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# Association Between Quorum-Sensing Genes (LasI, LasR) and Biofilm Genes (pelA, LecA) in Multidrug-Resistance *Pseudomonas aeruginosa* Isolated from Clinical Specimens

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**Abstract:** Nosocomial infections were detected by *P. aeruginosa* bacterial isolates, which had the ability to biofilm formation. Which are associated with the quorum sensing system that confers on bacterial isolates the possibility to cause chronic infections elicited change in antibiotic resistance and expression of virulence factors the connecting with QSP (lipase, protease, and pyocianin) that *P. aeruginosa* protection against body immunity and phagocytosis. Clinical isolates from nosocomial infection were A total of 77 isolates of *P. aeruginosa* from nosocomial infections were collected by sampling in wound, sputum, burn, urine, ear swab, bronchial, nasal, and operation room (environmental of hospital), subsequently identification by morphology test and biochemical test. In addition, the result of identification was confirmed by compound system Vitek-2, identified, and examined to determine the distribution of biofilm genes (*pelA* and *lecA*) and the genes responsible for QSP (*lasR*/*lasI*) residing in the genomic DNA of these isolates. Results showed that 20 (26%) of these isolates are multidrug resistant; the genomic DNA of all 20 isolates carries copies of the (*pelA, lecA, lasI, and lasR*). Moreover, detection of these genes found to be associated with antibiotic resistance in these clinical isolates. The data suggest that genes of biofilm and QSP are characteristic genes in the genome of multiresistant nosocomial *P. aeruginosa* isolates.

**Keywords**: *Pseudomonas aeruginosa*, multidrug resistant, Biofilm, Quorum sensing, *LasI*, *LasR*, *pelA* and *LecA*.

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## Introduction

*Pseudomonas aeruginosa*, as a causative organism of nosocomial infection, is now often multidrug resistant, causing acute and chronic infections resulting in significant morbidity (1)(2). The appearance of antibiotic resistance among bacterial pathogens is a major problem in the treatment of infectious disease in healthcare settings throughout the world (3). *P. aeruginosa* has acquired resistance to nearly all antibiotics used in clinical practice, especially with compromised immune systems, burn patients, wound infections, and respiratory infections. Whereas some resistance mechanisms are conferred by biofilm formation ability that means cause of chronic infections in various environments, such as ventilate devices

in hospital, implanted medical devices (stents for cardiac and urinary catheters and lenses)(4). Biofilm has ability to work specific genes responsible about virulent factors (exotoxin, lipase, protease and pyocianin) and antibiotic resistance by quorum sensing system that forms of communication by signal molecule when this signal reaches threshold density; therefore, the biofilm-related genes were began expression (5) This study focused on confer suitable environmental condition to resist antibiotics (3,16).

## Material and Method

### Bacterial isolates

A total of 200 sterile cotton swabs with media to collect clinical specimens were collected from wounds and burn specimens, while urine, sputum, and blood were cultured in MacConkey agar and blood agar at 37ºC for 24 hours for bacterial growth and isolation after transfer to a microbiology laboratory in a private Nursing Hospital in Baghdad. for patients with a wide range of ages attending the division of Microbiology/Private Nursing Hospital, Ghazi AL-Hariri Hospital for surgical specialties, the National Center for Educational Laboratories in the Baqubah, and Public Health Laboratories in Diyala during the period from Oct/2024 to Jun/2025. From these specimens,78 isolates were identified as Pseudomonas aeruginosa isolates and performed by standard microbiological and chemical methods (Gram stain to examination reaction with dye, bacterial isolate were ability to produce oxidase and catalase enzyme, and performed the type of ferment lactose, addition bacterial isolate were growth in selective media such as cetrimide agar were characterized based on morphology of colony(17), King A agar media to show pigment formation by bacterial isolates. Approved identification uses the Vitek2 system.

