Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 8, 3425-3436

# Evaluation Of Quorum Sening Lasr And Lasa Genes In Pseudomonus Aeruginosa And It`S Relation With Biofilm Formation

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**ABSTRACT** : 100 clinical samples in total were gathered from various clinical sources. , from different ages , the results of culture and biochemical tests showed that 65 isolate was P. aeruginosa. The source of these isolates which was burn swab n=22 (44%) ,urine culture n=11(22%) ,wound swab n=5(10%), sputum n=4(8%) ,ear swab, n=7(14%) and blood n=1(2%). Clinical specimen were collected through the period extending from September 2021 to January 2022, from Al-Yarmouk Teaching hospital and AL-Imameen Al-Kazmeen medical city.

In addition, various classical and modern culturing with biochemical tests were conducted to differentiate these isolates from another nosocomial infection bacterium. On the other hand the study was also aimed to identify existence, dispersion and occurrence rate of Las A and Las R genes by conventional PCR technique, and this Genes believed that it have arelation In regards to the capacity of isolates to produce biofilms using the microtiter plate method (MTP), Bacteria exhibit two social phenomena: quorum sensing and biofilm development. The study of the relationship between quorum sensing and biofilms is known as sociomicrobiology.

The results showed that76% of isolates was biofilm producers on the other hand LasR Gene was revealed in 58% of the samples while LasA Gene was estimated in 56% in p.aeruginosa isolates. and these gene especially Las R was found in most producer biofilm isolates. The presence of the investigated Genes and the isolates' capacity to produce biofilms were significantly correlated.

**Key words**: Biofilm, burns, Las R gene, LasA gene, Conventional PCR

## Introduction

An aerobic, gram-negative rod known as Pseudomonas aeruginosa does not produce spores that may infect both immune competent and immune compromised hosts. (1) Its proclivity for infecting immunocompromised hosts, extraordinary adaptability, It is a challenging organism to treat in modern medicine due to antibiotic resistance and a wide variety of dynamic defenses.(2)

It is a frequent reason why people get hospital-acquired infections, and it can be particularly problematic in intensive care facilities. Several groups, such as individuals with cystic fibrosis, chronic obstructive pulmonary disease (COPD), or healthcare-associated pneumonia (CF), are affected by its infections, which are linked to significant morbidity and death (3)

Pseudomonas aeruginosa can create biofilms, which are complex structures

made up of bacteria. The most essential aspect of biofilm infections is antibiotic resistance. The production of biofilms is a complicated process that is influenced in part by quorum sensing signals.. Extracellular DNA, for example, plays a role in the initial adherence of cells to surfaces as well as the development of the biofilm matrix (eDNA) (4), While both Pseudomonas aeruginosa and Staphylococcus aureus infect CF patients during childhood, Pseudomonas aeruginosa predominates in adulthood, causing to a loss in lung function.(5) , Drug resistance in recurrent P. aeruginosa infections is mostly brought on by the accumulation of pathoadaptive mutations or by the horizontal gene transfer of antibiotic resistance genes (ARGs).(6), Antibiotic-resistant P. aeruginosa strains are becoming more common in communities and hospitals, resulting in severe infections and a therapeutic stalemate.(7)

It is a widespread infection that can infect almost all tissues. Its significance in burn wounds, lung, eye, urinary tract, joint, gastro-intestinal, and a variety of systemic infections is influenced by a wide range of virulence factors (8), Because of its capacity to carry multiple-resistance plasmids, Pseudomnas strains resistant to all known antibiotics have emerged. (9).

