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Detection of Quorum Sensing Genes of *Pseudomonas aeruginosa* Isolated from Different Areas in Iraq

Huda Abbas Mohammed[1], Ayaid Khadem Zgair[2*]
[1]*Environmental Research and Studies Center, Babylon University, Babylon, Iraq*
[2]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq*

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen. Quorum sensing (QS) is one of processes that are responsible for biofilm formation. *P. aeruginosa* can live in different environments, some of which are pathogenic (clinical isolates) and some that are found outside the body (environmental isolates). The present study aimed to determine the presence of a number of genes responsible for QS in clinical and environmental isolates of *P. aeruginosa*. In the present study full DNA was separated from all environmental and clinical isolates that contained seven genes (*rhlA*, *rhlR*, *rhlI*, *lasR*, *lasI*, *lasB*, *phzA1*) associated with QS occurrence. The total DNA was separated from all environmental and clinical isolates (PAE1, PAE2, PAE3, PAE4, PAE5, PAE6, PAE7, PAE8, PAE10, PAE11, PAE12, PAE14, PAC1, PAC2, PAC5, PAC7, PAC8, PAC9 and PAC10). This study found that all studied environmental and clinical isolates contained the seven genes *rhlA*, *rhlR*, *rhlI*, *lasR*, *lasI*, *lasB*, *phzA1*, which was associated with QS occurrence.

Keywords: *Pseudomonas aeruginosa*, biofilm formation, PCR, quorum sensing, DNA extraction.

الكشف عن جينات استشعار النصاب في *Pseudomonas aeruginosa* المعزولة من مناطق مختلفة في العراق

هدى عباس محمد[1] أياد كاظم زغير[2*]
[1] مركز البحوث والدراسات البيئية، جامعة بابل، بابل، العراق
[2] كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

ان بكتريا الزائفة الزنجارية هي من مسببات الأمراض الانتهازية. يعتبر استشعار النصاب (QS) أحد العمليات المسؤولة عن تكوين الأغشية الحيوية الرقيقة حيث يمكنها من العيش في بيئات مختلفة ، بعضها ممرض (عزلات سريرية) وبعضها موجود خارج الجسم (عزلات بيئية). تهدف الدراسة الحالية إلى تحديد وجود عدد من الجينات المسؤولة عن QS في العزلات السريرية والبيئية لبكتريا *P. aeruginosa*. في هذه الدراسة ، تم فصل كل الحمض النووي عن جميع العزلات البيئية والسريرية التي تحتوي على سبعة جينات تشمل *rhlA* ، *rhlR*، *rhlI*، *lasR*، *lasI*، *lasB*، *phzA1* ، والتي ارتبطت بحدوث QS. تم فصل الحمض النووي الكلي عن جميع العزلات البيئية والسريرية (PAE1، PAE2، PAE3، PAE4، PAE5، PAE6،

*Email: ayaid.zgair@sc.uobaghdad.edu.iq

PAC5، PAC2، PAC1، PAE14، PAE12، PAE11، PAE10، PAE8، PAE7، PAC7، PAC8، PAC9 و PAC10) . توصلت الدراسة الى ان جميع العزلات البيئية والسريرية قد احتوت على الجينات السبعة *rhlA* و *rhlR* و *rhlI* و *lasR* و *lasI* و *lasB* و *phzA1* ، والتي ارتبطت بحدوث QS.

Introduction

P. aeruginosa is a stick-shaped gram-negative pathogen that is associated with serious illnesses and infections such as pneumonia and various sepsis syndromes in humans, as well as it causes lung cancer. In addition, *P. aeruginosa* isolates infect immune compromised people, burns patients and individuals suffering from cystic fibrosis [1]. Its infection treatment is a big challenge as this bacteria is highly resistant to a wide spectrum of antibiotics [2]. *P. aeruginosa* swarming requires releasing of two external factors, rhamnolipids and 3-(3-hydroxyalkanoyloxy alkanoic acids) that play role as wetting factors [3]. However, a tiny chemical molecule called auto-inducer has the main role in connecting and leading the bacterial cell to combine and form a biofilm [4]. There is a correlation between the ability of bacteria to form biofilm and its resistance to antibiotics [2]. Previous studies have showed that environmental isolates of *P. aeruginosa* have an ability to form biofilm [5]. Quorum sensing is a process of gene organizing that uses the chemical indicators by many hosts-related bacteria for observing accumulation of population of bacteria in a certain area [6]. It is well known that the quorum sensing style differs from bacterium to bacterium, i.e.,gram negative bacteria use acyl-homoserine lactone (AHL) [7]. The adhesion part of biofilm formation and quorum sensing regulation to synthesis the signal such as *lasI* in *Pseudomonas* bacteria which have role to synthesis quorum sensing signal regulates by many genes [8]. In biofilms, the gene expression depends on bacterial response to the native ecological conditions.

