JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

A complex hierarchical quorum-sensing circuitry modulates phenazine gene expression in *Pseudomonas aeruginosa*

Norhan K Abd El-Aziz[1], Marwa I Abd El-Hamid[1], El-sayed Y El-Naenaeey[1]

[1] *Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*

Abstract

Introduction: *Pseudomonas aeruginosa* (*P. aeruginosa*) modulates the expression of a myriad of virulence factors via two complicated hierarchical quorum-sensing (QS) cascade. This study shed light on the interrelation between *P. aeruginosa* QS systems and pyocyanin production.

Methodology: Transcription analysis of *las*R, *rhl*R, *rhl*I and *phz* genes using quantitative real time-reverse transcriptase PCR (qRT–PCR) assay, followed by sequencing of the autoinducer synthase (*las*I gene) were applied for 15 *P. aeruginosa* strains recovered from diverse animal clinical sources.

Results: Expression studies revealed that most *P. aeruginosa* strains demonstrated statistically significant differences ($p < 0.05$) with a very wide range of transcript levels of QS and *phz* genes in comparison to *P. aeruginosa* ATCC 27853. We have identified significant positive correlations ($r \geq 0.3$) between the expressions of QS and *phz* genes in eleven analyzed strains, whereas pyocyanin production positively correlated with the expression of *las*R only in three strains ($r \geq 0.6$). We further found that there was a negative correlation between the transcript levels of QS and *phz* genes in one bacterial strain. Analysis of *las*I sequences showed point mutations explaining the alterations in pyocyanin expression. The deficiencies of *las*I, *las*R and *rhl*I with *rhl*R-dependent expression of *phz* in one strain were also recorded.

Conclusions: These results provided new insights to the pivotal role of QS signal molecules on pyocyanin production presenting the las system as the dominant regulator.

Key words: Quorum sensing; pyocyanin; *P. aeruginosa*; real time-reverse transcriptase PCR.

J Infect Dev Ctries 2017; 11(12):919-925. doi:10.3855/jidc.8775

(Received 16 May 2016 – Accepted 15 August 2016)

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a ubiquitous Gram-negative bacterium and an important opportunistic pathogen with elevated metabolic and physiological versatility. Generally, *P. aeruginosa* is capable of expressing an impressive array of virulence determinants including extracellular secreted factors such as pyocyanin and pyoverdine pigments with potential roles in pathogenicity, mainly through a variety of iron acquisition mechanisms. Notably, it has since become clear that the coordinated expression of around 600 genes and many virulence-related characteristics such as pyocyanin synthesisis regulated by the utilization of cell-to cell communication system based on signal molecules known as quorum sensing in cell density dependent fashion, where certain target genes are induced or repressed [1,2]. In *P. aeruginosa*, at least two well-defined intertwined hierarchical QS systems, namely, las and rhl have been characterized. Each signaling QS system is comprised of two components, the autoinducer synthases (*las*I and *rhl*I) and their cognate transcriptional regulators (*las*R and *rhl*R), respectively [3]. The las system triggers the expression of elastase, exotoxin A and alkaline protease, while rhl system enhances the expression of rhamnolipid biosynthesis, alkaline protease, elastase, cyanide and pyocyanin production [4]. The two systems are intimately connected being hierarchically organized with lasI/R system regulating rhlI-rhlR transcription [5]. *P. aeruginosa* is distinguished by the generation of highly diffusible pigmented toxic secondary metabolites, known as phenazines. Pyocyanin is the major soluble bluish green phenazine derivative that is unique for *P. aeruginosa* suppurative infections [6].

Herein, a long-term transcriptional study was carried out with the objective of relative quantification of mRNA transcribed by *las*R, *rhl*R and *rhl*I to elucidate the correlation between the expressions of QS systems and *phz* gene using qRT–PCR assay. Afterwards, we gain insight in analyzing the presence of mutations that may affect the QS synthetase gene, *las*I, among a subset of clinically relevant *P. aeruginosa* strains.