Using a complex quorum-sensing (QS) network, the bacterial pathogen Pseudomonas aeruginosa stimulates expression of several virulence genes in a cell density-dependent way. In P. aeruginosa, the acyl-homoserine-lactone circuits LasI-LasR and RhlI-RhlR mediate QS. Numerous genes, including those that code for RhlI-RhlR, must be activated for LasI-LasR to function (10) The Pseudomonas quinolone signal (PQS), a third QS system, and the recently discovered integrated QS (IQS) system, a fourth QS system, are both present in P. aeruginosa. (11), Additionally, P. aeruginosa is recognized as a "model organism" in the field of quorum sensing, which has been well studied. There is evidence that quorum sensing regulates several bacterial genes. Nearly 300 genes are controlled by quorum sensing in P. aeruginosa.(12)

A second elastase called LasA is transcriptionally regulated by the Las R gene. It was the first gene from Pseudomonas aeruginosa that had been cloned and sequenced that was involved in proteolysis and elastolysis. Despite earlier claims to the contrary, whether or not lasB elastase was present, the size of the LasA protein was the same. As demonstrated by the production of lasA in the lasR-negative mutant PAO-R1, the LasA protein is produced in an active state in the absence of (lasB) elastase or alkaline protease and is also a protease with elastolytic activity. (13). This research sought to ascertain the capacity of p. aeruginosa isolates from burn, wound, urine, sputum, and blood sources from hospitals in Baghdad, Iraq, to produce biofilms, as well as the presence of quarum detecting genes LasA and LasR.

## 2. **methodology**

### 2.1 Identification of bacterial isolates:

Clinical specimen were collected through the period extending from September 2021 to January 2022, from Al-Yarmouk Teaching hospital, AL-Kadhmiya , 100 samples from numerous clinical sources, representing varied ages The samples came from a variety of clinical sources , the

results of culture and biochemical tests showed that 50 isolate was P. aeruginosa. The source of these isolates which was burn swab n=22 (44) % ,urine culture n=11(22%) ,wound swab n=5(10%), sputum n=4(8%) ,ear swab, n=7(14%) and blood n=1(2%),under sterile conditions from all genders and ages. Transformed media were used to collect and transfer specimens (brain heart broth). they were cultured on the Petri dishes, blood agar, MacConkeys Agar and Cetrimide( pseudomonas selective Agar), and incubated aerobically at a temperature 37 ïC for 24 hours. .

Microscopic examination was carried out by soaking a smear of bacterial growth in distilled water, heat coloring it with Gram stain, and then examining it using a light microscope with an oil immersion lens to assess the Gram response, cell shape, and arrangement.(14).The isolates were identified by using vitek2 (bioMérieux) (15)

### 2.2 **Biofilm production by Microtiter Plat Method(MTP):**

The capacity of 50 isolates to produce biofilms was evaluated by 96 –well micro titer assay then the microtiter plate was measured spectrobolometrically at620 nm . According to the following formula, the optical density cut-of value (ODc) was determined to be three standard deviations (SD) above the mean optical density (OD) of the negative control. The formula is : ODc = average OD of negative control + (3×SD of negative control). The outcomes were categorized into four groups based on their optical densities. (16)

### 2.3 **conventional polymerase chine reaction:**

50 isolats were tested using cPCR to find the presence of the Las A and Las R genes. .The positive colonies for the culturing and biochemical examination were extracted by using the Wizard Genomic DNA Purification Kit from Promega.

as per the manufacturer's instructions , aconventional polymerase chain reaction (C-PCR) is not quantitative, but rather qualitative method for detection of microorganisms, We used c-pcr to detect two quarum sensing Gene,two primers for LasA Gene (forwared primer: 5'-CGA CAA GAG CGA ATA CCT GGAG-3') and (reveres primer: 5'-CAA CTG GTA TTC CTC GAA ACC GTA- 3')and two primers for LasR Gene(forwared primer: 5’ aagtggaaaattggagtggag 3’)and (reveres primer: 5’ gtagttgccgacgacgatgaag 3’)

The primers were designed using a Perl primer program, and National Center for Biotechnology Information (https://www.ncbi.nlm. nih.gov/). the product size for LasA Gene were 226bp and

130bp for Las R Gene pcr reaction cared out by adding 13 microliter of master mix from (Intron/Korea) to one microliter of both forwared and revers primers and three microliter from DNA then the volume was completed to 25 microliter by adding 7microleter from de-aeunized free water.

