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Original article

Insight into quorum sensing genes *lasr* and *rhlr*, their related virulence factors and antibiotic resistance pattern in *Pseudomonas aeruginosa* isolated from ocular infections

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ABSTRACT

Background: *Pseudomonas aeruginosa* can cause several ocular infections that are associated with adverse outcomes, owing to the production of different virulence factors that are regulated by quorum-sensing (QS) mechanisms. The current study aims to detect different QS dependent virulence factors, antibiotic resistance patterns in *P. aeruginosa* associated with ocular infections to correlate them with QS genes and to assess their impact on visual outcome. **Methods:** several ocular specimens were collected for isolation of *P. aeruginosa***.** Antibiotic susceptibility was evaluated**.** The presence of QS genes (*rhlR* and *lasR)* was identified by PCR**.** The isolates were assessed for their capability to produce virulence factors such as pyocyanin, protease, twitching motility, exopolysaccharides, and biofilm. **Results**: Out of 55 *P. aeruginosa* isolates, 38.2% were MDR, 29.1% were XDR, and 12.7% were PDR. The highest sensitivity was to meropenem 67.3% while the lowest sensitivity was to ceftazidime18.2%. The frequency of studied virulence factors, exopolysaccharides production, biofilm formation, twitching motility, protease, and pyocyanin production was 94.5%, 89.1%, 83.6%, 81.8%, and 78.2% respectively. Quorum-sensing genes *lasR* and *rhlR* were identified in 89% and 81.8% of the isolates respectively. The assessed virulence factors and antibiotic resistance pattern were significantly correlated with the presence of QS system. **Conclusion**: This study emphasized the importance of the QS system in regulating the formation of virulence factors and antimicrobial resistance in *P. aeruginosa*. Consequently, anti-QS and anti-virulence therapy can be a promising substitute to antibiotic therapy currently available.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is the most frequent Gram-negative organism accused in severe ocular infections including endophthalmitis, infectious keratitis and conjunctivitis [1,2], which mostly caused by an exogenous source of infection as *P. aeruginosa* is not a part of the normal bacterial flora [3].

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Endophthalmitis is a catastrophic vision threatening complication to both patients and ophthalmic surgeons [4,5]. Two forms of endophthalmitis coexist, the first one is the endogenous form that usually occurs in immunocompromised patients associated with systemic infections and the second one is the exogenous form that can occur following ocular surgeries especially cataract and glaucoma surgeries, intravitreal injection, and penetrating ocular trauma [6].

Pseudomonas keratitis is another devastating ocular infection that mostly occurs in contact lens wearers and following ocular trauma, it can rapidly progress to corneal perforation and melting with subsequent visual loss, therefore, early diagnosis and treatment is crucial in such cases [7]. Moreover, *P. aeruginosa* conjunctivitis is another form of serious ocular infection, as *P. aeruginosa* does not normally exist on the ocular surface, cases of *P. aeruginosa* conjunctivitis usually develop in patients with artificial devices in the eye [8,9].

Different virulence factors work together to determine the pathogenesis and fate of *P. aeruginosa* ocular infection including its ability to adapt to different environmental conditions, to produce variable virulence factors as pyocyanin, protease, and biofilm formation, along with development of multi drug resistance (MDR), extensive drug resistance (XDR) and pan drug resistance (PDR). All these factors contribute to extensive damage to the eye, decreasing the efficacy of antibiotic use with subsequent loss of vision [10,11].

Quorum sensing (QS) plays an important role in cell-cell communication, information transmission, interaction, and control of the production of different virulence factors through what are called auto-inducers [12]. *las and rhl* are the two main components of the pathways involved in regulation of QS in *P. aeruginosa* which are composed of auto-inducer synthases (*lasI and rhlI*, respectively) and their cognate transcriptional regulators (*lasR and rhlR*, respectively). Expression of multiple virulence factors is attributed to these systems [13]. The study was designed to detect different QS virulence factors, antibiotic resistance patterns in *P. aeruginosa* associated with ocular infections to correlate them with QS genes, and to assess their impact on visual outcome.

Methods

Study setting and study subjects

This cross-sectional study was carried out at the Medical Microbiology and Immunology Department in collaboration with Ophthalmology Department, Faculty of Medicine, Tanta University over nine months duration from April 2022 to December 2022. Prior to commencing the study, ethical clearance was obtained from Tanta University's Research Ethics Committee faculty of medicine (approval code 35417/4/22). This study was performed in accordance with The Declaration of Helsinki guidelines. All cases who had symptoms and signs of ocular infections with culture-confirmed *P. aeruginosa* infections (Conjunctivitis, keratitis, endophthalmitis) were enrolled in this research. Written informed consent was attained from each participant in the study.

Sample collection

The type of sample was collected from each patient according to the type of the clinical infection. A 23-G pars plana vitrectomy (PPV) was performed by a single experienced surgeon (AEN). After creation of vitrectomy ports, aspiration of the vitreous fluid (at least 50 ul) was performed using a vitrectomy cutter either while turning off the infusion cannula or under air to maintain suitable pressure in the eye. Corneal scraping was performed under topical anesthesia at the slit lamp. Instruments like cotton tipped applicator, metallic kimura spatula or surgical blade were used. Before scraping, loose mucus, discharge or debris were removed to avoid contamination. Scraping should be directed at the edges and the base of the ulcer. Conjunctival sample was collected from the inferior fornix by using sterile cotton swabs. To be revised by ophthalmologist

Identification of *P. aeruginosa* isolates

The ocular specimens were cultivated on blood, MacConkey's agar, and nutrient agar plates (Oxoid UK) and then incubated at 37◦C for 24 h aerobically. The presumed colonies of *P. aeruginosa* were studied according standard microbiological and biochemical tests by being glucose and lactose non fermenter, oxidase, catalase positive, and along with the distinctive fruity [14]. The isolates were confirmed by the automatic VITEK 2 (BioMérieux, Marcy l'Étoile, France). *Pseudomonas aeruginosa* strains were stored at -80 °C in brain heart infusion broth containing 20% glycerol and sub-cultured prior testing.

Antibiotic susceptibility testing

The standardized Kirby-Bauer disc-diffusion method was done on Mueller-Hinton agar plate (Oxoid, UK). As per clinical and laboratory standards institute (CLSI) guidelines [15]. The used antibiotics were (Oxoid UK); tobramycin (10µg) amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), imipenem (10 µg), piperacillin/tazobactam (100 µg /10 µg), cefepime (30 µg), aztreonam (30 µg), levofloxacin (5 µg), ceftazidime (30 µg), meropenem (10 µg). Multi-drug resistance was interpreted if *P. aeruginosa* isolate was resistant to at least one agent in three or more antibiotic classes, while XDR phenotype was characterized if the strain was resistant to at least one agent in every antimicrobial category except two or fewer (i.e., only one or two antimicrobials remain active against the bacteria). Pan drug resistant is categorized if the *P. aeruginosa* isolates were resistant to all agents in all antimicrobial classes [16].