Methodology

Bacterial strains and growth conditions

The present study was performed on 15 *P. aeruginosa* strains recovered from two animal clinical sources [bovine mastitis (5) and broiler breeders with respiratory problems (10)]. All pseudomonas strains were routinely enriched in brain heart infusion broth (Oxoid, Hampshire, UK) and immediately streaked on the selective medium of pseudomonas agar base with pseudomonas selective supplements (Oxoid, Hampshire, UK) to assess pigment production. The observed colonies were presumptively identified as *P. aeruginosa* according to their colonial pigmentation and conventional biochemical tests using the standard microbiological methods [7]. Subsequently, molecular characterization was performed using a *P. aeruginosa*-specific PCR amplification of *opr*L gene following a published protocol [8]. All the strains were stored frozen at -20°C in individual aliquots in brain heart infusion broth with 25% glycerol until further analysis.

RNA extraction and qRT–PCR

Total RNAs from bacterial cells were extracted according to the manufacturer's protocol of the QIAamp RNeasy mini kit (QIAGEN, Hilden, Germany). Quantitative RT-PCR was applied for examining the expression of *las*R, *rhl*R, *rhl*I and *phz* genes using SYBR green RT-PCR platform; the housekeeping gene *rpo*D was used as the normalizing gene [9]. Gene specific primer pairs were synthesized in the Reference laboratory for Veterinary Quality Control on Poultry Production, Egypt based on the previously published sequences [10,11].

In MX3005P real time PCR machine (Stratagene, La Jolla, USA), reverse transcription was performed at 50°C for 30 min. After a preliminary denaturation step, the reaction mixture was subjected to 40 cycles of 94°C, 50°C and 72°C, 30 seconds each for *las*R gene;94°C, 50°C (*rhl*R) or 53°C (*rhl*I) and 72°C, 45 seconds each and 94°C, 64°C and 72°C,one minute each for *phz* gene. Melting curves were then analyzed in one cycle of 94°C, 50°C (*las*R and *rhl*R) or 53°C (*rhl*I) or 64°C (*phz)* and 94°C, one minute each. Amplification curves and cycle threshold (CT) values were determined by Stratagene MX3005P software. Normalized expression was calibrated against corresponding mRNA expression by *P. aeruginosa* ATCC 27853. The CT values for each strain were converted into fold differences according to the relative quantification method described previously [12].

LasI *system amplification and sequencing studies*

Chromosomal DNA was extracted from *P. aeruginosa* strains using a commercial available QIAamp DNA Mini kit (Qiagen, Hilden, Germany,) in accordance with the manufacturers' recommendations. PCR amplification of *las*I quorum sensing synthetase gene was performed using specific primers and protocol described elsewhere [10]. PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia,USA) according to the manufacturers' instructions and referred to automated sequencing reactions using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, PerkinElmer, Foster City, USA) in ABI 3130 automated DNA Sequencer (Applied BioSystems,Foster City, USA). The resultant product sequences were compared with *P. aeruginosa* PAO1 wild type strain (accession number NC_002516.2) using the BLAST program from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST/). Sequence analysis was then performed by the use of MEGA5 program, product version 5.1 (http://www.megasoftware.net). All sequence data reported here were deposited into the GenBank databases with the following accession numbers: KP998820-KP998824 and KR020718-KR020726.

Statistical analysis

The relative expression levels of mRNA transcripts were analyzed statistically using Statistical Package for Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, USA). Comparison analysis was applied using independent samples t-test and Bonferroni's multiple comparison test and homogeneity of variances was analyzed by the Levene's test. Correlation analysis was performed using Pearson's correlation test. The data were expressed as SEM (mean ± standard error) and all *p* values were based on a 2-tailed distribution. The *p* values of < 0.05 were considered statistically significant.

Results

A las/rhl box functions in pyocyanin transcriptional activation

Since *P. aeruginosa* strains were selected for their range of pigment activities, different levels of pigmentation on isolation media were observed due to varying amounts of pyocyanin produced. This phenotypic behavior suggests the potential activity of QS machinery in a synergistic manner.

