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Study of quorum-sensing *LasR* and *RhlR* genes and their dependent virulence factors in *Pseudomonas aeruginosa* isolates from infected burn wounds

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Abstract

Background. *Pseudomonas aeruginosa (P. aeruginosa)* is an opportunistic pathogen responsible for burn-wound infection. High incidence, infection severity and increasing resistance characterize *P. aeruginosa*-induced burn infection.

Purpose. To estimate quorum-sensing (QS)-dependent virulence factors of *P. aeruginosa* isolates from burn wounds and correlate it to the presence of QS genes.

Methods. A cross-sectional descriptive study included 50 *P. aeruginosa* isolates from burn patients in Mansoura University Plastic and Burn Hospital, Egypt. Antibiotic sensitivity tests were done. All isolates were tested for their ability to produce biofilm using a micro-titration assay method. Protease, pyocyanin and rhamnolipid virulence factors were determined using skimmed milk agar, King's A medium and CTAB agar test, respectively. The identity of QS *lasR* and *rhlR* genes was confirmed using PCR.

Results. In total, 86% of isolates had proteolytic activity. Production of pyocyanin pigment was manifested in 66% of isolates. Altogether, 76% of isolates were rhamnolipid producers. Biofilm formation was detected in 96% of isolates. QS *lasR* and *rhlR* genes were harboured by nearly all isolates except three isolates were negative for both *lasR* and *rhlR* genes and two isolates were positive for *lasR* gene and negative for *rhlR* gene. Forty-nine isolates were considered as extremely QS-proficient strains as they produced QS-dependent virulence factors. In contrast, one isolate was a QS deficient strain.

Conclusions. QS affects *P. aeruginosa* virulence-factor production and biofilm in burn wounds. Isolates containing *lasR* and *rhlR* seem to be a crucial regulator of virulence factors and biofilm formation in *P. aeruginosa* whereas the *lasR* gene positively regulates biofilm formation, proteolytic activity, pyocyanin production and rhamnolipid biosurfactant synthesis. The QS regulatory *RhlR* gene affects protease and rhamnolipid production positively.

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is a Gram-negative bacillus, which is considered one of the most important opportunistic pathogens, and establishes itself in vulnerable immunocompromised patients causing urinary tract infections, respiratory system infections, bacteremia and burn-wound infections. In patients with severe burn wounds, it is difficult to treat and can cause high mortality [1]. However, this bacillus can be found on the skin, throat and stool of non-hospitalized patients [2].

Multiply resistant organisms, including *Pseudomonas* and *Acinetobacter*, are the leading causes of death from infection after burns [3]. Numerous *P. aeruginosa* virulence factors contribute to the pathogenesis of burn-wound infection such as pili, flagella, biofilm formation besides the protease, pyocyanin and rhamnolipid extracellular virulence factors. Due to a range of mechanisms for virulence and antibiotic resistance, understanding the regulatory mechanisms regulating the expression of virulence genes is critical for the development of alternative therapeutic approaches in order

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Keywords: *Pseudomonas aeruginosa*; virulence; QS; biofilm.
Abbreviations: CTAB, cetyl trimethyl ammonium bromide; KIA, kligler iron agar medium; MIO, motility indole ornithine reaction; OD, optic density; QS, quorum sensing.

to monitor and prevent these pseudomonal infections [4]. The creation of biofilms and the bacterial activity have been regulated by quorum sensing (QS) and often render *Pseudomonas* infections difficult to eradicate [5].

QS refers to the ability of a bacterium to sense information from other cells in the population when they reach a critical concentration (i.e. a quorum) and communicates with them to regulate the production of variable virulence factors using small, signalling molecules called autoinducers [6]. *P. aeruginosa* contains two main QS systems, las and rhl. Each system consists of two components, the autoinducer synthases (*lasI* and *rhlI*, respectively) and their cognate transcriptional regulators (*lasR* and *rhlR*, respectively). *P. aeruginosa* also possesses a third QS system called *Pseudomonas* quinolone signal (PQS) and a recently identified fourth QS system termed as the integrated QS (IQS) system [7].

These systems control expression of many extracellular virulence factors that play crucial roles in colonizing host tissues and are regulated by the las system and the rhl system [8]

Our aim was to estimate QS-dependent virulence factors as protease, pyocyanin, rhamnolipid and biofilm-producing ability of *P. aeruginosa* isolates from burn wounds and correlate it to the presence of QS genes.

METHODS

This is a cross-sectional descriptive study of 100 patients with burns who were admitted to the plastic surgery and burn centre at Mansoura University Hospital (MUH), from September 2017 to August 2018, included 65 males and 35 females with ages ranging from 2 to 54 years (mean±SD 27.74±12.93).

Swabs were collected using Levine's technique by rotating manoeuvre over 1 cm^2 area of the wound with sufficient pressure to extract fluid from within the wound tissue [9] and were transported aseptically in Stuart's transport media. Primary plating of all samples was performed quantitatively on nutrient, blood and MacConkey's agar, incubated aerobically at 37 °C. The infected wound was defined as those containing 1×10^5 organisms or more per swab [9]. The colonial forms were observed, and the characteristic odour of the organism was detected. Colonies were examined for pigment production on cetrimide agar at 42 °C. However, some strains, proved to be *P. aeruginosa* but were not fluorescent. Gram-stained smears from suspected colonies showed non-sporeforming Gram-negative bacilli.