Over the last decade, several researchers studied AHL indicators that are used by gram negative bacteria in the quorum sensing. The results clarified that *P. aeruginosa* genome expression of extracellular virulence agents created by *P. aeruginosa* is organized through quorum sensing [9]. Other studies found that quorum sensing has two specific patterns in *P. aeruginosa* of the *lasI* gene in the *las* quorum sensing patterns that are responsible for the diffusible extracellular indicator, N-(3-oxododecanoyl)-L-HSL.The first pattern is a *las* system, while the second pattern is *rhl* system that reacts together with *LasR* [10] to operate virulence genes including *lasB, lasA*, *apr*, *toxA* and *lasI* [11]. Composition of the siderophore pyoverdine also is stimulate by the *las* system[12],The 3OC12-AHL seems to division into cell membranes, and *P. aeruginosa* efflux pumps support divide the movement of this signal to the external environment although 3OC12-HSL is diffusible . The synthesis of N-butyryl-L-HSL (C4-AHL) catalyzes by *rhlI* product[13].Pyocyanin,Cyanide,and Chitinase are virulence factors, they are positively organized by the *rhl* system[14].

Previous studies highlighted the role of several genes in QS directly and indirectly such as *las* and *rhlR* [15]. These systems are coiled in a hierarchical manner, *las* system controls *rhl* system together, the transcriptional and posttranslational fields, It also has a role in the production and development of biofilms, In addition to antibiotic resistance[16] .A complicate phenazin biosynthetic lane found in *P. aeruginosa*, that consist of two symmetric core loci included phzA1B1C1D1E1F1G1, phzA2B2C2D2E2F2G2, they are responsible for the production of phenazine-1-carboxylic acid in addition to other three supplemental genes inclusive *phzM*, *phzS* and *phzH* encoding individual enzymes implicated in the transformation of phenazine-1-carboxylic acid to pyocyanin,1-hydroxyphenazine, in addition to phenazine-1-carboxamide [17]. The genes that are responsible for QS had been covered by several studies but the question raised here is regarding the existence of QS genes in clinical isolates of *P. aeruginosa* in parallel with QS genes that are present in environmental isolates of *P.*

aeruginosa. The current study provided an answer to this question

Materials and Methods

Samples Collection

Fifty-three samples were collected from burn wound, sputum of patients suffering from respiratory tract infections and ear infections. Samples were collected from indoor patients after getting an ethical approval from the Ethical Committee in the Department of Biology, College of Science, University of Baghdad and signed consents from the patients. Samples were cultured onto nutrient agar under sterile conditions. Subsequently, seventy samples collected from Shatt Al-Hillah and the Al-Yahudia rivers, were placed in sterile glass containers and 0.1 ml from liquid specimens were cultured onto nutrient agar [18].

*P. aeruginosa*Isolation and Identification

Suspicious isolates of *P. aeruginosa* were cultured onto cetrimide agar. The isolated colonies of suspected *P. aeruginosa* appeared yellow-green and blue-green under fluorescent down ultra violet rays light. Standard method was followed to identify bacterial isolates. VITEK 2 DensiCheck tool (bioMe´rieux) (ID-GNB card) was used in the present study [19, 20].

QS genes Detection

Nucleic acid was extracted to detect several genes (*rhlA*, *rhlR*, *rhlI*, *lasR*, *lasI*, *lasB*, and *phzA1)* that are responsible for QS and biofilm formation in the clinical and environmental isolates of *P. aeruginosa*. DNA was extracted according to the manufacturer's instructions of G-spin^{TM}iNtRON kit. Primers were used for the detection of genes by polymerase chain reaction (PCR). Sequences of primers used in the current study are mentioned in Table 1.