Relative expressions (fold-changes or fold-differences of expression levels) of QS systems and *phz*

gene of tested strains to a reference strain of *P. aeruginosa* ATCC 27853, which is assigned a value of 1, were detected. Nine isolates showed increased expressions (> one-fold increase) of *las*R (up to 15.67-fold), *rhl*R (up to 11.47-fold) and *rhl*I (up to 9.34-fold), which were accompanied by 1.13 to 6.73-fold increase in the transcription of *phz* gene. However, three isolates demonstrated increased expression of *phz* gene (up to 2.35-fold); one of them (No. 14) exhibited increased expressions of both *las*R (4.20-fold) and *rhl*R (1.14-fold) and the remaining two isolates (No. 2, 12) had increased expressions of *lasR* only (up to 3.16-fold). Another isolate (No. 8) without elevated *phz* expression had increased expressions of *las*R (2.77-fold), *rhl*I (1.57-fold) and *rhl*R (1.15-fold), suggesting other factors involved.

The contribution of las system in regulating the expression of pyocyanin production was verified. Indeed, all previously described thirteen isolates showed various point mutations in *las*I gene; nine of them (69.23%) possessed sense mutations with substitutions at lys^{13} position and only one isolate (No. 10) with an additional substitution at Asp^{12} position. These mutations probably explained the variations in pyocyanin expression in tested strains comparable to that produced by *P. aeruginosa* ATCC 27853.

A notable exception was recorded in the isolate No. 9, which possessed five point mutations in *las*I gene; two of them were evolved in the substitution of Lys^{13} to Arg. These mutations account for the decreased expressions of Qs systems conferring consequently a down regulation of *phz* gene expression. A striking observation in the present study was the deficiency of *las*I, *las*R and *rhl*I in an isolate No. 15; but *rhl*R, which is the known regulator of *phz* gene, was expressed in a low level (0.27-fold) producing a negligible expression level of pyocyanin (0.19-fold) in comparison with the reference strain. Detailed information on the relative expressions of QS systems and phenazine gene compared to *P. aeruginosa* ATCC 27853 as well as mutations in *las*I gene of *P. aeruginosa* strains under study are given in Table 1.

Table 1. Transcript levels of QS circuit and *phz* genes and *las*I mutations in *P. aeruginosa* strains

Strain number	Source	Relative expression [a]				*las*I mutations	Accession number
		*las*R	*rhl*R	*rhl*I	*phz*		
1	Bovine mastitis	5.74**	2.17**	2.34**	6.68**	Silent mutations (n=3)	KP998820
2	Bovine mastitis	1.95**	**0.30****	**0.07****	1.05	lys^{13} (AAA)→Glu (GAA) Silent mutations (n=3)	KP998821
3	Broiler breeders	2.58**	2.00**	1.60**	1.13	lys^{13} (AAA)→Glu (GAA) Silent mutations (n=3)	KP998822
4	Broiler breeders	3.68**	4.26**	8.17**	1.66**	lys^{13} (AAA)→Gly (GGA) A silent mutation	KP998823
5	Broiler breeders	11.31**	8.16**	7.55**	5.24**	lys^{13} (AAA)→Glu (GAA) A silent mutation	KP998824
6	Broiler breeders	9.51**	7.31**	5.23**	3.03**	lys^{13} (AAA)→Iso (ATA) A silent mutation	KR020722
7	Bovine mastitis	9.45**	8.51**	6.50**	1.91**	Silent mutations (n=3)	KR020721
8	Broiler breeders	2.77**	1.15	1.57**	**0.93**	lys^{13} (AAA)→Arg (CGA) Silent mutations (n=3)	KR020720
9	Broiler breeders	**0.30****	**0.30****	**0.30****	**0.24****	lys^{13} (AAA)→Arg (CGA) Silent mutations (n=3)	KR020719
10	Broiler breeders	3.92**	2.58**	1.97**	2.25**	Asp^{12} (GAT)→Val (GTC) lys^{13} (AAA)→Glu (GAA) A silent mutation	KR020718
11	Broiler breeders	14.12**	11.47**	7.62**	5.43**	Silent mutations (n=2)	KR020723
12	Broiler breeders	3.16**	**0.42****	**0.75****	2.08**	lys^{13} (AAA)→Asp (GAT) Silent mutations (n=2)	KR020724
13	Bovine mastitis	15.67**	7.52**	9.34**	6.73**	Silent mutations (n=2)	KR020725
14	Broiler breeders	4.20**	1.14*	**0.26****	2.35**	lys^{13} (AAA)→Glu (GAA) Silent mutations (n=2)	KR020726
15	Bovine mastitis	-	**0.27****	-	**0.19****	Deficient	-