Colonies were reexamined using different biochemical tests; oxidase-positive colonies became dark blue within a few seconds, catalase-positive isolates detected by the presence of immediate effervescence, Fig. 1.

The tested organism was subcultured and incubated on Kligler iron agar (KIA) medium; it was examined for the colour change caused by sugar fermentation, gas and H_2S production, *P. aeruginosa* isolates are neither glucose nor lactose fermenter, detected by pink red slant and butt with

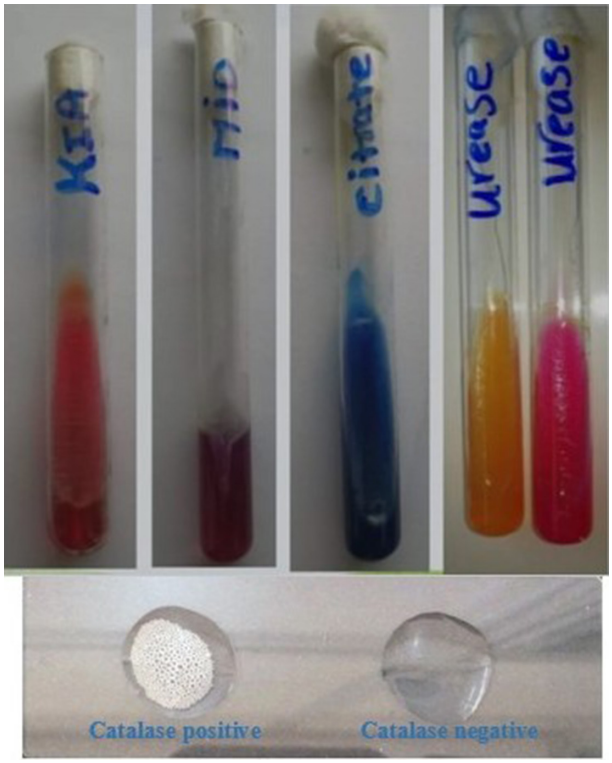

Fig. 1. Different biochemical reactions of *P. aeruginosa* isolates (KIA=Kligler iron agar medium, MIO=motility indole ornithine reaction).

no gas or H_2S production. Motility indole ornithine (MIO) reaction allows detection of the organism's motility along the stab line of inoculation, Fig. 1.

n's citrate medium was inoculated by the tested organism; *P. aeruginosa* isolates produce positive citrate utilization test, the presence of alkaline product is proved by the changing colour into blue. Speaking of which, the test strain was streaked on urea agar slant where *P. aeruginosa* isolates are either urease positive or urease negative Fig. 1.

Furthermore, the automatic VITEK 2 (bioMérieux) was used to identify the bacterial species, antibiogram profile of the isolates were deduced by disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) [10].

Biofilm production of *P. aeruginosa* isolates was assessed by using a micro-titration assay method previously described by Stepanović *et al.* [11]. Biofilm-forming capacity was calculated at OD 492 nm using a microtitre plate reader. The taken value was the mean of three readings for each clinical isolate. The tested isolates were classified into three categories according to the value of OD 492 nm as strong, moderate and weak biofilm adherence.

The assessment of *P. aeruginosa* proteolytic activity was performed using skimmed milk agar. The previously identified *P. aeruginosa* strains were streaked on this media, the clearance of skimmed milk occurred by hydrolysis of casein (a primary milk protein) [12]. Pyocyanin quantification was

Table 1. Primers that were used for detection of *lasR* and *rhlR* genes by PCR [15]

Gene type	Primer direction	Primer sequence (5′ to 3′)	Amplicon size (bp)
LasR	**Fw**	**5′-ATGGCCTTGGTTGACGGTT-3′**	**725**
	Rev	**5′-GCAAGATCAGAGAGTAATAAGACCCA-3′**	
RhlR	**Fw**	**5′-CAATGAGGAATGACGGAGGC-3′**	**730**
	Rev	**5′-GCTTCAGATGAGGCCCAGC-3′**	

executed using King's A broth media that enhances pyocyanin pigment production. Pyocyanin was extracted using chloroform and its absorbance was measured in the acidic form. The pyocyanin amount throughout the supernatant fractions of *P. aeruginosa* isolates is evaluated by the absorbance value at 520 nm. A higher absorption value implies higher production of pigments [13].

Rhamnolipid was assayed using cetyl trimethyl ammonium bromide (CTAB) assay method using M9 minimal media. Overall, 50 µl of supernatant from 24 h incubated bacterial broth at 37 °C were inoculated in performed agar wells, then the plates were incubated for 24–48 h at 37 °C [14].

PCR analysis was carried out using internal primers of QS genes (*lasR* and *rhlR*) as described previously by Cotar *et al.* [15], Table 1. Bacterial genomic DNA was extracted using aGeneJET Genomic DNA purification kits (Thermo Fisher Scientific, EU) from all phenotypically and biochemically tested strains, as well as from the reference positive control strain *P. aeruginosa* (PAO_1). By the use of DNA thermal cycler, PCR reaction was carried out in a 25 µl total volume. The cycling conditions incorporated initial denaturation followed by 35 cycles at 94 °C for 30 s for DNA denaturation, followed by annealing temperature at 50 °C for 30 s, extension at 72 °C for 2 min and finally the process was ended by final extension at 72 °C for 5 min [15].

