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The Expression Response of *las*I Quorum Sensing Gene Upon Exposure to Some Microbicides in *Pseudomonas aeruginosa* Isolated from Local Clinical Specimens

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Abstract: Microbicides are frequently used to manage surface bacterial contamination in a variety of settings and to prohibit infections from spreading to individuals as nosocomial infections. Due of its numerous virulence factors, which are controlled by the crucial quorum-sensing (QS) network, *Pseudomonas aeruginosa* can withstand hostile environments. This work aims to determine the minimum inhibitory concentration (MIC) of microbicide agents and explore the role of the *las*I gene in *P. aeruginosa* biofilm development in order to gain a better understanding of how these bacteria may survive in such a kind of environment.As well as measure the target gene expression before and after using microbicides.One hundred clinical sampleswere collected during the period from June 2023 until November 2023 from patients attending Baghdad hospitals. The VITEK-2 compact system was used to confirm the isolates of *P. aeruginosa*. Micro titer plate technique was employed to assess the isolates' capacity to produce biofilms in vitro. Out of collected samples, 38 (38%) *P. aeruginosa* isolates were identified. The highest percentages (47.37% and 23.68%) were assigned to burn and wound infections, while the lowest frequency (10.53% and 18.42%) was assigned to ear and UTI infections. The results indicated that the biofilm formation was strong in (79.41%), moderate in (14.17%) and weak in (5.88%). The antibiotics test showed the highest resistance to the fluoroquinolonesabout 80%.The QSgene was detected in 34 (89.47%) of the isolates.In conclusion, the expression level of *las*I gene showed down regulation of concentrations of microbicides treatment average (32-64 µg/ml).Significant correlations were found between the *las*I gene and the use of microbicides and biofilm development.

Keywords: *Pseudomonas aeruginosa*, Quorum Sensing, *las*I, Microbicides, Biofilm.

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Introduction

Hospital-acquired infections are an emerging issue that affects medical staff, hospitals, and patients (1). *Pseudomonas aeruginosa (P. aeruginosa)* is a multi-drug resistant (MDR) bacteria that is primarily responsible for hospital-acquired infections because of its innate and plasmid-mediated resistance to various antibiotics (2). Microbicides can be used to control these diseases, which helps to get rid of or lower the amount of pathogens in hospital environments (3). Disinfectants and antiseptics are examples of microbicides that are used extensively in the fight against

nosocomial spreading of infectious pathogens. Biguanides like chlorhexidine digluconate, quaternary ammonium compounds, and benzalkonium chloride are among the more commonly used types (4). On the other hand, microbicides are frequently used to manage surface bacterial contamination in a variety of settings and to prohibit infections from spreading to individuals or animals. Chemical microbicides are usually effective on several targets, in contrast to antibiotics, which frequently have a specific mode of action focusing on one or a small number of bacterial targets (5). Because quorum sensing (QS) regulates a multitude of virulence components, *P. aeruginosa* can withstand extreme environments (6). Not only that, but *P. aeruginosa* is a pathogen that can express a range of virulence factors, adapt to its surroundings, and display a pattern of multidrug resistance, making the treatment of infections caused by this bacteria complex and demanding (7). In order to respond to environmental stress signals and modify gene expression, the QS pathways are carried out up of populations of bacteria, signal molecules, and behavioral genes. Autoinducers, extracellular signaling molecules, are the mediating agents of this intercellular communication (8). These molecules serve as mirrors, reflecting the inoculum's density. They control the expression of associated genes once the growth threshold is met (9). Bacterial populations start to recognize the chemicals once they reach a specific threshold concentration. The result is the activation of response genes in the bacteria, which control a variety of behaviors like pathogenicity, competence (the capacity to take up DNA), and horizontal gene transfer (6). By overexpressing efflux pumps, environmental pressure might cause temporary resistance that eventually results in bacterial persistence. Consequently, there are more spontaneous mutations and persistent resistance as a result of this (10). *P. aeruginosa* regulates communities' behavior by actually, two N-acyl homoserine lactone (AHL) -dependent QS systems known as LasI/R and RhlI/R regulate the transcriptional regulation of many virulence genes. The LasI/R system controls the expression of target genes. N-(3-oxo-dodecanoyl)-homoserine lactone (3-oxo-C12-HSL) issynthesized by LasI, and it binds to and activates the homologous response regulator LasR. (11). Therefore, the purpose of the current study is to assess the expression of the *las*I Quorum Sensing gene in clinically isolated strains of*P. aeruginosa* in response to some microbicides.

