Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2024, 37(3)

Detecting the Efflux Pumps Gene (mexB and oprM) in MDR Pseudomonas Aeruginosa Isolated from Wound Infection

Wael Abbas Hameed AL-Mhesin[1], Okan Ürker[2] and Rana Mujahid Abdullah[3*]

[1,2]Department of Biology, Graduate School of Natural and Applied Sciences, Çankiri Karatekin University.
[3]Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

Received:27 April 2023 Accepted:19 June 2023 Published:20 July 2024
doi.org/10.30526/37.3.3447

Abstract

The large illnesses caused by *Pseudomonas aeruginosa* and the more challenging therapy for antimicrobial resistance are both due to the organism's extensive spectrum of virulence factors and several mechanisms for antibiotic resistance. The aim of the study is to detect phenotypic and genotypic efflux pump genes in *Pseudomonas aeruginosa*. In the current study, 100 wound samples were collected from both sexes and different ages for a period from May 2022 to November 2022 from Teaching Laboratories Hospital/Medical City, Martyr Ghazi Hariri Hospital, and Baghdad Teaching Hospital. 19 isolates of *Pseudomonas aeruginosa* were taken from wound infections. The samples were cultured on cetrimide agar and MacConkey agar for diagnosis, and then they underwent several microscopic, phenotypic, and biochemical tests, where included Oxidase, Indole, Urease, Glucose and Triple sugar-iron test (TSI). *Pseudomonas aeruginosa* susceptibility to 11 antibiotics was tested using the disc diffusion method. It has been shown that *Pseudomonas aeruginosa* has multi-antibiotic resistance. It showed that the bacteria isolates are resistant to Levofloxim 42%, Ciprofloxacin 40%, Piperacillin tazobactam 35%, Amicacin 32%, Ceftazidime 31%, Gentamicin 31%, Cefepime 30%, Piperacillin 29%, Tobramycin 29%, Imipenem 17%, and Meropenem 16%. The ability of bacteria to produce efflux pumps was also studied using the Ethidium Bromide Agar Cartwheel Method, where it was found that 8/19 isolates (42%) were positive**.** The efflux pump genes *mexB and oprM* were studied. The results of this study showed that all isolates of *Pseudomonas aeruginosa* possess *mexB* at 100%. While the oprM increased by 57.8%
Keywords: *Pseudomonas aeruginosa*; Antibiotic resistance; Efflux pumps.

1.Indroduction

The word "pseudomonas" has Latin and Greek roots that mean "false unit." When referring to germs in the early history of microbiology, the stem word mon was used [1].The Latin title for the species, aeruginosa, which refers to the blue-green hue of the species' laboratory cultures, means copper rust. Pyocyanin and pyoverdine, two *Pseudomonas aeruginosa* compounds that give cultures their distinctive blue-green hue, are combined to create this blue-green pigment.