### Virulent Factor that connecting with PQS

### Lipase examination

Spirit blue agar was prepared by add 32.15 to 1000 ml of DW. Dissolved and blended in hot plate stirrer until the media completed dissolved and justified pH at 7. Sterilized by autoclave at 1Gole to 121º C for 15 min. After cooling media add 100 ml of Olive oil. Boring media in petri dish and straighten bacterial isolates on the media, inoculate at 37ºC for 24 H.

### Protease examination

Skimming Milk agar media was propertied by suspended 50.40 gm in 950 ml DW. Dissolved, Justified, sterilized and cooling as mentioned above. Added 50ml skimmy milk mixed well then poured in petri dish and straighten bacterial isolates on the media, inoculate at 37ºC for 24 hours.

### Biofilm formation

1. *P. aeruginosa* isolates were cultured in LB broth at 37ºC for 24 hours.
2. Diluted 1:100 in to BHI broth media at 37ºC for 24 hours.
3. Add 100 µL of BHI in 96 well dish, uses 3 replicates well for each treatment then the microtiter was incubated at 37ºC for 24 hours.
4. Shaking out the liquid then washing by pipet. Shaking out. repeated third time.
5. Crystal violates solution was Added by 125 µL to all wells for 15 min then the plate was rinsed three times, microtiter plate was turned down to dry few hours.
6. Acetic acid was added 125 µL of 30% concentration to each well for 15 min.
7. Plate was dread at 550nm.

### Pyocianin production

King A agar media was prepared by dissolve 41 gm in 990ml DW ,10ml

glycerol, sterilized by autoclave at 1Gole to 121º C for 15 min. plates were incubated at 37ºC for 24 hours.

**Antibiotic susceptibility**

*P. aeruginosa* isolates were tested for their susceptibility to antimicrobial agents by Kirby-Bauer method on MHA according to criteria recommended by Clinical and laboratory standards Institute/2023(18).

**Genotyping Detection**

**DNA Template Preparation by boiling method**

DNA template was prepared by a boiling method as described by (19). Briefly, 5 isolated colonies of overnight growth bacteria were suspended thoroughly in 2 ml distilled water and boiled in a water bath, for 10 min. After centrifugation, the supernatant was used as template DNA for the PCR.

**PCR Amplification**

The PCR amplification procedure for the genetic level to detecting *Pseudomonas aeruginosa* local isolates table (1) by follows step:

**Table (1) Primers Oligonucleotide Sequences Used in This Study.**

| Primer | | 5 → 3 | Product size/bp | Program | Reference |
|---|---|---|---|---|---|
| ***LasI*** | F | CACATCTGGGAACTCAGC | 160 | Initial Denaturation 95 C for 10 min, followed by 40 cycles at 95 C for 30 s, 56 C for 30 s, and 72 C for 30 s. Final Extension 72C for 5 min. | (20) |
| | R | ACGGATCATCATCTTCTCC | | | |
| ***LasR*** | F | TGCCGATTTTCTGGGAACC | 401 | Initial Denaturation 95C 2 min, followed by 35 cycles at 95C for 30 sec, 59C for 30 min. and 72Cfor 1 min. Final Extension 72C for 5 min. | (20) |
| | R | CCGCCGAATATTTCCCATATG | | | |
| ***pelA*** | F | CCTTCAGCCATCCGTTCTTCT | 118 | Initial Denaturation 95 5 min. followed by 35 cycles at 94C for 30 sec, 52C for 40 sec, 72C for 50 sec. Final Extension 72C for 5 min. | (21) |
| | R | TCGCGTACGAAGTCGACCTT | | | |
| ***LecA*** | F | CACCATTGTGTTTCCTGGCGTTCA | 100 | Initial Denaturation 95 C for 10 min, followed by 40 cycles at 95 C for 30 s, 56 C for 30 s, Final Extension 72 C for 30 s. | (10) |
| | R | AGAAGGCAACGTCGACTCGTTGAT | | | |

by a boiling method as described by (19). Briefly, 5 isolated colonies of overnight growth bacteria were suspended thoroughly in 2 ml distilled water and boiled in a water bath, for 10 min. After centrifugation, the supernatant was used as template DNA for the PCR.