The amplified DNA obtained after PCRprocedure was electrophoresesusing1.5% agarose gel, then stained with safety red DNA staining and visualized under the UV light

( **Table -1): primers sequence of genes used in this study**

| Primers | Nucleotide sequences(5`to3`) | Length | Size of amplified product (bp) |
|---|---|---|---|
| **Las A-F** | 5'-CGA CAA GAG CGA ATA CCT GGAG-3' | 22 | 226bp |
| **Las A-R** | 5'-CAA CTG GTA TTC CTC GAA ACC GTA- 3' | 24 | |
| **Las R -F** | 5’ aagtggaaaattggagtggag 3’ | 21 | 130bp |
| **Las R-R** | 5’ gtagttgccgacgacgatgaag 3’ | 22 | |

### 3.Statistical Analysis:

To identify the impact of various factors on study parameters, the Statistical Analysis System- SAS (2018) application (17) was utilized. Chi-square test was used to compare percentages (0.05 and 0.01 probability) significantly, and Least Significant Difference test (ANOVA) was used to compare means significantly. Estimation of the correlation coefficients-r between some study variables

### 4.RESULTS

3.1 the identifying of isolates

Clinical specimen were collected through the period extending from September 2021 to January 2022, from Al-Yarmouk Teaching hospital and AL-Imameen Al-Kazimeen Medical city , 100 sample were collected from various clinical sources, the results of culture and biochemical tests showed that50 isolate was P. aeruginosa. The source of these isolates which was burn swab n=22 (44) % ,urine culture n=11(22%) ,wound swab n=5(10%), sputum n=4(8%) ,ear swab, n=7(14%) and blood n=1(2%) , and the difference here was significant (P≤ 0.05). as shows result in table-2

**table -2: Isolation percentage of p.aeruginosa**

| Specimen type | Specimen no. | Isolates no. |
|---|---|---|
| **Burn** | 31 (62%) | 22 (44%) |
| **Urine** | 20 (40%) | 11 (22%) |
| **Wound** | 18 (36%) | 5 (10%) |
| **Sputum** | 17 (34%) | 4 (8%) |
| **Ear** | 10 (20%) | 7 (14%) |
| **Blood** | 4(8%) | 1 (2%) |

| | | |
|---|---|---|
| **Total** | 100 | 50 (100%) |

Samples were collected and transferred by transformed media (brain heart broth) and inoculated on MacConkey agar, Blood agar and nutrient agar at 37 C for 24 hr. The colonies on MacConkey agar appeared as a pale color because this pathogen is not fermenting lactose and it produces a diffusing green pigment in the agar .On blood agar gave the β type of hemolysis while some gave A single colony was injected on Cetrimide agar, a selective and differential medium used for the identification of P. aeruginosa, following the type on blood agar after 24 hours at 37 C were selected. Increased colonies, brilliant green growth, and a grape-like odor are all characteristics of P. aeruginosa. Acetyl trimethyl ammonium bromide, which serves as a quaternary ammonium cationic detergent and prevents the growth of other microorganisms by releasing N and P from microorganisms, is employed in this medium to test an organism's capacity to grow in its presence. (18), On cetrimide, the majority of non-Pseudomonas species are inhibited.

