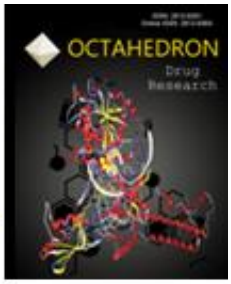
Octahedron Drug Research 3 (2023) 18-29

ISSN: 2812-6351
Online ISSN: 2812-636X

OCTAHEDRON
Drug Research
Vol. 3

Research Article




# Disruption of the quorum-sensing system in *Pseudomonas aeruginosa* using the PPI esomeprazole: An exceptional and promising role

**Moustafa M. Saleh[1]*, Mohammed Ramadan[2], Islam Zaki[3], Mohammed Salah[1]**

[1] Microbiology and Immunology Department, Faculty of Pharmacy, Port Said University, Egypt.

[2] Microbiology and Immunology Department, Faculty of Pharmacy, Al-Azhar University, Assiut branch, Egypt

[3] Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Port Said University, Egypt

* Correspondence: Email: Mostafa.mohamed@pharm.psu.edu.eg Tel: +201225998747

**ARTICLE INFO**

Article history:
Received 10 March 2023
Received in revised form
25 March 2023
Accepted 1 April 2023
Available online 1 April 2023

***Keywords:*** *Pseudomonas aeruginosa*; quorum-sensing; esomeprazole; gene expression; virulence factors



**ABSTRACT**

Quorum-sensing is a phenomenon of intercellular messages in microorganisms through secretion of small signaling molecules, which spread between cells, facilitating communication, and regulating virulence factors production. Quorum-sensing system disruption in *Pseudomonas aeruginosa* through identifying new inhibitors is a promising approach. Herein, we test the possible quorum-sensing inhibitory effect of esomeprazole, a proton pump inhibitor, on the standard strain *Pseudomonas aeruginosa* PAO1. The production of the quorum-sensing controlled violacein pigment in the biosensor microorganism *Chromobacterium violaceum*- CV026 was significantly decreased by using 1/4 minimal inhibitory concentration of esomeprazole. For *Pseudomonas aeruginosa* PAO1, such concentration of esomeprazole inhibited the swarming motility and reduced the production of the quorum-sensing dependent virulence factors proteases, elastase, pyoverdin, and pyocyanin. qRT-PCR confirmed that esomeprazole significantly decreased the relative expression levels of the quorum-sensing regulated genes *LasI*, *LasR*, *RhlI*, *RhlR*, *PqsA*, and *PqsR* which control virulence factors production. Docking study revealed potential strong binding capability of esomeprazole with LasR, RhlR and PqsR receptors. To summarize, this study showed that esomeprazole could be used as a valuable quorum-sensing inhibitor that can help develop a new approach for identifying alternative solutions to treat pseudomonal infections that may not respond to traditional antibiotics.

## 1. Introduction

*Pseudomonas aeruginosa* (*P. aeruginosa*) plays a crucial role in Gram-negative bacterial infections, particularly in seriously sick patients [1]. *P. aeruginosa* is ranked as one of the topmost common microorganisms that cause long-term illness among immunocompromised patients such as those with severe burns, wounds, and cystic fibrosis (CF), where it establishes chronic infections [2, 3]. The severity of *P. aeruginosa* infections is thought to be due to production of a variety of virulence factors and pathogenicity determinants, including proteases, elastase, pyocyanin, pyoverdin, and exotoxin A, in addition to its ability to exhibit different types of motilities that facilitate the invasion of host tissues resulting in hazardous infections [4].

Fighting the associated infections of *P. aeruginosa* can be aided by understanding the mechanisms by which this pathogen regulates the production of various virulence factors [5]. Extensive research has reported that *P. aeruginosa* exhibits a cell-to-cell interconnection mechanism known as quorum sensing (QS) that permits the bacterium to control the expression and production of its virulence factors [6]. The QS machinery of *P. aeruginosa* was successfully explored at a large scale, and it is considered as a model organism in QS research [7]. It was found that the QS system in *P. aeruginosa* consists primarily of three circuits, namely, (LasI/R), RhlI/R, and PqsA/R, which are interwoven hierarchically to regulate the virulence behavior of *P. aeruginosa*. A fourth QS system, known as integrated quorum sensing system (IQS), has been discovered but the exact roles of IQS need more investigations (**Figure 1**) [8, 9]. Antimicrobial resistance caused due to misuse of antibiotics has resulted in a massive restriction of treatment options for *P. aeruginosa* infections, which in turn has emerged as a critical and fatal concern [10]. An exceptional idea in the battle against bacterial diseases concentrates on producing or identifying agents that inhibit bacterial virulence rather than bacterial viability, which is known as anti-virulence therapy or anti-QS [11]. In the last few years, several FDA-approved drugs have been confirmed to be efficacious as QS inhibitors with no toxicity as found with several synthetic chemical compounds with a QS inhibitory effect that cannot be used in humans [12, 13].

Proton pump inhibitors, which include esomeprazole, are frequently used in clinical practice as a supplement to antibiotics in the treatment of peptic ulcers with microbial etiology [14]. Importantly, a previous study reported that esomeprazole significantly inhibited biofilm formation, which is regulated by QS in *P. aeruginosa* [15]. Based on this previously reported biofilm formation inhibitory effect of esomeprazole in *P. aeruginosa* proved by Singh et al. 2012, this study aimed to see whether esomeprazole had any QS inhibitory activity on various virulence factors of *P. aeruginosa* PAO

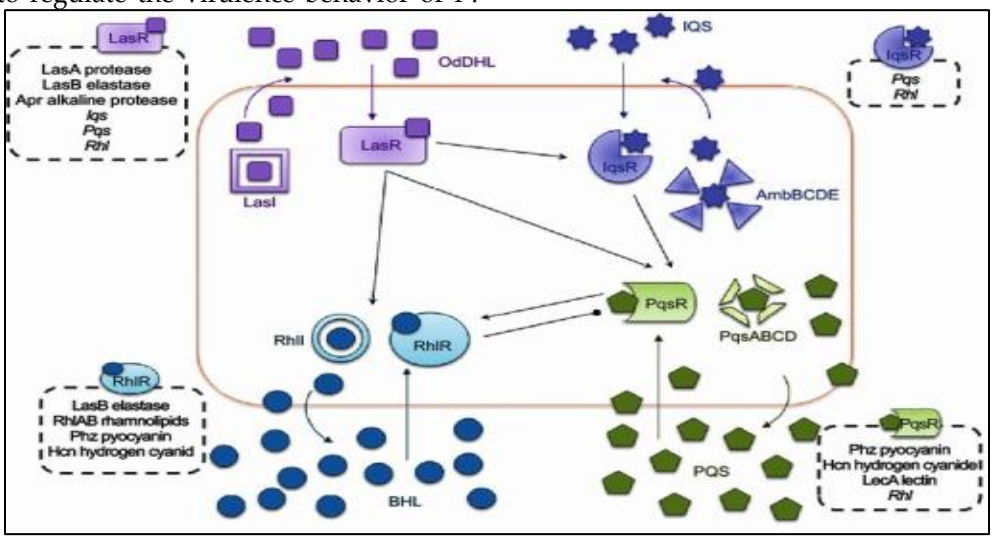



**Figure 1.** Schematic representation of the four QS signaling networks in *P. aeruginosa* and their respective regulons [9].