Extracellular protease activity

Protease production was assessed by streaking *P. aeruginosa* isolate onto skim milk agar plates (25% nutrient broth (Oxoid UK), 10% defatted milk granules, and 2% agar), and incubated at 37 °C for 24 h. The presence of a clear zone around the colonies was indicative of proteolytic activity [11].

Motility assay

The motility was evaluated as described by **Kus et al.** each isolate was stabbed to the bottom 1% Luria–Bertani agar plate (Tryptone, yeast extract, sodium chloride and agar) (Oxoid UK) using a sterile toothpick and then after overnight incubation at 37°C. Twitching motility was assessed by the occurrence of a hazy zone around the point of inoculation [17].

Pyocyanin pigment production

Pyocyanin production was determined qualitatively by inoculation of *P. aeruginosa* in modified *Pseudomonas* broth medium that contain the following ingredients (4 g of *Pseudomonas* broth (Oxoid, UK), 20 g of dextrose, 1.4 g of magnesium Chloride, 20 g of peptone, 50 ml of glycerol, and 10 g of potassium sulfate) incubation for 2–3 days at 35 ◦C with 150 *rpm* shaking. The alteration in color of the pigment to blush green was indicative for pigment production [18].

Exopolysaccharide production assay

Pseudomonas aeruginosa isolate's capacity to produce exopolysaccharides was evaluated as described by **Nassar et al.,** *P. aeruginosa* isolates were streaked onto Congo red agar (Brain heart infusion broth (37 gm/l), sucrose (5 gm/l), agar (10 gm/l) and Congo red dye (0.8 gm/l). (Sigma - Aldrich Co., USA) and then incubated at 37°C for 24 hours. Exopolysaccharide-producing strains formed black colonies, while non-exopolysaccharide-producing isolates yielded red colonies [19].

Biofilm detection

The ability of *P. aeruginosa* to develop biofilm was evaluated using tissue culture plate method as previously explained by **Stepanovic et al.** [20] Briefly, tryptic soy broth (Oxoid UK) containing 1% glucose (TSBG) was inoculated with the tested bacteria and incubated for 24 hours at 37 ◦C. Then, the suspension of each isolate was standardized to 0.2 at OD600nm with TSBG medium and transferred to sterile flat-bottomed 96-well microtiter plates. Following overnight incubation at 37 ◦C. Each well was aspirated and washed three times using phosphate buffered saline (200 µl) to get rid of planktonic non adherent bacteria, the wells were dried in air. Absolute methanol was added to fix the adhered biofilms for 15 minutes. Methanol was then removed, and the fixed biofilm was stained for 10-15 minutes with of 1% crystal violet (Sigma -Aldrich Co., USA). The plate was rinsed with water and left to air dry. 2 mL of 95% ethyl alcohol was added to solubilize the biofilm. Wells containing only growth medium without culture inoculation were used as negative controls. The optical density (OD) was measured at 570 nm with a microtiter plate reader (BioTek, USA). The isolates were categorized as non-biofilm producer (no variation in OD over the control), weak biofilm producer (up to a 2-fold change), moderate biofilm producer (up to 4-fold change), or strong biofilm producer (more than 4-fold change). As described by **Di Domenico et al.** [21]

Molecular detection of quorum-sensing genes *lasR* and *rhlR*

Quorum-sensing genes *lasR* and *rhlR* were detected by PCR as described by **Zhu et al.,** using a panel of primer *lasR* -F 5'- AAGTGGAAAATTGGAGTGGAG -3'; *lasR* -R 5'- GTAGTTGCCGACGACGATGAAG-3' and *rhlR* -F 5'- TGCATTTTATCGATCAGGGC -3'; *rhlR* -R 5'- CACTTCCTTTTCCAGGACG-3' [22]. Primers were supplied by (Invitrogen Thermofisher Scientific). The genomic DNA of *P. aeruginosa* isolates were extracted using a DNA extraction kit (QIAprep Spin Miniprep Kit) according to the

manufacturer's instructions. The PCR amplification was performed in a thermocycler. The reaction mixtures were prepared in a total volume of 25µl. Amplification cycles parameters were denaturation at 94°C for 1 minute, primers annealing at 52°C for 1 minute, and then primer extension at 72°C for 1.5 minutes, for 30 cycles [22]. Amplified DNA was detected by agarose gel electrophoresis. PCR products were compared to 100bp DNA ladder markers (Euroclone, UK catalog number EMR814100) to determine their molecular size.

Statistical analysis

Data processing and analysis were done using IBM SPSS Statistics for Windows, Version 25.0. (IBM Corp, 2017). Qualitative data were presented as numbers and percentage of total. Chi-Square test was used for comparing categorical data between 2 groups. Fisher's Exact Test was used to compare categorical data between 2 groups when the expected count is less than 5. *p* value ≤ 0.05 was considered statistically significant.

Results

The present study included 55 cases of microbiologically confirmed *P. aeruginosa* ocular infections: 30 cases of endophthalmitis (20 post traumatic, 8 post operative and 2 following intravitreal injection), 20 cases of *pseudomonas* keratitis and 5 cases of *pseudomonas* conjunctivitis. There was a male gender predominance representing 56,4% of cases. The mean ± SD of the best corrected visual acuity (BCVA) by log MAR was 1.4 ± 0.4. Evisceration was performed in 14.5% of cases. The baseline demographic and clinical data was illustrated in **table (1)**.

Antibiotic sensitivity tests were done to all *P. aeruginosa* isolates, the highest sensitivity was detected to meropenem followed by piperacillin-tazobactam, imipenem, amikacin, levofloxacin, tobramycin, gentamicin, aztreonam, ciprofloxacin and cefepime (67.3%, 61.8%, 60%, 45.5%, 43.6%, 43.6%, 41.8%, 41.8%, 36.4 and 25.5% respectively) while the lowest sensitivity was reported to ceftazidime (18.2%). Most of the isolates were found to be MDR representing 38.2% as shown in **table (2)**. Also, the heat map was conducted to show the distribution of antibiotics resistance in all *P. aeruginosa* isolates as shown in **figure (1a)**.

Regarding the ability of the isolates to produce different virulence factors, most of them were biofilm producers, motile and had proteolytic activity representing 89.1%, 83.6% and 81.8% respectively. Pyocyanin and exopolysaccharides production were detected in 78.2% and 94.5% respectively as illustrated in **table (3)** and **figure (1b)**.