Table 1: Description of primers used in PCR technology to detect some of the studied QS and biofilm genes [21]

Amplicon	Primer	Sequence 5'-3'	Position	Length (bp)
rhlA	rt_rhlA-F	AACATTCAACGTGGTGCTG	155	100
	rt_rhlA-R	ATTTCCACCTCGTCGTCCTT	254	
rhlR	rt_rhlR-F2	CTGGGCTTCGATTACTACGC	112	124
	rt_rhlR-R2	CCCGTAGTTCTGCATCTGGT	215	
rhlI	rt_rhlI-F	GGAGCGCTATTTCGTTCG	429	100
	rt_rhlI-R	GTAGGCCGGGAAGCTGAT	528	
lasR	rt_ lasR –F	CGGTTTTCTTGAGCTGGAAC	15	100
	rt_ lasR –R	GCCGAACAGGATCTTCGAG	114	
lasI	rt_ lasI –F	GGCTGGGACGTTAGTGTCAT	94	100
	rt_ lasI –R	CCTGGGCTTCAGGAGTGTCAT	193	
lasB	rt_ lasB –F	AACCGTGCGTTCTACCTGTT	1279	100
	rt_ lasB –R	TGGTGGCGGTCCAGTAGTA	1378	
phzA1	rt_ phzA1 –F	AACCACTACATCCATTCCTTCG	355	100
	rt_ phzA1 –R	CGGCTATTCCCAATGCAC	454	

Two microliter of extracted DNA (extracted from all clinical and environmental isolates of P. aeruginosa) was mixed with 8 µl of the master mix (BioNeers,USA), 7.5 µl of free water ddH_2O and 0.5 µl of $MgCl_2$. After adding 2µl of primer to the final mix and it was then placed in PCR machine (Biometra, Germany) for incubation at 94°C for 5 min. The temperatures and duration of each cycle (35 cycles) is shown in Table 2.

Table 2: PCR reaction schedule for 35 cycles that were applied for detecting *rhlA, rhlR, rhlI, lasR, lasI, lasB,* and *phzA1* in the clinical and environmental isolates of *P. aeruginosa*.

Stage	Temperature (°C)	Time	Number of Cycle
Initial denaturation	94	5 min	1
Denaturation	94	30 sec	35
Annealing	55	30 sec	
Elongation	72	30 sec	
Final elongation	72	5 min	1

Gel Electrophoresis

0.8 gram of the agarose was added to 80 ml of the Tris-borate-EDTA (TBE) buffer. The mixture was heated using microwave oven. Subsequently, the mixture temperature was decreased to 50-60°C. Finally, 4 µl of Red Safe stain (iNtRON, Korea) was added to agarose prior to solidifying. Comb was put at one end of the gel template and then the agarose mixture was poured into template. Next it was left at room temperature for 30 minutes. After that the tank tray was loaded with TBE buffer. Finally, after releasing the comb from the template, the template was placed into the tank tray. DNA samples were prepared by mixing 1 µl of bacterial DNA by blending 1 µl of dye. The samples and leader were loaded in the agarose wells and then the electrophoresis device (BIORAD, UK) was turned on at 75 volt/cm, 20 mA. Gel documentation system was used to get the pictures of bands [22].

Results and Discussion

In the current study, a number of clinical samples were collected from patients suffering from burns wound infection, respiratory tract infections, otitis and a number of river water samples. A number of *P. aeruginosa* isolates were separated and diagnosed based on some chemical tests and the colony shapes on the culture media, where 10 isolates (PAC1, PAC2, PAC3, PAC4, PAC5, PAC6, PAC7, PAC8, PAC9 and PAC10) were pre-identified as *P. aeruginosa* isolated from clinical samples and 12 isolates (PAE1, PAE2, PAE3, PAE4, PAE5, PAE6, PAE7, PAE8, PAE9, PAE10, PAE11 and PAE12) were pre-identified as *P. aeruginosa* isolated from river water. In order to obtain a more accurate diagnosis, all isolates were re-diagnosed using the VITEK 2 DensiCheck technique. From the 10 clinical isolates only 7 were confirmed as *P. aeruginosa* (PAC1; PAC2; PAC5; PAC7; PAC8; PAC9; and PAC10*)* and from the 12 environmental isolates only 11 (PAE1; PAE2; PAE3; PAE4; PAE5; PAE6; PAE7; PAE8; PAE10; PAE11; PAE12) were confirmed as *P. aeruginosa.*

In the current study, DNA was extracted from both clinical and environmental isolates of *P. aeruginosa*. PCR technique was applied to determine the presence of seven genes (*rhlA, rhlR, rhlI, lasR, lasI, lasB,* and *phzA1*) associated with the QS and biofilm phenomenon.The

results showed that the studied genes were found in all clinical and environmental isolates of *P. aeruginosa*.

Figures 1-7 show an electrophoresis image from PCR results that were taken by gel documentation system which showed the amplification genes by PCR technology. The figures show that all the identified genes had the number of base pairs (bp) around 100, except *rhIR* gene where the number of base pairs was 120.

