Original Article

Gene Expression Evaluation of *mexT* and *mexF* Genes of *Pseudomonas aeruginosa* under Antibiotic Stress

Roaa Abd Al-Rahman Abdulla, Rasmiya Abd Aburesha

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Background: *Pseudomonas aeruginosa* is a major cause of mortality and morbidity in those with immunodeficiencies and those who have cystic fibrosis and antibiotic resistance. **Objectives:** To investigate the role of genes responsible for pump efflux of antibiotics (*mexF* and *mexT*) and their relation to antibiotic resistance. **Materials and Methods:** A total of 100 clinical swabs were collected from different hospitals in Baghdad, Iraq. The identification of bacterial isolates was confirmed using the VITEK2 compact system and *16s rRNA*. Drug susceptibility tests were performed by the VITEK2 compact system. Conventional polymerase chain reaction (PCR) was used for the detection of efflux pump (*mexT* and *mexF*) genes for the isolates. Reverse transcription (RT)-PCR was used to detect gene expression and the effect of ceftazidime and amikacin antibiotics on the *mexT* gene for five isolates. **Results:** The results of culture and biochemical tests showed that 50 isolates were *P. aeruginosa*. They were resistant to cefotaxime (71.43%), ceftazidime (71.43%), cefepime (71.43%), meropenem (65.71%), imipenem (62.86%), amikacin (62.86%), gentamicin (62.86%), norfloxacin (60%), and ciprofloxacin (51.43%). The result of conventional PCR showed that *mexT* 20 (57.1%) was positive, whereas *mexF* 18 (51.4%) and the results of RT-PCR indicated that all isolates exhibited downregulation of the *mexT* gene. **Conclusion:** There is a positive correlation between *mexT* and *mexF* genes and antibiotic resistance.

Keywords: Antibiotic resistance, efflux gene, *mexF*, *mexT*, *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is a Gram-negative, heterotrophic, rod-shaped bacterium.[1,2] *P. aeruginosa* causes diseases in animals and plants as well as humans. It is an opportunistic bacteria, which is a major cause of mortality and morbidity in individuals with immunodeficiencies and those who have cystic fibrosis.[3]

With a wide range of species, antibiotic resistance is becoming more prevalent.[4] It can survive in a variety of other natural and artificial settings due to its adaptability and high intrinsic antibiotic resistance.[5] Numerous biological control programs have included *Pseudomonas* species.[6]

Efflux pumps can play a role in bacterial pathogenicity by conferring resistance to antibiotics and other antimicrobial compounds. They can also transport molecules important for bacterial virulence, such as quorum-sensing signals and toxins. Efflux pumps have been implicated in the virulence of a range of bacterial pathogens, including *Salmonella enterica*, *Staphylococcus aureus*, and *P. aeruginosa*. Therefore, targeting efflux pumps is an attractive strategy for developing new antimicrobial agents that could be used for treating multidrug-resistant (MDR) bacterial infections.[7]

The *mexT* gene regulates a variety of targets that mediate phenotypic change, suggesting that it may have a more extensive impact on *P. aeruginosa* virulence than has previously been reported.[7] The *mexF* gene encodes a cytoplasmic-membrane protein that is, thought to be the efflux pump.[8] *MexT* is a universal *LysR* transcriptional

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_776_23

Address for correspondence: Roaa Abd Al-Rahman Abdulla, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
E-mail: ruaa.abd1998r@gmail.com

Submission: 17-Jun-2023 **Accepted:** 16-Aug-2023 **Published:** 29-Mar-2025

How to cite this article: Abdulla RAA, Aburesha RA. Gene expression evaluation of *mexT* and *mexF* genes of *Pseudomonas aeruginosa* under antibiotic stress. Med J Babylon 2025;22:282-8.

regulator that regulates *P. aeruginosa* virulence and antibiotic resistance.[9] The *mexT* expression is controlled by several factors, including the MexEF-OprN efflux pumps themselves and the two-component system, PmrAB. In the absence of MexEF-OprN, *mexT* expression is increased, leading to upregulation of other efflux pumps and MDR. On the other hand, *MexT* expression increases when the PmrAB system is activated, leading to the upregulation of efflux pump genes.[10]

Materials and Methods

Collection of specimens

A total of 100 clinical specimens of urine, burn swabs, wound swabs, sputum, blood, and ear swabs were obtained throughout the period from September 2022 to January 2023. Then, it was inoculated and subsequently incubated for 24 h at 37°C on MacConkey agar. The pale, nonlactose fermenting colonies were chosen, and one colony was inoculated on a cetrimide medium for biochemical assays.