Following amplification, aliquots (10 µl) were removed from each reaction mixture and examined by means of gel electrophoresis composed of 1.5% agarose in TBE buffer (40 mM Tris, 20 mM boric acid, 1 mM EDTA, pH 8.3). Then, the gels were Image graphed under UV lights and the DNA bands were visualized by ethidium bromide staining [15]. The PCR products were estimated by comparison with the 100 bp DNA ladder molecular size markers.

Statistical analysis

Data were analysed using the Statistical Package of Social Science (SPSS) programme for Windows (Standard version 21). The normality of data was first tested with one-sample Kolmogorov–Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Fischer's exact test when expected cell count was less than 5. Continuous variables were presented as mean±SD for parametric data and median (min-max) for non-parametric data. The two groups were compared with Student's *t*-test for parametric data and Mann–Whitney test for non-parametric data. Probability levels≤0.05 were considered statistically significant.

RESULTS

In the present study, 110 isolates from 100 patients with burns were collected. *P. aeruginosa* was the most common pathogen that was isolated from 50 swabs (45%) followed by *Klebsiella pneumoniae* isolated from 26 swabs (24%), *Staphylococcus aureus* isolated from 12 swabs (11%), *Escherichia coli* isolated from nine swabs (8%) and *Proteus mirabilis* isolated from seven swabs (6%), Table 2.

Identification of all the collected *Pseudomonas* specimens was confirmed. The physiological properties of all isolates proved their ability to produce pigment, grow at 42 °C, being oxidase positive and catalase positive. Different biochemical tests were used to identify *P. aeruginosa* isolates as KIA where they were sugar non-fermenters. MIO test showed that all isolates are motile. Isolates of *P. aeruginosa* showed their ability to utilize citrate and variable urease enzyme production.

The isolates were reported to be sensitive, intermediate and resistant from the respective interpretation charts recommended by CLSI [10] where the highest sensitivity of *P. aeruginosa* isolates was to colistin (100%) followed by polymyxin B (96%). Meanwhile, the least sensitivity was recorded against ceftazidime (6%). Forty-six (92%) of isolates were MDR as displayed in Table 3.

Biofilm-producing ability was detected by way of strain that was twice that of the negative control OD, considered positive. Seven (14%) *P. aeruginosa* isolates exhibited strong adherence as they produced biofilm with OD 492 nm ≥0.272 compared with PAO1 that was used as a positive control and had an OD 0.374. Nineteen (38%) isolates were moderately adherent as they produced biofilm ranged between $0.136<OD_T\leq0.272$, the remaining isolates (44%) with biofilm ranged between $0.068<OD_T\leq0.136$ reflected weak producers. However, two (4%) isolates were non-biofilm producers, Figs. 2 and 3.

The majority of *P. aeruginosa* isolates 43 (86%) had proteolytic activity whereas 7 (14%) showed no proteolytic activity, Figs 4 and 5. Quantification of pyocyanin production by the isolates occurred as described before by Essar *et al.* [13]. Thirty three (66%) *P. aeruginosa* isolates produced pyocyanin pigment and 17 (34%) lack the ability for pyocyanin production, five

Table 2. Bacterial isolates from burn wounds

Organism	No. [Total no.=110]	Percentage
Pseudomonas aeruginosa	50	45%
Klebsiella pneumoniae	26	24%
Staphylococcus aureus	12 [6 *MRSA and 6 MSSA*]	11%
Escherichia coli	9	8%
Proteus mirabilis	7	6%
Enterococci	3	3%
Citrobacter species	2	2%
Streptococcal pyogenes	1	1%

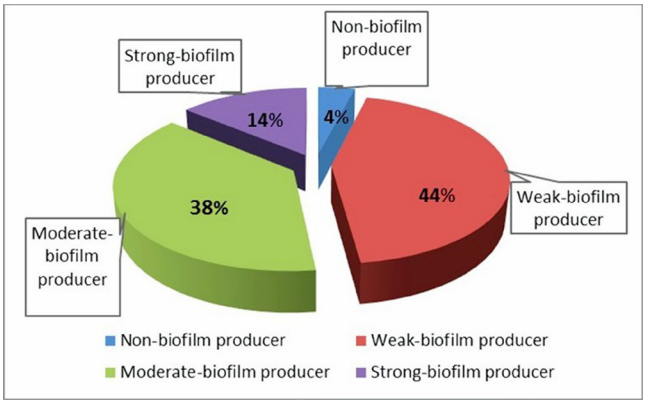

Fig. 2. The percentage of degrees of biofilm producers among *P. aeruginosa* isolates.

isolates produced a high amount of pyocyanin ≥8.365 µg ml^{-1} compared with the positive control PAO1 and 28 isolates produced a moderate amount of pyocyanin ranged between the negative control (double mutant PAO-JP2) (1.536 µg ml^{-1}) and positive control PAO1 (8.365 µg ml^{-1}), Fig. 6. An evidence of rhamnolipid production was detected in 38 (76%) isolates that were rhamnolipid producers, on the other hand, the remaining 12 (24%) isolates were categorized as rhamnolipid non-producers, Fig. 7.