**PCR Amplification**

The PCR amplification procedure for the genetic level to detecting Pseudomonas aeruginosa local isolates table (1) by follows step. Final volume for PCR mixture was 25 µl (12.5 of Master Mix 2x, 5 µl template DNA, 1 µl primers for each forward and reverse primer, finally, 5.5 µl nuclease free water) in uniplex PCR Eppendorf tubes but amount changed in multiplex PCR, mixed briefly via vortex then been placed in thermocycler polymerase chain reaction.

**Statistical Analysis**

Data of current study were analyzed by using Chi-square (X2) test to compared between percentages. Odd ratio was used to measure strength association between presence factor and appear happened. A level of significance of α=0.01 was applied to test. (SPSSv.22) programs used to analyze current data.

**Result and discussion**

**Incidence of *P. aeruginosa***

The *P. aeruginosa* isolates were identified based on how they responded to the Gram stain dye, where the bacterial isolates were negative gram that rod shape, biochemical characteristics as they were results positive for non-ferment of lactose for 200 samples were 118(59%) positive and 54 (27%) negatives. Given the low p-value (0.01) to be statistically significant association between the source of specimens and lactose fermentation, production of each all oxidase and peroxidase, colony morphology on cetrimide agar greenish-yellow, mucoid colony, it also has distinctive odors such as fruit when cultured in nutrient agar, when bacterial isolates were cultured in specific card to vitick2 compound system confirm the identify with probability 99%.

The prevalence of these isolates among different clinical specimens is in urinary tract infections: 14 out of 77 individuals examined (18.1%), wound 10 (12.9%), sputum 7 (9.0%), burn 23 (29.8%), ear swab (10.8%), operation room 11 (14.2%), and bronchial nasal 3 (3.8%). *P. aeruginosa* is now a common pathogen that has been successfully established in the hospital environment.

**Antibiotic susceptibility**

The antibiotic susceptibility pattern of *P. aeruginosa* isolates was studied. Results indicated in figure (1) showed that multi-drug resistance was spread in these clinical isolates, it was found that most of the bacterial isolates (100%) were resistant to Ampicillin and, 99% were resistant to Ceftazidime, 90% to Colistin, 77% to Gentamicin and Polymycin, ,45% to Cefepime and Piperacillin, 42% to Imipenem, 40% to Ofloxacin and Ciprofloxacin,37% to Meropenem was resistance showed result in figure (1). 20 isolates were taken that were more resistant to antibiotics. These results are in agreement with Shen *et al.* (2022), who found all its isolates were MDR and XRD; in addition, they threatened the lives of patients. Another study optioned by Motevasel *et al.* (2024) found *P. aeruginosa* isolates. Biofilm production was evaluated by the qualitative microplate method, which revealed 50.84% strong biofilm producers, 23.72% moderate producers, 6.77% weak producers, and 18.84% non-biofilm producers of *P. aeruginosa*. The distribution of biofilm-producing *P. aeruginosa* isolates from different clinical specimens. Identification of antibiotic resistance genes provides valuable information; however, knowledge about the sixth most common cause of hospital-acquired infections was *P. aeruginosa* because of their widespread resistance to several antibiotic groups, multi drug resistance and extensively drug-resistant *P.*

*aeruginosa* pose significant problems in healthcare settings, increasing rates of morbidity and medical expenses. On the other hand, the statistical description of these results highlights a significant challenge in treating *P. aeruginosa* infection and this result may be due to factors include to:

1. increased use of broad-spectrum antibiotics like aminoglycosides, carbapenems, and cephalosporins, the prevalence of MRD and XRD bacterial isolates.
2. Extended hospitalizations.
3. Heightened patient's susceptibility.