Tables 4, 5 and **(Figures 1c,2,3)** show the prevalence of *lasR* and *rhlR* genes by PCR, *lasR* was detected in 49 out of 55 PA isolates while 45 isolates harbored *rhlR* genes. There was statistically significant difference (*p* value <0.001) between *lasR* positive and negative and *rhlR* positive and negative isolates as regarding motility, biofilm, protease, pyocyanin production and drug resistance. Moreover, exopolysaccharides production was statistically significant different between *lasR* positive and negative and *rhlR* positive and negative isolates (*p* value was 0.001 and 0.005 respectively)

Table 6 shows the impact of different virulence factors, *lasR* gene and *rhIR* gene on the visual outcome in the studied patients. It was reported that production of these factors including biofilm, pyocyanin, motility, protease and exopolysaccharide was associated with worse visual outcomes with recorded statistical significance (*p* value of 0.001 regarding biofilm and <0.001 concerning other factors). In addition, the presence of *lasR* and *rhIR* genes resulted in poorer BCVA with *p* value <0.001.

Table 1. Demographic data of studied patients.

Variables	**N (%) N =55 / Mean ± SD**
Age median (Min – Max)	41(6-95)
Sex	
Male	31(56.4)
Female	24(43.6)
BCVA	1.4 ± 0.4
Etiology	
Post endophthalmitis	
Post traumatic.	20 (36.4)
Post operative.	8 (14.5)
Post intravitreal injection.	2 (3.6)
Pseudomonas keratitis	20 (36.4)
Conjunctivitis	5 (9.1)

BCVA: Best Corrected Visual Acuity.

Table 2. Antibiotics susceptibility pattern in the isolated *P. aeruginosa***.**

Antibiotic	**Resistance**	**Sensitive**
	N (%) N =55	
Imipenem	22 (40)	33(60)
Meropenem	18(32.7)	37(67.3)
Amikacin	30(54.5)	25(45.5)
Ceftazidime	45(81.8)	10(18.2)
Ciprofloxacin	35(63.6)	20(36.4)
Aztreonam	32(58.2)	23(41.8)
piperacillin/Tazobactam	21(3.2)	34(61.8)
Gentamycin	32(58.2)	23(41.8)
Cefepime	41(74.5)	14(25.5)
Levofloxacin	31(56.4)	24(43.6)
Tobramycin	31(56.4)	24(43.6)
Drug resistance		
Non MDR	11(20)	
PDR	7(12.7)	
MDR	21(38.2)	
XDR	16(29.1)	

Table 3. Different virulence factors detected in *P aeruginosa* isolates.

Virulence factors	**Positive**	**Negative**
	N (%) N =55	
Biofilm	49(89.1)	6(10.9)
Pyocyanin	43(78.2)	12(21.8)
Motility	46(83.6)	9(16.4)
Protease	45(81.8)	10(18.2)
Exopolysaccharides	52(94.5)	3(5.5)

Table 4. Correlation between virulence factors, antibiotics resistance and *LasR* gene in the isolated *P.aeruginosa.*

Factors	***LasR***		***p* value**
	Positive N= 49	**Negative N=6**	
	N (%)		
Biofilm (+ve)	48(98)	1(16.7)	<0.001
Pyocyanin (+ve)	43(87.8)	0	<0.001
Motility (+ve)	45(91.8)	1(16.7)	<0.001
Protease (+ve)	45(91.8)	0	<0.001
Exopolysaccharides (+ve)	49(100)	3(50)	0.001
Drug resistance			
Non MDR	6(12.2)	5(83.3)	<0.001
PDR	6(12.2)	1(16.7)	
MDR	21(42.9)	0	
XDR	16(32.7)	0	

+ve: positive

Table 5. Correlation between virulence factors, antibiotics resistance and *RhlR* gene in the isolated *P aeruginosa.*

Factors	***RhlR***		***p* value**
	Positive N= 45	**Negative N= 10**	
	N (%)		
Biofilm (+ve)	45(100)	4(40)	<0.001
Pyocyanin (+ve)	43(95.6)	0	<0.001
Motility (+ve)	44(97.8)	2(20)	<0.001
Protease (+ve)	43(95.6)	2(20)	<0.001
Exopolysaccharides (+ve)	45(100)	7(70)	0.005
Drug resistance			
Non MDR	4(8.9)	7(70)	<0.001
PDR	7(15.6)	0	
MDR	19(42.2)	2(20)	
XDR	15(33.3)	1(10)	

+ve: positive

Table 6. Comparison of BCVA according to virulence factors, *LasR* gene and *rhlR* gene in the studied patients.

Factors	BCVA Mean ± SD	*p* value
Biofilm		
Negative	0.9 ± 0.3	0.001
Positive	1.5 ± 0.4	
Pyocyanin		
Negative	0.9 ± 0.3	<0.001
Positive	1.6 ± 0.3	
Motility		
Negative	0.9 ± 0.2	<0.001
Positive	1.5 ± 0.3	
Protease		
Negative	0.87 ± 0.2	<0.001
Positive	1.6 ± 0.3	
Exopolysaccharides		
Negative	0.7 ± 0.2	<0.001
Positive	1.5 ± 0.4	
LAS R		
Negative	0.8 ± 0.2	<0.001
Positive	1.5 ± 0.4	
RhlR		
Negative	0.96 ± 0.3	<0.001
Positive	1.5 ± 0.4	

BCVA: Best Corrected Visual Acuity.

Figure 1. Heatmap of *P.aeruginosa* isolates (a): hierarchical clustering of antibiotic resistance where red indicating resistant and blue indicating sensitive. (b): hierarchical clustering of virulence factors where red indicating positive and blue indicating negative. (c): hierarchical clustering of LasR and RhIR genes where red indicating positive and blue indicating negative.

