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The Effect of Qourum Sensing genes (*lasI, rhlI*) in Some Virulence Factors of *Pseudomonas aeruginosa* Isolated from Different Clinical Sources

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Abstract

Background and Objective: Quorum sensing (QS) is a chemical language of bacteria, and use of N-acyl-homoserine lactone (AHL) signals is one of the most known mechanisms by which bacteria can communicate with each other to regulate the gene expression based on bacterial density. Previous studies suggested that QS has an important role in pathogenicity of *P.aeruginosa*, so that the present study investigation the correlation of QS genes in local isolate of *P.aeruginosa* with production of virulence factors.

Materials and Method: The study included collection of 126 samples from various clinical sources and, these samples included wounds, burns, ear, cystic fibrosis and urinary tract infections (UTI). Pyocyanin and biofilm production were determined spectrophotometrically, QS genes were detected by PCR based on specific sequences for *lasI and rhlI* genes.

Results: Final diagnosis showed that 51 isolates belong to target bacteria and, the phenotypic detection of some virulence factors showed that 46 isolates (90%) showed variance in the strength of Pyocyanin production, and 44 (86%) of the isolates showed a differences in their ability to biofilm formation and, screed QS genes showed that 48 (94%) of isolates were positive for *las*I gene, while 44 (86%) of isolates have *rhlI* gene, and this study pointed that 9 isolates have one of QS genes *lasI* or *rhlI*, and also have one or more of virulence factors, as well as the results showed that 1 isolate out of 51 negative for both QS genes and weakly in production of virulence factors.

Conclusion: Most of local isolates were positive for QS genes and, QS plays an important role in the pathogenesis, not all of the virulence factors controlled by QS. The study indicated that the isolates which have both QS genes were more virulence than isolates that have single gene.

Keywords: *Pseudomonas aeruginosa, Quorum sensing, virulence factors, Biofilm, Pyocyanin.*

Introduction

Pseudomonas aeruginosa is a successful opportunistic secondary pathogen and most predominant species in community infection[(1)]. Immunosuppressed people are the individuals remain at high risk of this infection such as those with severe burns, cancer, cystic fibrosis and acquired immune deficiency syndromes (AIDS)[(2)]. The virulence factors of *P. aeruginosa* play an important role in the pathological state, the survival of the bacteria and the invasion of host tissues[(3)].

Bacteria have chemical molecule which mediated cell to cell communication system to regulate gene expression and group activates within communities[(4)].

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Quorum sensing (QS) was first discovered in the marine bioluminescent bacterium *Vibrio fisheri* in the period early 1970 [5]. QS is widely spread in the bacteria, and considered as a "speaking" systems, QS plays major role in virulence factor production, and biofilm formation [6]. The communication occurs through small molecules called acyl homoserine lactones (AHL) also known as "Autoinducers" that diffuse freely across the membranes of bacteria when bacterial density increase, these molecules when reach to threshold concentration act as a cofactors of transcriptional regulators [7].

P.aeruginosa has two of QS system *las* and *rhl* system[8]. The las system consist of *lasI*, which is responsible for the synthesis of N-(3-oxododecanoyl)-L-homoserine lactone (3O-C12-HSL), and the transcriptional activator *LasR* [9], the *las*R binds to (3-oxo-C12-HSL) molecule and regulates expression of specific genes [10]. While another QS system in *P. aeruginosa* consists of the *rhlI* and *rhlR* genes [11,12]. The *rhlI* synthase responsible for produce of the AHL (N-butyryl-L-homoserine lactone (C4-HSL), and *rhlR* is the transcriptional regulator only when *rhlR* is complexed with C4-HSL does it regulate the expression of several genes (rhamnolipid,elastase cyanide,alkaline protease, and pyocyanin production [13]. Briefly *lasI* and *rhlI* genes activate of *lasR* and *rhlR* gene to activate encoding virulent genes.

The QS system contributes in increase virulent of *P. aeruginosa*. However, only a few local studies are available concerning the role of *P.aeruginosa* QS systems in various clinical infections. So that the present study try to understanding the role of QS gene in production of virulence factors (pathogenicity) in local isolates of *P.aeruginosa*.

Methodology

The study samples (126) were collected from clinical state including both sexes with different ages, who suffered from; cystic fibrosis, urinary tract infections, wounds, burns and ear infection from different teaching hospitals in Baghdad city. The study was carried out through May 2016 till May 2017.

Laboratory and Molecular Diagnosis: The samples cultured in Pseudomonas agar and Cetramide agar. Lab. diagnosis was done according to Holt *et al* [14] while the molecular diagnosis was done based on *16SrRNA* gene as a detection gene.

Detection of some Virulence Factors: Pyocyanin production determinate according to Parsons *et al* [16] and, biofilm formation method was done according to Bose *et al* [17].