PCR detection of *lasR* and *rhlR* genes showed that they were harboured by nearly all isolates except three isolates with amplicon sizes of 725 and 730 bp, respectively. Regarding *lasR*, it was detected in 47 (94%) out of 50 *P. aeruginosa* isolates collected as only three isolates did not harbour the gene, Fig. 8. There was a statistically significant difference between *lasR*-positive isolates and *lasR*-negative isolates regarding biofilm production (weak, moderate and strong) (*P* value=0.031), Table 4. Also, there was a highly statistically significant difference between *lasR*-positive isolates and *lasR*-negative isolates regarding protease production (*P* value=<0.001). Once more, there was a statistically significant difference between *lasR*-positive isolates and *lasR*-negative isolates regarding pyocyanin production (*P* value=0.035). Equally there was a statistically significant difference between

Table 3. The antibiotic susceptibility pattern of *P. aeruginosa* isolates

	Sensitive		Resistant	
	Number	Percentage %	Number	Percentage %
Piperacillin (PRL)	4	8%	46	92%
Piperacillin-tazobactam (TPZ)	23	46%	27	54%
Ceftazidime (CAZ)	3	6%	47	94%
Cefepime (FEP)	4	8%	46	92%
Aztreonam (ATM)	4	8%	46	92%
Imipenem (IMP)	21	42%	29	58%
Colistin (CT)	50	100%	0	0%
Polymyxin B (POB)	48	96%	2	4%
Gentamycin (CN)	8	16%	42	84%
Tobramycin (TOB)	13	26%	37	74%
Amikacin (AK)	8	16%	42	84%
Ciprofloxacin (CIP)	16	32%	34	68%
Levofloxacin (LEV)	19	8%	46	92%
MDR Yes No		46(92%) 4(8%)		

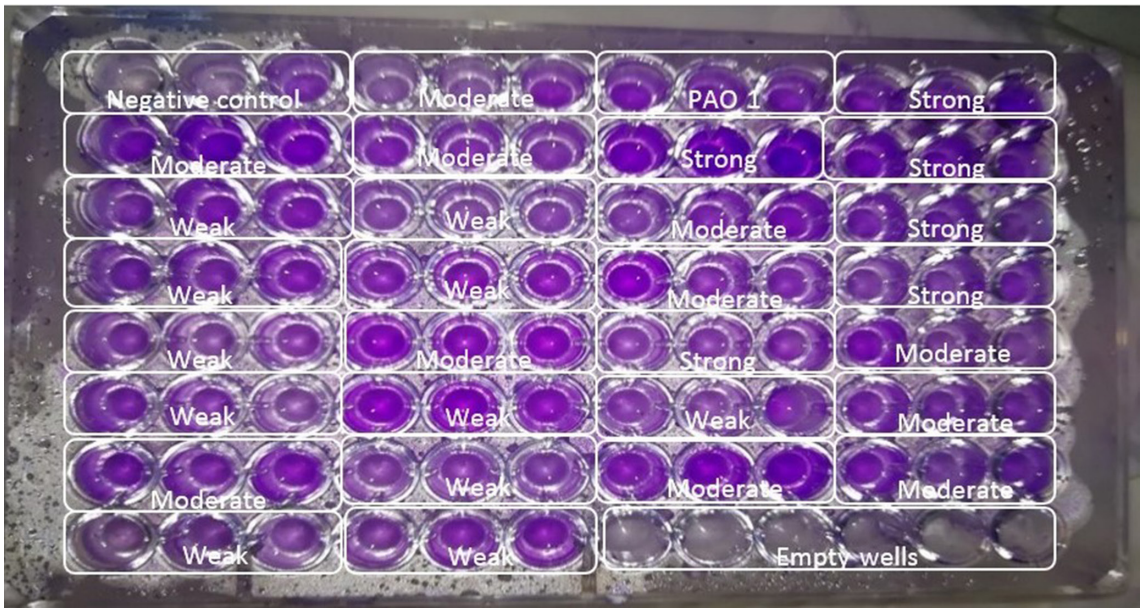

Fig. 3. Micro-titration plate showing different grades of *P. aeruginosa* biofilm.

lasR-positive isolates and *lasR*-negative isolates regarding rhamnolipid production (*P* value=0.011).

The *rhlR* gene was detected in 45 (90%) out of 50 *P. aeruginosa* isolates collected, while only five (10%) isolates did not harbour the gene, Fig. 9**.** There was no statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding biofilm production (weak, moderate and strong) (*P* value=0.163), Table 5. Identically, there was statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding protease production (*P* value=0.016). Dissimilarly, there was no statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding pyocyanin production (*P* value=0.321). There was a highly statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding rhamnolipid production (*P* value=<0.001).

Forty-nine (98%) isolates displayed the QS-proficient phenotype and one (2%) isolate was found to be lacking all tested virulence factors. Variable production of virulence factors is shown in Table 6.

DISCUSSION

P. aeruginosa is an opportunistic human pathogen capable of causing a wide array of extracellular virulence factors besides antibiotic resistance that continues to evolve [16]. These virulence factors regulated by QS help bacteria evade the host immune system. Strategies for QS disruption have become an attractive target for the development of new therapeutic measures.