Bacterial identification

Identification of *P. aeruginosa* isolates was performed using standard microbiological and biochemical tests.[11,12]

Antimicrobial susceptibility using the VITEK2 system

The VITEK 2 method (bioMérieux, Marcy-l'Étoile, France) is a novel automated method for detecting bacterial susceptibility to antibiotics using fluorescence-based technologies.

The bacterial isolates were grown in nutrient agar using the streak technique and incubated at 37°C for 24 h.

The test tube was filled with sterile saline (3.0 mL), and a sterile swab was used to transfer a pure colony and suspend the isolated colony in the sterile saline.

The DensiCHEK™ (bioMérieux) turbidity meter was used to adjust the bacterial suspension. The results were obtained after 4–6 h.

DNA extraction from bacteria

High-antibiotic resistance isolates had their deoxyribonucleic acid (DNA) extracted using the Easy-Pure® Genomic DNA Kit's (TransGen Biotech Co., Beijing, China) instructions.

Detection of *mexT* gene in *P. aeruginosa* isolate

Amplification of the tested gene was performed by conventional polymerase chain reaction (PCR), and the primer sequence was designed by used as bioinformatics software. The final optimized PCR reaction consisted of 1.5 µL of forward primer of *mexT* gene (GACAGGTGGGCGAAGATTTCC) and 1.5 µL of reverse primer (GTGTTCGAGACCCTGATGCAC), forward primer of *mexF* gene (GATCGGAGGCATCGTTTCGTT) and reverse primer (GCGAGGACATGTACAGCATCC), 10 pmol/µL from each primer, Green master mix (17.5 µL), 5 µL DNA (4.5 µL) nuclease-free water polymerase (NEB® England), to give a final volume of 25 µL. Adjustment of the cycling program for the *mexT* gene was: initial denaturation (94°C) for 5 min, denaturation at 94°C for 30 s, annealing at 56°C for 45 s, extension at 72°C for 45 s, and final extension at 72°C for 7 min.

mexT gene expression before and after treatment with antibiotics (amikacin and ceftazidime) at sub-minimum inhibitory concentration (MIC) values.

RNA extraction from bacteria

The RNA of the isolates that gave high-antibiotic resistance, was extracted according to the protocol of Qubit™ RNA HS Assay Kit (Q32852) Thermo Fisher® (Waltham, MA, USA).

Reverse transcription (RT)-quantitative PCR protocol

The main step in this project is divided into two parts. The first part involves using RNA to produce complementary DNA (cDNA). A ProtoScript cDNA Synthesis Kit and specific primers for the *mexT* and 16S ribosomal ribonucleic acid (16S rRNA) transcripts 5 µL of the total sample's extracted RNA, 10 µL of a ProtoScript reaction mix containing deoxynucleotide triphosphates, buffer, and 1 µL of reverse transcriptase enzyme were added to the reaction for each sample. About 1 µL of random primer was added, and adding 3 µL of free water raised the volume to 20 µL. Incubated for 10 min at 25°C for binding primer and 25 min at 42°C by using a thermocycler for activation of the enzyme, 85°C for inactivation of the enzyme.