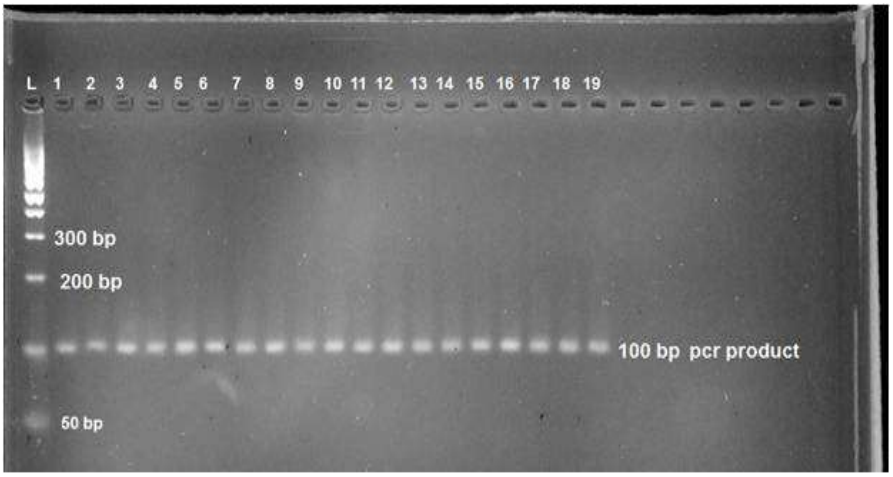

Figure 1: Agarose gel electrophoresis photograph for *rhlA* amplified by PCR technology. The number of base pairs was 100. The samples were run at 75 volt and 20 mA for 1.5 hours, the concentration of agarose was 10 gm/l.

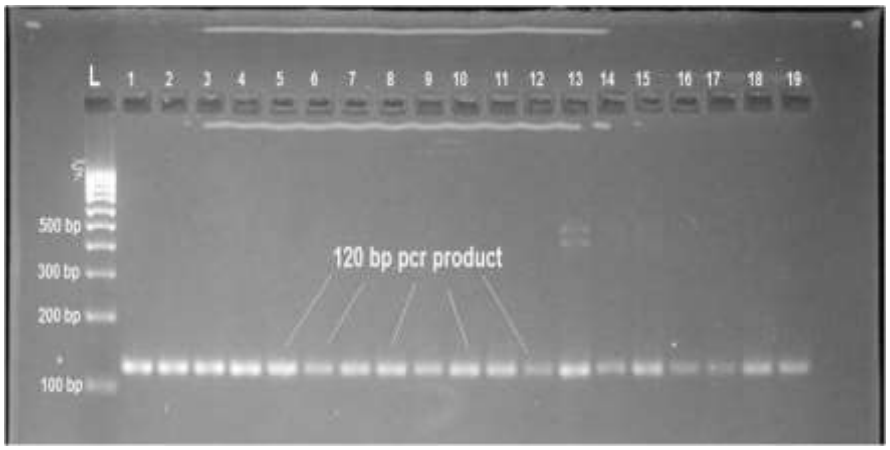

Figure 2: Agarose gel electrophoresis photograph for *rhlR* amplified by PCR technology. The number of base pairs was 120. The samples were run at 75 volt and 20 mA for 1.5 hours, the concentration of agarose was 10 gm/l.

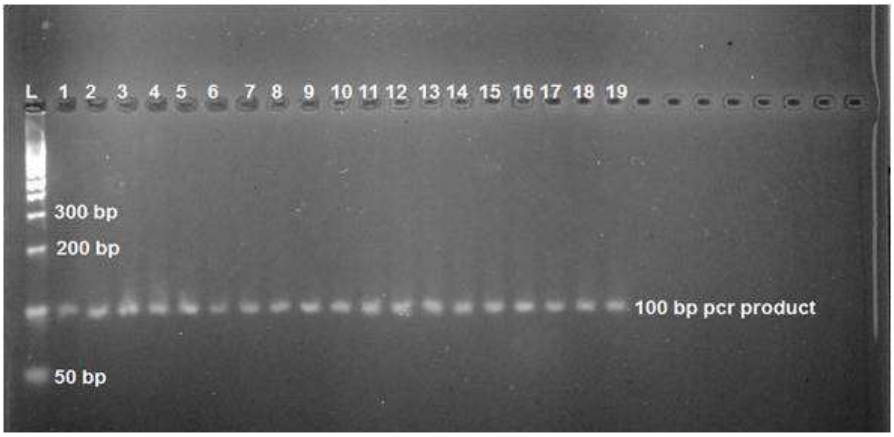

Figure 3: Agarose gel electrophoresis photograph for *rhlI* amplified by PCR technology. The number of base pairs was 100. The samples were run at 75 volt and 20 mA for 1.5 hours, the concentration of agarose was 10 gm/l.

