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Study gene expression for *the PelF gene responsible for the biofilm formation of Pseudomonas aeruginosa using qPCR.*

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Abstract
The bacterial isolates results explained that there are 66 bacterial isolates belonging to the bacterium P. aeruginosa in reality, 46 infection isolation and 20 environmental isolation. They confirmed the diagnosis using genetic diagnostics and the use of 16SrDNA and observed the appearance of one band in the drilling of the gel at the same level for all isolates.
The Micro titration plates method (MTP) was used to detect isolates' ability to form the biofilm; this method gives a numerical value for the absorbency using an ELISA reader device, considered a quantitative assay, more accurate and sensitive than other methods. The isolates were diverse in forming biofilm between productive with high efficiency, medium efficiency, and low efficiency.
For gene expression study for these isolates, extracted RNA was done for all isolates, then the amount of gene expression by qRT- PCR technology of *PelF* gene that responsible for biofilm formation for studied samples that represented by 56 isolates as treatment isolates and ten isolates as control isolates of each gene using the One Step Real-time–PCR and all samples were genetically expressive, with varied results of gene expression between high samples of expression and medium of expression and a weak expression, with highly significant differences and probability (P <0.001).
Keywords: gene expression, *biofilm, PelF gene & Pseudomonas aeruginosa*

INTRODUCTION
The bacterium Pseudomonas aeruginosa of the most significant types of negative bacteria dye gram pathogenic in human history because they are many virulence that have factors has an impact with Pathogenicity, which is associated with cell Flagellam and pillies of the called Pili IV used by the attaching process on epithelial and settlement cells and then formation biofilm infection in a position of injury, after the gathering in large numbers, and biofilm protects it from the body's defenses and increase resistance to antibiotics[1] , So the membrane bio style is of important growth patterns in the rapid physiological adaptive organisms pathological, it helps biofilm bacteria to survive in the harsh coditions in the host , and longer appendages filamentous peptide Fimbria surrounding the bacterial cell of the most important bacterial components that have a role in attaching qualitative that develops to the formation of biofilm[2] , It was noted that these appendages receptors quality glycoprotein in cells that are connected by surfaces, occurs adhesion between the surface of the bacteria molecules quality and their receptors in the surfaces objectives

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cells, and the formation contiguous clusters court to resist the environmental conditions and unfavorable factors and characterized these gatherings have cannels, during which the transfer nutrients and cellular secretions[3], Many of the bacteria tend to form biofilm which is a layer of external polysaccharides and different expression in the composition of the layer depending on the conditions is appropriate, such as a lack of nutrients and lower the pH, as well as to provide the necessary protection to bacterial populations of the host defenses. Biofilm is an essential adjective for the continuation of infection[4]. With formation, the bacteria show high antibiotic resistance, and by up to 1000 times than it is in the species, bacteria is composed of biofilm. Therefore, there is a variation in the expression of a bacterial cell to another depending on the conditions surrounding and the virulence of those bacteria[5]; as the biofilm formation, bacteria P.aeruginosa helps increase the virulence and survival for a long time[6].

It is often the expression of phenotypic mode through the synthesis of proteins that control the shape of the organism, or control of the enzymes stimulate the metabolic pathways that characterize specific organism[7], The bacterial cells work and constantly work requires that express the genes in them and the word Expression means the gene products that are usually RNA or protein, carries DNA original genetic information is used to store this information and are not indicated as a direct work of a living cell, but instead working copies of the gene factor RNA is the process of transferring genetic information from DNA to RNA is called cloning Transcription then use portable information on the mRNA strand to give a series of amino acids in a polypeptide this includes the translation process Translation and which are converted the genetic code to protein reading[8], And thus are mainly the regulation of gene expression levels copies of the genetic material and construction of the new version of the mRNA and also the translation levels that control metabolism of organisms that depend on environmental conditions and the needs of the cell of nutrients conditions surrounding[9].

The Real-time PCR technology is the latest technological invention which allows an average of amplifying DNA, which is measured by detection of the probe or the dye-tagged that are flash radiator at each cycle of the interaction cycles[10], And that the purpose of estimating the amount of DNA where in-based dye SYBR Green detection screening is formed by the dye link in tow strands the DNA instead of linking with strand and one is flash formed monitored once for each cycle after the production of the elongation of the strand of DNA and increases flash after each cycle depending on the concentration of the initial template for DNA[11], It can also start with small amounts of DNA and determined final product accurately and moreover, there is no need for processing by PCR, which provides resources and time these benefits in addition to the particles sent to fluorescence upon which the Rt-PCR brought about a complete revolution in the estimation of the quantity of each of the the DNA and RNA, as it can not happen contamination because the interaction systems are in a closed system all these advantages have enabled the use of such technology in several areas to determine the extent of gene expression to determine the efficiency of medicine, forensic medicine, bio-medicine, microbiology, veterinary science, agriculture, biotechnology, science Toxicology[12].