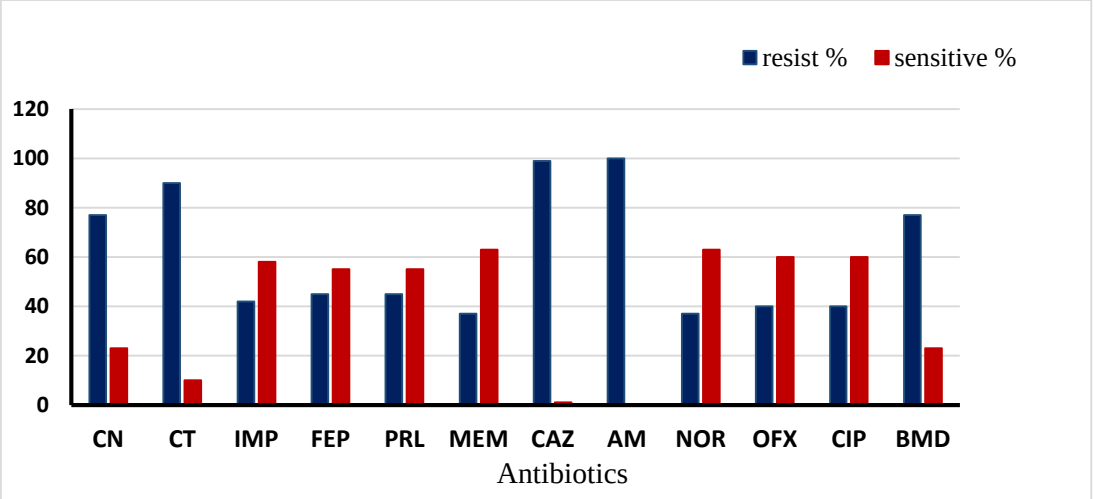


**Figure (1): Antiprogram of *P. aeruginosa* isolates and the susceptibility percentage against different antibiotics. GN: gentamycin; CT: Colistin; IMP: Imipenem; FEP: Cefepime; PRL: Piperacillin; MEM: Meropenem; CAZ: Ceftazidime; AM: Ampicillin; NOR: Nofloxacin; OFX: Ofloxacin; CIP: Ciprofloxacin; BMD: polymycin.**

## Virulent Factor that connecting with PQS

The 20 bacterial isolates were evaluated for their capacity to produce protease by cultured on skimming milk agar by the appearance of a transparent area surrounding the colonies. Where 18(90%) *P. aeruginosa* isolates were producing protease enzyme, another study agreement with the result study by Moonnee *et al*. (2024) found all *P. aeruginosa* isolates were producing enzyme protease. According in figure (2).

Particularly *P. aeruginosa* produces protease, directly contributes to bacterial invasion and necrosis. Protease, which is an essential enzyme because it causes the matrix protein to split, which promotes microbial adhesion and colonization (22).

In the other hand showed result of cultured *P. aeruginosa* isolates on spirit blue agar is (100%) and also showed transpared zone around isolates which this indicates the production of the lipase, that has the ability to hydrolysis of fat tissues.


**Figure (2*): P. aeruginosa* growth in skimmy milk agar show transparent area surrounding the colonies (produce protease).**


**Figure (3): *P. aeruginosa* growth in spirit blue agar shows transparent area surrounding the colonies (produce lipase).**

**Figure (4): P. aeruginosa growth in king A agar shows produce pyocianin pigment**

An essential property of lipase is their capacity to create ester linkages in non-aqueous media in addition to hydrolyzing them(23), as in figure (3). *P. aeruginosa* was produced lipase as virulent factor A study was introduced by Rawi *et al* (2023) showed the lipase production (100%) agreement with this study(24) .That is similar to the findings of Abubakar *et al* (2024) all

*P.aeruginosa* isolates produce lipase(25).In addition, 20 bacterial isolates were cultured on King A agar media, the result were 15 (75%) isolates were producing blue green pigment that show to ability pyocianin forms such like in figure (4). Pyocianin was redox active secondary metabolite. It gives the culture a definite blue dye created during stationary phase. By actively participating in reduction mechanisms that facilitate the release of iron from transferrin, it plays a crucial function in iron metabolism. Through the generation of reactive oxygen species and consequent cell death, it has been demonstrated to have harmful effects on a number of organs(26).