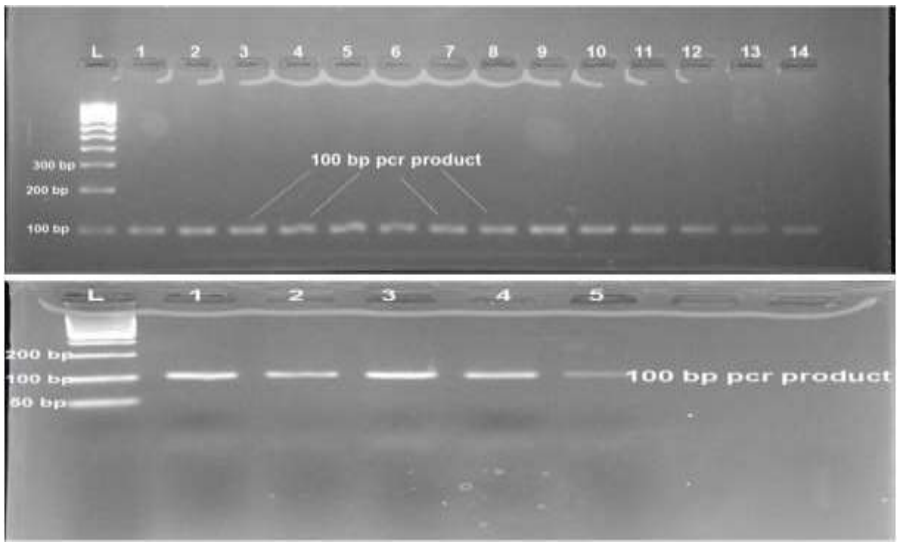

Figure 4: Agarose gel electrophoresis photograph for *lasR* amplified by PCR technology. The number of base pairs was 100. The samples were run at 75 volt and 20 mA for 1.5 hours, the concentration of agarose was 10 gm/l.

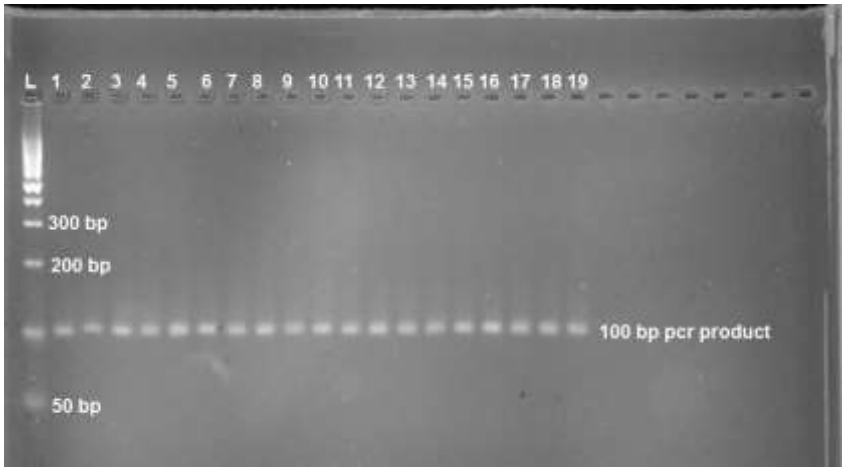

Figure 5: Agarose gel electrophoresis photograph for *lasI* amplified by PCR technology. The number of base pairs was 100. The samples were run at 75 volt and 20 mA for 1.5 hours, the concentration of agarose was 10 gm/l.