In the second step of this protocol, cDNA samples from patients and controls are chosen for the same run. Three PCR tubes are used for each sample, one for the *mexT* gene, *mexF* gene, and one for the housekeeping gene *16S rRNA* (Forward ACTCCTACGGGA GGCAGCAGTand Reverse: TATTACCGCGGCTGCTGGG in this study. Based on SyberGreen's fluorescent power, quantity detection. The following ingredients are present in the reaction mixture in the amounts consisting of Luna Universal qPCR Master Mix (10 µL; NEB®, UK), forward primer (10 µM) 0.5 µL, reverse primer (10 µM) 0.5 µL of each primer, template DNA (RNA) 5 µL, Nuclease-free Water 4 µL (NEB®). The final volume of the reaction mixture is 20 µL. Adjustment of the cycling program for the *mexT* gene and housekeeping gene 16S rRNA was: (1 cycle). Initial Denaturation 94°C for 5 min (35×cycle). Denaturation 94°C for 30 s. Annealing 56°C, 50°C for 45 s. "Extension 72°C for 45 s" (1 cycle, final "Extension 72°C for 7 min."

Statistical analysis

Data entry and analysis were performed using the Statistical Package for the Social Sciences, version 21 (IBM Company, Chicago, IL, USA). In this cross-sectional study, the odds ratio (OR) was estimated to define the association between the presence of bacterial genes and antibiotic resistance. The analysis of variance test was used to assess the significance level of different laboratory parameters among the study groups. Furthermore, the Kruskal–Wallis test was used to assess the significance level.

Ethical approval

The study was conducted following ethical principles. It was carried out with patients' verbal consent from patients before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by the Baghdad University, College of Dentistry's local ethics committee according to the document number CSEC\0922\0075 on September 25, 2022.

Results

In this study, the results of biochemical tests and culture showed that 50 isolates were *P. aeruginosa*. A total of 35 isolates were confirmed by identification using the VITEK2 compact system and *16srRNA* the results showed that all the isolates were *P. aeruginosa*, as shown in Figure 1.

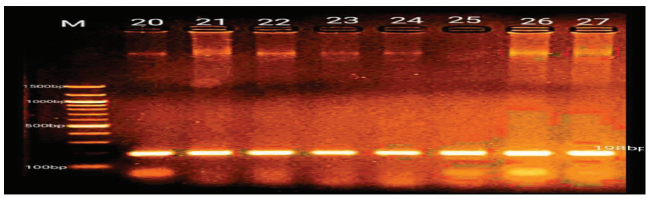


Figure 1: Gel electrophoresis of amplified PCR product of 16srRNA gene (198 bp) of *P. aeruginosa*. About 1.5% agarose gel electrophoresis stained with RedSafe dye (10 mg/mL).100 v/m Amp for 75 min. Tris/borate/ethylenediaminetetraacetic acid buffer (1×). M:100 bp DNA marker

The detection of the resistant isolates was conducted by the VITEK2 compact system and the percentage of resistance is shown in Figure 2.

These results revealed that the percentages of resistant isolates were as follows: cefotaxime 25 (71.43%), ceftazidime 25 (71.43%), cefepime 25 (71.43%), meropenem 23 (65.71%), imipenem 22 (62.86%) and 22 (62.86%), gentamicin 22 (62.86%), norfloxacin 21 (60%), and ciprofloxacin 18 (51.43%).

The result showed that there was a significant difference in resistance to the antibiotics cefotaxime, ceftazidime, and cefepime, which means a high number of resistant isolates. While the resistance of the other isolates to imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, and norfloxacin showed nonsignificant results, which means a low number of resistant isolates. The reason for this difference because no. of isolates was sensitivity to the antibiotics.

Molecular detection was done for the genes (*mexT and mexF*) in 35 isolates by conventional PCR Technique [Table 1; Figures 3 and 4].

Molecular detection was done to gene (*mexT* and *mexF*) for 35 isolates by conventional PCR technique. The results showed that 20 (57.1%) of the isolates were carrying the *mexT* gene, and there was a nonsignificant difference between *P. aeruginosa* isolates, whereas the *mexF* gene was 18 (51.4%).

Correlation between the *mexT* gene in bacteria isolated from different clinical specimens [Figure 5].