## 2. Materials and Methods

### *2.1. Bacterial Strains*

Both the *P. aeruginosa* PAO1 standard strain and the biosensor mutant strain *Chromobacterium violaceum* CV026, developed and described by McClean et al.

(1997) [16] were received as gifts from the culture bank collection of the Microbiology and Immunology Department, Faculty of Pharmacy, Zagazig University, Egypt.

*2.2. Determination of Esomeprazole Minimal Inhibitory Concentration*

To determine the minimal inhibitory concentration (MIC) of esomeprazole obtained from the International Drug Agency for Pharmaceutical Industry (IDI, Port Said, Egypt), the agar dilution technique was performed following the Clinical and Laboratory Standards Institute (CLSI) procedures [17]. Briefly, various concentrations of esomeprazole were added to molten nutrient agar at 55°C–60°C, mixed well and poured into Petri plates to reach the final concentrations of 64, 32, 16, 8, 4, 2, 1 and 0.5 mg/mL, after which the agar plates were allowed to harden. Then, 10 µL of the tested isolate PAO1 solution containing approximately $10^8$ CFU/mL was placed onto the surface of the plates. The plates were incubated at 37°C overnight. The MIC value was calculated as the lowermost concentration of esomeprazole which exhibited no observable growth of the tested PAO1 strain.

*2.3. Determination of the Effect of Sub-inhibitory Concentration of Esomeprazole on Bacterial Growth*

To confirm that esomeprazole sub-inhibitory concentration does not have any effect on the growth of the tested isolate PAO1, the turbidity of PAO1 in presence and absence of 1/4 MIC of esomeprazole was determined according to Abbas et al. [18]. In brief, Luria-Bertani (LB) broth [19] was inoculated with overnight cultures of PAO1 to reach turbidity of 0.5 McFarland. The tested cultures with and without esomeprazole were incubated for 24 h at 37°C. The tested cultures were incubated for 24 hours at 37°C, both with and without esomeprazole. A spectrofluorometer was used to assess the turbidities of cultures that had been exposed to esomeprazole and cultures that had not at 600 nm. Also, the viable counts following incubation at 37 °C 24 h were evaluated.

*2.4. Testing for Esomeprazole QS Inhibitory Activity Using Violacein Inhibition Test*

The capability of esomeprazole to inhibit the activity of the QS-regulated violacein pigment was investigated according to the method of Tan et al. [20]. The biosensor strain *Chromobacterium violaceum* CV026 was grown in LB broth and incubated overnight at 37°C. The optical density of the bacterial suspension was adjusted to 1 at 600 nm, and then 15 mL of the overnight bacterial culture was added to 200 mL of molten LB agar that was supplemented with 0.25 µg/mL N-hexanoyl homoserine lactone (Sigma-Aldrich, Steinheim, Germany). Petri plates were filled with *Chromobacterium violaceum* CV026 agar suspension and let to harden and wells were made in the agar plates. An aliquot of 30 µL of 1/4 MIC of esomeprazole was added into a well and solvent (DMSO) was used as the negative control. The plates were incubated for 24 h at 28°C. The formation of colorless halo on a purple background around the esomeprazole- containing cup indicates QS inhibitory activity

*2.5. The effect of esomeprazole on Quorum Sensing-dependent Virulence Factors*

*2.5.1. Swarming Motility Assay*

The method performed by Abbas et al. to study the possible inhibitory effect of esomeprazole on the swarming motility of PAO1 strain [21] was used. Briefly, 0.5% LB swarming agar plates were prepared and allowed to solidify at room temperature. The swarming plates were spotted in the center by 2 µL of 0.5 McFarland equivalent suspension of an overnight culture of the tested PAO1 strain with and without 1/4 MIC of esomeprazole and incubated at 37ºC for 24 h. The diameter of the swarming colony on the agar surface was recorded in mm.

*2.5.2. Elastase Estimation*

The elastin Congo red (ECR) assay described by Abbas et al. [18] was used. Cell-free supernatant of the tested *P. aeruginosa* PAO1 strain was prepared by centrifugation of overnight bacterial cultures in LB broth with and without 1/4 MIC of esomeprazole at 10,000 rpm for 10 min. A volume of 500 µL of ECR solution (10 mg/ml) (Sigma, USA) prepared in Tris buffer (pH 7.0) was mixed with 250 µL of cell-free supernatants of *P. aeruginosa* PAO1. The mixtures were shaken and incubated for 6 h at 37°C before any insoluble ECR was centrifuged out, then the absorbance of the red color of ECR released in the supernatants was measured at 495 nm using a spectrofluorometer.

*2.5.3. Total Proteases Assay*

The milk agar method as described by Abbas et al. [18] was carried out. The supernatants were separated after centrifuging overnight cultures of PAO1 in LB broth at 10,000 rpm for 15 min with and without 1/4 MIC of esomeprazole. Supernatants were applied in volumes of 100 µL to the wells made on 5% skim milk

agar plates. The plates were incubated at 37°C for 24 h, after which the clear zones around the wells representing the proteolytic activity of the tested PAO1 were measured in mm.

*2.5.4. Pyocyanin estimation*

The modified method of Das and Manefield was utilized for pyocyanin estimation [22]. The PAO1 strain was grown in LB broth and incubated for 24 h at 37°C. The bacterial suspension was adjusted to an approximate turbidity of 0.3-0.4 at 600 nm. Sterile tubes each containing 1 mL LB broth in the presence of 1/4 MIC of esomeprazole and control tubes contain 1 mL LB broth without 1/4 MIC of esomeprazole were prepared and inoculated with 10 µL of the prepared bacterial suspension. After incubation at 37°C for 48 h, the tubes were centrifuged at 10,000 rpm for 10 min and the supernatants were separated. Using a spectrofluorometer, the absorbance at 691 nm was utilized to quantify the pyocyanin pigment produced.