According to our antibiogram results, the antibiotic susceptibility pattern of isolated *P. aeruginosa* showed the highest sensitivity of *P. aeruginosa* isolates was to colistin (100%) followed by polymyxin B (96%) and the least sensitivity was recorded against ceftazidime (6%). These results showed that *P. aeruginosa* has become more resistant to drugs that were thought to be efficient previously. This was in accordance with another study carried out by Choudhary *et al.* [17] that revealed the resistance of *P. aeruginosa* was alarmingly high, where the sensitivity for piperacillin-tazobactam was

Fig. 4. Positive protease on skimmed milk agar.

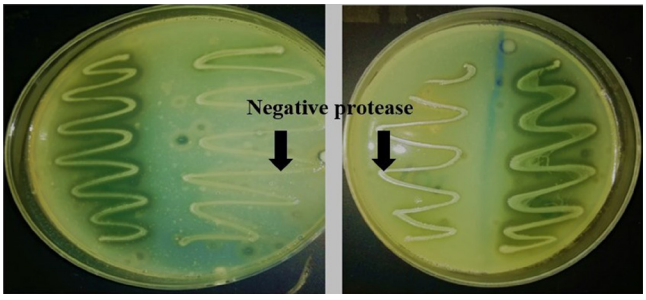

Fig. 5. Negative protease on skimmed milk agar.

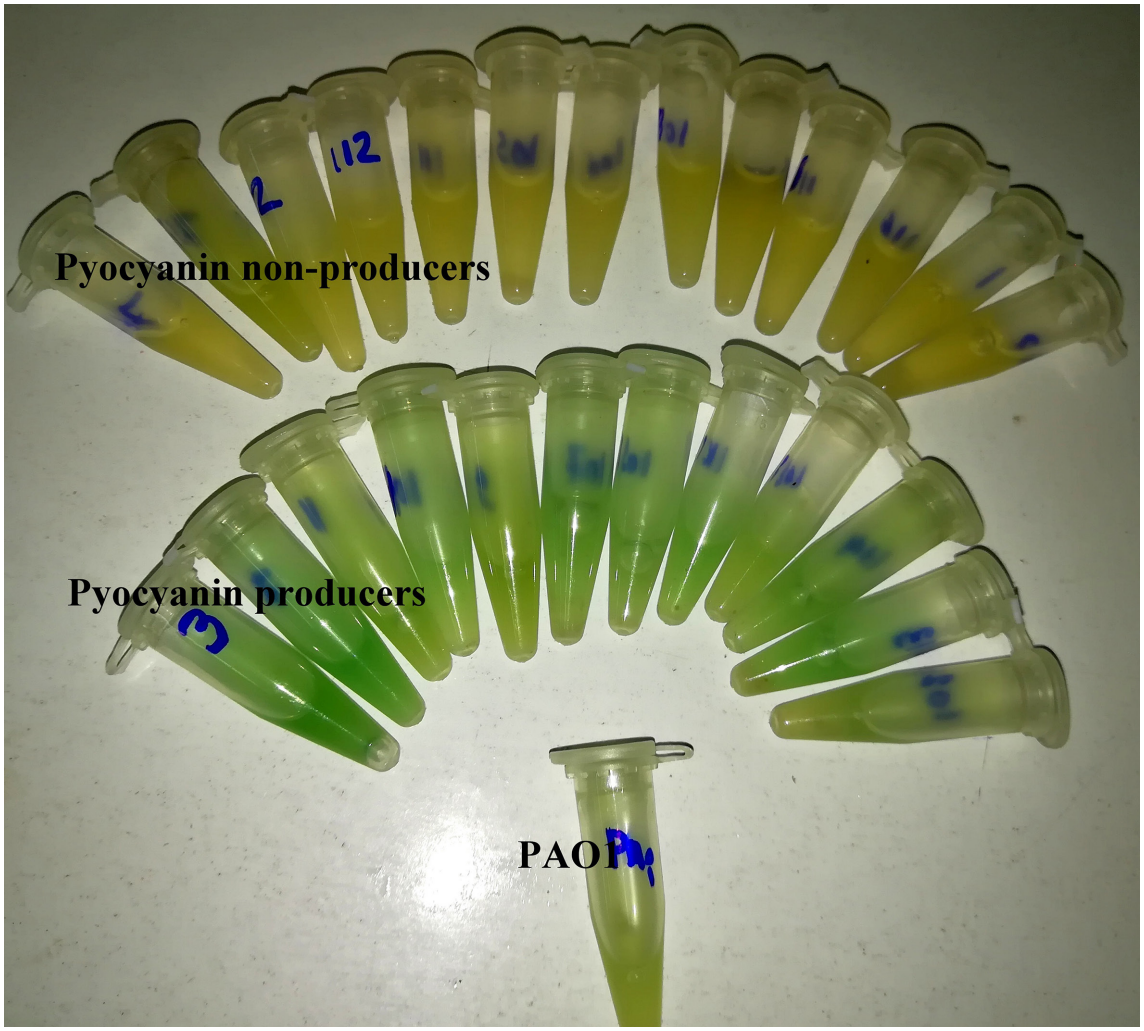

Fig. 6. Pyocyanin pigment detection.

38.9%, amikacin 25%, imipenem 23.4%, meropenem 19%, aztreonam 19.4%, ceftazidime 8.8% and for cefepime 8.3%. In disagreement to this study, another study by Revathi *et al.* [18] reported *P. aeruginosa* isolates were susceptible to commonly used antibiotics. This may be due to increased emergency of resistant *Pseudomonas* strains due to increased use of antibiotics to treat infections.