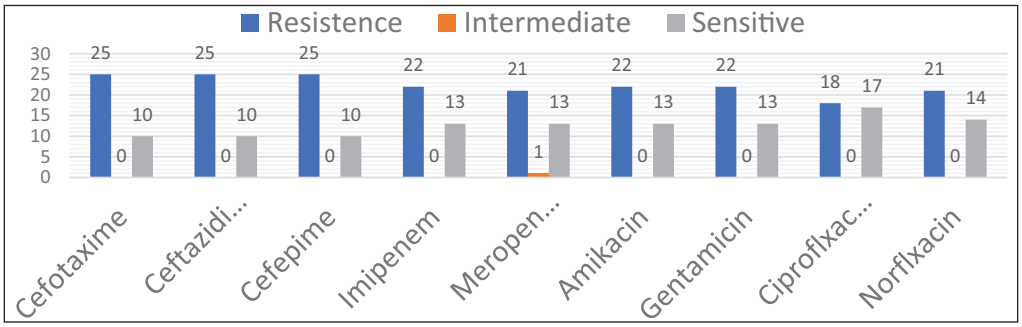


Figure 2: Antibiotic susceptibility percentage of the resistant isolates

Table 1: Percentage of *mexT* and gene in bacterial isolate

Gene	Positive	Negative	*P* value
mexT	20 (57.1%)	15 (42.9%)	0.162 NS
mexF	18 (51.4%)	17 (48.6%)	0.841 NS

NS: nonsignificant

Five *P. aeruginosa* isolates were used to detect gene expression to evaluate the effect of ceftazidime and amikacin, which were from different groups of antibiotics and have the highest resistance rate, on the gene expression of the efflux pump *mexT* gene. The used isolates were from burn sources. MDR isolates were resistant to ceftazidime and amikacin causing downregulation of the *mexT* gene in all isolates. The results show that the treatments with sub-MIC (64 mg/mL) of ceftazidime, whereas sub-MIC (32 mg/mL) of amikacin [Tables 2–4].

The results showed a decrease in the folding of gene expression of the *mexT* gene in all isolates when treated with amikacin and ceftazidime at sub-MIC.

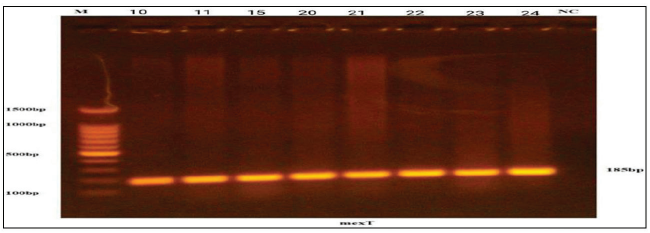


Figure 3: Amplified PCR product of the *P. aeruginosa mexT* gene (185 bp) electrophoresed on a gel. Electrophoresis of 1.5% agarose stained with RedSafe die (10 mg/mL). About 75-min tris/borate/ethylenediaminetetraacetic acid buffer at 100 v/m Amp. 100 bp DNA marker Lane 1

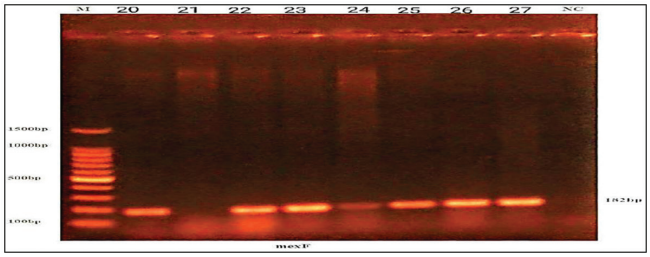
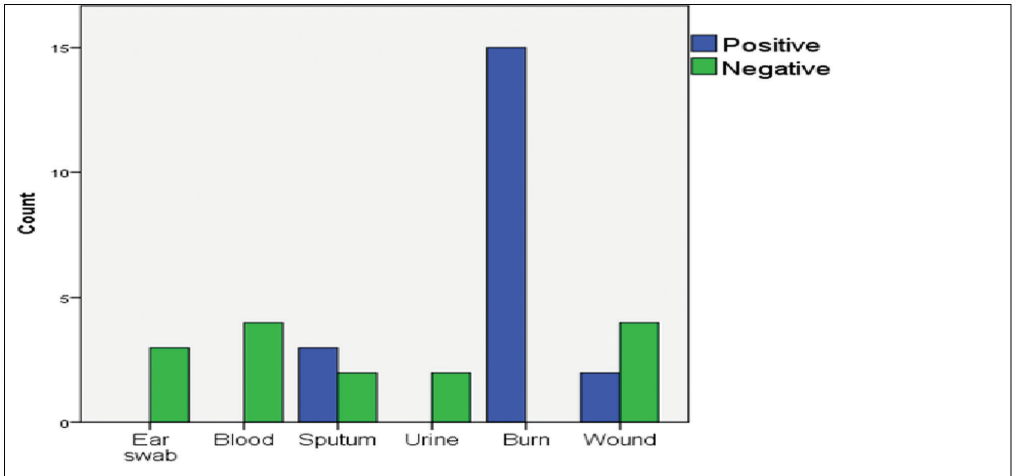