Aeruginosa may have originated from the Greek prefix ae-, which means "ancient or elderly," and the suffix ruginosa, which implies wrinkled or bumpy, according to a 1956 claim [2] A Gram-negative rod bacterium known as *Pseudomonas aeruginosa* is motile, aerobic, asporogenous, and monoflagellated. which is part of the Pseudomonadaceae family [3,4] it can thrive in a wide range of temperatures from 4 to 42 ℃, although it can grow effectively around 37 ℃ [5]. *Pseudomonas aeruginosa* is an opportunistic multi-drug resistant (MDR) bacteria that can infect immunocompromised individuals with COPD, cystic fibrosis, cancer, wounds, burns, sepsis, and ventilator-associated pneumonia (VAP), especially COVID-19-related VAP, acutely or repeatedly [6].The large variety of illnesses produced by *Pseudomonas aeruginosa* and the more challenging therapy for the ensuing antimicrobial resistance are both due to the organism's extensive spectrum of virulence factors and several mechanisms for antibiotic resistance. Numerous mechanisms, including inherent antibiotic resistance, efflux systems, and antibiotic-inactivating enzyme, have been connected to pseudomonas antibiotic resistance [3].Because *Pseudomonas aeruginosa* may colonize catheters, burns and wounds, it can be discovered in a variety of medical settings, including the urology unit, burn unit, and critical care unit. [7] The production of endotoxin and exotoxin, two components of bacterial virulence, and the ensuing aberrant immune response may be the main reasons for *Pseudomonas aeruginosa*'s pathogenicity. The exotoxin pyocyanin is one of the key virulence factors generated by *Pseudomonas aeruginosa*. The quorum sensing (QS) system, a crucial communication mechanism that regulates survival, pathogenicity, and biofilm development in bacterial colonies, regulates pyocyanin [8]. The las, rhl, PQS, and IQS systems are the four QS network sub-systems that effectively control the QS system. A crucial element in the control of virulence gene expression is the hierarchical QS network [9].*Pseudomonas aeruginosa* may cause a variety of conditions, including chronic or acute infections in immunocompromised patients , cystic fibrosis, cancer, wounds, burns, sepsis, and ventilator-associated pneumonia (VAP), particularly those caused by COVID-19 [6]. *Pseudomonas aeruginosa* was the most frequently isolated bacteria in ICU illnesses, according to a study done in India [10]. Nosocomial UTIs are frequently brought on by *Pseudomonas aeruginosa*, which is responsible for up to 16.3% of UTIs in ICU patients and 9% of UTIs across the hospital. Patients with indwelling urinary catheters get nosocomial UTIs with *Pseudomonas aeruginosa* more commonly than those without these devices (10.5% vs. 4.1%) [11].It was originally thought that efflux pumps and proteins of the outer membrane did not work together to reduce intracellular drug concentrations. However, a current investigation of Burkholderia thailandensis has discovered a link between active efflux pumps and the permeability barrier of the outer membrane [12]. In truth, the development of both antimicrobial and multidrug resistance is greatly aided by the overexpression of efflux pumps. For the creation of efflux pump inhibitors, understanding the molecular makeup of efflux pumps and their critical drug-binding sites is essential [13, 14]. They are protein transporters that are located in the cell membrane, and because they are essential for removing various chemicals from the cell to prevent harmful effects, they are a significant contributor to bacterial antibiotic resistance. Besides being hydrophilic, hydrophobic, or amphipathic compounds, these carriers also carry fatty acids, antiseptics, and colors like acriflavine, violet crystal, and ethidium bromide outside of the cell. Disinfectants, sterilizers, disinfectants, and bromide Heavy metals, organic solvents, and antibiotics such as novobiocin, chloramphenicol, lactams, macrolides, and tetracyclines [15, 16].The main efflux pump responsible for *Pseudomonas aeruginosa's* clinical drug resistance is MexAB-OprM [17]. The MFP, RND transporter, and OMF in this pump are represented by the

proteins MexA, MexB, and OprM, respectively. The aim of this study is to detect phenotypic and genotypic efflux pump genes in MDR *Pseudomonas aeruginosa* isolates from wound infection.

2. Materials and Methods

2.1. Sample collection

One hundred clinical samples were collected from wound infections for a period from May 2022 to September 2022 from Teaching Laboratories Hospital/Medical City, Martyr Ghazi Hariri Hospital, and Baghdad Teaching Hospital.

2.2.Identification of bacterial isolates

2.2.1.Morphological examination

The phenotypic characteristics of Pseudomonas aeruginosa were grown on (Cetrimide and MacConkey agar to diagnose characteristics of colony shape, color, size, and smell [18].

2.2.2.Microscope examination:

a microscopic examination by stained with Gram stain to identifying cells shape, aggregation and interaction with the Gram stain [19].

2.2.3.Biochemical Tests

Clinical samples were diagnostic by biochemical tests (Oxidase test, Indol production test, Urease test, Glucose and Triple sugar- iron agar test (TSI) [20].

2.3.Antibiotic Susceptibility Test

Using the Kirby-Bauer method, a sensitivity test for bacterial isolates. The inhibition zone was measured in millimeters. The findings were compared with CLSI [21].

2.4.Morphological Detection of Efflux Pump

Trypton Soy Agar Medium used ethidium bromide stain in different concentrations to detect the efflux pump [22].

2.5.Genomic DNA extraction

Using the ABIOpure Extraction technique, genomic DNA was extracted from bacterial growth.

2.6. Quantitation of DNA

Using a Quantus Fluorometer, the concentration of the DNA that was extracted was determined to determine the amount of DNA needed for further applications. Quantifluor Dye in a diluted form (200 µl) was combined with 1 µl of DNA. DNA concentration measurements were discovered after 5 minutes.