**Biofilm formation**

The Microtiter plate (96 wells) with method were used with ELISA device which provides a numerical value for absorbance at a wavelength of 630nm, the capacity of 20 *P. aeruginosa* isolates to form biofilms was examined at a rate of three replicates per isolate. The result was obtained proved that isolates were 100% productive of bioactivity and different levels of intensity by comparing them with the negative control, the results showed that 8 isolates, at a rate 40% of strongly adherence, while 50% (10) were moderately adherence, and 2 isolates, at a rate 10% were weakly adherence as shown in diagram (1).

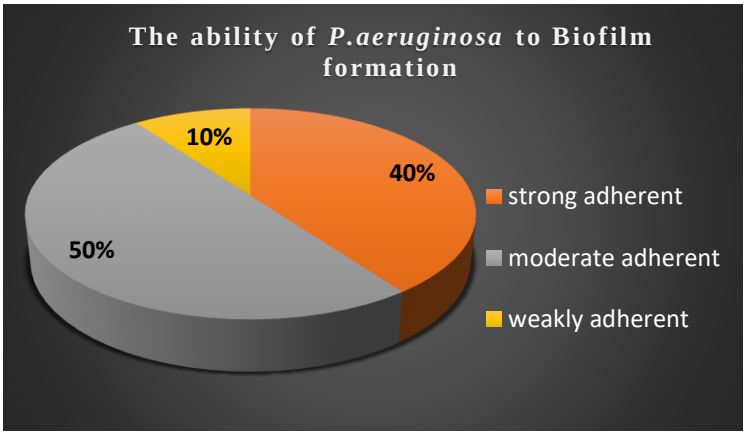


**Diagram (1): show ration to the ability of *P. aeruginosa* to biofilm formation.**

The analysis reveals that a very high proportion of *P. aeruginosa* isolates (90% combine moderate and strong adherence) exhibit a significant ability to form biofilms. This characteristic is clinically relevant because biofilms act as protective barriers for bacteria, shielding them from antibiotics and the host immune system. The high prevalence of strong and moderate biofilm producers among *P. aeruginosa* isolates suggests that biofilm-mediated resistance is a widespread concern in the context of infections caused by this pathogen. According to the study by Chimi *et al.* (2024), which reported 50% strong adherence and 37% moderate adherence, finally, 13% of the isolates were classified as weak biofilm producers. Another study optioned by Motevasel *et al.* (2024) found *P. aeruginosa* isolates. Biofilm production was evaluated by the qualitative microplate method, which revealed (50.84%) strong biofilm producers, (23.72%) moderate producers, (6.77%) weak producers, and (18.84%) no biofilm producers of *P. aeruginosa.* The distribution of biofilm producing *P. aeruginosa* isolates from different clinical specimens. The microtiter plate method is an important method to

establish the early stages of biofilm formation and to differentiate between strong, moderate and weak biofilm production. Fortunately, because it provides production from macrophages and helps in the onset of infection and the occurrence of pathogenic diseases (27).

**Molecular Study of *P. aeruginosa* DNA**

In this study, we wanted to investigate whether pathogenic *P. aeruginosa* differ with respect to the presence of biofilm genes and QSP genes in their genomes. This genes was activated were biofilm formation(12). Results showed that gene *LecA* was detected in the genome of all *P. aeruginosa* clinical isolates, a study in Saarland in Germany done in 2024 to inhibitors *Las B* and *lec A* found that all isolates positive results(8), mentioned Shen *et al* (2024) done in China that detection lecA in all urine isolates(10), show figure (5).