DNA extraction method: The bacterial DNA extracted according to Genomic DNA mini Kit which provides by Geneaid Company.

PCR technique used for Detection of QS Genes and *16SrRNA*gene

Primers (table1) were designed by Primer3 program according to **NCBI** and supplied by the Bioneer Company as a lyophilized product of different picomol concentrations.

Table 1: Sequence of primers and their size

Gene		Sequence of forward and reverse Primer(5´- 3´)	Size (bp)	Annealing Temp.°C
16SrRNA	F	GGGGGATCTTCGGACCTCA	956	58
	R	TCCTTAGAGTGCCCACCCG		
Las I	F	GCGCGAAGAGTTCGATAAAA	537	60
	R	ATCTGGGTCTTGGCATTGAG		
Rhl I	F	CTTGGTCATGATCGAATTGCTC	626	61
	R	ACGGCTGACGACCTCACAC		

F: Forward sequences, R: Reverse sequences

Sequencing of *lasI* and *rhlI* genes: To search for the presence of mutations that may affect the QS genes, *lasI and rhlI* underwent sequencing according to Senturk (23).

Results

The collected samples were cultured in some media agar to initially diagnosis based on morphological characteristics of the colonies. Molecular detection used to confirm final diagnosis of all isolates. The result of gel electrophoresis (figure 1) for amplification PCR product showed that presence of bands in same level for all isolates. Final diagnosis of *P.aeruginosa* showed that 51 isolates belong to target bacteria.

Pyocyanin pigment estimated by measure of absorbance in ELISA reader equipment (VERSAmax microplate reader, Molecular Devices, USA) at 690nm, the results showed that 46 (90%) of isolates have ability to produce pyocyanin through 3 days of incubation but in varying degrees (figure 2). While the results of biofilm formation showed that 44 (86.2%) of isolates produce biofilm**.**

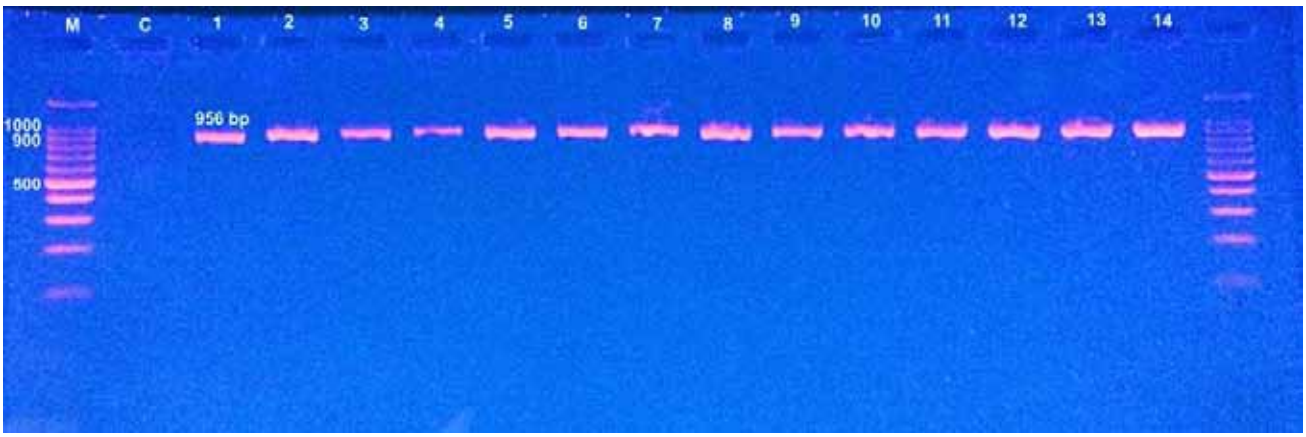

Figure 1: Agarose gel electrophoresis (1.5%agarose, 7 V/cm^2 for 90min) of *16SrRNA* gene (956bp). Lane M: represent 100bp DNA ladder, Lane C: represent negative control, Lanes 1-13: represent bands of *P.aeruginosa* gene.