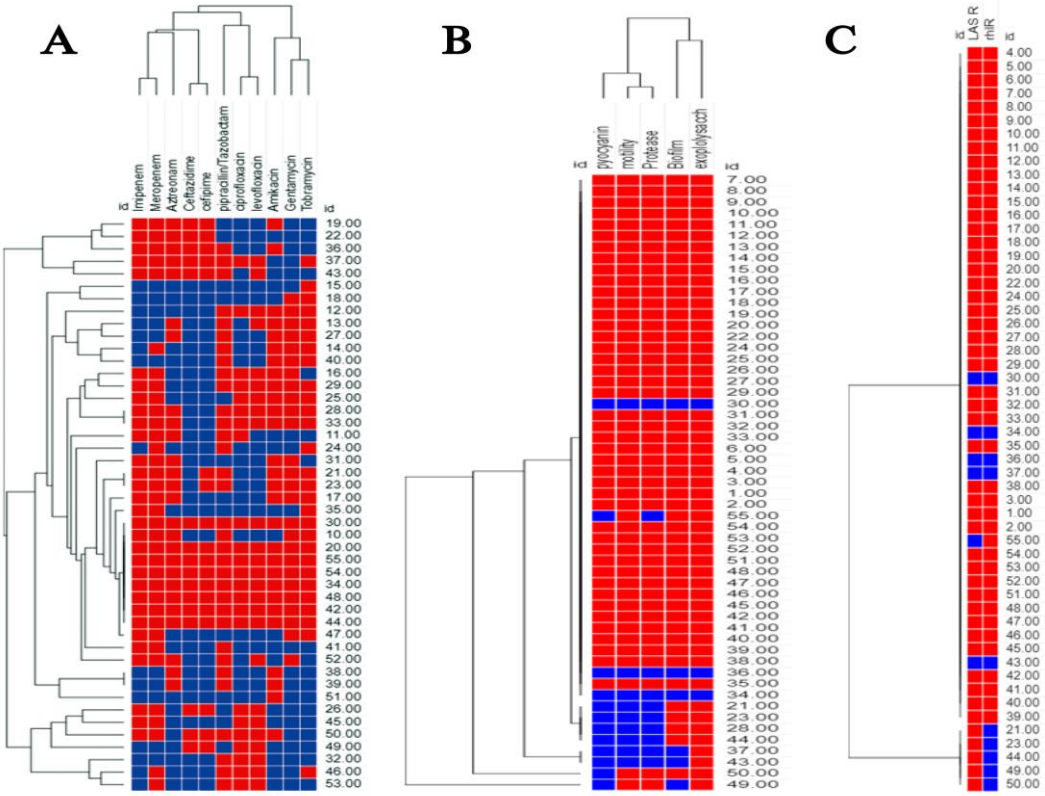


Figure 2. Agarose gel electrophoresis of LasR gene. lane(M): 100 bp DNA ladder. Lane (1,2,3,4,5,6,8,9) positive for LasR gene (130 bp).

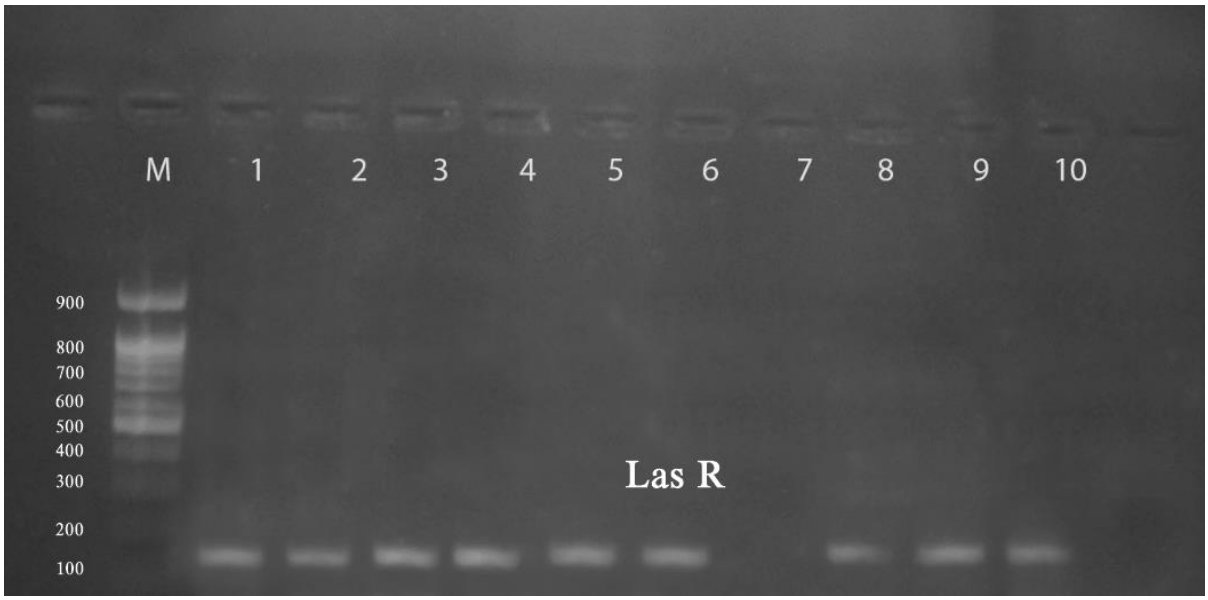
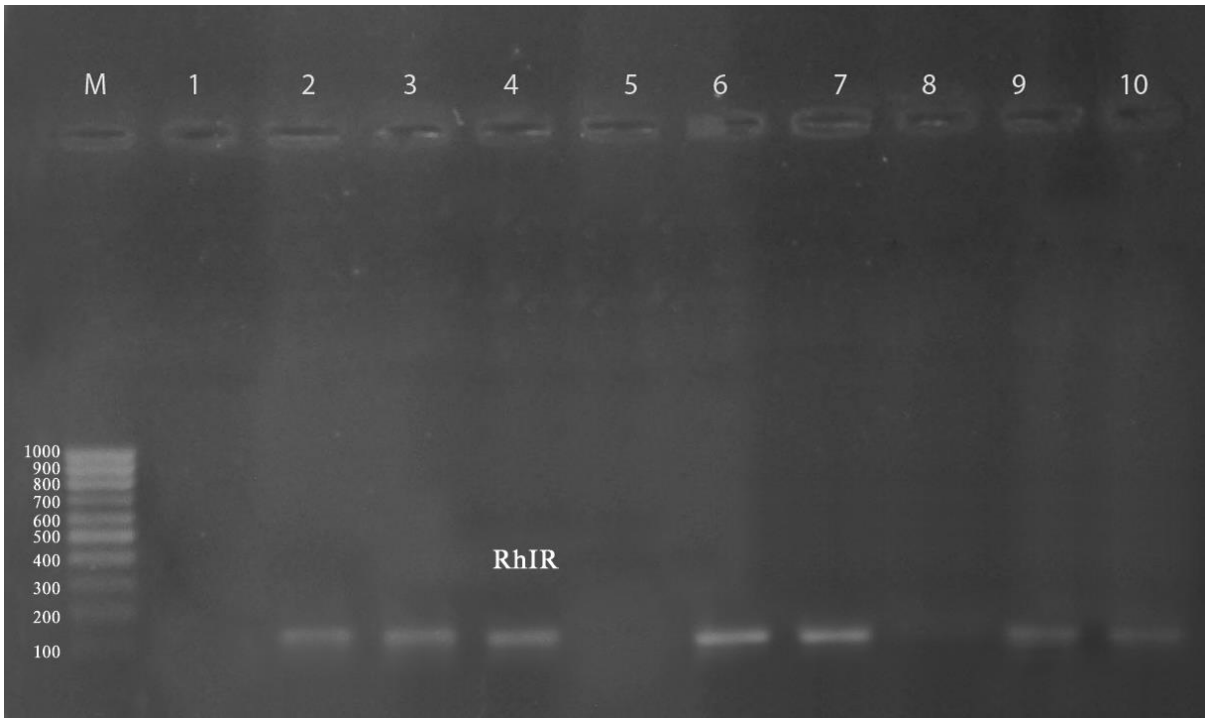