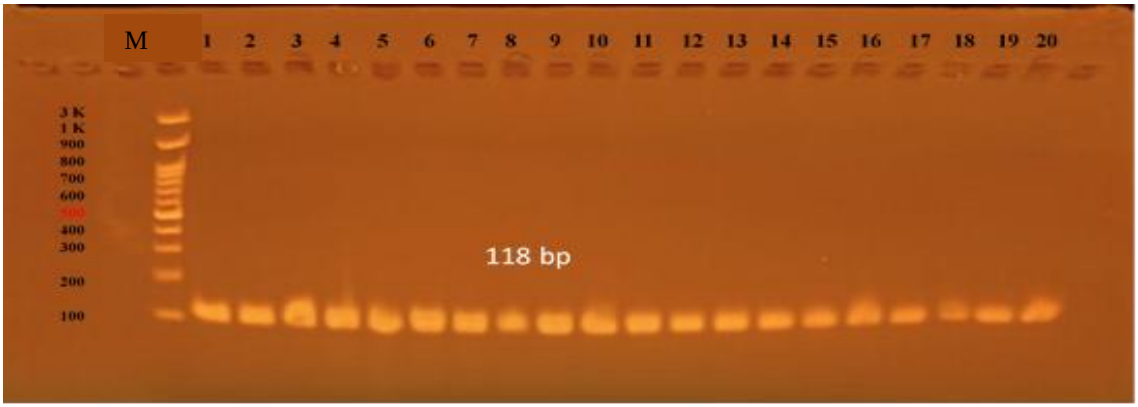


**Figure (5): Agarose gel electrophoresis (1.5% agarose,7v/cm2 for 60 min) for LecA gene (100 bp amplicon) lane 100 bp DNA Ladder.**

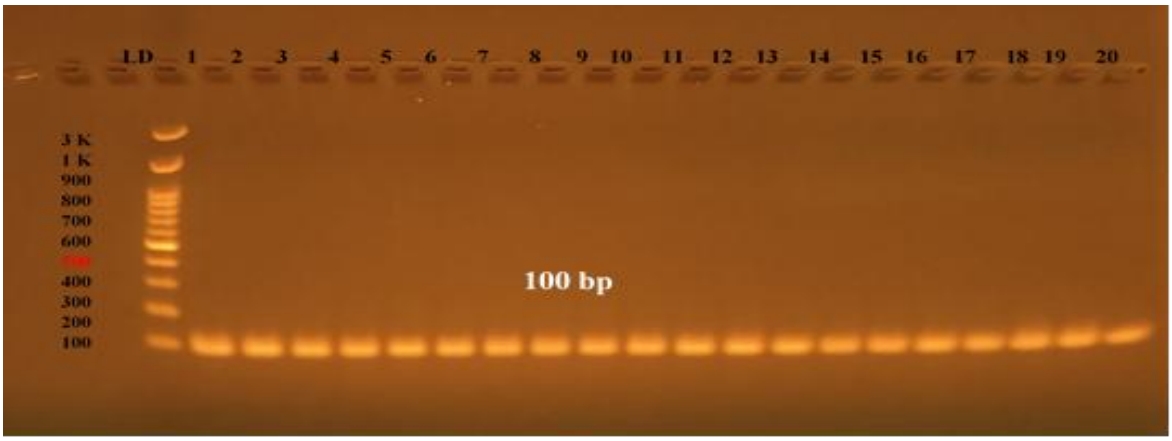


**Figure (6): Agarose gel electrophoresis (1.5% agarose,7v/cm2 for 60 min) for pelA gene (118 bp amplicon), lane 100 bp DNA Ladder.**

While *pelA* was detected in each all isolates. On the other hand, results illustrated in figure (6) showed that Agarose gel electrophoresis , In study done by Van *et al*(2024) in Canada showed that pelA genes is part of essential genome of *P.aeruginosa*(7).

that Agarose gel electrophoresis, this study agreement with study done in USA, Oregon State University,

Corvallis, treated integrate experiments with mathematical modelling to quantitatively analyses the *LasI*/*LasR* quorum sensing pathway in the opportunistic pathogen (28).