Materials and methods

Sample scollection

100 clinical samples (burns, ear infections, wound infections, and UTIs), were obtained during the period from June 2023 until November 2023 for both sexes of different ages from Baghdad Medical City (Specialized burns hospital, Ghazi Al-Hariri hospital and The National Center of Teaching Laboratories) were incorporated in the study.

Bacterial identification

To identify the bacterial isolates at first, the colonies' appearance on nutritional agar, Mac Conkey agar, and cetrimide agar were utilized. Analyses were done on the color, texture, and shape of the colony. In addition to biochemical tests using the oxidase and catalase tests. The VITEK 2 compact

system's conformation used.

Antimicrobial susceptibility test

Growth-based methods were employed in the automated microbiology compact system VITEK 2 using colorimetric reagent cards that were automatically interpreted after being incubated, it was utilized to detect the presence of bacteria, test for antibiotic susceptibility, and identify resistance mechanisms. Lists of antibiotic categories recommended for antimicrobial susceptibility testing were created using data and breakpoints from the Clinical Laboratory Standards Institute (CLSI).

Biofilm quantitation

The microtiter plate (MTP) is a quantitative method used with a microtiter plate reader to evaluate biofilm production (12). The strains were separated into three categories based on their capacity to form biofilm: weak, moderate, and robust biofilm makers. in contrast to the control group, which consisted mainly of broth media-filled wells.

Microbicides susceptibility testing by agar well diffusion

This method was used to evaluate the susceptibility of *P. aeruginosa* isolates tothe microbicides agents. To prepare each microbicides that used in this study which are (chlorhexidine digluconate (20%) and benzalkonium chloride (>95%) (Sigma-Aldrich, USA), dissolved in sterile distilled water following the CLSI protocol as a stock solution (13).

Minimum inhibitory concentration of microbicides

MIC is the lowest antimicrobial agent concentration that may inhibit apparent bacterial growth but not kill it. Microbicides were dissolved in D.W. to a final concentration of 1024 µg/ml and create a stock solution. According to (13) Muller-Hinton broth was used in 96-well microtiter plates to perform two fold serial dilutions from the stock solution to gate concentrations ranging from 512 µg/ml to 2µg/ml.

Genetic identification

Using an extraction kit methodology, genomic DNA was isolated from a pure culture of *P. aeruginosa* bacteria (FavorPrep Total DNA Mini Kits, Korea). This kit used the bacterial procedure (for gram negative bacteria) to extract DNA.To identify *P. aeruginosa*, a particular primer was utilized in this investigation. According to information provided by the supplier, Macrogen, it was manufactured in a lyophilized form. Tables 1 and 2 display the gene employed in this work, together with its amplicon size, genetic sequence, and PCR amplification program.

Table (1): Primers utilized in this research

Gene	Primer Sequence5`to 3`	Product Size bp	Ref.
***las*I**	**F**-CGCACATCTGGGAACTCA	177	(14)
	R-CGGCACGGATCATCATCT		
***16S r*RNA**	**F**-CAGCTCGTGTCGTGAGATGT	150	(15)
	R-CGTAAGGGCCATGATGACTT		

Table(2):The PCR program utilized in this investigation to amplify the gene

Steps	Temperature (ºC)	Time	Cycles
Initial denaturation	94	5 min	1
Denaturation	94	30 sec	30
Annealing	57	30 sec	45
Extension	72	30 sec	45

Final extension	72	7 min	1
Hold	4	10 min	1

Using electrophoresis and a 2% agarose gel dyed with Red Safe, the PCR products were seen.

Quantitative real time-PCR

RNA extraction

Before and after applying the microbicides, RNA was extracted, and subMIC dosages of the agents were used to encourage bacterial growth. TRIzolTM Reagent was utilized to extract total RNA.