Figure 3. Agarose gel electrophoresis of RhIR gene. lane(M): 100 bp DNA ladder. Lane (2,3,4,6,7, 9,10) positive for RhIR gene (133bp)



Discussion

Pseudomonas aeruginosa is an opportunistic pathogen which can produce different virulence factors that are regulated by quorum sensing process and cause several ocular infections with adverse outcomes [23]. Identification of *P. aeruginosa* virulence factors is critical to recognize the pathogenesis of this pathogen and to explore novel antimicrobial policies in MDR strains [24]. In recent years, antibacterial strategies have been focusing on virulence traits as potential alternatives to antibiotics without triggering antibiotic resistance, signifying anti-QS agents can be used as an alternative to antibiotics and allow the host's immune system to clear the infection before it causes significant tissue damage [19]. Therefore, this study was designed to detect antibiotic

resistance patterns of *P. aeruginosa* isolated from different ocular infections, different QS virulence factors and to correlate them with QS genes, along with assessment of their impact on visual outcome.

The current study evaluated 55 cases of *P. aeruginosa* induced ocular infections that were divided into 30 cases of endophthalmitis, 20 cases of keratitis and 5 cases of conjunctivitis. Most *P. aeruginosa* endophthalmitis patients occurred on top of trauma, this is quite similar to **Lin J et al.** who detected trauma as the most frequent cause of *P. aeruginosa* endophthalmitis followed by corneal ulcers and ocular surgeries representing 41.7%, 25% and 13.9% respectively [25]. However, trauma was the least common cause of *P. aeruginosa* endophthalmitis in other studies [26,27]. Furthermore, other studies detected no cases of trauma in *P. aeruginosa* endophthalmitis [28,29]. The BCVA was markedly diminished, and evisceration was performed in 14.5% of enrolled cases. **Parchand et al.** performed a study on case series of *P. aeruginosa* endophthalmitis following cataract surgery with similar poor visual outcome, only 14.5% of these cases achieved BCVA better than 20/200, in contrast, a higher rate of evisceration 30.6% was reported [30]. Other studies reported similar or slightly higher rates of evisceration [31,32]. The poor visual outcome of *P. aeruginosa* infections is attributed to its ability to produce multiple toxins and proteolytic enzymes that increase its invasion capability with subsequent cellular destruction. In addition, the presence of glycocalyx and beta-lactamase production by *P. aeruginosa* renders it highly resistant to antibiotics [33,34].

In our study, the antibiogram of *P. aeruginosa* isolates showed the highest sensitivity to meropenem (67.3%) while ceftazidime exhibited the highest resistance (81.8%). This is nearly comparable with previous studies carried out by **Elnegery et al.** and **Choudhary et al.** in which *P. aeruginosa* isolates were resistant to the most frequently used antibiotics which were effective previously with the lowest susceptibility to ceftazidime (6%) [11,35]. Besides, many other studies detected increased resistance of *P. aeruginosa* isolates against different antibiotics used [36-38].

The emergence of MDR, XDR and PDR *P. aeruginosa* is considered a major health care obstacle with subsequent difficulty in the treatment with poor outcome [39]. In the current study, 80% of the isolates were either MDR, XDR and PDR. This is comparable to the resistance rates reported by **Rodulfo et al, Gonçalves et al,** and **El-Mahdy et al** representing (71.9%, 73.9% and 72.5% respectively) [40-42]. However, in other studies performed by **Hashem et al.** and **Sonbol et al.,** lower resistance rates were detected [43,44]. The higher resistance rate in our study may be probably due to the widespread use of broad-spectrum antibiotics in the treatment of different types of infections. So, selection of proper antibiotic regimen is necessary to manage *P. aeruginosa* infection according to the site of infection [45].

In the present study, Concerning the ability of *P. aeruginosa* isolates to produce different virulence factors, 89.1% of them were biofilm producers. This finding is in line with previous studies [11,42,46,47]. On the other hand, **Heydari et al.** documented that only 43.5% of *P. aeruginosa* isolates formed biofilms [48]. Interestingly, **Saffari et al.** and **Elhabibi et al.,** found that all tested isolates were biofilm producers [49,50]. There are several factors involved in the development of biofilms in *P. aeruginosa*, including culture conditions, the presence of genes implicated in biofilm formation, the rate at which genes are expressed and flagella-mediated motility [51].

Production of pyocyanin aids in both acute and chronic pseudomonas infections, by reducing the host-response and causing neutrophil apoptosis [52]. In the current study, among the tested *P. aeruginosa* isolates, 78.2% were pyocyanin-producers. On the contrary, **Nassar et al.,** and **Aboushleib et al**. found that 59.2% and 38% produced pyocyanin pigment [19,53]. While, **Kalaiarasan et al.** reported that the pyocyanin production was detected only in 14% of isolates [52].

Concerning the current study, 81.8% of *P. aeruginosa* isolates were able to produce protease enzyme, which is consistent with results from previous studies [11,54]. However, **Naik et al.** detected protease activity in 62.5% of *P. aeruginosa* isolates [55]. Twitching motility was observed in 83.6% of *P. aeruginosa* isolates which was in the same line with **El-Mahdy et al**. [42].

Regarding the molecular detection of *lasR* and *rhlR* genes by PCR in our study, *lasR* was detected in 89% of *P. aeruginosa* isolates while 81.8% of the isolates harbored *rhlR* gene. Our finding is coincident with that reported by **Elnegery et al.** [11] and **El-Khashaab et al.** [56]. On the other

hand, **El-Mahdy et al.** informed that *lasR* and *rhlR* genes were identified in all *P. aeruginosa* isolates [42]. However, **Aboushleib et al.** found that 40% of *P. aeruginosa* isolates were positive for the *lasR* gene and the *rhlR* gene was detected in 36% of isolates [53].

In the current study, there was statistically significant difference between *lasR* and *rhlR* positive and negative isolates as regarding motility, biofilm, protease, pyocyanin production, exopolysaccharides production as well as antibiotic resistance pattern. Our findings agree with **Sonbol et al.** who observed that *P. aeruginosa* isolates with a QS deficiency produced significantly less pyocyanin, protease, biofilm formation, and swimming motility [57]. Moreover, **Aboushleib et al.** reported that twitching motility and biofilm production were significantly correlated with the presence of *lasR* gene, while pyocyanin synthesis was not significantly correlated with the *lasR* gene [53]. Also, **Li et al.** reported that biofilm production was significantly associated with the presence of *lasR*-gene [58]. However, **Elnegery et al.,** found a significant correlation between the presence of *lasR*-gene and pyocyanin production but there was no statistically significant difference between the presence of *rhlR* gene and pyocyanin and biofilm production [11].