Figure 4: Amplified PCR product of the *P. aeruginosa mexF* gene (182 bp) electrophoresed on a gel. Electrophoresis of 1.5% agarose stained with RedSafe die (10 mg/mL). About 75-min tris/borate/ethylenediaminetetraacetic acid buffer at 100 v/m Amp. 100 bp DNA marker Lane 1

Discussion

Medical microbiology laboratories have employed different methods for microbial detection to screen for and detect microbial resistance in clinical specimens. Cetrimide agar used to isolate *P. aeruginosa* appears on agar and produces fluorescein and pyocyanin.[13] *Pseudomonas* lactose is a nonfermentor; therefore, colonies on MacConkey agar will appear pale.[14] The positive result for the oxidase test bacterial colony showed a change of a blue-purple color within 10 s, and was positive result for the catalase test bubble formation was observed within 5–10 s.[15] About 21 (32%) of the 65 clinical ear swabs examined in another clinical study tested positive for *P. aeruginosa*.[16] *Pseudomonas* sp. isolated 23 different strains, with 11 (47.8%) coming from wounds and 12 (52.2%) from burns.[17] According to other study results of the antibiotic susceptibility test, all isolates showed signs of being highly resistant to the B-lactam group, ceftazidime (76%) and imipenem (59%).[18] This study is in agreement with the study of Sarah and Zainab.[19] This study does not agree



	Ear swab	Blood	Sputum	Urine	Burn	Wound	Total
Mex T gene Positive	0 (0%)	0 (0%)	3 (60%)	0 (0%)	15 (100%)	2 (33.33%)	20 (57.14%)
Mex T gene Negative	3 (100%)	4 (100%)	2 (40%)	2 (100%)	0 (0%)	4 (66.67%)	15 (42.86%)

Figure 5: Correlation between *mexT* gene in bacteria isolation from different clinical specimens

Table 2: *P. aeruginosa* (*mexT*) gene expression at sub-MIC before antibiotic treatment

Bacterial isolate before treatment	Ct of *16 sRNA* gene	Ct of *mex*T gene	ΔΔCt	Folding before treatment
P.24	12.7	15.7	0.0	1.00
P.22	13.6	13.7	0.0	1.00
P.25	15.4	14.5	0.0	1.00
P.23	9.1	16.1	0.0	1.00
P.20	15	14.1	0.0	1.00

Table 3: Effect of amikacin at sub-MIC on gene expression *mexT* gene of *P. aeruginosa* isolate

Bacterial isolate after treatment with amikacin	Ct of *16 sRNA* gene	Ct of *mexT* gene	ΔΔCt	Folding after treatment with amikacin
P.24	13.3	28.1	11.8	0.02
P.22	13.0	30.2	17.0	0.007
P.25	13.2	28.3	16.0	0.001
P.23	13.4	32.2	11.8	0.02
P.20	6.9	17.4	11.4	0.03