In this study, *P. aeruginosa* isolates showed biofilm-forming capacity. Out of 50 *P. aeruginosa* isolates, 48 (96%) isolates were biofilm producers [7 (14%) isolates were strong biofilm producers, 19 (38%) were moderate biofilm producers and 22 (44%) were weak biofilm producers], whereas two (4%) isolates were non-biofilm producers. Our results were consistent with Jabalameli *et al.* [19] who reported a high percentage of *P. aeruginosa* biofilm formers, in which positive *P. aeruginosa* isolates for biofilm were about 96% [47, 26 and 22.9% were strong, moderate and weak biofilm producers, respectively]. In disagreement with our results, Heydari and Eftekhar reported biofilm formation in only 43.5% of *P. aeruginosa* isolates (66.7% strong and 33.3% weak biofilm formers) [20].

Proteases enzymes participate significantly in the *P. aeruginosa* pathogenesis; degrade host tissues and enhance the bacterial growth and invasiveness in burnt patients [21]. In the current study, 50 *P. aeruginosa* isolates were screened for total protease production; 43 (86%) isolates were positive protease while seven (14%) isolates were negative protease. Our results were in agreement with Khalil *et al.* who reported a high percentage (95%) of protease production in *P. aeruginosa* isolates from burn wounds [22]. This promotes the opinion that says in acute *P. aeruginosa* infections, protease activity is seen more and reduces when infection become chronic [23].

Pyocyanin has the ability to arrest different micro-organism's electron transport chain and to exhibit a wide range of cell damage and antimicrobial activity [24]. We assessed the ability of *P. aeruginosa* isolates to produce pyocyanin and we detected a high rate of pyocyanin production, where 33

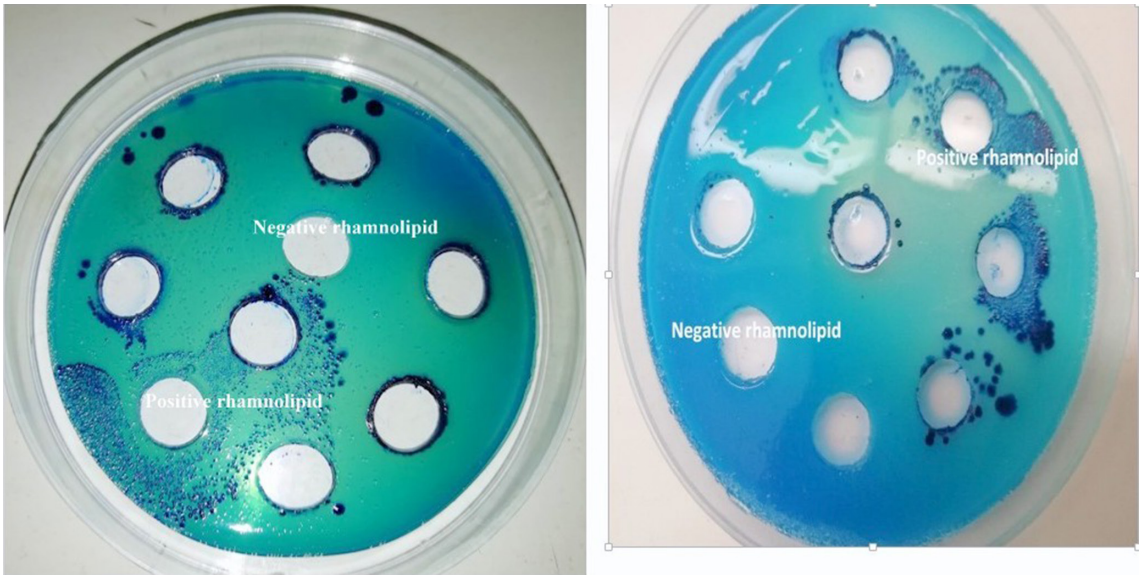

Fig. 7. Qualitative detection of rhamnolipid produced by *P. aeruginosa* isolates using cetyl trimethyl ammonium bromide (CTAB) assay method.

(66%) isolates were positive pyocyanin producers and 17 (34%) isolates were negative pyocyanin producers. This result corresponds to the study conducted by Khadim and Marjani [25] who proved high pyocyanin production in 35% of *P. aeruginosa* isolates of burn patients.

Rhamnolipids influence the biofilm architecture [26]. Rhamnolipid was found positive in 38 (76%) of the *P. aeruginosa* isolates in our study. This is similar to a study done by Khalil *et al.* [22] who documented 80% positive rhamnolipid in *P. aeruginosa* isolates collected from burn patients.