Quantitative Real-time PCR Assay

The master amplification reaction given in Tables (3, 4 and 5) and the EsayScript® 1-Step RT-qPCR System were used to amplify mRNA fragments.

Table (3): The components were added to obtain cDNA

Component	Volume
Random Primer	1 µl
2×ES Reaction Mix	10 µl
Easy Script®RT/R.I. Enzyme Mix	1 µl
RNase-free Water	Up to 20 µl
Eluted RNA	5 µl
Final volume	25 µl

Table (4): Program PCR converted RNA to cDNA

Step	Temperature (°C)	Time (minutes)	Cycles
Priming	25	11	1
Reverse transcriptase	42	35	1
RT inactivation	80	5	1

Table (5): Reaction Mix for Quantitative RT-PCR

Cycle Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	60 sec	1
Denaturation	95	15 sec	40 - 45
Annealing	60	30 sec	
Melt Curve	60-95	40 min	1

Delta delta Ct method

In order to obtain relative quantification, the delta delta Ct (ΔΔCt) technique necessitates sample selection and a direct comparison of Ct values for the target gene and the reference gene. The calibrator can be any sample that acts as a guideline by which other samples can be measured. The Livak formula was utilized to calculate the mean Ct value statistical approach, which was then applied to three duplicate reactions to determine the levels of gene expression.

Statistical analysis

We utilized SAS (2018) from the Statistical Analysis System to examine the effects of the experimental variables. The analysis of variance (ANOVA) test with the least significant difference (LSD) was used to compare the means in a meaningful way. The chi-square test was used in this investigation to assess the statistical significance of an outcome difference between 5% and 1%.

Results and discussion

Identification of *P. aeruginosa*

The identity of (38) clinical isolates as *P. aeruginosa* was confirmed using the Gram-negative strain identification

card that came with the VITEK 2 compact system. Numerous prior research has demonstrated the diagnostic and validation efficacy of biochemical tests using this approach. This automated tool can be used to determine the antibiograms of *P. aeruginosa* isolates.

P. aeruginosa distribution based on type of sample

Out of 100 clinical samples, 38% included P.aeruginosa, according to the results displayed in Table (6). The samples were taken from a variety of sites, including burns, wounds, UTIs, and ear infections in hospitalized patients.

Table (6): _P. aeruginosa_ isolates distribution throughout the body's various regions

Source of samples	No. of samples	No. and % *P. aeruginosa*
Burns	27	18 (47.37%)
Wounds	29	9 (23.68%)
Ear infection	21	4 (10.53%)
UTI	23	7 (18.42%)
Total	100	38
Chi-square (χ^2)	---	11.473 **
P-value	---	0.0094
** (P≤0.01).		

Based on the percentages of distribution of isolated *P. aeruginosa* from various places in the body, the current study's findings showed statistically significant variations (P≤ 0.01). The highest percentages (47.37% and 23.68%) were assigned to burn and wound infections, while the lowest frequency (10.53% and 18.42%) were assigned to ear and UTI infections. A rise in patients with compromised immune systems due to diseases, exposure to the environment, or prolonged hospital admissions may be the cause of these numbers. Studies conducted in Baghdad City (16) and Al-Muthanna (17) are comparable to this one. According to these investigations, burns and wound infections at Al Muthanna hospitals resulted in the diagnosis of over 55 (68.75%) *P. aeruginosa* isolates. Comparably, burns accounted for (60.9%) of *P. aeruginosa* isolates, while wound infections accounted for (42.1%).

Pattern of antibiotics susceptibly

All 38 isolates had their antibiotic resistance examined using the automated VITEK 2 Compact device. Every isolate was cultivated on MacCkonkey agar plates using a McFarland 0.5 standard suspension. Liquid containing each of the isolated compounds was loaded into the VITEK device. Using the Gram-negative susceptibility card in the VITEK 2 Compact device, the efficiency of 24 antibiotics in getting rid of certain bacteria was evaluated.The study's findings showed that *P. aeruginosa* isolates with the highest percentage of antibiotic resistance were also the least resistant, whereas sensitive isolates showed the lowest percentages. It was discovered that these variations were statistically significant (P≤ 0.01). We found that most isolates of *P. aeruginosa* were resistant to multiple medications.