2.7 Primer preparation

These primers were available from The Macrogen Company in lyophilized form. Primers that had been lyophilized were dissolved in water devoid of nuclease to create a stock solution that had a final concentration of 100 pmol/µl. The primer stock solution, which was kept at -20 C° in the freezer, was combined with 90 ml of nuclease-free water to create a usable primer solution with a concentration of 10 pmol/µl. **Table 1** shows the primers used in this study.

Table 1. Primer used in this study

Primer	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)	References
mexB-F	GTGTTCGGCTCGCAGTACTC	56	214	(Pourakbari, *et al*., 2016) [23]
mexB-R	AACCGTCGGGATTGACCTTG			
oprM-F	CCATGAGCCGCCAACTGTC	57	180	(Pourakbari, *et al*., 2016) [23]
oprM-R	CCTGGAACGCCGTCTGGAT			

2.8. PCR Program

The PCR mixture consisted of 10 µl of Master Mix supplied by the company Bioneer (Korea), 2 µl of the DNA of the isolates, 1 µl of Primer-F, 1 µl of Primer-R, and 6 µl of Deionized Sterile Distilled Water supplied by the company Bioneer (Korea). The contents of the PCR were then mixed in a vortex and then in a microcentrifuge, and then put in the PCR machine for an hour. After that, blend for 5 seconds with the mixer. Based on the ideal heat settings for the cycles, transfer the tubes to a thermocycler for the polymerase chain reaction for the DNA amplification procedure. A thermocycler was used to perform the polymerization chain reaction after being designed to perform the reaction depicted in **Table 2**.

Table 2. Optimal conditions for (PCR)

Steps	°C		m: s	Cycle
Initial Denaturation	95		05:00	1
Denaturation	95		00:30	30 mu
	Primer	°C		
Annealing	*MexB*	56	00:30	
	oprM	57		
Extension	72		00:30	
Final extension	72		07:00	1
Hold	10		10:00	

2.9. Agarose Gel Electrophoresis:

Transfer 5µl of the gene product to the electrophoresis on the prepared 2% agarose gel at a voltage of 100 V for 45 minutes.

3. Results and discussion

3.1. Isolation and diagnosis

One hundred wound samples were collected from both sexes and different ages for a period from May 2022 to September 2022 from Teaching Laboratories Hospital/MMedical City, Martyr Ghazi Hariri Hospital, and Baghdad Teaching Hospital. The diagnosis has been made based on biochemical tests. The phenotypic diagnosis of *Pseudomonas aeruginosa* on cetrimide agar shows greenish coloration due to the production of pyocyanin [22]. The bacterial culture appears pale in color and is not lactose-fermented in MacConkey agar [20]. Microscopic diagnosis of *Pseudomonas aeruginosa* is based on gram-recoloring morphology appearing gram-negative [24]. The biochemical test for all isolations showed positive results in oxidase [25]. A negative result is of the indole, urease, and glucose. As well as growing in a medium (TSI) without changing the color of the medium or producing H_2S [26]. From the biochemical diagnosis of the samples, it appears that there were 19 isolates of *Pseudomonas aeruginosa* from 100 samples collected after diagnosis. The result of the biochemical diagnosis of *Pseudomonas aeruginosa* is shown in **Table 3**.

Table 3. Biochemical diagnosis of *Pseudomonas aeruginosa*

The Test	The Result
MacConkey agar	Non lactose fermented
Cetrimide agar	greenish coloration due to production of pigment
Microscopic Exam.	G-ve
Oxidase	positive
Indole	Negative
Urease	Negative
Glucose	Acid (Oxidative)
Triple sugar-iron test (TSI)	K/K (Alkaline/Alkaline)