Las system is involved in early stages of biofilm formation where O'Toole and Kolter [27] found that mutation in the las synthase gene resulted in defective, uniform, flat and undifferentiated biofilms. The rhl system shares biofilm maturation through deposition of rhamnolipid [28]. Protease and elastase

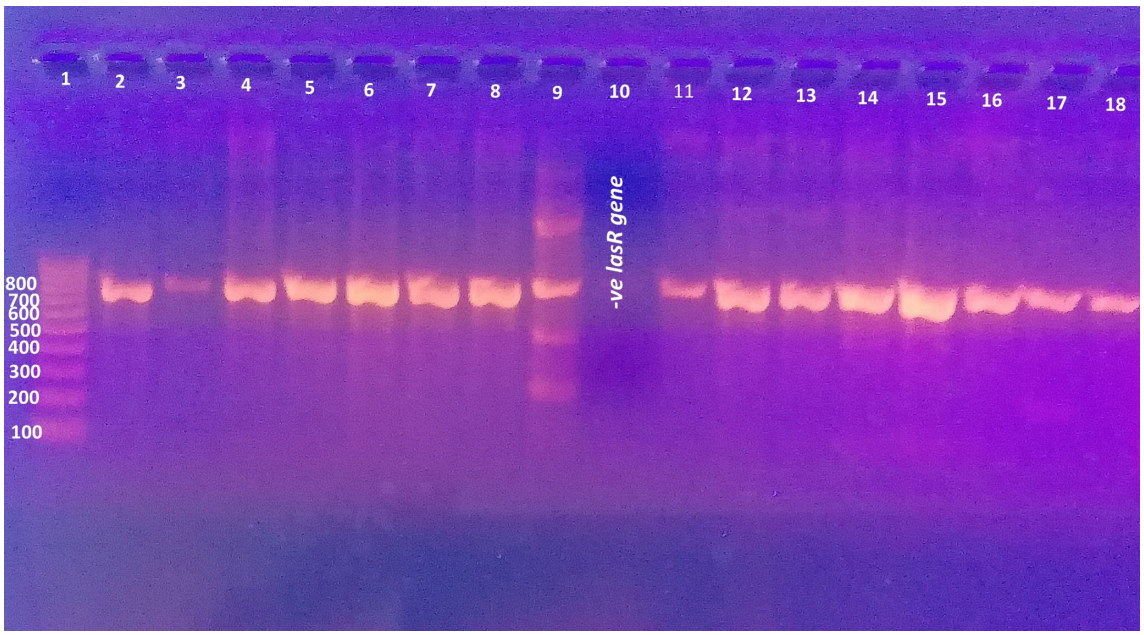

Fig. 8. Agarose gel electrophoresis for *lasR* amplicons in *P. aeruginosa* isolates.

Table 4. *LasR* gene and biofilm formation in *P. aeruginosa* isolates

	Positive *lasR* gene (*n*=47)		Negative *lasR* gene (*n*=3)		*P* value
	No	%	No	%	
Biofilm formation					**0.031***
Non-biofilm former	1	2.1	1	33.3	
Weak-biofilm former	20	42.6	2	66.7	
Moderate-biofilm former	19	40.4	0	0.0	
Strong-biofilm former	7	14.9	0	0.0	

are regulated by the las system, however pyocyanin and rhamnolipid are regulated by the rhl system [29].

Deficient *lasR* strains were found to be less virulent [30]. In our study of QS *lasR* and *rhlR* genes, the *lasR* gene was detected in 94% of *P. aeruginosa* isolates. This result is consistent with El-Khashaab *et al.* [31] who reported 94.3% of *P. aeruginosa* isolates that were positive for the *lasR* gene.

In the current study, all biofilm-producer isolates were found to harbour the *lasR* gene with statistically significant difference between *lasR*-positive isolates and *lasR*-negative isolates regarding biofilm production (*P* value=0.031). This is similar to data published by *Li et al.* [32].

C12-HSL/*lasR* regulates the production of different virulence factors including elastase (*lasB*), *toxA* and *lasA* protease [33]. Our results revealed a highly statistically significant difference between *lasR*-positive isolates and *lasR*-negative isolates regarding protease production (*P* value=<0.001).

Certainly, pyocyanin secretion is under the control of the rhl systems. As well, PQS controls rhl-dependent virulence-factor production such as pyocyanin [34], as regards *lasR*, the study done by Abou shleib and his colleagues found that there was no statistical significant value between pyocyanin production and the studied *lasR* gene [35]. In contrast, in our study we found a statistically significant difference between *lasR*-positive isolates and *lasR*-negative isolates regarding pyocyanin production (*P* value=0.035).

Rhamnolipid is under rhl system control as well as PQS [34]. We found a statistically significant difference between *lasR*-positive isolates and *lasR*-negative isolates regarding rhamnolipid production (*P* value=0.011). This is in agreement with a study done by Henkel *et al.* [36] who reported a role of AHL in regulating rhamnolipid production. In addition, las system controls the rhl system.

In this study, there was no statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding biofilm production (*P* value=0.163), which is dissimilar to the study conducted by Li *et al.* [32] who reported positive statistically significant value between *rhlR* expression and biofilm formation only on day 14 (*P*<0.05).

In this study, there was statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding protease production (*P* value=0.016). In agreement