Table 7 summarizes that among all 24 tested antibiotics, the highest resistance was found against levofloxacin (86.84%), ciprofloxacin (84.21%), and ticarcillin (81.57%).According to these findings, *P. aeruginosa* isolates have demonstrated strong resistance to the fluoroquinolones ciprofloxacin and levofloxacin, which is consistent with research (18). *P. aeruginosa* was most common in burn patients, and the most common kind of resistance was to fluoroquinolones, such as Ciprofloxacin and Norfloxacin.Study (19) revealed significant resistance to amikacin (94.3%), gentamicin (86.7%), and tobramycin (84.9%). On the other hand, study (20) found reduced resistance to ciprofloxacin (32.6%) and levofloxacin (15.2%) in the quinolones class.Despite the fact that pseudomonas species use horizontal gene transfer to obtain new resistance genes (integrons, plasmids, or transposons), they yet have a variety of significant virulence factors, this agreed with study (21).

Table (7): Antimicrobial susceptibility test of 38 *P.aeruginosa* isolates

Antibiotics	Resistant	Sensitive	Intermediate	P-value
Ticarcillin	31(81.57)	0	5(13.15)_	0.0001 **
Ticarcillin/ clavulanic acid	17(44.73)	1(2.63)	19(50)	0.0001 **
Piperacillin	28(73.68)	0	8(21.05)	0.0001 **
Piperacillin/ tazobactam	13(34.21)	1(2.63)	17(44.73)	0.0001 **
Cefixime	15(39.47)	2(5.26)	4(10.52)	0.0006 **
Cefazolin	14(36.84)	0	2(5.26)	0.0001 **
Ceftazidime	10(26.31)	0	27(71.05)	0.0001 **
Cefepime	8(21.05)	1(2.63)	14(36.84)	0.0001 **
Cefpodoxime	9(23.68)	0	1(2.63)	0.0001 **
Ceftriaxone	10(26.31)	0	1(2.63)	0.0001 **
Cefotaxime	14(36.84)	0	7(18.42)	0.0001 **
Ertapenem	21(55.26)	0	0	0.0001 **
Doripenem	8(21.05)	0	11(28.94)	0.0001 **
Imopenem	16(42.10)	1(2.63)	19(50)	0.0001 **
Meropenem	18(47.36)	2(5.26)	16(42.10)	0.0001 **
Amikacin	13(34.21)	4(10.52)	19(50)	0.0001 **
Gentamicin	17(44.73)	8(21.05)	10(26.31)	0.0008 **
Netilmicin	11(28.94)	0	13(34.21)	0.0001 **
Tobramycin	8(21.05)	4(10.52)	13(34.21)	0.0063 **
Norfloxacin	18(47.36)	0	3(7.89)	0.0001 **
Ciprofloxacin	32(84.21)	1(2.63)	4(10.52)	0.0001 **
Levofloxacin	33(86.84)	0	4(10.52)	0.0001 **
Moxifloxacin	9(23.68)	0	0	0.0001 **
Ofloxacin	6(15.78)	0	3(7.89)	0.0071 **
Colistin	21(55.26)	1(2.63)	16(42.10)	0.0001 **
P-value	0.0001 **	0.0026 **	0.0001 **	---
** ($P \leq 0.01$).				

Biofilm Formation

The results of the investigation showed that the 38 *P. aeruginosa* isolates adhesion and the capacity to form beneficial biofilms. The outcomes were divided into categories as follows: (79.41%) exhibited strong biofilm development, (14.17%) moderate biofilm formation, 5.88% weak biofilm formation. These data correspond with a study by (22), which found that (18.1%) of samples had significant biofilm

development, 21.7% had moderate biofilm formation, and 28.9% had weak biofilm formation.Biofilm development is one of these bacteria's strategies for reducing the effect of antibiotic treatment. The findings showed that the majority of the isolates were capable of forming biofilm, though to differing degrees from the negative control. The isolates that were able to form biofilm fell into three categories: high, medium, and weak adherent. The high productivity of biofilm creation could be attributed to the method's sensitivity in measuring the small amounts that are generated, which is regarded as a crucial approach in researching the early phases of biofilm development.By measuring the absorbance at 630 nm using ELISA reader equipment, the optical density of biofilm formation was found to differ statistically significantly between all isolates. These results corroborated research (22).