*2.5.5. Pyoverdin estimation*

The production of pyoverdin was determined using the method described by Abbas, 2015 [23]. In brief, overnight cultures of PAO1 in LB broth were prepared with and without 1/4 MIC of esomeprazole and then centrifuged at 10,000 rpm for 10 min. The supernatants were diluted 10-fold in Tris-HCl buffer (pH 7.4). Then, by using a spectrofluorometer, a volume of 100 µL was poured into 96-well microtiter plates on ice, and the pyoverdin concentration was measured based on the supernatant's fluorescence at an excitation wavelength of 405 nm and an emission wavelength of 465 nm.

*2.6. Extraction of total RNA for qRT-PCR and Quantitative RT-PCR of QS genes regulating the production of virulence factors*

Total RNA of esomeprazole-treated and untreated *P. aeruginosa* PAO1 isolates was extracted using the RNA Purification Kit GeneJET (Thermo-Scientific, USA) following the manufacturer's instruction. The relative expression levels of the QS genes *LasI*, *LasR*, *RhlI*, *RhlR*, *PqsA*, and *PqsR* were evaluated in esomeprazole-treated and untreated PAO1 by qRT-PCR. The housekeeping gene *RopD* was used to normalize the level of relative expression of each tested gene because there are no differences in this gene's expression level between treated and untreated PAO1 isolates. [12, 24]. The primers used in this study are shown in Table 1.

**Table 1.** Primers used in qRT-PCR.

| Name of gene | Sequence of primers | Amplicon base pair size | Reference |
|---|---|---|---|
| *ropD* (F[1]) | 5'-CGAACTGCTTGCCGACTT-3' | 131 | [12] |
| *ropD* (R[2]) | 5'-GCGAGAGCCTCAAGGATAC-3' | | |
| *lasI*(F) | 5'-CGCACATCTGGGAACTCA-3' | 176 | [12] |
| *lasI*(R) | 5'-CGGCACGGATCATCATCT-3' | | |
| *lasR*(F) | 5'- CTGTGGATGCTCAAGGACTAC-3' | 133 | [12] |
| *lasR*(R) | 5'- AACTGGTCTTGCCGATGG-3' | | |
| *rhlI*(F) | 5'- GTAGCGGGTTTGCGGATG-3' | 101 | [12] |
| *rhlI*(R) | 5'- CGGCATCAGGTCTTCATCG-3' | | |
| *rhlR*(F) | 5'- GCCAGCGTCTTGTTCGG-3' | 160 | [12] |
| *rhlR*(R) | 5'- CGGTCTGCCTGAGCCATC-3' | | |
| *pqsA*(F) | 5'- GACCGGCTGTATTCGATTC-3' | 74 | [12] |
| *pqsA*(R) | 5'- GCTGAACCAGGGAAAGAAC-3' | | |
| *pqsR*(F) | 5'- CTGATCTGCCGGTAATTGG-3' | 142 | [12] |
| *pqsR*(R) | 5'- ATCGACGAGGAACTGAAGA-3' | | |

[1]= Forward, [2]= Reverse.

*2.7. The docking studies.*

The protocol described in the SensiFAST™ SYBR® Hi-ROX One-Step Kit (Bioline, UK) was applied for this analysis according to the manufacturer's instructions using the StepOne Real-Time PCR system (Applied Biosystem, USA). The relative gene expression was estimated by applying the comparative threshold cycle approach ($\Delta\Delta Ct$) reported by Livak and Schmittgen [25]. The experiment was performed with three technical replicates. To find out the possible molecular interaction of esomeprazole with the *P. aeruginosa* LasR, RhlR and PqsR a docking study was conducted. The crystal structure of the targets LasR, RhlR and PqsR were

downloaded from the protein data bank PDB code: 2UV0, 6CC0 and 4JVD, respectively [1, 26]. All molecular docking calculations and docking studies into the receptor active site of LasR, RhlR and PqsR of our tested agent esomeprazole and the classical compound C30 furanone; the most active known standard QS inhibitor, were performed using Molecular Operating Environment (MOE 2009.10) software. The 3D structures of the ligands esomeprazole and C30 furanone were built using MOE and subjected to the following step: (i) 3D protonation of the structure (ii) hide of the hydrogen (iii) selecting the least energetic conformer. Also, the targets LasR, RhlR and PqsR were prepared for docking studies as follow; water molecules were discarded; (ii) hydrogen atoms were added to the enzyme (iii) MOE alpha site finder was used for the active site search (iv) removal of the co-crystalline ligand and docking of the targeted ligands.

*2.8. Statistical analysis*

The GraphPad Prism7 software using unpaired *t*-test and one-way ANOVA according to Dunnett's multiple comparison tests was used to detect the significance of the inhibitory effect of esomeprazole on bacterial growth, swarming motility, and production of elastase, total protease, pyocyanin, and pyoverdin. P values <0.05 were considered to be statistically significant. The mean ± standard errors of the three biological experiments with three technical replicates were used to calculate the results.

## 3. Results

*3.1. Determination of Esomeprazole MIC against P. aeruginosa PAO1*

It was found that esomeprazole exhibited growth inhibitory effect against *P. aeruginosa* PAO1 at a concentration of 8 mg/mL and a concentration of 1/4 MIC (2 mg/mL) was chosen to evaluate the effect of esomeprazole on virulence factors and QS genes of PAO1, as lower concentrations (1 and 0.5 mg/ml) did not show any inhibitory effect on virulence factors production using phenotypic tests.

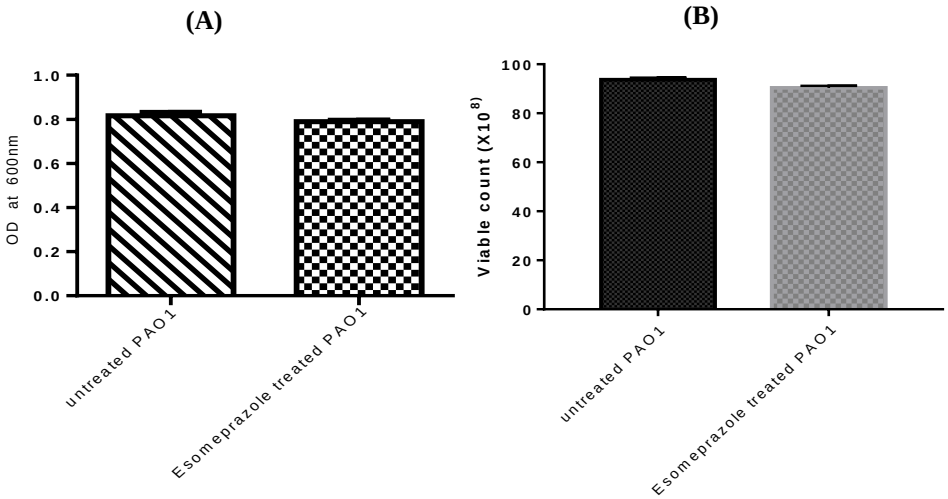



**Figure 2.** (A) The effect of 1/4 MIC of esomeprazole on the growth and (B) viable counts of PAO1 culture after 24 hours.