Table 2. Correlation between phz and QS systems expression levels of *P. aeruginosa* strains.

***P. aeruginosa* strains**	**Correlation between *phz* and expression levels of**					
	***las*R**		***rhl*R**		***rhl*I**	
	r	**P**	**r**	**P**	**r**	**P**
1	0.912	0.015	0.539	0.054	0.545	0.053
2	0.801	0.023	-0.301	0.779	-0.175	0.396
3	0.722	0.019	0.841	0.016	0.963	0.014
4	0.683	0.042	0.584	0.052	0.306	0.054
5	0.304	0.051	0.478	0.055	0.593	0.042
6	0.397	0.051	0.488	0.052	0.742	0.045
7	0.311	0.051	0.383	0.053	0.535	0.057
8	-0.257	0.055	-0.931	0.068	-0.914	0.056
9	0.978	0.012	0.977	0.013	0.985	0.011
10	0.826	0.015	0.935	0.011	0.974	0.014
11	0.323	0.051	0.412	0.057	0.874	0.045
12	0.857	0.081	-0.815	0.274	-0.767	0.219
13	0.313	0.056	0.921	0.024	0.765	0.042
14	0.607	0.082	0.821	0.099	-0.934	0.565
15	-	-	0.917	0.013	-	-

Transcriptional analysis of QS communication circuit and controlled phenazine gene

Results of this study have a significant clinical impact. In particular, the changes in transcript levels of QS components and *phz* gene were significantly higher than those produced by the reference strain ($p < 0.05$) in majority of analyzed *P. aeruginosa* strains (Table 1).

Moreover, to determine if there was any relationship between the transcript levels of pyocyanin and the key QS regulators, a correlation analysis was performed. Indeed, there were significant positive ($p < 0.05$) correlations of *phz* transcript levels and QS signal molecules in 73.3% (11/15) of *P. aeruginosa* strains ($r \geq 0.3$). However, in three strains (No. 2, 12, 14), pyocyanin production positively correlated with the expression of *las*R only ($r \geq 0.6$), but there were no real correlations in the changes in the transcript levels of phenazine with those of *rhl*R and *rhl*I in strains No. 2 and 12 nor with that of *rhl*I in strain No. 14. Conversely, in *P. aeruginosa* strain No. 8, the target gene transcript level negatively correlated with the expression of its regulators suggesting that factors other than QS-based regulation of *phz* transcription appeared to have a more common effect than did the QS system transcript levels. Relationships between transcription levels of phenazine and those of individual QS systems are shown in Table 2.

It is conceivable that *las*I mutations associate with the relative expression levels of pyocyanin. Overall, there were reasonably high significant variations between the mean levels of *las*R, *rhl*R, *rhl*I, *phz* transcripts and mutation rates among 15 tested *P. aeruginosa* strains ($p < 0.0001$) (Table 3). Interestingly, transcription levels of highly mutating strains are more influenced than those with low mutations.

Actually, there were modestly significant differences noted in the levels of *las*R transcripts in *P. aeruginosa* isolates from bovine mastitis and broiler breeders with respiratory disorders ($t = 2.19$, $p = 0.032$). It is unclear if this association between clinical source and *las*R transcript level is of significance in regard to virulence; in spite of the bovine mastitis *P. aeruginosa* strains having higher levels of *las*R-regulated transcripts for *rhl*I and *phz* genes than those of the

Table 3. Relationship between mean of transcript levels of QS circuits and target genes and *las*I mutation rates.