In the current research there was a statistically significant correlation between the presence of *lasR* and *rhlR* in *P. aeruginosa* isolates and antibiotic resistance pattern. Similarly, **Haque et al.** and **Jiang et al.** reported an association between the QS system and antibiotic resistance [59,60]. This may be attributed to biofilm formation and drug efflux pump which are regulated by QS system which are considered important mechanisms of antibiotics resistance in *P. aeruginosa* [61,62]. In contrast, **Crémet et al.** found that resistance to ceftazidime was strongly correlated with QS deficiency [63]. In addition, **Karatuna et al**. found that QS-deficient *P. aeruginosa* isolates tend to be less sensitive to antimicrobials [64]. While **El-Mahdy et al,** found that carbapenem resistance was not correlated with QS genes lasR and *rhlR* [42].

Regarding the present study, the presence of multiple virulence factors like motility and production of biofilm, pyocyanin, protease and exopolysaccharides in *P. aeruginosa* was associated with worse BCVA. The result of the current study agrees with **Karami et al.** who confirmed the presence of biofilm as an important factor in MDR and XDR *P. aeruginosa* strains [65]. Moreover, other studies reported worse visual prognosis in *P. aeruginosa* endophthalmitis with the previously mentioned virulence factors which also coincide with our study [55-67]. Concerning *P. aeruginosa* keratitis, a previous report confirmed the role of proteases produced by *P. aeruginosa* in impairment of the host connective tissue and immunological factors with subsequent visual loss [68].

To the best of our knowledge, this is the first study to correlate *las R* and *RhIR* genes with BCVA in PA ocular infections. According to this study, the existence of *las R* and *RhlR* genes was associated with worse visual outcomes denoting the importance of these two genes as determinants of virulence in *P. aeruginosa* ocular infections. In agreement with the current study performed by Storey *et al*. confirmed the role of *las R* gene in the expression of multiple virulence factors in the lungs of patients with cystic fibrosis [69], another study emphasized the importance of *Rh1R* gene and its connection to virulence [70]. Furthermore, a recent study documented the role of the two genes in *P. aeruginosa* virulence and resistance to antibiotics [71].

Conclusion

This study emphasized the importance of the QS system in regulating the formation of virulence factors and antimicrobial resistance in *P. aeruginosa*. Consequently, anti-QS and anti-virulence therapy can be a promising substitute to antibiotic therapy currently available.

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References

1-**Galvis V, Tello A, Guerra A, Acuña MF, Villarreal D.** Antibiotic susceptibility patterns of bacteria isolated from keratitis and intraocular infections at Fundación Oftalmológica de Santander (FOSCAL), Floridablanca. Colombia Biomedica 2014; 34(0):123–133.

2-**Summaiya M, Sangita R, Neeta K.** Ocular infections: rational approach to antibiotic therapy. Natl J Med Res 2012; 2:122–124.

3-**Kresloff MS, Castellarin AA, Zarbin MA.** Endophthalmitis. Surv Ophthalmol 1998;43(3):193-224.

4-**Ramappa M, Majji AB, Murthy SI, Balne PK, Nalamada S, Garudadri C, et al.** An outbreak of acute post-cataract surgery Pseudomonas sp. Endophthalmitis caused by contaminated hydrophilic intraocular lens solution. Ophthalmology. 2012;119(3):564-70.

5- **Pinna A, Usai D, Sechi LA, Zanetti S, Jesudasan NC, Thomas PA, et al.** An outbreak of post-cataract surgery endophthalmitis caused by Pseudomonas aeruginosa. Ophthalmology 2009;116(12):2321-6.

6-**Durand ML.** Endophthalmitis. Clinical Microbiology and Infection 2017; 19:227–234.

7-**Thibodeaux BA, Caballero AR, Marquart ME, Tommassen J, O'Callaghan RJ.** Corneal virulence of Pseudomonas aeruginosa elastase B and alkaline protease produced by Pseudomonas putida. Curr Eye Res 2007;32(4):373–86.

8-**Rich RM, Smiddy WE, Davis JL**. Infectious scleritis after retinal surgery. Am J Ophthalmol 2008, 145(4):695–699.

9-**Wirostko WJ, Covert DJ, Han DP, Connor TB Jr, Kim JE, Hammersley J, et al.** Microbiological spectrum of organisms isolated from explanted scleral buckles. Ophthalmic Surg Laser Imaging 2009, 40:201–202.

10-**Zhao H, Clevenger AL, Coburn PS, Callegan MC, Rybenkov VV.** Condensins are essential for *Pseudomonas aeruginosa* corneal virulence through their control of lifestyle and virulence programs. Molecular Microbiology 2022;117(4):937-57.

11-**Elnegery AA, Mowafy WK, Zahra TA, Abou El-Khier NT.** Study of quorum-sensing LasR and RhlR genes and their dependent virulence factors in Pseudomonas aeruginosa isolates from infected burn wounds. Access Microbiology 2021;3(3).

12- **Rutherford ST, Bassler BL.** Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2012;2:a012427

13-**Luo J, Kong J-L, Dong B Y, Huang H, Wang K, Wu LH, et al.** Baicalein attenuates the quorum sensing-controlled virulence factors of Pseudomonas aeruginosa and relieves the inflammatory response in P. aeruginosa-infected macrophages by downregulating the MAPK and NFκB signal-transduction pathways. Drug Des Devel Ther 2016; 10:183–20.

14-**Mahon C, Lehman D, Manuselis GJMEHS Jr.** Textbook of Diagnostic Microbiology Maryland Hieghts. Philadelphia: W.B. Saunders; 2014:249–303.

15-**Clinical and Laboratory Standards Institute**. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI Supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA. 2022.

16-**Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al**. Multidrug-resistant, extensively drug-resistant, and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection 2012;18(3):268-81.

17-**Kus JV, Tullis E, Cvitkovitch DG, Burrows LLJM.** Significant differences in type IV pilin allele distribution among Pseudomonas

aeruginosa isolates from cystic fibrosis (CF) versus non-CF patients. Microbiology 2004;150(5):1315–1326.

18-**Mullaiselvan I, Kanagaraj V, Dharmar B, Balaraman M, Meignanalakshmi S.** Production, characterization, and cytotoxic evaluation of pyocyanin pigment extracted from Pseudomonas aeruginosa Isolated from industrial soil resources. Int J Curr Microbiol App Sci 2020;9(3):2117-30.

19-**Nassar O, Desouky SE, El-Sherbiny GM, Abu-Elghait M.** Correlation between phenotypic virulence traits and antibiotic resistance in *Pseudomonas aeruginosa* clinical isolates. Microbial Pathogenesis 2022 ;162:105339.