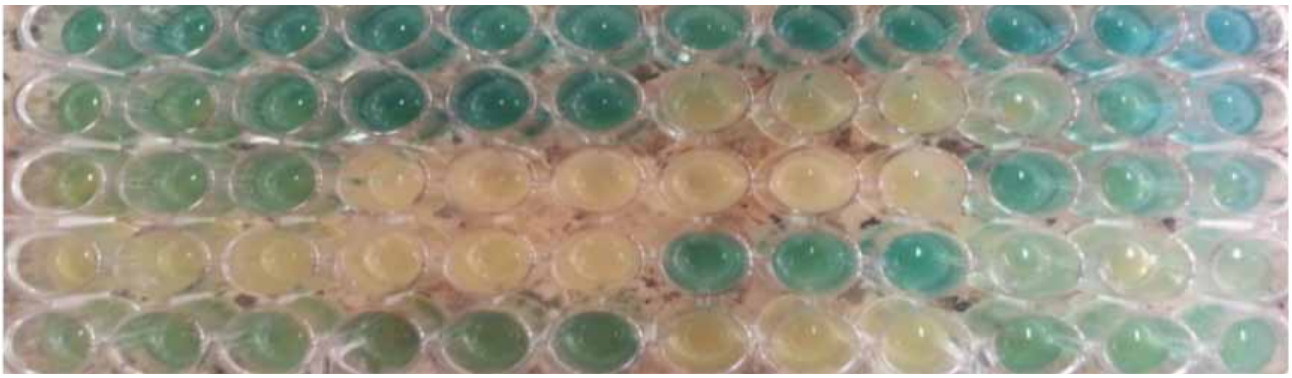

Figure 2: Produce of pyocyanin pigment by *P.aeruginosa* isolates.

Molecular detection of (QS) genes by used specific primers for *lasI and rhlI* genes showed that 48 (94.1%) of isolates were positive for this gene, where are the bands appeared within the expected size of the gene (537bp) for all positive isolates (Figure 3) .While 44 (86.2%) of isolates have *rhlI* gene (Figure 3), the results pointed that 9 isolates have one of QS genes *lasI* or *rhlI*, and also have one or more of virulence factors, and one isolates out of 51 negative for both QS genes. To determine if QS genes have mutations in some isolate, sequence analysis of the PCR products showed that no difference in sequences where the percent of matching 100% for *rhlI* gene and 99% for *lasI* gene when comparing with the information bank within the site www.ncbi.nlm.gov.