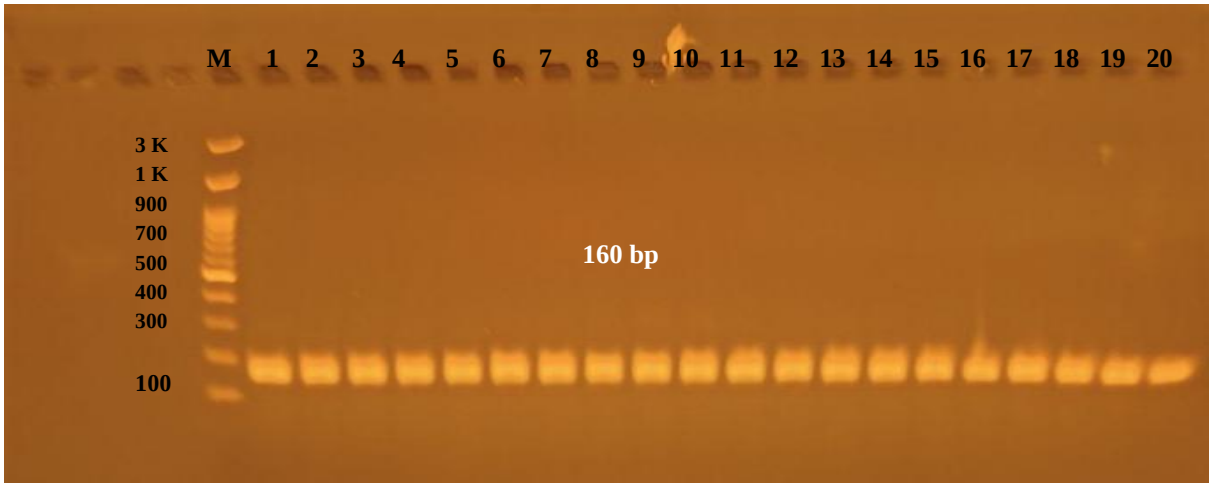


**Figure (7): Agarose gel electrophoresis (1.5% agarose,7v/cm2 for 60 min) for LasI gene (160 bp amplicon) , lane 100 bp DNA Ladder.**