### 3.2 Results of biochemical tests

The results of the biochemical tests showed that all bacterial isolates gave a positive result for the analysis of oxides and catalase enzymes, and negative result of Urease. All isolates gave negative results for the methyl red and voges proskauer test, also ability of bacteria to consume the cimmon citrate agaras a single source of carbon, by turning the color of the media to blue color (19)

### 3.3 Biofilm formation ability of the isolates

The result of biofilm detection show that 38(76%) specimen was biofilm producers (14 isolates heigh producers,14 isolates moderate and 10 isolates was weak producers)while the remining 12 (24%)spacemens were biofilm non-produceres as shown in the( table-3) .among 14(28%) strong biofilm isolates 12(24%) were burn isolates and 2(4%) were urin isolates and among 14 (28%) moderate biofilm isolates were 9(18%) were burn ,3(6%)were urin,1(2%) sputum and 1(2%) ear and among 10(20%)weak isolates were 4(8%) urine ,2(4%) wound 3(6%) seputum and 1(2%) ear on the other hand among the 12 non-biofilm producers 1(2%)was burn,2(4%) urin,4(8%)wound,3(6%)sputum and 2(4%)was ear isolates

**( Table-3) :results of bacteria ability to form biofilm**

| **Biofilm producer isolates NO. (%)** | | | **Non-biofilm producer isolates NO. (%)** |
|---|---|---|---|
| **Strong biofilm isolates NO. (%)** | **Moderate biofilm Isolates NO. (%)** | **Weak biofilm Isolates NO. (%)** | |
| **14 (28%)** | **14 (28%)** | **10 (20%)** | **12 (24%)** |

### 3.4 Conventional Polymerase Chine Reaction (C-PCR).

The Las A Gene was detected in 28 isolates out off 50 isolates while LasR Gene was detected in 29 isolates out off 50 ( figure -1),(Figure -2) on the other hand these isolates show different results of biofilm strong , moderate, weak and nonbiofilm producers and these isolates were from different sources, the strong biofilm isolates were from burns ,the moderate biofilm isolates were from burns and urine, while weak isolates for biofilm production were from wound ,sputum ,ear and urine .Las A Gene is aquorum sensing gene so belived that it was have an effect on biofilm production ,among 28 isolates have positive results for LasA gene, 7 (25%)were strong biofilm and 7(25%)were moderate biofilm, also LasR Gene is aregulatory Gene in LasI-Las R QS system so it`s pracence in isolates may increase its ability to produce biofilm ,among 29 isolates 9(31%)was strong biofilm and 6(20.6%) was moderate biofilm .the association between virulence and the capacity for biofilm formation According to statistical analysis, the gene was extremely significant. as shown in( table -4), (table -5)and (table-6)

**(table-4) Correlation between strong biofilm producer isolates and sources of isolation with Gene`s distribution**.

| Strong biofilm isolates | Isolates sources | LasR Gene | LasA Gene |
|---|---|---|---|
| **B2** | Burn | +ve | -ve |
| **B3** | Burn | +ve | -ve |
| **B4** | Burn | +ve | -ve |
| **B5** | Burn | +ve | -ve |
| **B8** | Burn | -ve | +ve |
| **B9** | Burn | -ve | +ve |
| **B10** | Burn | -ve | -ve |
| **B12** | Burn | -ve | +ve |
| **B14** | Burn | -ve | -ve |
| **B17** | Burn | -ve | +ve |
| **B19** | Burn | +ve | +ve |
| **B20** | Burn | +ve | -ve |
| **B24** | Urine | +ve | -ve |
| **B25** | Urine | +ve | +ve |
| 14 (28%) | | 8(16%) | 6(12%) |

Among The 14 (28%) strong biofilm isolates was 8(16%) positive for LasR and 6(12%) positive for LasA gene .that mean Las R gene may be the resone for biofilm formation ability for the isolates because it`s quroum sensing regulatory gene also las A gene is aqurum sensing and the second elastase gene so it may also related to isolates ability to form biofilm.