QS circuits and their target gene	***las*I mutation rates**			**F-value**	**p-value**
	Deficient strains (n = 1)	**Low mutating strains (n = 12)**	**High mutating strains (n =2)**		
*las*R	-	7.11 ± 0.59^{a}	1.54 ± 0.41^{b}	59.80	< 0.0001
*rhl*R	0.27 ± 0.03^{b}	4.68 ± 0.47^{a}	0.94 ± 0.22^{b}	47.39	< 0.0001
rhl1	-	4.26 ± 0.43^{a}	0.72 ± 0.14^{b}	59.68	< 0.0001
phz	0.19 ± 0.04^{b}	3.29 ± 0.26^{a}	0.58 ± 0.12^{b}	68.19	< 0.0001

broiler breeders' strains, there were no significant associations ($p > 0.05$) between levels of the *rhl*R ($p = 0.823$), *rhl*I ($p = 0.203$) or *phz* ($p = 0.167$) genes and source of isolation (Figure 1).

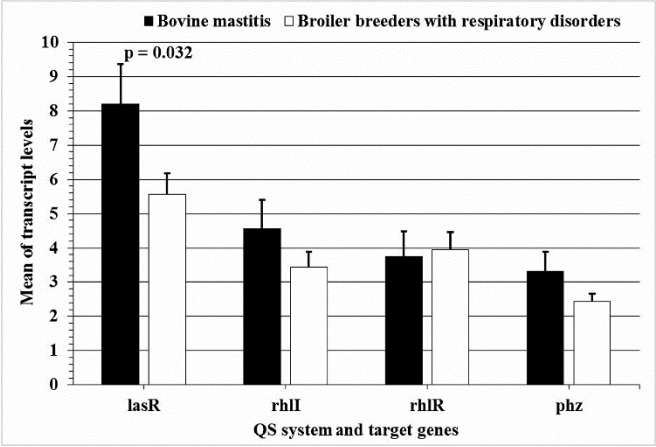
Discussion

Quorum sensing signaling systems play a vital role in the regulation of cell physiology of *P. aeruginosa* in a population density-dependent fashion [13]. The present study was undertaken to principally define the QS responses in *P. aeruginosa* clinical isolates by analyzing the relationship between transcriptional analysis of individual QS inducer or regulator component on the expression of pyocyanin production.

Our results provide additional evidence supporting this hypothesis; an extensive analysis of transcriptional responses using qRT-PCR indicated that most *P. aeruginosa* strains showed significantly higher expression levels of QS systems and their regulated genes in comparison to the reference strain, while they were parallel to that produced by the wild type in a previous study [14]. There were variations in transcript levels of the key QS regulators (*rhl*R and *las*R), *rhl*I and even among the QS-regulated transcriptional responses regarding *phz* expressions in comparable *P. aeruginosa* strains. In addition, statistical analysis revealed positive moderate-to-high correlations in the transcript levels between QS and *phz* genes in more than one half of the isolates demonstrating that both las and rhl QS systems contributes equally to pyocyanin production. In the light of the published genomic data, further support for the idea that the las system positively regulates the expression of both *rhl*R and *rhl*I have been defined [5]. Other studies reported the regulation of pyocyanin by QS previously [10,15,16]. It was also documented that pseudomonas strains can express increased pyocyanin levels despite decreased expressions in one or both QS systems. Moreover, in strains No. 2, 12, pyocyanin production positively correlated with the expression of *las*R only. The existence of such strains would indicate that *phz* production is not stringently controlled by all QS systems. In addition the QS systems are not absolutely essential for *P. aeruginosa* to establish infection, and other QS independent factors can substitute for the loss of QS-controlled mechanisms [14].

It is clear that the regulation of QS systems is not limited only to lasI/R and rhlI/R, but many other global regulators are interconnected with the QS circuitry. The activation of QS requires a critical cell density, although a quorum of bacteria is not sufficient by itself to trigger the QS-regulated genes as available elsewhere

Figure 1. Comparison of the expression levels of QS communication circuit and controlled phenazine gene in *P. aeruginosa* strains from bovine mastitis and broiler breeders with respiratory disorders.