Table 4: Effect of ceftazidime at sub-MIC on gene expression *mexT* gene of *P. aeruginosa* isolate

Bacterial isolate after treatment with ceftazidime	Ct of *16 sRNA* gene	Ct of *mexT* gene	ΔΔCt	Folding after treatment with ceftazidime
P.24	9.8	18.8	6	0.01
P.22	10.4	14.4	3.8	0.07
P.25	10.6	14.6	4.9	0.03
P.23	9.8	17.3	0.5	0.7
P.20	12.8	15.1	3.2	0.1

with another study (ceftazidime and imipenem), the isolates showed low resistance.[20] Another study finds that imipenem and meropenem are highly sensitive to *P. aeruginosa*.[21] Over the past few decades, it has been noted that *P. aeruginosa* has become increasingly resistant to various medications[22] and other research studies. More than 94% of our isolates were resistant to ciprofloxacin, gentamicin, imipenem, and ticarcillin, while the isolates showed high sensitivity to colistin, followed by ceftazidime (100% and 16%, respectively).[23] Other studies revealed that the two antibiotics with the highest rates of resistance were ceftazidime (90.5%) and gentamicin (88.5%), and the most effective drug for treating *P. aeruginosa* was imipenem.[24] In another study, about 4 out of 10 isolates (40%) with conventional PCR results had the efflux pump gene.[25] In another study, the efflux pump gene was only found in 8/28 (28.5%) isolates, whereas another efflux gene was found in 25 (89.5%) isolates. Al-Jubori *et al*.[26] discovered efflux pump genes were found in 56.7% (34 strains) and 46.7% (28 strains) of the tested isolates. Abdel-Salam *et al*.[27] discovered greater burn size means more exposed body surface and an increased probability of colonization[28] in another study. The majority came from infections of the respiratory tract (15.09%), swabs (18.6%), urinary tract (22.11%), and wound/pus (22.46%).[29] This results when bacterial isolates are challenged to antibiotic concentration in value near to MIC. The regulatory gene *mexT* decreases in expression maybe this gene is the effect of concentration that causes inhibitor for expression, and there is no time for the gene to express, and there may be another regulatory gene effect on the efflux pump. Thus these isolates were resistant to the antibiotic.

Therefore, if the *mexT* gene had a mutation, it was a likely candidate that caused the mexEF-oprN operon to be overexpressed. PCR was used to amplify the *mexT* region from the isolate, which contains the structural gene and the regulatory region.[30] Lister *et al*.[31] discovered that mutations affecting various regulatory genes may lead to the overexpression of both efflux systems. Moreover, when the expression of *AmpC* was significantly elevated, *P. aeruginosa* demonstrated except for carbapenems, almost all -lactam antibiotic types, except for carbapenems.[31] The findings of previous research indicate that these genes were overexpressed at a rate exceeding 50%.[32] In another research, the gene expression of some efflux pump genes occurred to increase and a decrease for the efflux gene expression of some other pump genes was revealed.[33] the analysis of relative gene expression demonstrated that the frequencies of overexpression were highest at the following percentages: 100%, 100%, 87.5%, 81.25%, and 56.25% of the efflux pump gene.[34]