*3.2. The Effect of Esomeprazole on the Production of Violacein from Chromobacterium violaceum CV026*

It has been reported that the production of violacein pigment in the biosensor strain *Chromobacterium violaceum* CV026 is controlled by the QS machinery; therefore, the anti-QS effect of 1/4 MIC of esomeprazole on PAO1 inhibited QS in *Chromobacterium violaceum* CV026, demonstrated as clear zone or a halo formation on a purple background around the well containing esomeprazole in the agar medium seeded with the biosensor organism (**Figure 3**)

*3.3. Inhibition of Swarming Motility*

Importantly, the swarming motility of *P. aeruginosa* PAO1 was significantly decreased by (73.8%) in treated PAO1. The average swarming colony diameters were

reduced from 38 mm in untreated PAO1 to 10 mm under the effect of 1/4 MIC of esomeprazole (**Figure 4**).

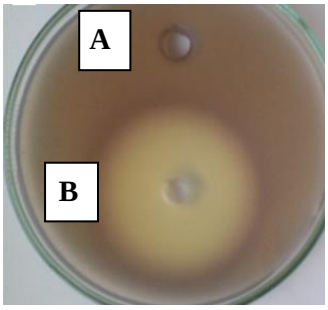



**Figure 3.** Inhibition of violacein pigment production of *Chromobacterium violaceum* CV026. **(A)** Negative control (DMSO) and **(B)** 1/4 MIC of esomeprazole.

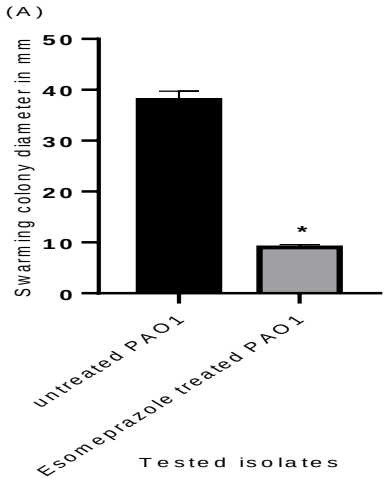



**Figure 4.** Inhibition of swarming motility of PAO1 by 1/4 MIC of esomeprazole in treated PAO1 in comparison with untreated PAO1. $P < 0.05$ was significant.

*3.4. The Effect of Esomeprazole on the Production of QS-dependent Virulence Factors*

As illustrated in (**Figure 5A**), esomeprazole significantly diminished the production of total proteases, elastase, pyocyanin, and pyoverdin from 100% in untreated PAO1 to 26.6%, 25.2%, 35.2% and 9.7% in esomeprazole-treated PAO1, respectively.

*3.5. The effect of Esomeprazole on the Expression Levels of QS Genes in P. aeruginosa by qRT-PCR*

The expression levels of QS genes were evaluated in esomeprazole-treated and untreated PAO1 using the $2^{-\Delta\Delta Ct}$ method. A significant reduction in the levels of expression of *LasI*, *LasR*, *RhlI*, *RhlR*, *PqsA*, and *PqsR* genes was detected in esomeprazole-treated PAO1 compared to that in control untreated PAO1. The relative expression levels of QS genes were significantly diminished from 100% in untreated PAO1 to 26.7% for *LasI*, 25% for *LasR*, 48.7% for *RhlI*, 44.6% for *RhlR*, 42.7% for *PqsA* and 49.4% for *PqsR* in esomeprazole-treated PAO1 (**Figure 5B**)

*3.6. The Docking Study*

Esomeprazole and C30 furanone were investigated for molecular docking into the active site of the LasR, RhlR and PqsR receptor (PDB code: 2UV0, 6CC0 and 4JVD, respectively) using Molecular Operating Environment (MOE) to compare the binding mode of these two compounds. The results of plausible binding interaction suggested stronger binding of esomeprazole with the active site of the targets LasR, RhlR and PqsR receptor in comparison to the classical QS inhibitor C30 furanone.

Esomeprazole could interact with the key amino acid of LasR by hydrogen bonds and hydrophobic interactions when compared with the classical QS inhibitor C30 furanone which has only one hydrogen bond with LasR, (**Table 2** and **Figures** 6 A, B, C and D).

In addition, esomeprazole could interact by its NH group of benzimidazole ring as H-bond donor with the key amino acid Ser129 of RhlR. As H-bond acceptor, it may interact with its sulphoxide group (S=O) with Ser38 and Ser129 of the RhlR receptor. The O of methoxy group interacted as H-bond acceptor with Trp62. On the other hand, the carbonyl group of C30 furanone showed only one possible hydrogen bond interaction with Ser129 of RhlR, (**Table 3** and **Figures** 7 A, B, C and D).