20-**Stepanovic S, Vukovic D, Hola V, Bonaventura GD, Djukic S, Cirkovic I, et al.** Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 2007; 115(8):891–9

21-**Di Domenico EG, Toma L, Provot C, Ascenzioni F, Sperduti I, Prignano G, et al.** Development of an in vitro assay, based on the biofilm ring test®, for rapid profiling of biofilm-growing bacteria. Frontiers in Microbiology 2016; 7:1429.

22-**Zhu H, Bandara R, Conibear TC, Thuruthyil SJ, Rice SA, Kjelleberg S, et al.** *Pseudomonas aeruginosa* with lasI quorum-sensing deficiency during corneal infection. Investigative ophthalmology & visual science 2004;45(6):1897-903.

23-**Cocotl-Yañez M, Soto-Aceves MP, González-Valdez A, Servín-González L, Soberón-Chávez G.** Virulence factors regulation by the quorum-sensing and Rsm systems in the marine strain Pseudomonas aeruginosa ID4365, a natural mutant in lasR. FEMS Microbiology Letters 2020 ;367(12):fnaa092.

24-**Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M.** Bacterial profile of ocular infections: a systematic review. BMC ophthalmology 2017 Dec;17(1):1-9.

25-**Lin J, Huang S, Liu M, Lin L, Gu J, Duan F.** Endophthalmitis Caused by Pseudomonas aeruginosa: Clinical Characteristics, Outcomes, and Antibiotics Sensitivities. Journal of Ophthalmology 2022;14

26- **Eifrig CW, Scott IU, Flynn Jr HW, Miller D.** Endophthalmitis caused by Pseudomonas aeruginosa. Ophthalmology 2003;110(9):1714-7.

27- **Chen KJ, Sun MH, Lai CC, Wu WC, Chen TL, Kuo YH, et al.** Endophthalmitis caused by *Pseudomonas aeruginosa* in Taiwan. Retina 2011;31(6):1193-8.

28- **Falavarjani KG, Alemzadeh SA, Habibi A, Hadavandkhani A, Askari S, Pourhabibi A.** *Pseudomonas aeruginosa* endophthalmitis: clinical outcomes and antibiotic susceptibilities. Ocular Immunology and Inflammation 2017;25(3):377-81.

29- **Sridhar J, Kuriyan AE, Flynn Jr HW, Miller D.** Endophthalmitis caused by *Pseudomonas aeruginosa*: clinical features, antibiotic susceptibilities, and treatment outcomes. Retina 2015;35(6):1101-6.

30-**Parchand SM, Agrawal D, Chatterjee S, Gangwe A, Mishra M, Agrawal D.** Post-cataract surgery cluster endophthalmitis due to multidrug-resistant Pseudomonas aeruginosa: A retrospective cohort study of six clusters. Indian Journal of Ophthalmology 2020;68(7):1424.

31-**Irvine WD, FlynnHW Jr, Miller D, Pflugfelder SC.** Endophthalmitis caused by Gram-negative organisms. Arch Ophthalmol 1992;110(10):1450-4.

32-**Guerra RL, FreitasBde P, Parcero CM, MaiaOde OJr, Marback RL.** An outbreak of forty-five cases of Pseudomonas aeruginosa acute endophthalmitis after phacoemulsification. Arq Bras Oftalmol 2012; 75:344-7.

33-**Kreger AS, Griffin OK.** Physicochemical fractionation of extracellular cornea-damaging proteases of *Pseudomonas aeruginosa*. Infection and Immunity 1974;9(5):828-34.

34-**Mayo MS, Cook WL, Schlitzer RL, Ward MA, Wilson LA, Ahearn DG.** Antibiograms, serotypes and plasmid profiles of Pseudomonas aeruginosa associated with corneal ulcers and contact lens wear. J Clin Microbiol 1986;24(3):372-6.

35-**Choudhary V, Pal N, Hooja S.** Prevalence, and antibiotic resistance pattern of metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolates from clinical specimens in a tertiary care hospital. Journal of Mahatma Gandhi Institute of Medical Sciences 2019;24(1):19

36-**Banar M, Emaneini M, Satarzadeh M, Abdellahi N, Beigverdi R, Leeuwen WB, et al.** Evaluation of mannosidase and trypsin enzymes effects on biofilm production of Pseudomonas aeruginosa isolated from burn wound infections. PloS one 2016 Oct 13;11(10):e0164622.

37-**Mohamed A, Abdelhamid F.** Antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from different clinical sources. Zagazig Journal of Pharmaceutical Sciences 2020;28(2):10-7.

38-**Abdulhaq N, Nawaz Z, Zahoor MA, Siddique AB.** Association of biofilm formation with multi drug resistance in clinical isolates of Pseudomonas aeruginosa. EXCLI journal 2020;19:201.

39-**Weinstein RA, Gaynes R, Edwards JR.** National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. Clinical infectious diseases 2005;41(6):848-54.

40-**Rodulfo H, Arcia A, Hernández A, Michelli E, Martinez DD, Guzman M, et al.** Virulence factors and integrons are associated with MDR and XDR phenotypes in nosocomial strains of *Pseudomonas aeruginosa* in a Venezuelan university hospital. Revista do Instituto de Medicina Tropical de São Paulo 2019 ;4;61.

41-**Gonçalves IR, Dantas RC, Ferreira ML, Batistão DW, Gontijo-Filho PP, Ribas RM**. Carbapenem-resistant *Pseudomonas aeruginosa*: association with virulence genes and biofilm formation. brazilian journal of microbiology 2017; 48:211-7.

42-El-Mahdy R, El-Kannishy G. Virulence factors of carbapenem-resistant *Pseudomonas aeruginosa* in hospital-acquired infections in Mansoura, Egypt. Infection and drug resistance. 2019;12:3455.

43-**Hashem H, Hanora A, Abdalla S, Shaeky A, Saad A.** Dissemination of metallo-β-lactamase in *Pseudomonas aeruginosa* isolates in Egypt: mutation in bla VIM-4. Apmis. 2017 May;125(5):499-505.

44-**Sonbol FI, Khalil MA, Mohamed AB, Ali SS.** Correlation between antibiotic resistance and virulence of *Pseudomonas aeruginosa* clinical isolates. Turkish journal of medical sciences 2015;45(3):568-77.

45-**El-sayed H, Fahmy Y.** Correlation between biofilm formation and multidrug resistance in

clinical isolates of *Pseudomonas aeruginosa*. Microbes and Infectious Diseases 2021 ;2(3):541-9.

46-**Badawy MS, Riad OK, Taher FA, Zaki SA.** Chitosan and chitosan-zinc oxide nanocomposite inhibit expression of LasI and RhlI genes and quorum sensing dependent virulence factors of *Pseudomonas aeruginosa*. International journal of biological macromolecules 2020;149:1109-17.