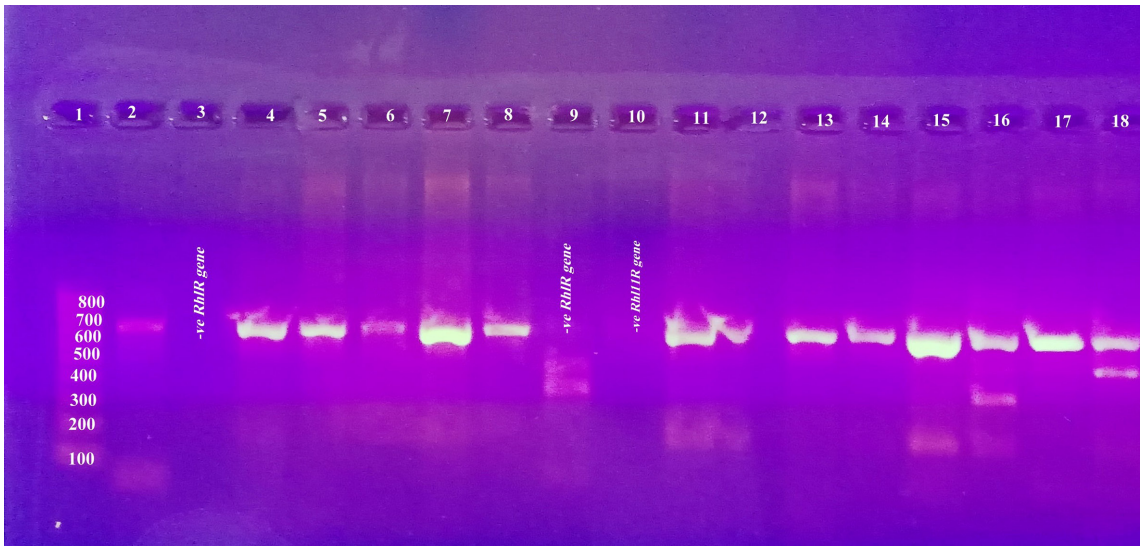

Fig. 9. Agarose gel electrophoresis for *rhlR* amplicons in *P. aeruginosa* isolates.

Table 5. *RhlR* gene and biofilm formation in *P. aeruginosa* isolates

Resistant	Positive *rhlR* gene (*n*=45)		Negative *rhlR* gene (*n*=5)		*P* value
	No	%	No	%	
Biofilm formation					0.163
Non-biofilm former	1	2.2	1	20.0	
Weak-biofilm former	19	42.2	3	60.0	
Moderate-biofilm former	18	40.0	1	20.0	
Strong-biofilm former	7	15.6	0	0.0	

to our results, Van Delden and Iglewski [37] reported that an intact rhl system is required for the restoration of elastase in the absence of *lasR*. But, we were unable to use this approach to understand what happened with the *rhlR*, *lasI* or *rhlI* mutant, since such mutants still produce enough elastase.

Pyocyanin is a rhl-related virulence factor. In many studies conducted by Lee and Zhang [34] and Reuter *et al.* [38], they used the production of pyocyanin by *P. aeruginosa* as an indicator for rhl and PQS system activity. In the present study there was no statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding pyocyanin production (*P* value=0.321).

Another exoproduct controlled primarily by *rhlR* is rhamnolipid where its production relies on several environmental and nutritional factors, including nitrogen and iron diminution, pH and temperature [39]. In our results there was a highly statistically significant difference between *rhlR*-positive isolates and *rhlR*-negative isolates regarding rhamnolipid production (*P* value=<0.001).

Table 6. Virulence factors, QS *lasR* and *rhlR* genes in *P. aeruginosa* isolates

	Virulence factors				QS gene	
No. of isolates	**Biofilm**	**Total protease**	**Pyocyanin**	**Rhamnolipid**	***LasR* gene**	***RhlR* gene**
8	Weak producer	+	+	+	+	+
12	Moderate producer	+	+	+	+	+
5	Strong producer	+	+	+	+	+
2	Weak producer	–	+	+	+	+
1	Weak producer	+	+	–	+	+
1	Weak producer	+	+	–	+	–
1	Moderate producer	+	+	–	+	+
1	Moderate producer	+	+	–	+	–
1	Strong producer	+	+	–	+	+
1	Non-biofilm producer	+	+	–	+	+
2	Weak producer	+	–	–	+	+
1	Moderate producer	+	–	–	+	+
5	Weak producer	+	–	+	+	+
4	Moderate producer	+	–	+	+	+
1	Weak producer	–	–	+	+	+
1	Strong producer	–	–	+	+	+
2	Weak producer	–	–	–	–	–
1	Non-biofilm producer	–	–	–	–	–
Total=50						

Schaber *et al.* [40] reported one QS-deficient clinical isolate that caused a wound infection even in the absence of all tested virulence factors. QS-dependent phenotypes may be positive for all four QS genes as observed by Karatuna and Yagci [41]. However, Schaber and colleagues isolated *P. aeruginosa* strain with no elastase or pyocyanin activity, although having all QS genes [40].

Our study has some limitations, such as small sample size for survey, which can be validated by further large-scale studies. Methodology-related limitations included the swab sampling, which could be replaced by wound biopsy to provide definite evidence of burn-wound infection, and studying of some phenotypic virulence factors and only 2 QS genes ignoring the other genes.

CONCLUSIONS

QS affects *P. aeruginosa* virulence-factor production and biofilm in burn wounds. QS-deficient isolates failed to amplify *lasR* and *rhlR* genes however it is still capable of causing infections by other mechanisms. Isolates containing *lasR* and *rhlR* seem to be a crucial regulator of virulence factors and biofilm formation in *P. aeruginosa* whereas the *lasR* gene positively regulates biofilm formation, proteolytic activity, pyocyanin production and rhamnolipid biosurfactant synthesis. The QS regulatory *RhlR* gene affects protease and rhamnolipid production positively.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The protocol of this study was accepted by Institutional Review Board (IRB), Faculty of Medicine, Mansoura University; code number: R/16.12.81.

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