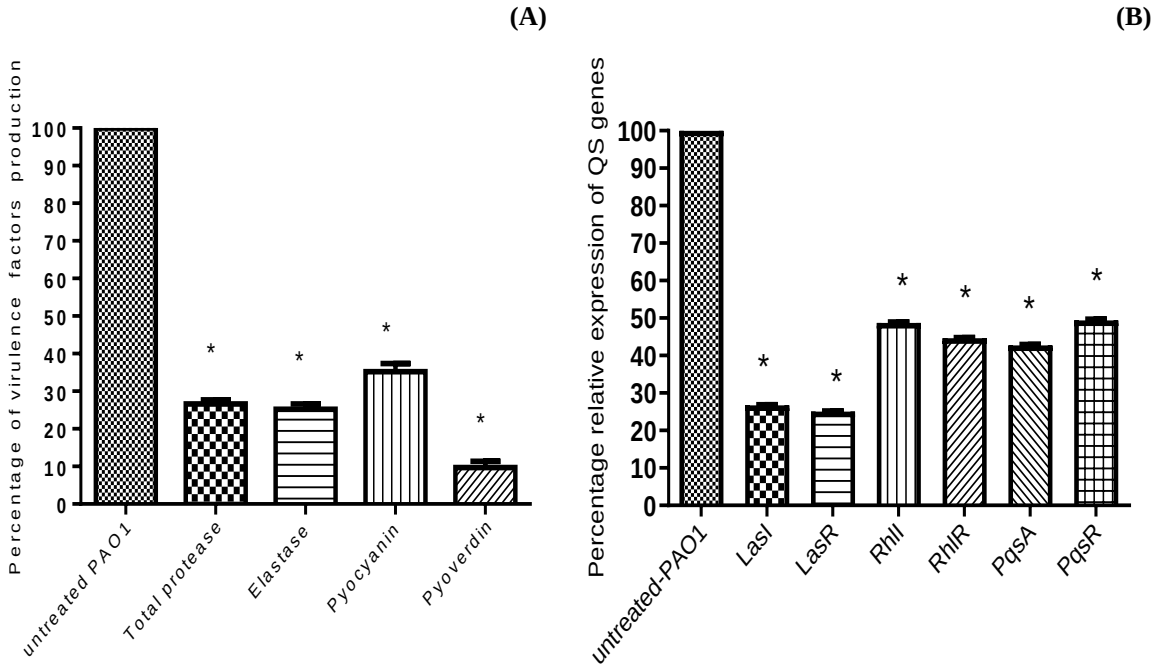



**Figure 5. (A)** the effect of 1/4 MIC of esomeprazole on the production of virulence factors (total proteases, elastase, pyocyanin, and pyoverdin) of PAO1 compared with control untreated-PAO1. The data shown reveals a significant ($P < 0.05$) reduction in the production of these virulence factors. **(B)** The effect of 1/4 MIC of esomeprazole on the level of expression of QS genes in PAO1 compared with untreated cells. The data shows a significant reduction ($P < 0.05$) in the level of expression of these genes.

**Table 2.** The docking score and possible binding interaction of esomeprazole and C30 furanone inside LasR (2UV0) active site.

| Compound | Docking score (Kcal/mol) | Amino acid | Interacting group | Type of interaction |
|---|---|---|---|---|
| Esomeprazole | -9.7471 | Thr 75 | Pyridine N | Hydrogen bond |
| | | Ser 129 | S=O | Hydrogen bond |
| | | Trp 88 | Pyridine ring | arene-arene |
| C30 furanone | -4.5537 | Arg 61 | Furane C=O | Hydrogen bond |

**Table 3.** The docking score and possible binding interaction of esomeprazole and C30 furanone inside RhlR (6CC0) active site.

| Compound | Docking score (Kcal/mol) | Amino acid | Interacting group | Type of interaction |
|---|---|---|---|---|
| Esomeprazole | -10.0829 | Ser 129 | imidazole NH | Hydrogen bond |
| | | Ser 129 | S=O | Hydrogen bond |
| | | Ser 38 | S=O | Hydrogen bond |
| | | Trp 62 | $OCH_3$ | Hydrogen bond |
| C30 furanone | -8.3619 | Ser129 | Furane C=O | Hydrogen bond |

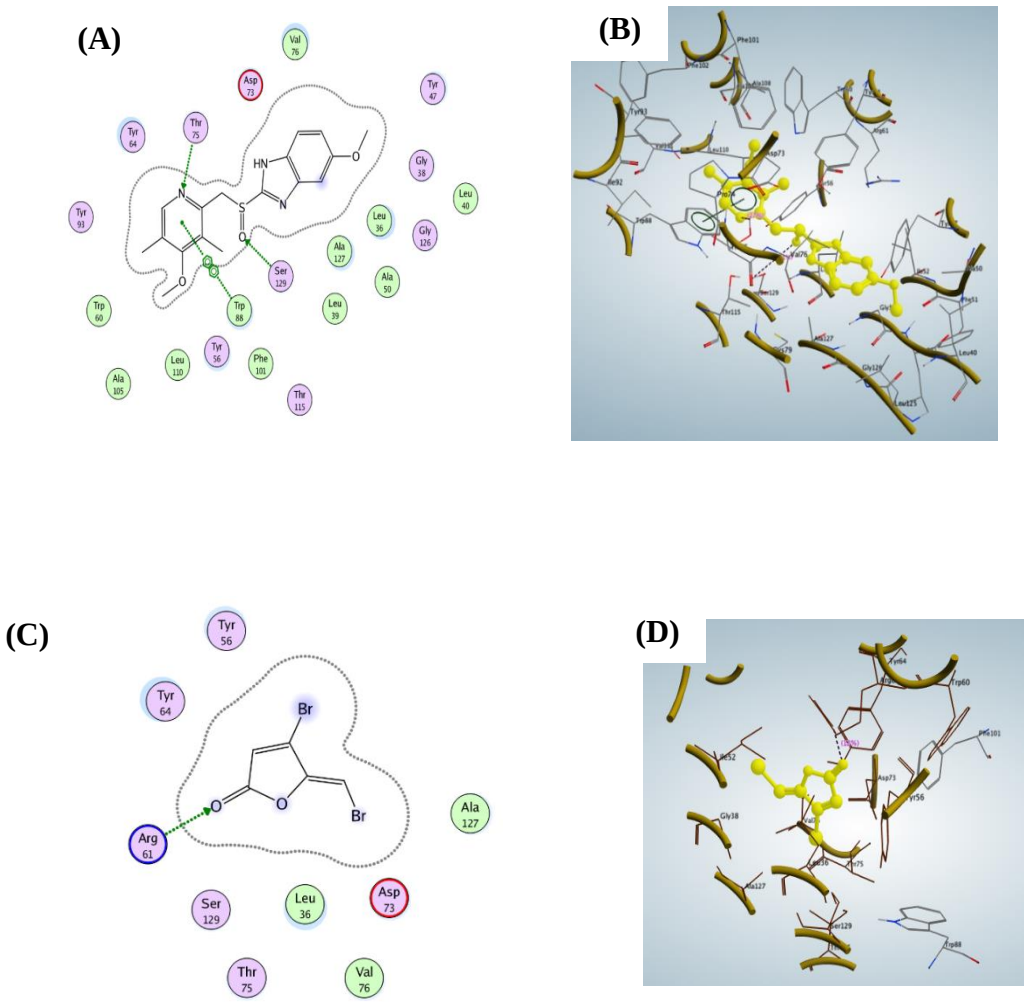



**Figure 6.** The 2D and 3D schematic of receptor-ligand interaction of LasR active site (PDB Code: 2UV0) with esomeprazole **(A, B)** and C-30 furanone **(C, D).**

**Table 4.** The docking score and possible binding interaction of esomeprazole and C30 furanone inside PqsR (4JVD) active site.

| Compound | Docking score (Kcal/mol) | Amino acid | Interacting group | Type of interaction |
|---|---|---|---|---|
| Esomeprazole | -9.5316 | Leu207 | imidazole NH | Hydrogen bond |
| | | Leu197 | $OCH_3$ | Hydrogen bond |
| C30 furanone | -7.6974 | Leu197 | Furane C=O | Hydrogen bond |