MATERIALS AND METHODS

Bacterial isolation and identification

Collected 207 samples from patients suffering from various disease states, including burns, wounds, cystic fibrosis, ear infections and eyes inflammation , Was obtained samples from the laboratories

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of some hospitals in Baghdad included the Central Child Hospital and the Canadi Teaching Hospital and the Hospital of the abn of the Albaladi for Women's and Children's Hospital and al-Numan, has been diagnosed specimens with the methods culture Using several media by observing their ability to grow on the mdia Pseudomonas agar, your specific media of bacteria Pseudomonas and Cetrimide agar that was selective media of bacteria pseudomonas aeruginosa and thriving culture on the media MacConkey agar and also culture on Blood base agar to diagnose characteristics culturally also as shape and color of the colonies[13], And the use of biochemical tests such as Oxidase and Catalase, and confirmed the diagnosis of genetic diagnosis and the use of gene 16S rDNA definitive diagnosis of bacterial samples.

ELISA use in the measurement of biofilm formation

The specimens were culture Microtitration plates method (MTP) and, after an incubation of 24 hours, washed with a solution of phosphate buffer to get rid of bacterial cells non-attaching and then colored dye (Crystal violet) and then washed wale with distilled water to remove dye then added 200 Micrometre of ethanol 95% to wales and measured absorption spectrum Optical density of the bacterial cells attached and coloring with (ELISA reader) by nm630 length[14].

Study Gene Expression

Isolation of RNA using an Accuzol Reagent

The extraction and purification were done using a total RNA extraction kit per the manufacturer's instructions (Bioneer).

Gene expression to form a biofilm

The study of gene expression using gene *PelF* responsible for the formation of biofilm bacteria *P.aeruginosa* using Real-Time PCR (one-step RT-qPCR) and Using the specific primer *PelF* gene with accountable for biofilm formation and primer *gyrB* gene user to calibrate the results as shown in the table(1) below:

Result (bp)	Primer sequence (5′–3′)	primer
200	TGTTCGAGCTGAGCAGTTG	**pelF–F**
	GATCAGCGGCACGAAGAA	**pelF–R**
190	GGCGTGGGTGTGGAAGTC	**gyrB –F**
	TGGTGGCGATCTTGAACTTCTT	**gyrB –R**

The reaction conditions used in the device q RT-PCR and by program contained in [15] as the following table (2):

Step	Temperature	Duration	Cycles
Reverse Transcription	42°C	5 min	Hold
Enzyme inactivation	95°C	3 min	Hold
Denaturation	95°C	3 sec	40 cycles
Annealing	59°C	20 sec	
Extension	72°C	20 sec	

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Calculate the value Ct.

Method Livak equation was used to calculate the value ct (Cycle Threshold) and then the gene expression value as follows:

ΔCt(treated sample) = Ct Gen – Ct *gyrB*

ΔCt(control) = Ct Gen – Ct *gyrB*

ΔΔCt = ΔCt(treat.) - ΔCt(con.).

Fold1= $2^{-\Delta\Delta Ct}$.

RESULTS AND DISCUSSION

After necessary analyses for diagnosing bacteria were obtained on 66 samples back to bacteria Pseudomonas aeruginosa, 207 samples of different disease and environmental states were collected.

Table (3) The number of specimens and percentages Distributed according to disease and environmental cases.

percentage %	Positive samples	Number of samples	Sample source	Sequence
%76.19	48	63	Burns	-1
%73.33	33	45	Wounds	-2
%70	14	20	Eyes	-3
% 60	18	30	Ear	-4
% 66.66	6	9	C.F	-5
% 90	18	20	Soil	-6
% 80	16	20	Water	-7
%73.91	153	207		Total

The results showed that all the bacterial specimens under study could produce biofilm using the Microtitration plates method (MTP) with a percentage of 100%, and Statistical analysis also showed significant differences apparent with significant levels ($P \leq 0.05$).

RNA was extracted for all bacterial specimens diagnosed with pathological and environmental cases and had 66 sample Figures (1). RNA was obtained at a concentration of high purity_ concentration ranged between (122-198) ng \ µm while purity measured ranged at (260 \ 280)between (1.38 - 2.82).