DNA extraction and*las*I detection

Using particular primer for the *las*I gene, the (QS) gene was detected molecularly in 34 (89.47%) of the isolates; all positive isolates had bands that occurred within the predicted gene size (177 bp) while 4 (10.52) isolates have not possessed the (*lasI*)by evaluating the bands on gel electrophoresis and comparing their molecular weight to the 100 bp DNA ladder, as indicated in Figure (1).

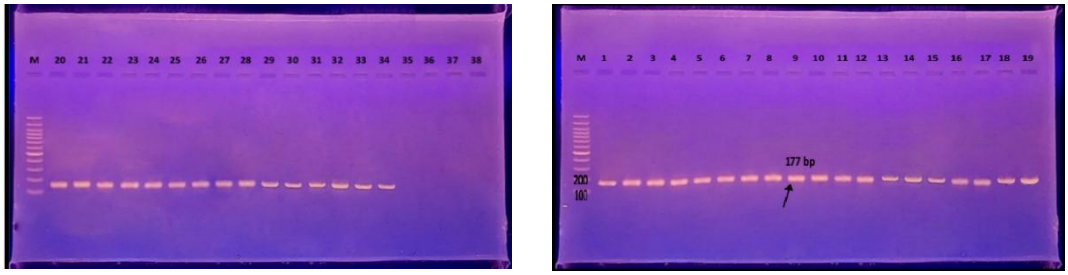

Figure (1): Electrophoresis Agarose gel of the amplification products of *las*I gene. Lane M: 100bp DNA ladder; Lanes 1-19: PCR product of samples with expected size 177 bp. (2% agarose at 70 volts for 60 min).

Numerous investigations revealed a close relationship between the QS system and biofilm development, with biofilm formation data indicating that (34) of isolates capable of forming biofilms were positive for QS genes. The *las*I gene is crucial for maintaining *P. aeruginosa* biofilm.LasI synthesizes the signaling 3-oxo-C12-HSL, which is necessary for the production of P. aeruginosa biofilm. In this context, *las*I is produced in a significant number of cells during the early phases of biofilm formation. On the other hand, a *las*I mutant produces a thin, flat biofilm. These findings agreed with study (23) whichindicated a significant relationship between the production of biofilm and the *lasI* gene. Also our results matched with study (24) which showedthe *las*I gene was not detected in isolate 15 which is a moderate biofilm producer.

Real time PCR quantification of *las*I expression

Before group treatment, the *las*I Ct value in isolates was (1± 0.12). Ct values for *las*I in Chlorhexidine digluconate -treated isolates fell within the range of (0.419± 0.11).

Ct values for Benzelalkonium cloride-treated isolates ranged from (0.591± 0.13) as shown in table 8.

Since the *16S r*RNA gene's

expression is consistent in the cells under investigation under all conditions, it was chosen as the housekeeping gene for this experiment.

Table (8): Fold change of *las*I gene expression in different treatments with microbicides

Group	Fold change ±SE of *las*I
Before treatment	1±0.12 a
After treatment 1 (Chlorhexidine digluconate)	0.419 ±0.11 b
After treatment 2 (Benzalkonium chloride)	0.591 ±0.13 b
P-value	0.0485
Means having with the different letters in same column differed significantly. * (P≤0.05).	

The findings demonstrate that there was a statistically significant variation in the groups' Ct values. The Ct values of isolates treated with Chlorhexidine digluconate were higher than those of isolates treated with Benzalkonium chloride and untreated isolates. This difference in Ct values suggests that the genes were present in the mRNA samples.These results demonstrate a relationship between the (down regulation) levels and the treatment groups for microbicides.Due to its position at the head of the QS signal transduction pathway, the LasI/R system has been highlighted in the majority of studies looking into QS and its role in *P. aeruginosa* pathogenicity. As a result, modifications to their expression would have an impact on the organism's phenotypic. These findings matched withstudy (25) which believed that biofilm development, compared to the expression of genes resistant to microbicides, may be the primary cause of microbicide tolerance.