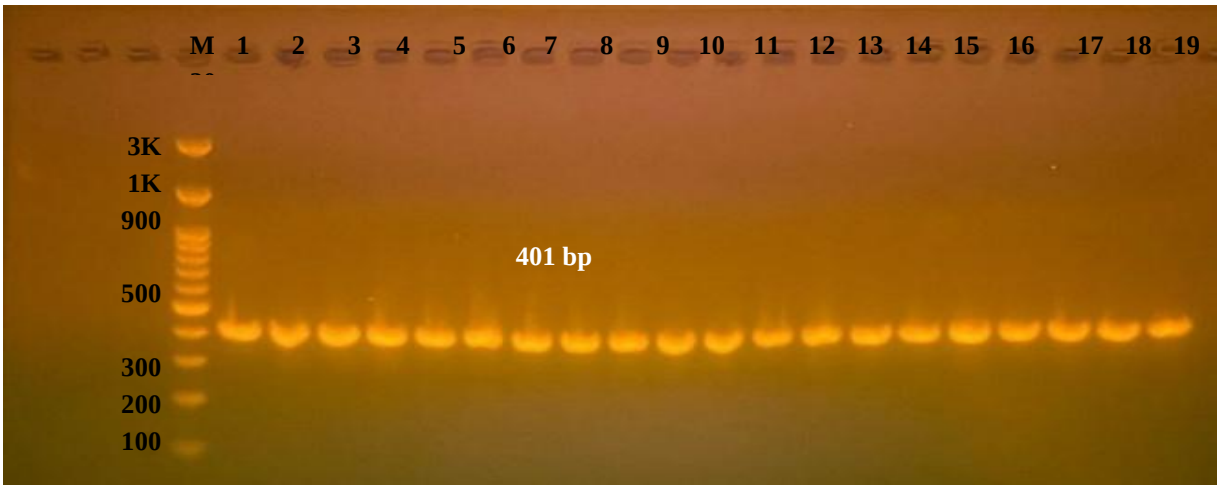


**Figure (8): Agarose gel electrophoresis (1.5% Agarose,7v/cm2 for 60 min) for LasR gene (401 bp amplicon); for VIM gene (390 bp amplicon); lane 100 bp DNA Ladder.**

**Table (2): Prevalence detection genes (*lecA, pelA, lasI and lasR*) in *P. aeruginosa* in different sources**

| Source | Number | *lec A* | *PelA* | *lasI* | *las R* |
|---|---|---|---|---|---|
| **Sputum** | 7 | Positive | Positive | Positive | Positive |
| **Urine** | 8 | Positive | Positive | Positive | Positive |
| **Bronchial Wash** | 1 | Positive | Positive | Positive | Positive |
| **Wound** | 2 | Positive | Positive | Positive | Positive |
| **Ear Swab** | 2 | Positive | Positive | Positive | Positive |

In the Same hand pelA was detected in each all isolates. results illustrated in figure (6) . In study done by Van et al (7) in Canada showed that pelA genes are part of essential genome of P. aeruginosa (7). Addition, LasI gene was prevalent in all isolates (100%) from all sources results illustrated in figure (7). In study done by Aghamollaei *et al* in (20) Iran found all the samples of P. aeruginosa have las I/las R (20). So, the same result was found to LasR genes responsible of QSP were found in all isolates in rate (100%) showed in figure (8).

Bottom line, all of the specimens studied contained biofilm genes (pelA and LecA) and QSP genes (LasI and LasR), indicating high levels of virulent bacterial isolates in the hospitals of Baghdad and Baqubah from which the specimens were Taken. The prevalence of genes in the bacterial isolates were as shown in table (2), which shows that each of the selected and obtained varied across the sources, with urine and sputum being the most common. This a dangerous indicator that must be pointed out in an attempt to find effective treatment methods. The result showed that *P. aeruginosa* isolates had a remarkably high induce of treatment resistant. In the same hand, the quorum sensing system was significantly correlated with drug resistance and biofilm formation, regulatory system depends on extracellular signals to determine cell density(12).

Examined bacterial isolates from various clinical samples (sputum, urine, bronchial wash, wound, and ear swab) that have all detected genes (*lec A, PelA, lasI*, and *las R*) are highly prevalent among the *P. aeruginosa* isolates in this study. The number of isolates suggesting that it play a crucial role in the development of nosocomial infections brought on by *P.aeruginosa* (29).This result because they are associated with higher rates of disease and death. Alternative approaches to treating infectious disorders linked to biofilms must be investigated and developed because biofilms often show poor responsiveness to antibiotics. One important mechanism that controls the expression of genes linked to virulence factors is QS. This

## Conclusions

The present study provides evidence that all *P. aeruginosa* isolates under study were highly resistant to antibiotics and formed biofilms with varying adhesion rates. They possess genes responsible for biofilm formation (*pelA, lecA*) and genes that encode the quorum sensing system (*las I/las R*)(27).

All of above leads to persistent infections and makes it more difficult to target individual bacterial cells.

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## Approval

It was approved as research unsheathed from a Ph.D. thesis by Biology Department, College of Education for Pure Sciences University of Dayala, Dayala, Iraq.

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