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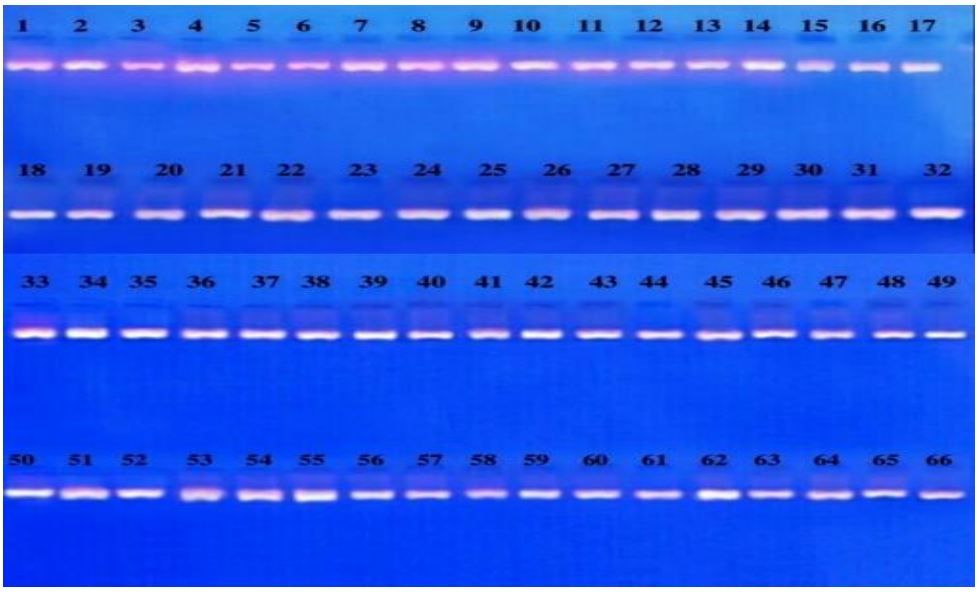

Figure(3-4): Agarose gel electrophoresis (1% agarose,7v/cm² for 60 min) RNA extracted from 66 samples of bacteria P. aeruginosa represents the path M (1-10) samples burns, M (11-20) samples eyes inflammation, M (21-30) samples Ear Infection, M (31-40) samples water, M (41-50) samples soil, M (51-60) samples wounds and M (61-66) samples cystic fibrosis.

The gene expression by technology qRT-PCR for gene PelF responsible for the formation of Biofilm samples studied represented 56 sample treatments and ten samples the control of the gene the amount of gene expression, which has been measured in a way One Step, which is transferred RNA to cDNA in the tube One. Measured way Relative Quantification, which measures gene expression in the sample depending on the reference sample known or control Calibration, has been using natural soil samples as Normal flora that was not exposed to any conditions; the value of the gene to measure gene expression in both samples also are compared to the results for each Ct sample with the results Ct of gene expression for gene *gyrB* genes, which represents the Housekeeping gene of the bacteria *pseudomonas aeruginosa* table (4):

Table (4) Ct values and ΔCt values of the PelF gene for control samples

PelF	16.42	17.22	17.96	17.29	16.16	16.47	17.38	16.43	17.92	18.94
gyrB	14.41	15.30	16.12	15.17	14.40	14.82	15.11	14.95	16.23	16.76
ΔCt	2.02	1.92	1.84	2.12	1.76	1.65	2.27	1.48	1.69	2.18

The results of gene expression for gene *pelF* responsible for production Biofilm varied values specimens studied, where he was the highest expression of *pelf* generate is for samples Source of ear infections, which expression rate is 2.13 came second in the order of burns samples at a rate of gene expression was 1.76, and followed samples cystic fibrosis at the rate of gene expression for gene *pelF*

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amounted to 1.57 followed the wounds sample with gene expression rate of 1.02 then eyes samples came at a rate of gene expression for gene *pelF* was 0.87 with less gene expression samples sourced the water reached a rate of gene expression 0.81, While statistical analysis showed that there were significant differences in the level of a high probability of (P <0.001) table(5)

Table(5) Gene expression for gene *pelF* responsible for the production Biofilm

Folding / Samples	1	2	3	4	5	6	7	8	9	10	Mean	P value
Burn	1.8	1.6	1.8	1.4	1.7	1.9	1.5	2	1.8	2.1	1.76	0.001
Ear	2.29	2.42	1.98	1.75	2.2	2.45	1.98	1.76	2.3	2.25	2.13	
Eyes	0.81	0.93	0.85	0.83	0.96	0.82	0.86	0.91	0.9	0.88	0.87	
Wound	1.02	1.1	0.98	1.04	1.12	1.17	0.92	0.9	1.03	1	1.02	
Water	0.83	0.81	0.75	0.8	0.85	0.79	0.77	0.83	0.86	0.81	0.81	
C.F	1.6	1.56	1.45	1.67	1.48	1.71	-	-	-	-	1.57	
Mean	1.391	1.403	1.301	1.248	1.385	1.473	1.206	1.280	1.378	1.408		
P value	0.001											