In many hospital and community settings, *Pseudomonas aeruginosa* is a prevalent nosocomial infection that causes serious illnesses [27]. These bacteria represent a threat to many patients suffering from burn and wound infections [28]. The current study showed 19 isolates of *Pseudomonas aeruginosa* from 100 samples collected from wound infections after biochemical diagnosis.The study in [29] showed that only 23 isolates from burn infections and 10 isolates from wound infections were isolated from 75 isolates of Pseudomonas aeruginosa. [30] show (69%) clinical sources, including wounds, 24 (35%), and burns, 45 (65%) of P. aeurginosa. These bacteria are a common cause of burns because they live in a humid environment, in the skin and intestines with small numbers, and in the wet environment in hospitals. Therefore, they are the main causes of both burns and wounds. The rate of infection with *P. aeruginosa* is very high in wounds and burns, and this is due to many reasons, including the ease of obtaining low-quality and ineffective antibiotics without a prescription, and it may be due to the contamination of the hospital environment and the permanent crowding of patients [31].Antibiotic resistance of *Pseudomonas aeruginosa*: According to the results of the current investigation, all 19 isolates of *Pseudomonas aeruginosa* had distinct variations in antibiotic resistance, as shown in **Table 4**. Antibiotics were used in the sensitivity test. Separates appeared moderately resistant to Levofloxacin (42%), Ciprofloxacin (40%), Piperacillin tazobactum (35%), Amikacin (32%), Ceftazidime (31%), Gentamicin (31%), Cefepime (30%), Tobramycin (29%), Piperacillin (29%), Imipenem (17%), and Meropenem (16%), and the MDR was 6 isolates (31%).

Table 4. Example of resistance of *Pseudomonas aeruginosa* to various antibiotics

Antibiotics	Resistance (%)
Piperacillin	29
Ceftazidime	31
Gentamicin	31
Amikacin	32
Ciprofloxacin	40
Piperacillin tazobactum	35
Cefepime	30
Imipenem	17
Meropenem	16
Tobramycin	29
Levofloxacin	42

Pseudomonas aeruginosa is resistant to Imipenem 17%, which is similar to [32]. It was found that the isolates were resistant to Imipenem (17.33%). As for resistance to amikacin, the percentage is 32%, which is similar to [33]. Who found resistant to amikacin in a percentage 28%. The results of the current study did not agree with [34]. It's indicated that the resistance rate of *Pseudomonas aeruginosa* to Ceftazidime is 81%, Tobramycin is 74%, Gentamicin is 72%, Amikacin is 70%, Ciprofloxacin is 74%, Meropenem is 70%, and Imipenem is 65%. The researcher [23] In Iran, it was indicated that the resistance rate of *Pseudomonas aeruginosa* to Meropenem was 73%, Imipenem was 66%, Ceftazidime was 62%, Cefepime was 64%, Gentamicin was 15%, and Tobramycin was 76%. These results are not similar to those reported in this study. While it was an approach with Ciprofloxacin at 31% and Amikacin at 29%, in the study by [35], it was shown that *Pseudomonas aeruginosa is* resistant 100% to Cephalothin, Carbencillin, Amikacin, Amoxicillin/clavulanic Acid, Ciprofloxacin, and Gentamicin. The study by [31] shows that *Pseudomonas aeruginosa* is highly resistant to cefepime, while the resistance to Ciprofloxacin and Gentamicin is 45.2%

for each of them. Moreover, resistance to Aztreonem was 33.33%, Ceftazidim was 28.5% for each of them, and Imipenem was 26.19%.

3.2. Detection of Efflux pump

Use the ethidium bromide-agar cartwheel technique for phenotypic detection used to identify efflux pumps in 19 bacterial isolates. The results showed that 8 isolates (42%) were positive. The result is positive when the bacterial growth fluoresces in the dishes treated with ethidium bromide stain under ultraviolet rays, as shown in **Table 5**.

In our study, 42% of *Pseudomonas aeruginosa* isolates had an efflux pump. And it was similar to what was obtained [36]. In Egypt, it has been proven that all isolates of *Pseudomonas aeruginosa* were 100% productive efflux pumps [37].

Table 5. Efflux Pump forming in *Pseudomonas aeruginosa*

Efflux Pump forming	The number of isolates	Persentage (%)
Forming Efflux Pump	8	42%
Not Forming Efflux Pump	11	58%
Total	19	100%

3.3 DNA extraction

The manufacturer's instructions were followed while extracting the DNA of bacterial isolates using a DNA kit (Promega, USA).

3.4 Measurement of DNA Concentration

The Quantus Fluorometer was used to gauge DNA concentrations. The outcomes showed that the extracted DNA concentrations ranged between 20 and 25 mg/µl.

3.5 Detection of efflux pump Genes

The detection of efflex pump genes among *Pseudomonas aeruginosa* isolates was done through PCR using Thermal Cycler. The PCR is based on the amplification of efflex pump genes with specific primers. These genes included *mexB and oprM*. The results showed that isolates of *Pseudomonas aeruginosa* possess these genes in different proportions, as shown in **Table 6**.