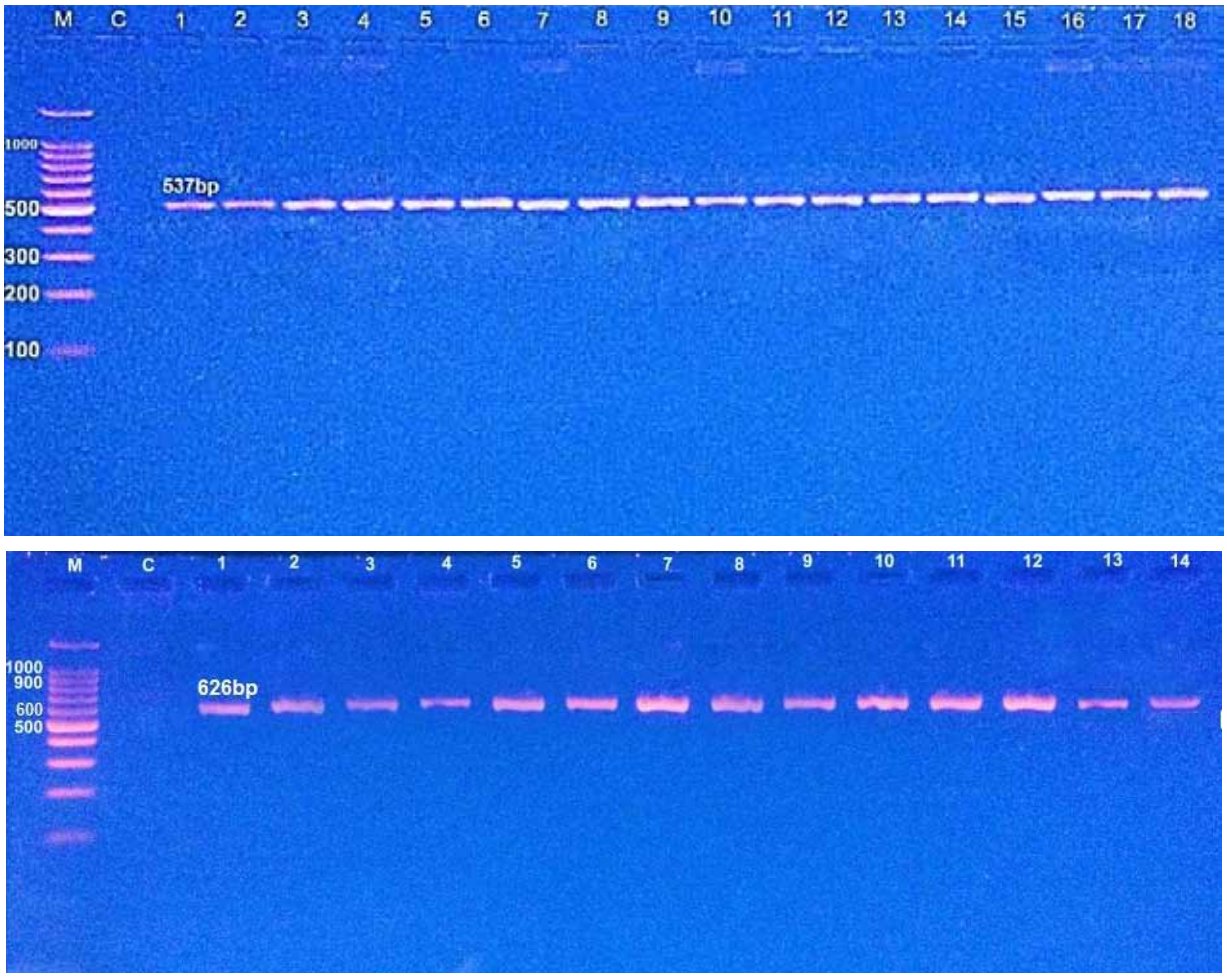

Figure 3: Agarose gel electrophoresis (1.5%agarose, 7 V/cm^2 for 90min) for (a) *rhlI* gene (626bp) and (b) *lasI* (537bp). Lane M: represent 100bp DNA ladder, Lane C: represent Negative control, Lanes 1-14, 18 represent bands of *P.aeruginosa* gene.

Discussion

In the present study examined the role of QS in production of some virulence factors of *P. aeruginosa* in various clinical infections. Final diagnosis based on molecular detection of *16SrRNA* gene which is considered one of the basic criteria in the classification because of highly constant and unable to change over time of its regions [15], showed that 51 isolates belong to target bacteria and, highest percentage of clinical isolates belong to burns followed by wounds, this may be explain that *P.aeruginosa* is one of the more bacterial species that cause burns and wounds infection in hospitals.

Virulence factors (Biofilm and Pyocyanin) were examined spectrophotometrically, result of biofilm formation indicated that most of isolate were able to biofilm formation, but in varying degrees with compare to negative control, the isolates capable to form biofilm ranged in intensity among high, medium and weak adherent. The high productivity of biofilm formation may be back to sensitivity of way to measure the few quantities formed, and considered important method in studying the early stages of biofilm formation [19], this results agree with Heydari and Eftekhar) [18]. While pyocyanin production increases over time, this explains the state of competition among bacterial cells on nutrients whereas the pigment consider is an antagonist, also our result nearly with Oleiwi [20].

Many studies and reports indicated that there is a strong correlation among QS system and biofilm formation, where the results of biofilm formation showed that (43/44) of isolates which have able to biofilm form were positive to QS genes. The *las* gene plays an

important role in maintenance of *P.aeruginosa* biofilm, where that the signaling 3-oxo-C12-HSL (synthesized by *LasI*) is necessary for the establishment of *P. aeruginosa* biofilm, whereas a *lasI* mutant forms a flat and thin biofilm, and *lasI* is expressed in a large number of cells during the initial stage of biofilm formation [22]. QS genes regulate the production of pyocyanin pigment, whereas results of pyocyanin production showed that 39/46 of isolates which are able to produce of pyocyanin were positive to *rhl*I gene.

Among of 51 isolates identified one isolate was defective in production of all virulence factors, but have QS gene*s lasI* and *rhl*I, this result may be indicated to absent of *lasR* or *rhlR* genes, because sequencing analyses revealed no mutation in *lasI* or *rhlI* gene of this isolate, or may be ways used in detect virulence factors were not sensitive to very few quantities produced by this isolate. This study pointed that 9 of isolates have one of QS genes *lasI* or *rhlI*, and also produce one or more of virulence factor. As well as the results showed that 1 isolate (P35) out of 51 negative for both QS genes and weakly in production of virulence factors these isolate from wound, this results agree with [24] who identified QS deficient in clinical isolate which lost all virulence factors tested, but still caused a wound infection, suggested that in addition to known virulence factors, there may be another factors yet uncharacterized involved in the pathogenesis of *P. aeruginosa.* Another possibility that may indicate or lead a QS deficient strain to cause infection is the presence of multiple strains of *P. aeruginosa* in the same infection site.

The present study confirm that important role of QS systems in Pathogensis of *P. aeruginosa* bacteria and also indicated that *P. aeruginosa* able to causing clinical infections in humans despite an weakness of QS system in some isolates. On the other hand these results do not contradict with theory that QS system plays a main role in *P. aeruginosa* pathogenicity, and not all virulence factor controlled by QS [21].

Conclusions

Most of local isolates of *P.aeruginosa* were positive for QS genes, also QS plays an important role in the pathogenesis of *P.aeruginosa* infection in varous, not all of the virulence factors controlled by QS system. The *lasI* gene has important role in the production of bioflim. Detection of bacteria by *16SrRNA* gene is very simple and rapid technique compare with other conventional method.

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Funding: Self

Ethical Clearance: Not required

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