P value<0.001

Isolation showed that the results of the most pathological specimens obtained were of burns and wounds, with 76.19% and 73.33%, respectively. The results of this study are compatible with a somewhat [16], As it found that the bacteria *P.aeruginosa* is the most isolated in samples burns and wounds taken from Baghdad's hospital's Percentage was 76%, with a study conducted in Iran[17], Where the rate of infection of wounds and burns *P.aeruginosa* is the most common, and with 73.1%, As it indicated[18] in the results of its study that the highest percentage of P. aeruginosa bacteria was 78.57% of infections and burns that are compatible with the current results of our research.

When the screening for specimens producing Biofilm, they were all productive and 100% agreed with the current study results of the study conducted by the[19] In the city of Ramadi on bacteria and P. aeruginosa bacteria Klebsiella spp. The productivity of P. aeruginosa bacteria biofilm in a way (MTP)As it were all of the specimens and 100% highly productive biofilm, The high percentage of viability samples bacteria P. aeruginosa on the production of bio-membrane explains the high rates

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of resistance by these specimens the direction of all antibiotics As it biofilm plays a significant role in the pathogenesis of bacteria and resistance to many antibiotics because they are immersed in the host proteins and layer microbial mucous, which provide a suitable place for the growth of bacteria and other organisms which increases the resistance to the treatment and this creates a big problem[20],[21].

All samples were expressive genetically of gene *pelF* responsible for biofilm formation results of our study identical a somewhat with[22] In China in the study of the virulence factors of the bacteria P. aeruginosa gene expression, which was the high expression of the results as the value of gene expression for of gene pelF reached value 5.5 and high a significant differences (P <0.005), Our results also agreed with the study occurred in America by[23] In the study of gene expression for of gene *pelf* technology Real-time PCR as the value of gene expression for of gene *pelf* 2.2 and difference was significant with level (P <0.05), When I was approached in the results of our study of[24] Which found that the value of gene expression for of gene pelf bacteria *P. aeruginosa* which was sourced from cystic fibrosis has reached 5.7, While our results differed with the study conducted in Australia by[25] In the study of biofilm of bacteria P. aeruginosa, which was the value of gene expression for of gene pelf are -1.3.

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Conflict of interests

The authors declare that there is no competing interest.

References

1. **Zubair, M.; Malik, A.; Ahmad, J.; Rizvi, M.; Farooqui, K.; Rizvi, M.** (2011). A study of biofilm production by gram-negative organisms isolated from diabetic foot ulcer patients. J. Bio. Med.; 3(2): 147-157.
2. **Yan, J., & Bassler, B. L.** (2019). Surviving as a community: antibiotic tolerance and persistence in bacterial biofilms. *Cell host & microbe*, *26*(1), 15-21.
3. **Su, L., Feng, Y., Wei, K., Xu, X., Liu, R., & Chen, G.** (2021). Carbohydrate-based macromolecular biomaterials. *Chemical Reviews*, *121*(18), 10950-11029.
4. **Pinna, D.** (2021). Microbial growth and its effects on inorganic heritage materials. *Microorganisms in the Deterioration and Preservation of Cultural Heritage*, *3*.
5. **Sharma, D., Misba, L., & Khan, A. U.** (2019). Antibiotics versus biofilm: an emerging battleground in microbial communities. *Antimicrobial Resistance & Infection Control*, *8*(1), 1-10.
6. **Romeu, M. J., Rodrigues, D., & Azeredo, J.** (2020). Effect of sub-lethal chemical disinfection on the biofilm forming ability, resistance to antibiotics and expression of virulence genes of Salmonella Enteritidis biofilm-surviving cells. *Biofouling*, *36*(1), 101-112.