**(table-5) Correlation between moderate biofilm producer isolates and sources of isolation with Gene`s distribution**

| Moderate biofilm isolates | Isolates sources | LasR Gene | LasA Gene |
|---|---|---|---|
| **B1** | Burn | +ve | -ve |
| **B6** | Burn | -ve | -ve |
| **B11** | Burn | +ve | -ve |
| **B13** | Burn | -ve | -ve |
| **B15** | Burn | +ve | +ve |
| **B16** | Burn | -ve | +ve |
| **B18** | Burn | -ve | +ve |
| **B21** | Burn | +ve | +ve |
| **B22** | Burn | +ve | +ve |
| **B23** | Urine | +ve | +ve |
| **B26** | Urine | -ve | -ve |
| **B27** | Urine | -ve | -ve |
| **B45** | Sputum | +ve | +ve |
| **B50** | Ear | -ve | -ve |
| 14(28%) | | 7(14%) | 7(14%) |

The 14(28%) moderate biofilm producers isolates have 7(14%) of both studied genes which less than the percentage of the genes in the strong biofilm producers.

**(table-6) Correlation between weak biofilm producer isolates and sources of isolation with Gene`s distribution.**

| Weak biofilm isolates | Isolates sources | LasR Gene | LasA Gene |
|---|---|---|---|
| **B28** | Urine | -ve | +ve |
| **B31** | Urine | +ve | +ve |
| **B32** | Urine | -ve | -ve |
| **B33** | Urine | +ve | -ve |
| **B34** | Wound | +ve | -ve |
| **B38** | Wound | -ve | +ve |
| **B40** | Sputum | -ve | +ve |
| **B43** | Sputum | +ve | +ve |
| **B46** | Sputum | +ve | +ve |
| **B47** | Ear | +ve | -ve |
| **10(20%)** | | **6(12%)** | **7(14%)** |

The 10(20%) weak biofilm isolates have the lowest percentage of the studied genes
That mean LasA and LasR genes related to biofilm formation ability for p.aeruginosa isolates especially in burn and urine isolates which was strong biofilm producers and have the highest percentage of two studied genes.

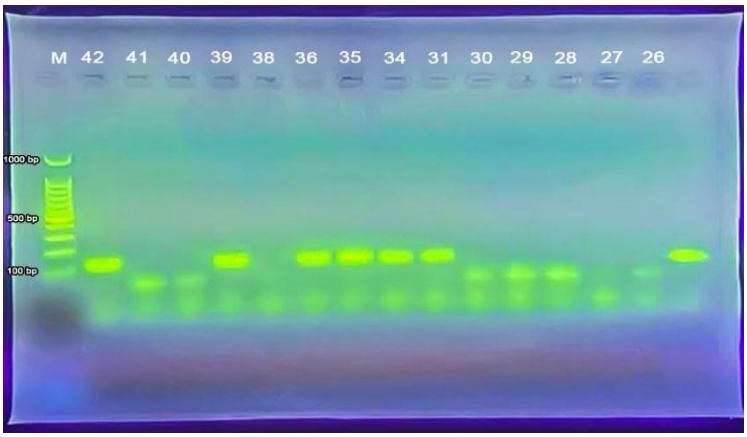


**figure-1-A**

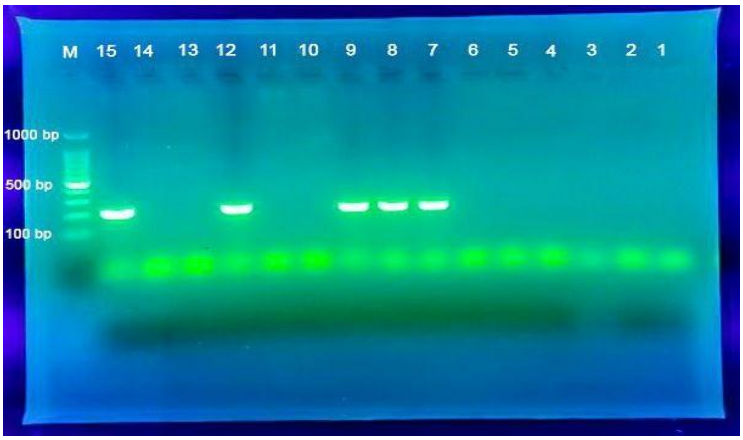


**figure-1-B**

**(Figure -1 ):** Figur -1,A :Gel electrophoresis of amplified PCR product of LasR Gene (130bp)of p.aeruginosa. 1.5% agarose gel electrophoresis stained with read save die (10mg/ml).100v/m Amp for 75min.TBE buffer (1x).Lane1:100bp DNA marker