47-**Lima JL, Alves LR, Jacomé PR, Bezerra Neto JP, Maciel MA, Morais MM.** Biofilm production by clinical isolates of *Pseudomonas aeruginosa* and structural changes in LasR protein of isolates non-biofilm-producing. Brazilian Journal of Infectious Diseases 2018; 22:129-36.

48-**Heydari S, Eftekhar F.** Biofilm formation and β-lactamase production in burn isolates of *Pseudomonas aeruginosa*. Jundishapur journal of microbiology 2015;8(3).

49-**Saffari M, Karami S, Firoozeh F, Sehat M.** Evaluation of biofilm-specific antimicrobial resistance genes in *Pseudomonas aeruginosa* isolates in Farabi Hospital. Journal of medical microbiology 2017;66(7):905-9.

50-**Elhabibi T, Ramzy S.** Biofilm Production by Multi Drug Resistant Bacterial Pathogens Isolated from Patients in Intensive Care Units in Egyptian Hospitals. J Microb Biochem Technol 2017; 9(4): 151-8.

51-**Elmanama AA, Al-Sheboul SA, Abu-Dan RI.** Antimicrobial resistance and biofilm formation of *Pseudomonas aeruginosa*: a short review article The International Arabic Journal of Antimicrobial Agents 2020, 10 (2): 3.

52-**Kalaiarasan E, Narasimha HB.** Antimicrobial resistance patterns and prevalence of virulence factors among biofilm producing strains of *Pseudomonas aeruginosa*. European Journal of Biotechnology and Bioscience 2016;4(11):26-8.

53-**Aboushleib HM, Omar HM, Abozahra R, Elsheredy A, Baraka K.** Correlation of quorum sensing and virulence factors in Pseudomonas aeruginosa isolates in Egypt. The Journal of Infection in Developing Countries 2015;9(10):1091-9.

54-**Khalil MAEF, Ibrahim Sonbol F, Mohamed AFB, Ali SS, Sonbol FI.** Comparative study of virulence factors among ESβL-producing and nonproducing *Pseudomonas aeruginosa* clinical isolates. Turk J Med Sci 2015; 45:60–69.

55-**Naik P, Pandey S, Gagan S, Biswas S, Joseph J.** Virulence factors in multidrug (MDR) and Pan-drug resistant (XDR) *Pseudomonas aeruginosa*: a cross-sectional study of isolates recovered from ocular infections in a high-incidence setting in southern India. Journal of Ophthalmic Inflammation and Infection 2021;11(1):1-1.

56-**EL-Khashaab TH, Erfan DM, Kamal A, El-Moussely LM, Ismail DK.** *Pseudomonas aeruginosa* biofilm formation and quorum sensing lasR gene in patients with wound infection. Egypt J Med Microbiol 2016; 25:101–108.

57-**Sonbol F, El-Banna T, Elgaml A, Aboelsuod KM.** Impact of Quorum Sensing System on Virulence Factors Production in *Pseudomonas aeruginosa*. *J Pure Appl Microbiol*. 2022;16(2):1226-123

58-**Li H, Li X, Wang Z, Fu Y, Ai Q, Dong Y, et al.** Autoinducer-2 regulates *Pseudomonas aeruginosa* PAO1 biofilm formation and virulence production in a dose-dependent manner. BMC microbiology 2015;15(1):1-8.

59-**Haque S, Ahmad F, Dar SA, Jawed A, Mandal RK, Wahid M, et al.** Developments in strategies for Quorum Sensing virulence factor inhibition to combat bacterial drug resistance. Microbial pathogenesis 2018; 121:293-302.

60-**Jiang Q, Chen J, Yang C, Yin Y, Yao K.** Quorum sensing: a prospective therapeutic target for bacterial diseases. BioMed Research International 2019 Oct;2019.

61-**Zhao X, Yu Z, Ding T.** Quorum-sensing regulation of antimicrobial resistance in bacteria. Microorganis 2020;8(3):425.

62-**Bäuerle T, Fischer A, Speck T, Bechinger C.** Self-organization of active particles by quorum sensing rules. Nature communications 2018;9(1):3232.

63-**Crémet L, Leroy AG, Muller D, Delanou S, Burghelea A, Broquet A, et al.** Antibiotic resistance heterogeneity and LasR diversity within Pseudomonas aeruginosa populations from pneumonia in intensive care unit patients. International Journal of Antimicrobial Agents 2021;57(6):106341.

64-**Karatuna O, Yagci AY.** Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in *Pseudomonas aeruginosa* respiratory isolates. Clinical Microbiology and Infection 2010;16(12):1770-5.

65-**Karami P, Khaledi A, Mashoof RY, Yaghoobi MH, Karami M, Dastan D, et al.** The correlation between biofilm formation capability and antibiotic resistance pattern in *Pseudomonas aeruginosa*. Gene Reports 2020; 18:100561.

66-**Subedi D, Vijay AK, Willcox M.** Overview of mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*: an ocular perspective. Clin Exp Optom 2018;101(2):2162–2171.

67-**Abo Elnour A, Negm S, Ismail A, Elhusseiny MA.** An outbreak of post cataract *Pseudomonas aeruginosa* acute endophthalmitis in Egypt. Bulletin of the National Research Centre 2019;43(1):1-7.

68-**Iglewski BH**. *Pseudomonas*. In: Baron S (ed) Medical Microbiology, 4th edn. University of Texas Medical Branch. Galveston, Galveston (TX) Chapter 27.1996.

69-**Storey DG, Ujack EE, Rabin HR, Mitchell I.** *Pseudomonas aeruginosa* lasR transcription correlates with the transcription of lasA, lasB, and toxA in chronic lung infections associated with cystic fibrosis. Infection and immunity 1998;66(6):2521-8.

70- **Simanek KA, Taylor IR, Richael EK, Lasek-Nesselquist E, Bassler BL, Paczkowski JE.** The PqsE-RhlR interaction regulates RhlR DNA binding to control virulence factor production in *Pseudomonas aeruginosa*. Microbiology spectrum.2022 ;10(1):e02108-21.

71-**Simanek KA, Paczkowski JE.** Resistance Is Not Futile: The Role of Quorum Sensing Plasticity in *Pseudomonas aeruginosa* Infections and Its Link to Intrinsic Mechanisms of Antibiotic Resistance. Microorganisms 2022;10(6):1247.

Mohamed EA, Nawar AE, Hegazy EE. Insight into quorum sensing genes *lasr* and *rhlr*, their related virulence factors and antibiotic resistance pattern in *Pseudomonas aeruginosa* isolated from ocular infections. Microbes Infect Dis 2023; 4(2): 575-589.