The bars represent the means of transcript levels and the error bars represent the standard errors of the means. The *p* value represents the significant differences in *las*R transcript levels using independent samples t-test.

[17]. Therefore, it is not surprising that one strain (No. 8) displayed an impaired QS-dependent phenotype as *phz* gene transcript level negatively correlated with the expression of all QS systems. Similar to our findings, another study in USA has also identified a *P. aeruginosa* isolate with no pyocyanin activity, which was negative for both autoinducers, but contained other QS genes [14].

Interestingly, there were moderately significant differences in the mean levels of *las*R transcripts in *P. aeruginosa* isolates from bovine mastitis and broiler breeders with respiratory disorders, thereby; there was no functional link between QS transcriptional response and source of isolation. On the basis of molecular aspects of QS cascade in present and previous studies, a definitely little impact of QS on the strains` sources has been suggested [18].

In light of these elements, the mean levels of *las*R, *rhl*R, *rhl*I and *phz* transcripts were observed to be statistically significant with *las*I mutations, which explain the plurality of QS-dependent phenotypes observed in 14 *P. aeruginosa* strains, in which all four QS genes were positive with PCR. The presence of PCR products does not exclude the possibility that QS genes may have inactivating mutations demonstrating that *las* mutation does not lead to loss of virulence factors as anecdotally observed [19-22].

Most intriguingly, the present study showed the deficiency of QS components (*las*I, *las*R and *rhl*I) but *rhl*R was expressed in a low level with inadequate

pyocyanin expression in isolate No. 15, which contradicts the prevailing concept that the rhl system is inactive in the absence of a functional *las*R [5]. Importantly, *P. aeruginosa* can circumvent the deficiency of one of its QS systems by allowing the other to take over so, rhlR is able to overcome the las system when the latter is deficient by activating specific lasR-controlled functions [15].

Conclusion

Our results do not contradict the theory that the expression of QS genes in *P. aeruginosa* is definitely linked with the pathogen communal behavior such as pyocyanin production and emphasize that some *P. aeruginosa* strains are capable of causing clinical infections despite an impaired QS system. In addition, this work demonstrates that the QS hierarchy is more complex than the model simply presenting the las system above the rhl system and provide for the future identification of new factors involved in QS regulation.

Authors' contributions

NKA and MIA contributed equally in the conception and design of the study, acquisition of data, analysis and interpretation of the results, writing the paper, revising it critically for important intellectual contents and final approval of the version to be submitted and EYE helped in analysis and interpretation of data and final approval of the version to be submitted