Table 6. Number and Percentage of Efflux pump genes in *Pseudomonas aeruginosa*

Sources	genes	Number of isolates have genes	Percentage (%)
Wound infection from	*mexB*	19	100%
Pseudomonas aeruginosa	*oprM*	11	57.8%

3.5.1.*mexB* detection

It also showed showed that 19 isolates of *Pseudomonas aeruginosa* at a rate of 100% possess a *mexB* gene. When comparing the doubled bundles with the ladder, it was found that the resulting bundles have a molecular weight of (214 bp), as shown in **Figure 1**.

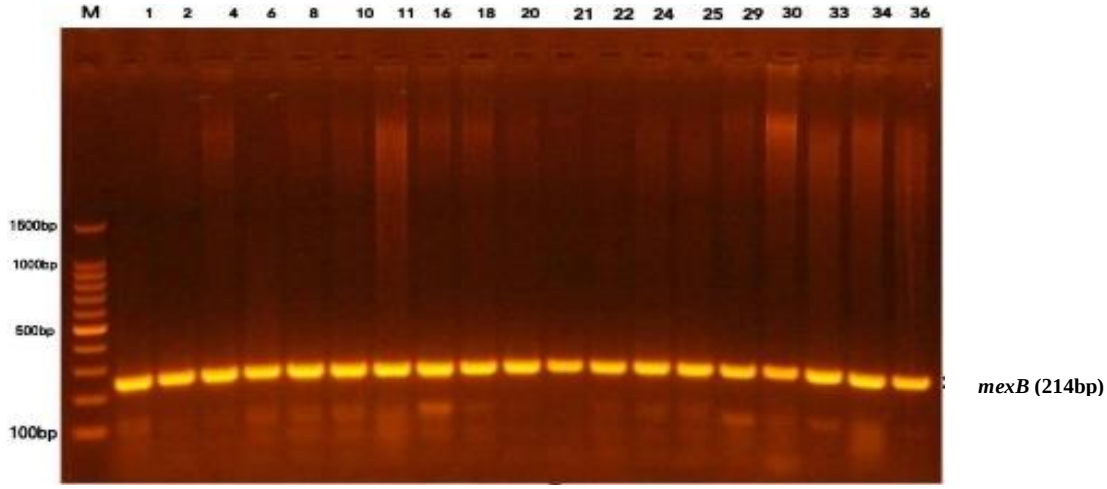

Figure 1. The amplification of the (*mexB*) gene of *Pseudomonas aeruginosa* was fractionated on 2 % agarose gel electrophoresis at a voltage of 100 volts for 45 minutes with an Eth. Br. M. 100-bp ladder marker. Lanes 1-36 The isolate has the *mexB* gene (214 bp).

3.5.2.*oprM* detection

As for the *oprM* gene, 11/19 isolates are due to *Pseudomonas aeruginosa* at a rate of 57.8%. While the isolates (21, 22, 25, 29, 30, 33, 34, and 36) do not contain the oprM gene, When comparing the doubled bundles with the ladder, it was found that the resulting bundles have a molecular weight of 180 bp, as shown in **figure 2**.