Conclusion

As with the rise of antibiotic resistance in pathogenic *P. aeruginosa*, the results of this study show that widespread use of microbicides in hospitals has raised concerns about the emergence and transmission of microbicide resistance genes. This may have been mediated by the direct action of the QS/LasI system product or by a decrease in the transcription of genes under Las system regulation. The QS genes are desirable targets for microbicide resistance techniques as a result of these discoveries. According to this study, the *las*I gene is essential for the synthesis of biofilms, and its absence corresponds to the absence of biofilm formation. The MIC concentrations of the microbicide agents were also concluded. These data make it evident that the target gene's maximum expression corresponds to the isolates' lowest mRNA copy number, and that the target gene's lowest expression islinked to the microbicides medications, where the mRNA copy number is highest.

References

1. Geraldes, C.; Tavares, L.; Gil, S. and Oliveira, M. (2023). Biocides in the Hospital Environment: Application and Tolerance Development. *Microbial Drug Resistance*, *29*(10), 456–476.
2. Al-Khikani, F. H. O. (2023). Virulence factors in Pseudomonas aeruginosa: The arms race between bacteria and humans. Microbes and Infectious Diseases, 4(1), 94–96.
3. Tashakor, A.; Abniki, R. and Mansury, D. (2023). Evaluating the Effectiveness of Benzalkonium Chloride on Gram-Positive and Gram-Negative Bacteria in Iran: A Systematic Review. Journal of Isfahan Medical School, 40(703), 1115–1120.

