MDR strain infection and this result was consistent with a research conducted by Rodulfo and his peers [38].

A substantial difference in biofilm production was observed between burned patients harboring MDR and non-MDRPA isolates, of which (90.2%) of MDR isolates demonstrated biofilm formation (p= 0.026) relative to non-MDR isolates. In line with the previous study, the majority of MDRPA isolates were found to have stronger biofilm formation[39].

Similarly, an Iranian study reported significantly higher levels of MDR isolates, which were relatively stronger producers of biofilms in the properly functioning media than non-MDR isolates [34].

No important difference between MDR and non-MDR isolates was noted in study results with regard to

various phenotypic virulence factors including hemolysin, lipase, proteases, esculinase, and gelatinase.

The same result was recently published in an El-Mahdy and El-Kannishy study [40] conducted in Egypt investigating the relationship between resistant and susceptible carbapenem isolates for either hemolysin, protease or twitching motility production.

CONCLUSION

From the present study we conclude that in additional to antibiotic resistance, a high percentage of *P. aeruginosa* burn wound isolates had a plentiful capacity for virulence enzymes, biofilm, and toxin production. Aligning the release of these pathogenicity factors along with patient data and burn wound clinical manifestations may allow the physician to adjust the burn unit's preventive and curative practices.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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