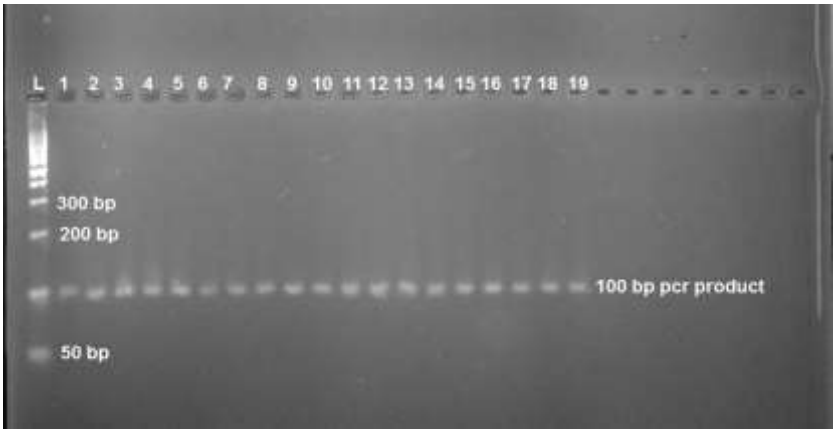

Figure 6: Agarose gel electrophoresis photograph for *lasB* amplified by PCR technology. The number of base pairs was 100. The samples were run at 75 volt and 20 mA for 1.5 hours, the concentration of agarose was 10 gm/l.

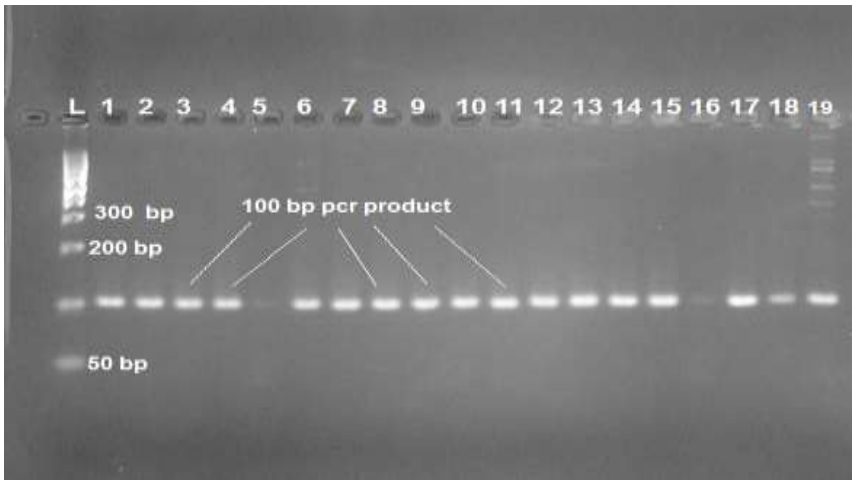

Figure 7: Agarose gel electrophoresis photograph for *lasB* amplified by PCR technology. The number of base pairs was 100. The samples were run at 75 volt and 20 mA for 1.5 hours, the concentration of agarose was 10 gm/l.

Several previous results showed the ability of *P. aeruginosa* to form biofilm [23, 24]. There are different factors that play an important role in its formation, QS phenomenon being one of them [25]. There are two pyramidal structures of the QS for *P. aeruginosa*, *las* and the *rhl* (26). The condensation of molecule signaling for 3-oxo-dodecanoyl of the homoserine lactone increased when the inhabitant's thickness grew in particular to form a complex with *lasR*. This complex operates the genes transcription as a *lasI*, *rhlR* and other genes [26].
rhl structure consists of the *rhlR* protein and the auto inducer synthesize *RhlI* which is incorporated to form auto inducer N-butyryl homoserine lactone. These structures are twisted in the manner of pyramidal structures. Indeed, the *las* structure is controlled by the *rhl* structure, in terms of transcriptional and also the next translational areas together. According to this fact, the condensation of marking molecule 3-oxo-dodecanoyl homoserine lactone which interacts with *LasR* at to activate a number of virulence genes such as *lasB*. These structures are wrapped up in the pyramidal manner.. Our results proved that proteases, exotoxin A, rhamnolipids and pyocyanin are considered as the virulence factors in the *P. aeruginosa*. This formation and organization is considered as an indicator of the QS, and also merge the formation and development of the biofilm. It also plays an important role in antibiotic resistance [16].

The results of the present study agree with recent research that indicates the selection genes chosen as targets, and also as one of the causes of epidemic, help spread of the *P. aeruginosa* bacteria. Further, the clinical isolates were able to form biofilm which had *lasR*, *rhlI*, and *rhlR* genes. As for the general, isolates included *lasI*. This finding also agrees with a previous study [27].

Conclusions:

The total DNA separated from all environmental and clinical isolates (PAE1, PAE2, PAE3, PAE4, PAE5, PAE6, PAE7, PAE8, PAE10, PAE11, PAE12, PAE14, PAC1, PAC2, PAC5, PAC7, PAC8, PAC9 and PAC10) contained the following seven genes: *rhlA*, *rhlR*, *rhlI*, *lasR*, *lasI*, *lasB*, *phzA1*, which are associated with QS and biofilm formation.