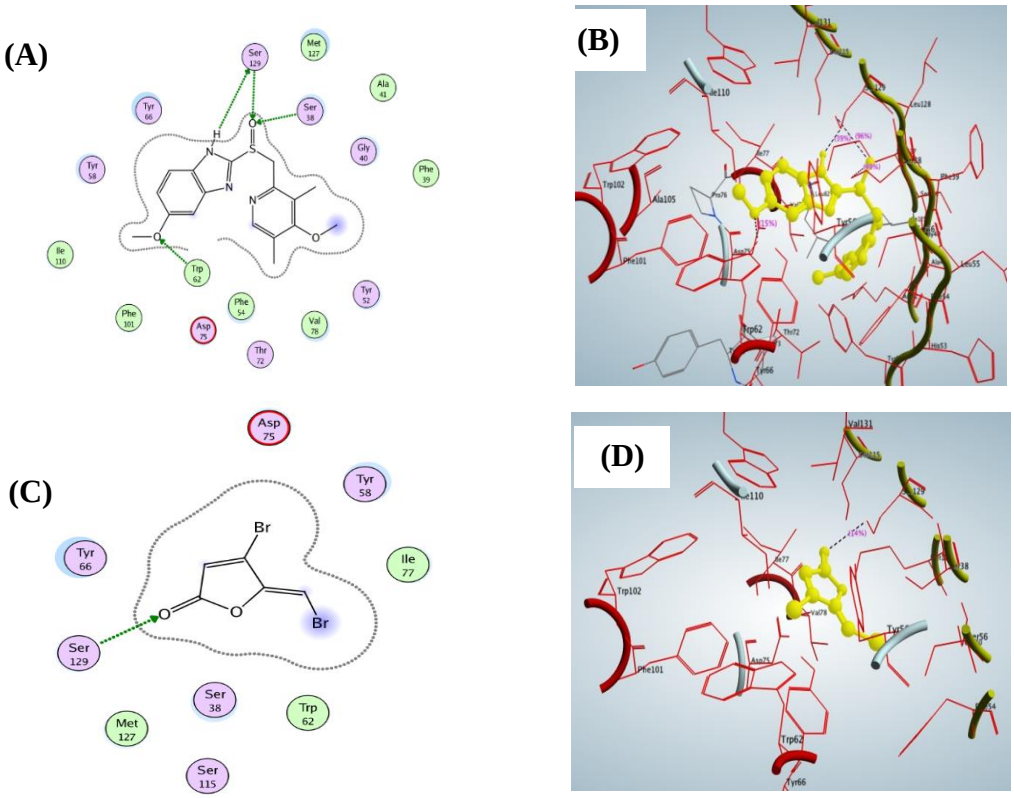



**Figure 7.** The 2D and 3D schematic of possible receptor-ligand interaction of RhlR active site (PDB Code: 6CC0) with esomeprazole **(A, B)** and C-30 furanone **(C, D).**

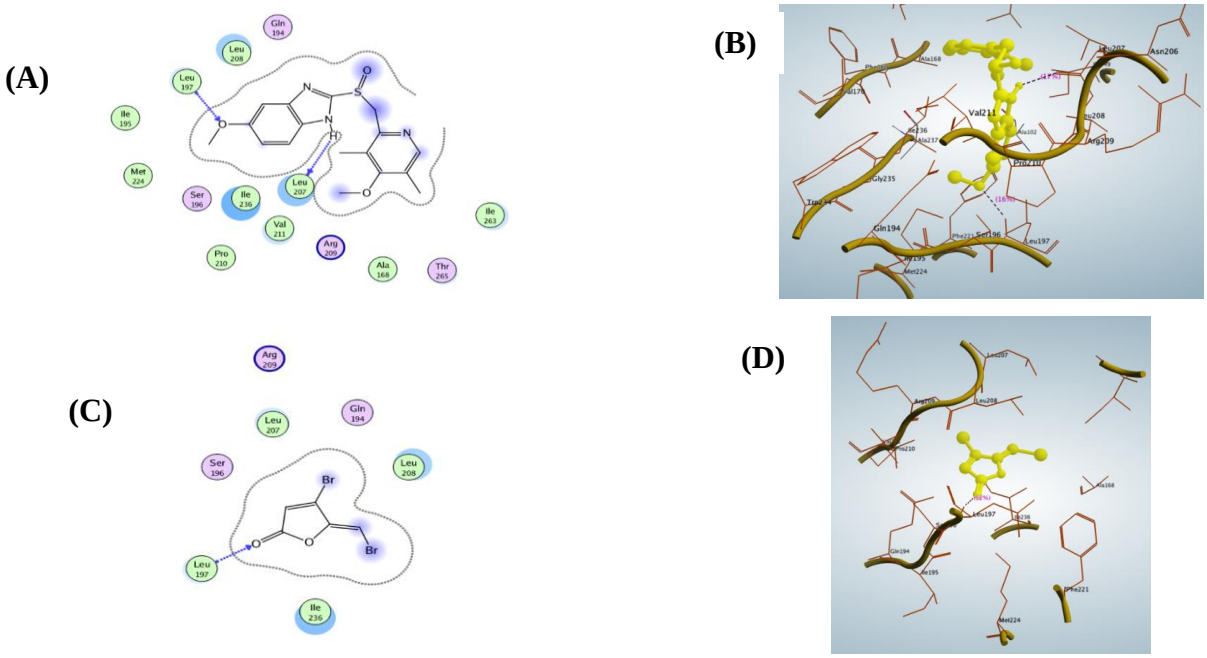



**Figure 8.** The 2D and 3D schematic of possible receptor-ligand interaction of PqsR active site (PDB Code: 4JVD) with esomeprazole **(A, B)** and C-30 furanone **(C, D).**

Moreover, the NH group benzimidazole ring of esomeprazole could allow for H-bond interaction with Leu207. The O of $OCH_3$ group may offer hydrogen bond acceptor with Leu197 of PqsR. On the other hand, the carbonyl group of C30 furanone showed one hydrogen bond interaction with Leu197 of PqsR, (Table 4 and **Figure**s 8 A, B, C and D).