4. Boyce, J. M. (2023). Quaternary ammonium disinfectants and antiseptics: tolerance, resistance and potential impact on antibiotic resistance. *Antimicrobial Resistance and Infection Control*, *12*(1), 1–14.
5. Maillard, J.-Y. Y. and Pascoe, M. (2024). Disinfectants and antiseptics: mechanisms of action and resistance. *Nature Reviews Microbiology*, *22*(1), 4–17.
6. Badawy, M. S. E. M.; Riad, O. K. M.; Harras, M. F.; Binsuwaidan, R.; Saleh, A. and Zaki, S. A. (2024). Chitosan–Aspirin Combination Inhibits Quorum-Sensing Synthases (lasI and rhlI) in Pseudomonas aeruginosa. Life, 14(4), 481.
7. Ghanem, S. M.; El-Baky, R. M. A.; Gamil, N. G. F. M.; Abourehab, M. A. S. and Fadl, G. F. M. (2023). Prevalence of Quorum Sensing and Virulence Factor Genes Among Pseudomonas aeruginosa Isolated from Patients Suffering from Different Infections and Their Association with Antimicrobial Resistance. Infection and Drug Resistance, 16(April), 2371–2385.
8. Leitão, M. M.; Vieira, T. F.; Sousa, S. F.; Borges, F.; Simões, M. and Borges, A. (2024). Dual action of benzaldehydes: Inhibiting quorum sensing and enhancing antibiotic efficacy for controlling Pseudomonas aeruginosa biofilms. Microbial Pathogenesis, 191(February).
9. Naga, N. G.; El-Badan, D. E.; Ghanem, K. M. and Shaaban, M. I. (2023). It is the time for quorum sensing inhibition as alternative strategy of antimicrobial therapy. Cell Communication and Signaling, 21(1), 1–14.
10. Abbood, H. M.; Hijazi, K. and Gould, I. M. (2023). Chlorhexidine Resistance or Cross-Resistance, That Is the Question. Antibiotics, 12(5), 1–17.
11. Schuster, M.; Li, C.; Smith, P. and Kuttler, C. (2023). Parameters, architecture and emergent properties of the Pseudomonas aeruginosa LasI/LasR quorum-sensing circuit. *Journal of the Royal Society Interface*, *20*(200).
12. Phuengmaung, P.; Somparn, P.; Panpetch, W.; Singkham-In, U.; Wannigama, D. L.; Chatsuwan, T. and Leelahavanichkul, A. (2020). Coexistence of Pseudomonas aeruginosa With Candida albicans Enhances Biofilm Thickness Through Alginate-Related Extracellular Matrix but Is Attenuated by N-acetyl-l-cysteine. *Frontiers in Cellular and Infection Microbiology*, *10*(November), 1–17.
13. Namaki, M.; Habibzadeh, S.; Vaez, H.; Arzanlou, M.; Safarirad, S.; Bazghandi, S. A.; Sahebkar, A. and Khademi, F. (2022). Prevalence of resistance genes to biocides in antibiotic-resistant Pseudomonas aeruginosa clinical isolates. *Molecular Biology Reports*, *49*(3), 2149–2155.
14. Fattah, R. A. F. A.; Fathy, F. E. Z. Y.; Mohamed, T. A. H. and Elsayed, M. S. (2021). Effect of chitosan nanoparticles on quorum sensing-controlled virulence factors and expression of lasi and rhli genes among pseudomonas aeruginosa clinical isolates. *AIMS Microbiology*, *7*(4), 415–430.
15. Abdul Razzaq, A. B.; Shami, A. M.; and Ghaima, K. K. (2022). Detection of vanA and vanB genes Among VancomycinResistant Staphylococcus aureus Isolated from Clinical Samples in Baghdad Hospitals. Iraqi Journal of Biotechnology. 21(1): 19-25.
16. Gawad, M. A. and Gharbi, W. A. (2022). Molecular Detection of oprI and oprL Virulence Genes of Pseudomonas aeruginosa Isolated from Burns and Wounds. Iraqi Journal of Biotechnology, 21(2), 215–224.
17. Mezher Kani, M.; Adil, Y. and Alabdali, J. (2022). Pseudomonas aeruginosa detection and identification in burn and wound clinical samples from Al Muthanna hospitals in Iraq using 16S rRNA gene sequencing and measurement of antibiotic resistance. Journal of Pharmaceutical Negative Results, 13(6), 1–7.
18. AL-Jesmany, E. H.; Abbas, O. and Hasan AlSaadi, B. (2022). Molecular technology for the detection of Pyoviridine gene in Pseudomonas aeruginosa isolated from burn cases. Mustansiriya Medical Journal, 21(1), 23.
19. Almusawy, N. M. and Al-hashimy, A. B. (2022). Role of Some Virulence Genes and Antibiotic Susceptibility of Pseudomonas aeruginosa Isolated from Different Clinical Samples. Iraqi Journal of Biotechnology, 21(2), 574–578.
20. Mays A. AL-Ameen, A. M. G. (2022). Prevalence of Colistin Resistance in Pseudomonas aeruginosa Isolated from Burn Patients in Sulaymaniyah City. Iraqi Journal of Biotechnology, 21(2), 713–722.
21. Alfarras, A. F. and Al-Daraghi, W. A. (2024). Characterization of Integron Genes of Clinical Isolates of Pseudomonas Aeruginosa Which Perform Resistance To Antibiotics and Biofilm Formation By These

Strains. *Microbiology Journal*, *86*(1), 3–13.
22. Shatti, H.; Al-Saeed, W. and Nader, M. (2022). Effect Biofilm Formation in Pseudomonas aeruginosa Resistance To Antibiotic. Mustansiriya Medical Journal, 21(1), 13.
23. Al-maeni, M. A. R. (2024). Detecting the Variation in the lasI Gene and Their Relation with Biofilm in Pseudomonas aeruginosa 89–79 ,(1)65.
24. Lahij, H. F.; Alkhater, A. H.; Hassan, M. H. and Yassir, L. A. (2021). The Effect of Qourum Sensing genes (lasI, rhlI) in Some Virulence Factors of Pseudomonas aeruginosa Isolated From Different Clinical Sources. Medico-Legal Update, 21(1), 303–308.
25. Betchen, M.; Giovinco, H. M.; Curry, M.; Luu, J.; Fraimow, H.; Carabetta, V. J. and Nahra, R. (2022). Evaluating the Effectiveness of Hospital Antiseptics on Multidrug-Resistant Acinetobacter baumannii: Understanding the Relationship between Microbicide and Antibiotic Resistance. Antibiotics, 11(5).