Conclusion

P. aeruginosa isolates from burns specimens are more frequent than other sources. The most isolates with antibiotic

resistance were from burns infections, urine, sputum, and wounds, respectively. In the molecular identification using *mexT* and *mexF* primers, 20 isolates were positive for *mexT* gene, and for *mexF*, 18 isolates were positive. The study found a correlation between the *mexT* and *mexF* genes and antibiotic resistance in *P. aeruginosa*, with highly significant differences for the *mexT* gene and significant differences for the *mexF* gene. Antibiotic sensitivity and MIC for *P. aeruginosa* isolates showed high resistance to ceftazidime and amikacin. The effect of the treatment with the sub-MIC of amikacin was downregulation for all isolates. On the other hand, the treatment with the sub-MIC of ceftazidime on gene expression of the *mexT* gene was downregulated for all isolates. Positive correlation between *mexT and mexF* genes with antibiotic resistance, indicating the role of this efflux pump gene and its effect on antibiotic resistance.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References
1. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: Mechanisms and alternative therapeutic strategies. Biotechnol Adv 2019;37:177-92.
2. Resha RA, Al-Zubaidy BB. Study the prophylactic role of anti-type IV Pili (fimbriae) antibody against pulmonary infection caused by P. aeruginosa in vivo (mice). Iraqi J Sci 2015;56:998-1008.
3. Al-Anssari MJ, Al-Charrakh AH. Flagellin B shifting the immune response against P. aeruginosa respiratory infections from chronic to cure state. Lat Am J Pharm 2023;42:55-60.
4. Al-Charrakh AH, Al-Enzi RM. Resistance to lead, copper, and mercury in P. aeruginosa isolated from hospitals in Iraq. Res J Pharm Biol Chem Sci 2016;7:2644-9.
5. Al-Charrakh AH, Al-Awadi, SJ, Mohammed AS. Detection of metallo-β-lactamase producing Pseudomonas aeruginosa isolated from public and private hospitals in Baghdad, Iraq. Acta Med Iran 2016;54:107-13.
6. Patel R, Mehta K, Prajapati J, Shukla A, Parmar P, Goswami D, *et al*. An anecdote of mechanics for Fusarium biocontrol by plant growth promoting microbes. Biol Control 2022;174:105012.
7. Tian ZX, Mac Aogain M, O'Connor HF, Fargier E, Mooij MJ, Adams C, *et al*. MexT modulates virulence determinants in *Pseudomonas aeruginosa* independent of the MexEF-OprN efflux pump. Microb Pathog 2009;47:237-41.
8. Li X, Nikaido H, Poole K. Role of mexA-mexB-oprM in antibiotic efflux in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1995;39:1948-53.
9. Liu Y, Ahator SD, Wang H, Feng Q, Xu Y, Li C, *et al*. Microevolution of the mexT and lasR reinforces the bias of quorum sensing system in laboratory strains of *Pseudomonas aeruginosa* PAO1. Front Microbiol 2022;13:821895.
10. De Gaetano GV, Lentini G, Famà A, Coppolino F, Beninati C. Antimicrobial resistance: Two-component regulatory systems and multidrug efflux pumps. Antibiotics 2023;12:965.
11. Sheikh AF, Ghanbari F, Afzali M, Shahin M. Isolation of oxidase-negative Pseudomonas aeruginosa from various specimens. Iran J Public Health 2020;49:1186-8.
12. Khatoon H, Anokhe A, Kalia V. Catalase test: A biochemical protocol for bacterial identification. AgriCos e-Newsletter 2022;3:53-5.
13. Rashad FF, Obaid SS, Al-Kadhi NA. Association of multidrug resistance with biofilm formation in *Pseudomonas aeruginosa* isolated from clinical samples in Kirkuk City. NTU J Pure Sci 2022;1:10-9.
14. Kamel A, Suleiman WB, Elfeky A, El-Sherbiny GM, Elhaw M. Characterization of bee venom and its synergistic effect combating antibiotic resistance of *Pseudomonas aeruginosa*. Egypt J Chem 2022;0:0-0.
15. Rawi NN, Ramzi MM, Rahman NIA, Ariffin F, Saidin J, Bhubalan K, *et al*. Antifouling potential of ethyl acetate extract of marine bacteria *Pseudomonas aeruginosa* strain RLimb. Life 2023;13:802.