The Peerian Journal
Open Access | Peer Reviewed
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ISSN (E): 2788-0303
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Email: editor@peerianjournal.com

7. **Montaño López, J., Duran, L., & Avalos, J. L**. (2022). Physiological limitations and opportunities in microbial metabolic engineering. *Nature Reviews Microbiology*, *20*(1), 35-48.
8. **Brueckner F, Armache KJ, Cheung A, et al.** (2009). Structure–function Acta Crystallogr; 65 (2):112–20.
9. **Acevedo, N., Alashkar Alhamwe, B., Caraballo, L., Ding, M., Ferrante, A., Garn, H., ... & van Esch, B. C.** (2021). Perinatal and early-life nutrition, epigenetics, and allergy. *Nutrients*, *13*(3), 724**.**
10. **Zhang, X., Fan, X., Wang, Y., Lei, F., Li, L., Liu, J., & Wu, P.** (2019). Highly Stable Colorimetric Sensing by Assembly of Gold Nanoparticles with SYBR Green I: from charge screening to charge neutralization. *Analytical chemistry*, *92*(1), 1455-1462.
11. **Atceken, N., Munzer Alseed, M., Dabbagh, S. R., Yetisen, A. K., & Tasoglu, S.** (2023). Point-of-Care Diagnostic Platforms for Loop-Mediated Isothermal Amplification. *Advanced Engineering Materials*, *25*(8), 2201174.
12. **Kaltenboeck** B,Wang C (2005). Advances in real-time PCR: application to clinical laboratory diagnostics. Adv Clin Chem; 40:219–49.
13. **Baron**, E. J. ; Finegold, S. M. and Peterson, I. L. R. (2007) .Bailey and Scotts Diagnostic Microbiology. 9th ed. *Mosby Company*. Missouri.
14. **Bose,** S. ; Khodke, M. ; Basak, S. ; Mallick, S.K.(2009).Detection of biofilm producing *Staphylococci*: need of the hour. *J. Clin. Diag. Res.*, 3:1915-1920.
15. **Dumas, J.L.; Delden, C.V.; Perron, K. and Delden, T.K.(**2006). Analysis of antibiotic resistance gene expression in *Pseudomonas aeruginosa* by quantitative real- time PCR. FEMS Microbiol. Lett.; 254:217-225.
16. , Al-Mustansiriya University.
17. **Rastegar, L.A.R.; Alaghehbandan, R. and Akhlaghi, L.** (2005). Burn wound infections and antimicrobial resistance in Tehran, Iran: an increasing problem. Annals of Burns and Fire Disasters; 453(2): 1-9.
18. **Alnuaimi, Ula Abdul Kareem Kadom.** (2015). Study the Effect of Probiotics on Biofilm Formation and Production of Protease Enzyme by Pseudomonas aeruginosa Isolated from Contaminated Burns and Wounds. College of Basic Education, Al-Mustansiriya University.
19. **Moteeb, H.** (2008). Quantitative and Qualitative assay of bacterial Biofilm produced by Pseudomonas aeruginosa and Klebsiella spp. J. of Al-Anbar University for pure sci.; 2(3): 2030-2033.
20. **Hassan, A.; Usman, J.; Kaleem, F.; Omair, M.; Khalid, A.; and Iqbal, M**.(2011). Evaluation of different methods of Biofilm formation in the clinical isolates. Braz. J. Infect Dis.15(40):305-311.
21. **Saxena, S.; Banerjee, G.; Garg, R. and Singh, M.** (2014). Comparative Study of Biofilm Formation in *Pseudomonas aeruginosa* Isolates from Pationts of Lower Respiratory Tract infection. J.Clin.and Dia.Res.8(5): 9-11.
22. **Ronghao Chen,YudingWeng, FengZhu, YongxinJin, ChangLiu, XiaoleiPan, Bin Xia, ZhihuiCheng, ShouguangJin and WeihuiWu**.(2016). Polynucleotide Phosphorylase Regulates Multiple Virulence Factors and the Stabilities of Small RNAs RsmY/Z in Pseudomonas aeruginosa; 7(247): 1-12.

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23. **Yun Kang, David T. Nguyen, Mike S. Son and Tung T. Hoang.** (2008). The *Pseudomonas aeruginosa* PsrA responds to long-chain fatty acid signals to regulate the fadBA5 b-oxidation operon. Microbiology; 154 (6):1584–1598.
24. **Melissa Starkey, Jason H. Hickman, Luyan Ma, Niu Zhang, Susan De Long, Aaron Hinz, Sergio Palacios, Colin Manoil, Mary Jo Kirisits, Timothy D. Starner, Daniel J. Wozniak, Caroline S. Harwood, Matthew R. Parsek**(2009). Pseudomonas aeruginosa rugose minor colony variants have adaptations likely to 6 promote persistence in the cystic fibrosis lung. Bacteriol.; 191(11): 3492-3503.
25. **Nicolas Barraud.** (2007). Nitric oxide-mediated differentiation and dispersal in bacterial biofilms. College of Science. New South Wales Sydney University.