Abbreviations

DNA:Deoxyribonucleic acid; *P. aeruginosa*: *Pseudomonas aeruginosa*; PAE1:*Pseudomonas aeruginosa* environmental isolate 1; PAE2: *Pseudomonas aeruginosa* environmental isolate 2; PAE3: *Pseudomonas aeruginosa* environmental isolate3; PAE4: *Pseudomonas aeruginosa* environmental isolate4; PAE5: *Pseudomonas aeruginosa* environmental isolate5; PAE6: *Pseudomonas aeruginosa* environmental isolate 6; PAE7: *Pseudomonas aeruginosa* environmental isolate 7; PAE8: *Pseudomonas aeruginosa* environmental isolate8; PAE10: *Pseudomonas aeruginosa* environmental isolate10;PAE11:*Pseudomonas aeruginosa* environmental isolate11; PAE12: *Pseudomonas aeruginosa* environmental isolate12, PAE14: *Pseudomonas aeruginosa* environmental isolate14; PAC1: *Pseudomonas aeruginosa* clinical isolate1; PAC2: *Pseudomonas aeruginosa* clinical isolate2; PAC5: *Pseudomonas aeruginosa* clinical isolate5; PAC7: *Pseudomonas aeruginosa* clinical isolate7; PAC8: *Pseudomonas aeruginosa* clinical isolate8; PAC9: *Pseudomonas aeruginosa* clinical isolate9;PAC10: *Pseudomonas aeruginosa* clinical isolate10; PCR:poly chain reaction; AHL:acyl homoserine lactone;HSL: homoserine lactone.

References

[1] A.K. Zgair, S. Chhibber, " Immunological and Biological Relationship among Flagellin of *Pseudomonasaeruginosa*, *Burkholderiacepacia*, and *Stenotrophomonas maltophilia*" *Microbiology*,vol.81, no.3, pp. 342–347, 2012 .

[2] H.A. Mohammed, A.K. Zgair, "The Effect of Silver Nanoparticles on Biofilm Formation in Environmental Isolates of Pseudomonas aeruginosa" *Indian Journal of Public Health Research & Development*, vol. 11, no.4, pp.1619-1624b, 2020.

[3] J. Tremblay, A.P. Richardson, F. Lépine, É. Déziel," Self-produced extracellular stimuli modulate the Pseudomonas aeruginosa swarming motility behavior" *Environmental Microbiology*, vol. 9, no.10, pp.2622-2630,2007.

[4] Y.H. Li, X.Tian, "Quorum sensing and bacterial social interactions in biofilms" *Sensors*, vol.12, pp. 2519-2538, 2012.

[5] H.A. Mohammed, A.K. Zgair. "Isolation and Identification Pseudomonas aeruginosa from Clinical Samples and Irrigation Water: Susceptibility to Common Antibiotics" *Indian Journal of Public Health Research & Development*, vol.11, no.4, pp.1474-1479a, 2020.

[6] W.C. Fuqua, S.C. Winans, E.P.Greenberg, "Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators" *Journal of Bacteriology*, vol.176, no.2, pp.269–275,1994.

[7] W.R. Galloway, J.T. Hodgkinson, S. Bowden, M. Welch, D.R. Spring, "Applications of small molecule activators and inhibitors of quorum sensing in Gram-negative bacteria" *Trends in Microbiology*, vol. 20, pp.449-458, 2012.

[8] K.K.Jefferson, "What drives bacteria to produce a biofilm" *FEMS Microbiology Letters* , vol. 236, no.2, pp.163-173, 2004.

[9] C. Fuqua, E.P. Greenberg, "Self-perception in bacteria: quorum sensing with acylated homoserine

lactones", *Curr. Opin. Microbiol*.vol.1, pp.183–189,1998.

[10] L. Passador, J.M.Cook, M.J. Gambello, L. Rust, B.H. Iglewski, "Expression of Pseudomonas aeruginosa virulence genes requires cell-to-cell communication" *Science*. vol. 260, no.5111, pp.1127–1130,1993.

[11] P.C. Seed, L. Passador, B.H. Iglewski, "Activation of the Pseudomonas aeruginosalasI gene by LasR and the Pseudomonasautoinducer PAI: an autoinduction regulatory hierarchy" *Journal of Bacteriology*, vol.177, no.3, pp.654–659,1995.