16. Ibrahim AH. Link between some virulence factors genes and antibacterial resistance of *Pseudomonas aeruginosa*. Iraqi J Agric Sci 2022;53:985-93.
17. Khudhair MK, Alaubydi MA. Determination the prevalence and antimicrobial susceptibility of bacteria isolated from burns and wounds. Iraqi J Agric Sci 2023;54:93-9.
18. Hamid OS, Mahmood SS. The synergistic effect of gold nanoparticle loaded with ceftazidium antibiotic against multidrug resistance pseudomonas aeruginosa. Iraqi J Agric Sci 2021;52:828-35.
19. Sarah NL, Zainab FM. Activity of marticaria chamomilla crude and total flavonoid extracts as anti-virulence factor for clinically isolated Pseudomonas aeruginosa. Iraqi J Agric Sci 2023;54:59-69.
20. Hasan SA, Najati AM, Abass KS. Prevalence and antibiotic resistance of "pseudomonas aeruginosa" isolated from clinical samples in Kirkuk City, Iraq. Eurasia J Biosci 2020;14:1821-5.
21. Lee Y, Ko W, Hsueh P. In vitro activities of ceftazidime–avibactam, ceftolozane–tazobactam, meropenem–vaborbactam, and other comparators against Pseudomonas aeruginosa isolates with discrepant resistance to carbapenems: Data from the Antimicrobial Testing Leadership and Surveillance (ATLAS) program, 2012–2021. Int J Antimicrob Agents 2023;62:106867.
22. Shakir ZA, Al-Draghi WAH, Al-Haboobi HMR. Molecular identification, prevalence, and antibiotic resistance of Pseudomonas aeruginosa isolated from clinical and medical waste samples in Baghdad City, Iraq. HIV Nurs 2023;23:1216-21.
23. Dai C, Lin J, Li H, Shen Z, Wang Y, Velkov T, *et al*. The natural product curcumin as an antibacterial agent: Current achievements and problems. Antioxidants 2022;11:459.
24. Zuhra S, Asad M, Sahar Z, Farrukh H, Bibi K, Tanveer T, *et al*. Simultaneous application of non-antibiotics with antibiotics for enhanced activity against multidrug resistant pseudomonas aeruginosa. J Pharm Negat Results 2023;14:4015-23.
25. Yaseen NN, Ahmed DA. Detection of mexB multidrug efflux gene in some local isolates of pseudomonas aeruginosa. Iraqi J Sci 2023;64:111-8.
26. Al-Jubori SS, Al-Jabiri HA, Al-Kadmy IMS. Molecular detection of aminoglycoside resistance mediated by efflux pump and modifying enzymes in pseudomonas aeruginosa Isolated From Iraqi Hospitals. Int'l Conference on Medical Genetics. Cellular & Molecular Biology, Pharmaceutical & Food Sciences, Istanbul. 2015.
27. Abdel-Salam S, Ahmed YM, Abdel Hamid DH, Fathy FEZ. Association between MexA/MexB efflux-pump genes with the resistance pattern among Pseudomonas aeruginosa isolates from Ain Shams University Hospitals. Microbes Infect Dis 2023;4:160-7.
28. Othman N, Babakir-Mina M, Noori CK, Rashid PY. Pseudomonas aeruginosa infection in burn patients in Sulaimaniyah, Iraq: risk factors and antibiotic resistance rates. J Infect Dev Ctries 2014;8:1498-502.
29. Al-Marzoqi AH, Al Taee ZM. Pseudomonas aeruginosa: Antibiotic resistance pattern to different isolates in Al-Hillah city, Iraq. J Nat Sci Res 2013;3:69-74.
30. Masuda N, Sakagawa E, Ohya S. Outer membrane proteins responsible for multiple drug resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 1995;39:645-9.
31. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22:582-610.

32. Mesaros N, Glupczynski Y, Avrain L, Caceres NE, Tulkens PM, Van Bambeke F. A combined phenotypic and genotypic method for the detection of Mex efflux pumps in Pseudomonas aeruginosa. J Antimicrob Chemother 2007;59:378-86.
33. Al Rashed N, Joji RM, Saeed NK, Bindayna KM. Detection of overexpression of efflux pump expression in fluoroquinolone-resistant Pseudomonas aeruginosa isolates. Int J Appl Basic Med Res 2020;10:37-42.
34. Llanes C, Hocquet D, Vogne C, Benali-Baitich D, Neuwirth C, Plésiat P. Clinical strains of Pseudomonas aeruginosa overproducing MexAB-OprM and MexXY efflux pumps simultaneously. Antimicrob Agents Chemother 2004;48:1797-802.