**4. Discussion**

*P. aeruginosa* is a universal versatile Gram-negative microorganism that regulates the expression and synthesis of its numerous virulence factors using the QS mechanism [27]. The QS system of *P. aeruginosa* is organized in a multilayer hierarchy comprising three interconnected circuits, Las, Rhl, and PQS, under the control of the Las circuit [5]. When the LasI/R circuit is stimulated, it turns on both the RhlI/R and PqsA/R systems to activate the production of QS-dependent virulence factors [6]. Trials exploring novel solutions to address the crisis of antibiotic resistance have largely considered the deterioration of *P. aeruginosa* virulence by targeting the QS system [1]. Unfortunately, numerous chemically synthesized and naturally extracted QS inhibitors have been reported to be toxic and cannot be used clinically [12, 28]. As previously reported, the strategy of identifying a novel use for some of the existing FDA approved drugs as QS inhibitors is beginning to produce promising outcomes [12, 29, 30]. The impact of the proton pump inhibitor esomeprazole as a possible QS inhibitor in *P. aeruginosa* was investigated in this study. Initially, the MIC of esomeprazole was estimated and was found to be 8 mg/mL. Sub-MIC (1/4 MIC or 2 mg/mL) was used in further tests on QS and its dependent virulence factors. When the sub-MIC of esomeprazole was evaluated for its activity against the growth of PAO1, no significant effect was found; therefore, any potential QS-inhibitory activity would not be due to any harmful activity against bacterial growth.*Chromobacterium violaceum* CV026 was reported to be a biosensor that can produce the violacein pigment in reaction to the presence of acyl-homoserine lactones in its culture medium, and under the regulation of the CVi/R QS system and any inhibition of this QS system will reduce the ability of *Chromobacterium violaceum* CV026 to produce violacein [31]. Importantly, the sub-MIC of esomeprazole significantly inhibited the violacein production which indicated the potential QS-inhibitory activity of esomeprazole. *P. aeruginosa* exhibits different types of motilities such as swarming and twitching in addition to the secretion of numerous extracellular virulence products such as proteases, elastase, pyocyanin, and pyoverdin which after colonization participate in tissue destruction, evasion of host immune system, and bloodstream invasion and facilitate dissemination through host cells [32, 33]. Interestingly, in this study, the assessment of the QS regulated virulence factors proteases, elastase, pyocyanin, and pyoverdin and the swarming motility under the effect of esomeprazole revealed a significant inhibition, with a calculated *P* value <0.05, which expands the possibility of esomeprazole to act as a valuable QS inhibitor. Our results are consistent with those of Singh et al. [15] who reported that esomeprazole significantly inhibited the ability of biofilm formation by *P. aeruginosa* which is also under the regulation of the QS system. In Addition, in this study, the inhibitory effect of the FDA approved drug esomeprazole on virulence factors production is matched to some extent with the results of Hegazy et al. [34] who reported that the FDA approved antidiabetic drugs sitagliptin, metformin and vildagliptin showed a significant reduction of virulence factors production in *P. aeruginosa*. Also, in accordance with our results Abbas et al. showed that Febuxostat decreased the production of virulence factors in *P. aeruginosa* [34]. For further confirmation of the phenotypic results, the qRT-PCR assay was conducted to evaluate the potential QS inhibitory effect of esomeprazole on the relative expression levels of the QS genes *LasI, LasR, RhlI, RhlR, PqsA*, and *PqsR*, which regulate the production of virulence factors. Importantly, esomeprazole significantly downregulated the relative expression levels of all the tested QS genes, a finding that provides a basis to explain the significant reduction in the production of QS-dependent virulence factors. We also detected a significant downregulation of the LasI/R circuit in the current study, which in turn affected the expression levels of the other QS circuits Rhl and PQS with a net result of significant QS inhibitory activity of esomeprazole and a potent decrease in the production of virulence factors. Previous research have shown that some FDA-approved drugs including sitagliptin, metformin, and vildagliptin are effective QSIs in *P. aeruginosa* at the molecular level, which is similar to our findings [21, 35]. Also, in accordance with our results El-Mowafy et al. showed that aspirin decreased the expression of the QS regulating genes in *P. aeruginosa* [36]. For more confirmation, a docking study of esomeprazole possible binding into the active site of LasR, RhlR and PqsR was conducted. The docking

results of esomeprazole and C30 furanone molecules with LasR showed energy scores of -9.7471 and -4.5537 Kcal/mol, respectively which indicates the potentially stronger binding of esomeprazole by two hydrogen bonds and one arene-arene bond in comparison with C30 furanone which binds with only one hydrogen bond. In addition, the docking results of esomeprazole and C30 furanone molecules with RhlR showed energy scores of -10.0829 and -8.3619 Kcal/mol, respectively which reveals the potentially stronger binding of esomeprazole by four hydrogen bonds in comparison only one hydrogen bond with C30 furanone. Also, the docking results of esomeprazole and C30 furanone molecules with PqsR showed energy scores of-9.5316 and -7.6974, respectively which indicates the possible stronger binding of esomeprazole by two hydrogen bonds in comparison with C30 furanone which binds with only one hydrogen bond. The current results of the docking study are consistent with the docking results of previous studies that reported the strong binding of FDA approved drugs as QSI such as sitagliptin, metformin and vildagliptin [21, 34] and QSI natural products such as 1-(4-Amino-2-hydroxyphenyl) ethanone from *Phomopsis liquidmbari* [1] with LasR, RhlR and PqsR receptors. In summary, the analysis of the docking results demonstrated that the docking study was consistent with the phenotypic and genotypic results of the potential QS inhibitory activity of esomeprazole.

## 5. Conclusion

In conclusion, the findings of the current study indicate that esomeprazole could be considered as a promising QS-inhibitor that can be used as an aid in the management of *P. aeruginosa* infections.

## Data Availability Statement

This article has all the data that were created or evaluated during this investigation.

## Ethical consideration

All the participants in this study gave their informed permission.

**Conflicts of Interest:** No conflicts of interest are disclosed by the authors.

## References

[1] Zhou, J.W.; Jia, A.Q.; Jiang, H.; et al. 1-(4-Amino-2-hydroxyphenyl) ethanone from *Phomopsis liquidambari* showed quorum sensing inhibitory activity against *Pseudomonas aeruginosa. Appl. Microbiol. Biotech*. 2020, 1-12.

[2] Verma, U.; Kulshreshtha, S.; Khatri, P. K. MDR *Pseudomonas aeruginosa* in nosocomial infection: Burden in ICU and burn units of a tertiary care hospital. *Int. J. Curr. Microbiol. Appl. Sci.* 2018, 7(1), 1267-1274.

[3] Bachta, K. E. R.; Allen, J. P.; Cheung, B. H.; et al. Systemic infection facilitates transmission of *Pseudomonas aeruginosa*. *Nat. Commun.* 2020, 11(543), 1-13.

[4] Rocha, A. J.; Barsottini, M. R. O.; Rocha, R. R.; et al. *Pseudomonas Aeruginosa*: Virulence factors and antibiotic resistance genes. *Braz. Arch. Biol. Technol.* 2019, 62, e19180503.

[5] Fong, J.; Mortensen, K. T.; Nørskov, A.; et al. Itaconimides as novel quorum sensing inhibitors of *Pseudomonas aeruginosa*. *Front. Cell Infect. Microbiol*. 2019, 8, 443.