References

1. Heurlier K, Dénervaud V, Haas D (2006) Impact of quorum sensing on fitness of *Pseudomonas aeruginosa*. Int J Med Microbiol 296: 93-102.
2. Ben Haj Khalifa A, Moissenet D, Vu Thien H, Khedher M (2011) Virulence factors in *Pseudomonas aeruginosa*: mechanisms and modes of regulation. Ann Biol Clin 69: 393-403.
3. Von Bodman SB, Willey JM, Diggle SP (2008) Cell-cell communication in bacteria: united we stand. J Bacteriol 190: 4377-4391.
4. Smith RS, Iglewski BH(2003) *P. aeruginosa* quorum-sensing systems and virulence. Curr Opin Microbiol 6: 56-60.
5. Latifi A, Foglino M, Tanaka K, Williams P, Lazdunski A (1996) A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators *Las*R and *Rhl*R (*Vsm*R) to expression of the stationary-phase sigma factor *Rpo*S. Mol Microbiol 21: 1137-1146.
6. Yahr TL, Parsek M*R* (2006) *Pseudomonas aeruginosa*, In Dworkin M, Falkow S, Rosenberg E, Schleifer K, Stackeprandt E,editors.The Prokaryotes, 3rd edition. New York: Springer Science+Business Media, LLC. 704-713.
7. Kiska DL, Gilligan PH (2003) Pseudomonas, In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. Manual of Clinical Microbiology, 8th edition. Washington, DC, USA: ASM Press. 719-728.
8. De Vos D, Lim AJr, Pirnay JP, Struelens M, Vandenvelde C, Duinslaeger L, Vanderkelen A, Cornelis P (1997) Direct detection and identification of *Pseudomonas aeruginosa* in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane lipoprotein genes, *opr*I and *opr*L. J Clin Microbiol 35: 1295-1299.
9. Savli H, Karadenizli A, Kolayli F, Gundes S, Ozbek U, Vahaboglu H (2003) 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 52: 403-408.
10. Bratu S, Gupta J, Quale J (2006) Expression of the las and rhl quorum-sensing systems in clinical isolates of *Pseudomonas aeruginosa* does not correlate with efflux pump expression or antimicrobial resistance.J Antimicrob Chemother 58: 1250-1253.
11. Jamunadevi S, Balashanmugam P, Muralitharan G, Kalaichelvan PT (2012) Molecular characterization of pathogenic and non-pathogenic *Pseudomonas aeruginosa* with special reference to phenazine gene. J Modern Biotechnol 1: 70-74.
12. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: e45.
13. Siehnela R, Traxlerb B, Anb DD, Parsek MR, Schaeferb AL, Singh PK (2010) Unique regulator controls the activation threshold of quorum-regulated genes in *Pseudomonas aeruginosa*. Proc Natl Acad Sci 107: 7916-7921.
14. Schaber JA, Carty NL, McDonald NA, Graham ED, Cheluvappa R, Griswold JA, Hamood AN (2004) Analysis of quorum sensing-deficient clinical isolates of *Pseudomonas aeruginosa*. J Med Microbiol 53: 841-853.
15. Dekimpe V, Déziel E (2009) Revisiting the quorum-sensing hierarchy in *Pseudomonas aeruginosa*: the transcriptional regulator *Rhl*R regulates *Las*R-specific factors. Microbiology 155: 712-723.

16. Karatuna O, Yagci A (2010) Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in *Pseudomonas aeruginosa* respiratory isolates. Clin Microbiol Infect 16: 1770-1775.
17. Venturi V (2006) Regulation of quorum sensing in pseudomonas. FEMS Microbiol Rev 30: 274-291.
18. Cabrol S, Olliver A, Pier GB, Andremont A, Ruimy R (2003) Transcription of quorum-sensing system genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. J Bacteriol 185: 7222-7230.
19. Déziel E, Gopalan S, Tampakaki AP, Le´ pine F, Padfield KE, Saucier M, Xiao G, Rahme LG (2005) The contribution of MvfR t*o Pseudomonas aeruginosa* pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting *las*RI*, rhl*RI or the production of N-acyl- L-homoserine lactones. Mol Microbiol 55: 998-1014.
20. Heurlier K, Denervaud V, Haenni M, Guy L, Krishnapillai V, Haas D (2005) Quorum-sensing-negative *(las*R) mutants of *Pseudomonas aeruginosa* avoid cell lysis and death. J Bacteriol 187: 4875-4883.
21. Salunkhe P, Smart CH, Morgan JA, Panagea S, Walshaw MJ, Hart CA, Geffers R, Tümmler B, Winstanley C (2005) A cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* displays enhanced virulence and antimicrobial resistance. J Bacteriol 187: 4908-4920.
22. Lujan AM, Moyano AJ, Segura I, Argarana CE Smania AM (2007) Quorum-sensing-deficient *(las*R) mutants emerge at high frequency from *a Pseudomonas aeruginosa* mutS strain. Microbiology 153: 225-237.

Corresponding author

Marwa Ibrahim Abd El-Hamid
Department of Microbiology
Faculty of Veterinary Medicine
Zagazig University, Egypt,
Saleh Abo Rahil Street 19, El-Nahal,
44519, Zagazig, Sharkia, Egypt
Phone: +20 055 2267009
Fax: +2 055 2283683
Email: mero_micro2006@yahoo.com

Conflict of interests: No conflict of interests is declared.