**Figure -1,B:** Gel electrophoresis of amplified PCR product of LasA Gene (226bp)of p.aeruginosa. 1.5% agarose gel electrophoresis stained with read save die (10mg/ml).100v/m Amp for 75min.TBE buffer (1x).Lane1:100bp DNA marker

## 5.Discussion

Pseudomonas aeruginosa is multifaceted in its pathogenicity, attacking host immune systems, damaging host tissues, and forming biofilms.(20),(21). P. aeruginosa is the cause of several types of human illnesses (22),here most of the isolates were collected from burn (40%), The greatest number of isolates were obtained from urine samples in previously published studies. (65,40.4%)

Pseudomonas aeruginosa isolates also produce several proteolytic enzymes such as Elastase which has the ability to degrade a wide range of plasma proteins, including immunoglobulins, coagulation factors, complement proteins , and alpha-proteinase inhibitors, and has tissue-damaging effects(23). Another important advance is the identification of the lasA gene and the observation that For the notable elastolytic activity, pseudomonas elastase and alkaline proteinase appear to collaborate with the LasA protein. (24),in current study Las A Gene was presented in 28 (56%) of the isolates Which is lower than Las R Gene(58%) in the isolates ,it`s another qourum sensing Gene act as regulatory Gene,another study found that 33 out of 35 isolates was positive for Las R Gene (25)another study by sabharwaI et al. (26) detected Las R Gene in75% isolates
The creation of fully distinct biofilms depends on the las QS system.(26)Clinical isolates of P. aeruginosa exhibit QS gene expression, particularly of the las signaling system, and this is significantly correlated with the development of biofilms. (27)

In this study, P. aeruginosa isolates were tested for their ability to create biofilms. Of the 38 (76%) isolates tested, biofilm production was found to be strong in 28%, moderate in 28%, and weak in 20% of the isolates, respectively. These results are consistent with EL-khashaab et al.2016(28) They discovered that 32 (91.4 percent) of P. aeruginosa isolates produced biofilms, with 25.7%, 40.7%, and 25.7% of those isolates producing strong, moderate, and mild biofilms, respectively, whereas 3 (8.7 percent) of the isolates did not create biofilms. Contrary to Zaranza et al. (29) who found that 86 percent of 100 P. aeruginosa isolates from various sources produced biofilms, with 22.1 percent producing robust, 47.7 percent producing moderate, and 30.2 percent producing weak biofilms. a local study conducted in 2021 by Ali et al (30) Biofilm was 35%, 12.5% and 5%, as strong,moderate and weak respectively another local study by fattema Ali 2022 (31)found that 70% of isolates was biofilm producer.while study of Mahdavi 2020 show only 15.5% of 161 was non biofilm producers(32)

The signaling molecule N-(3-oxododecanoyl)-L-homoserine lactones (3-oxo-C12-HSL), which is produced by the LasI synthase and recognized by its LuxR-type receptor protein LasR, causes transcription of target genes, which may be the second elastase-coding gene LasA, to occur. (33),in our study Las R was detected in 58% of the isolates while LasA detected in 56%of p.aeruginosa isolates the reason for the absence of Las A Gene in the isolates which was have Las R in our study could result from reduced gene expression brought on by environmental variables. The presence of two examined Genes was correlated with biofilm forming capacity in a highly significant manner. Finally, our findings show that p.aeruginosa isolates has astrong ability for biofilm formation and the QS studied Genes LasR,LasA are associated with biofilm formation ability for the isolates . More research on the molecular mechanisms underlying biofilm development, We could gain a better understanding of the pathophysiology of biofilm development by performing studies on the expression and sequencing of QS genes, for example.

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