[12] A. Stintzi, K. Evans, J. Meyer, K. Poole, "Quorum-sensing and siderophore biosynthesis in Pseudomonas aeruginosa: lasR/lasI mutants exhibit reduced pyoverdine biosynthesis" *FEMS Microbiology Letters*, vol.166, no.2, pp.341-345,1998.

[13] M.K. Winson, M. Camarat, A. Latifi, M. Foglino, S.R. Chhabra, et al. "Multiple N-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in Pseudomonas aeruginosa" *Proceeding of the National Academy of Sciences of the united states of America*, vol. 92, no.20, pp.9427–9431,1995.

[14] A.Latifi, K.M.Winson, M. Foglino, B.W. Bycroft, G.S.A.B. Stewart, A. Lazdunski, and P.Williams, "Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in Pseudomonas aeruginosa PAO1", *Molecular Microbiology*, vol.17, no. 2, pp. 333–344,1995.

[15] E.C. Pesci, J.P. Pearson, P.C. Seed, B.H. Iglewski, "Regulation of las and rhl quorum sensing in Pseudomonas aeruginosa", *Journal of Bacteriology*, vol. 179, no.10, pp.3127–3132,1997.

[16] D.G. Davies, M.R. Parsek, J.P. Pearson, B.H. Iglewski, J.W. Costerton, E.P. Greenberg, "The involvement of cell-to-cell signals in the development of a bacterial biofilm". *Science*, vol. 280, no.5361, pp.295–298,1998.

[17] D.V. Mavrodi, R.F. Bonsall, S.M. Delaney, M.J. Soule, G Phillips, L.S.Thomashow, "Functional Analysis of Genes for Biosynthesis of Pyocyanin and Phenazine-1-Carboxamide from Pseudomonas aeruginosa PAO1" *Journal of Bacteriology*, vol.183, no.21, pp.6454–6465,2001.

[18] J.F. MacFaddin. "*Biochemical Tests for Identification of Medical Bacteria" (3rd ed.): Williams and Wilkins, Baltimore, USA*, 2000.

[19] L.R.R. Perez, A.L. Barth, "Biofilm production using distinct media and antimicrobial susceptibility profile of Pseudomonas aeruginosa", *The Brazilian Journal of Infectious Diseases*, vol.15 , no.4, pp.1413-8670,2011.

[20] N.M. Ali, S.A. Mazhar, B. Mazhar, A. Imtiaz, S. Andleeb, "Antibacterial activity of different plant extracts and antibiotics on pathogenic bacterial isolates from wheat field water" *Pakistan Journal of Pharmaceutical Sciences*, vol.30, no.4, pp.1321–1325,2017.

[21] M.V. Grosso-Becerra, G. Croda-García, E. Merino, L. Servín-González, R. Mojica-Espinosa, G. Soberón-Chávez, "Regulation of Pseudomonas aeruginosa virulence factors by two novel RNA thermometers" *Proceeding of the National Academy of Sciences of the united states of America*, vol.111, no.43, pp.15562-15567, 2014.

[22] J.F. Sambrook, D.W. Russell, "*Molecular cloning: A laboratory manual",3rd ed. Cold spring harbor laboratory press*, vol.1,2,3, pp.2100,2001.

[23] S.S. Branda, S.Vik, R.Kolter, "Biofilms: the matrix revisited" *Trends Microbiol*.vol.13, no.1, pp.20-6,005.

[24] M.A. Abbasi, A. Zeb, A. Rehman, S.Z. Siddiqui, S.A.A.Shah, M.Shahid, H. Fatima, "Synthesis, Bacterial biofilm inhibition and cytotoxicity of new N-Alkyl/aralkyl-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4- nitrobenzene-sulfonamides" *Pakistan Journal of Pharmaceutical Sciences* ,vol. 33, pp.41-47,2020.

[25] M.J. Kirisits, M.R. Parsek, "Does Pseudomonas aeruginosa use intercellular signalling to build biofilm communities" *Cellular Microbiology*,vol.8, no.12, pp.1841-1849,2006.

[26] M. Schuster, E.P. Greenberg. "A network of networks: quorum sensing gene regulation in Pseudomonas aeruginosa". *International Journal of Medical Microbiology*, vol.296, no.2-3, pp.73-81,2006.

[27] J.L.C. Lima, L.R. Alves, P.R.L. Jacomé, J.P.B. Neto, M.A.V. Maciel, M.M.C. Morais. "Biofilm production by clinical isolates of Pseudomonas aeruginosa and structural changes in LasR protein of isolates non biofilm-producing" *The Brazilian Journal of Infectious Diseases*, vol.22, no.2, pp.129-136,2018.