[6] Birmes, F. S.; Säring, R.; Hauke, M. C.; et al. Interference with *Pseudomonas aeruginosa* quorum sensing and virulence by the mycobacterial 2 PQS dioxygenase AqdC in combination with the N-acyl homoserine lactone lactonase QsdA. *Infection and Immunity.* 2019, 87(10), e00278-19.

[7] Asfahl, K. L.; Schuster, M. Additive effects of quorum sensing anti-activators on *Pseudomonas aeruginosa* virulence traits and transcriptome. *Front. Cell Infect. Microbiol.* 2018, 8(2654), 1-20.

[8] Kostylev, M.; Kim, D.Y.; Smalley, N.E.; et al. Evolution of the *Pseudomonas aeruginosa* quorum-sensing hierarchy. *Proceedings of the National Academy of Sciences*. 2019, 116(14), 7027-7032.

[9] Lee, J.; Zhang, L. The hierarchy quorum sensing network in Pseudomonas aeruginosa. *Protein Cell*. 2015, 6(1), 26-41.

[10] Bassett,i M.; Vena A.; Croxatto, A.; et al. How to manage *Pseudomonas aeruginosa* infections. *Drugs in Context*. 2018, 7, 212527.

[11] Rampioni, G.; Visca, P.; Leoni, L.; et al. Drug repurposing for antivirulence therapy against opportunistic bacterial pathogens. *Emerging Topics in Life Sciences.* 2017, 1(1), 13-22.

[12] El-Mowafy, S. A.; Shaaban, M.; Abd El-Galil, K. H. Sodium ascorbate as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. *J. Appl. Microbiol.* 2014, 117, 1388-1399.

[13] Askoura, M.; Saleh, M.; Abbas, H. An innovative role for tenoxicam as a quorum sensing inhibitor in *Pseudomonas aeruginosa*. Archives of Microbiology. 2020, 202(3), 555-565.

[14] Fallone, C.A.; Moss, S.F.; Malfertheiner, P. Reconciliation of recent *Helicobacter pylori* treatment guidelines in a time of increasing resistance to antibiotics. *Gastroenterology*. 2019, 157(1), 44-53.

[15] Singh, V.; Arora, V.; Alam, M. J.; et al. Inhibition of biofilm formation by esomeprazole in *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 2012, 56(8), 4360-4364.

[16] McClean, K. H.; Winson, M. K.; Fish, L.; et al. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acyl homoserine lactones. *Microbiology.* 1997, 143(12), 3703-3711.

[17] Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard, 2012, CLSI Document M07-A9, Vol. 32, No. 3. Wayne, PA, USA.

[18] Abbas, H.A.; Elsherbini, A.M.; Shaldam, M.A. Repurposing metformin as a quorum sensing inhibitor in *Pseudomonas aeruginosa*. *African health sciences*. 2017, 17(3), 808-819.

[19] Bertani, G. Lysogeny at mid-twentieth century. *J. Bacteriol.* 2004, 186, 595-600.

[20] Tan L. Y.; Yin, W.; Chan, K. Silencing quorum sensing through extracts of *Melicopelunu ankenda*. *Sensors.* 2012, 12, 4339-4351.

[21] Abbas, H.A.; Shaldam, M.A.; Eldamasi, D. Curtailing Quorum Sensing in *Pseudomonas aeruginosa* by Sitagliptin. *Current Microbiology*. 2020, 1-10.

[22] Das, T.; Manefield, M. Pyocyanin promotes extracellular DNA release in *Pseudomonas aeruginosa*. *PLoS ONE.* 2012, 7, e46718.

[23] Abbas, H.A. Inhibition of virulence factors of *Pseudomonas aeruginosa* by diclofenac sodium. *Rom*. *Arch*. *Microbiol*. *Immun*. 2015,74(3), 79-85.

[32] Kipnis, E.; Sawa, T.; Wiener-Kronish, J. Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis. *Med Mal Infect*. 2006, 3678–91.

[33] Khan, F.; Lee, J. W.; Pham, D. T. N.; et al. Streptomycin mediated biofilm inhibition and suppression of virulence properties in *Pseudomonas aeruginosa* PAO1. *Appl. Microbio. Biotech*. 2020, 104(2), 799-816.

[34] Abbas, H. A.; Soliman, W. E.; Shaldam, M.A. Perturbation of Quorum Sensing in *Pseudomonas aeruginosa* by Febuxostat. *Advances Microbiol*. 2018, 8, 650-664.

[24] Savli, H.; Karadenizli, A.; Kolayli, F.; et al. Expression stability of six housekeeping genes: a proposal for resistance gene quantification studies of *Pseudomonas aeruginosa* by real-time quantitative RT-PCR. *J. Med. Microbiol.* 2003, 52, 403–408.

[25] Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods.* 2001, 25, 402-408.

[26] Bottomley, M.J.; Muraglia, E.; Bazzo, R.; et al. Molecular insights into quorum sensing in the human pathogen *Pseudomonas aeruginosa* from the structure of the virulence regulator LasR bound to its autoinducer. *J. Biolog. Chem.* 2007, 282, 13592-600.

[27] Vadakkan, K. Molecular Mechanism of Bacterial Quorum Sensing and Its Inhibition by Target Specific Approaches. In *Quorum Sensing: Microbial Rules of Life*. 2020, 221-234. American Chemical Society.

[28] Galloway, W. R.; Hodgkinson, J. T.; Bowden, S.; et al. Applications of small molecule activators and inhibitors of quorum sensing in Gram-negative bacteria. *Trends Microbiol.* 2012, 20, 449-458.

[29] Saleh, M. M.; Abbas, H. A.; Askoura, M. M. Repositioning secnidazole as a novel virulence factors attenuating agent in *Pseudomonas aeruginosa*. *Microb. Pathog.* 2018, 127, 31-38.

[30] Saleh, M.M.; Refa't A, S.; Latif, H.K.A.; et al. Zinc oxide nanoparticles inhibits quorum sensing and virulence in *Pseudomonas aeruginosa*. *African Health Sciences*. 2019, 19(2), 2043-2055.

[31] Stauff, D. L.; Bassler, B. L. Quorum sensing in *Chromobacterium violaceum*: DNA recognition and gene regulation by the CviR receptor. *J. Bacteriol.* 2011, 193, 3871-3878.

[35] Hegazy, W. A.; Khayat, M. T.; Ibrahim, T. S.; et al. Repurposing Anti-diabetic Drugs to Cripple Quorum Sensing in *Pseudomonas aeruginosa*. *Microorganisms*. 2020, 8(9), 1285.

[36] El-Mowafy, S. A.; Abd El-Galil, K. H.; El-Messry, S. M. et al. Aspirin is an efficient inhibitor of quorum sensing, virulence and toxins in *Pseudomonas aeruginosa*. *Microb*. *Pathog*. 2014b; 74, 25-32.