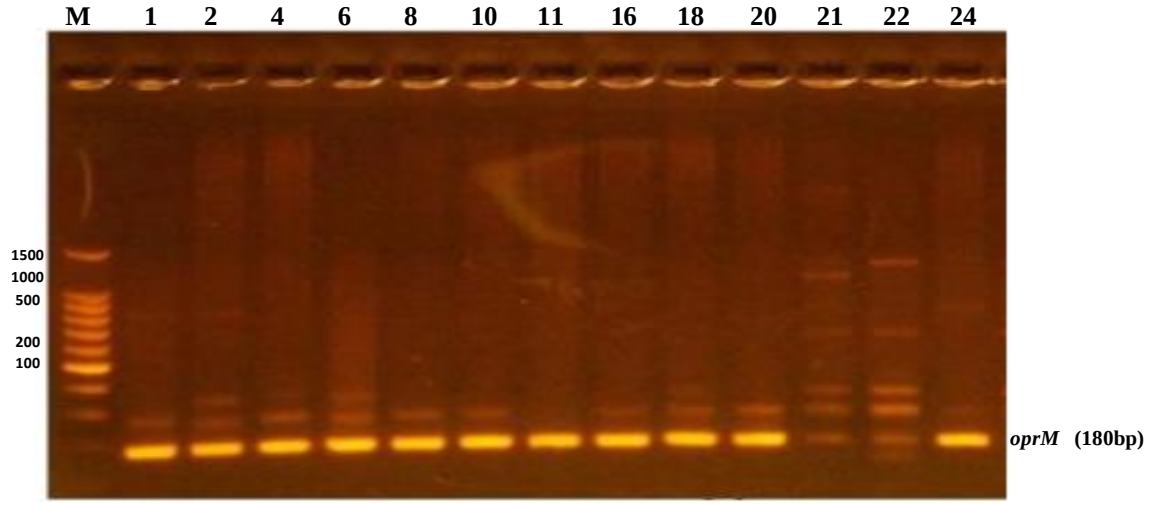

Figure 2. The amplification of the *oprM* gene of *Pseudomonas aeruginosa* was fractionated on 2% agarose gel electrophoresis at a voltage of 100 volts for 45 minutes with an Eth. Br. M. 100 bp ladder marker. Lanes 1-36 The isolate has the *oprM* gene (180 bp).

Our research showed that all 19 isolates of *Pseudomonas aeruginosa* in this study were 100% positive for the mexB efflux pump genes. And this is similar to [38]. It was proven that 98.3% (58/59) of *Pseudomonas aeruginosa isolates* possess *mexB, and* 100% (59/59) of *Pseudomonas aeruginosa isolates possess mexB.* The researcher [39] showed that the percentage of *the mexB* gene was 38.37%. This percentage does not agree with the results of the current study. The results by [39] showed that the percentage of the mexB *gene* was 38.37%. This percentage does not agree with the results of the current study. And showed that the percentage of *the mexB* gene

was 51.4%. in 18/23 isolates. also This percentage does not agree with the results of the current study.In this study, results showed that 11/19 isolates of *Pseudomonas aeruginosa* were found to be 57.8% positive for the *oprM* efflux pump gene [39], showing that the percentage of the oprM *gene* was 70.93%. This percentage does not agree with the results of the current study. The results by [40] showed that the percentage of *the oprM* gene was 8.6%. in the 3/23 isolates.
MexAB-OprM contributes significantly to multidrug resistance by expelling different drug molecules, one of the factors contributing to nosocomial infections drug production and efflux [41].

4. Conclution

Pseudomonas aeruginosa isolated from wounds was found to be multi-resistant to antibiotics, show the highest resistance to levofloxacin, and have the lowest resistance to meropenem. *Pseudomonas aeruginosa* isolates showed positive results for the phenotypic efflux pump by 42%. All isolates possessed *the mexB and oprM* genes.

Acknowledgment

Authors would like to thank all worker at Teaching Laboratories Hospital, Medical City, Baghdad Teaching Hospital, and Martyr Ghazi Hariri Hospital for their assistance in samples collection.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

No funding**.**

Ethical Clearance

The samples were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health.

References

1. Henry, R. Etymologia: *Pseudomonas*. Emerging Infectious Diseases, **2012,** *18* (*8*), 1241. DOI: http://dx.doi.org 10.3201/eid1808.et1808.
2. Tayeb**,** L.A.; Ageron, E.; Grimont, F.; Grimont, P.A. Molecular phylogeny of the genus Pseudomonas based on *rpoB* sequences and application for the identification of isolates. *Research in Microbiology*, **2005**, *156(5)*,763-73. DOI: 10.1016/j.resmic.2005.02.009.
3. Pang, Z.; Raudonis, R.; Glick, B.R.; Lin, T.J.; Cheng, Z. **(2019)**. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnology advances, **2019**, 37(1),* 177-192. http://dx.doi.org/10.1016/j.biotechadv.2018.11.013
4. Al-Shwaikh, R.M.A.; AL-Shuwaikh A.M.A.; Alornaaouti, A.F. BOX-PCR Fingerprinting for molecular genotyping of *P. aeruginosa. Asian Jr. of Microbiol. Biotech. Env. Sc*., **2018**, *20(3),* 738-743.
5. Diggle, S.P.; Whiteley, M. Microbe Profile: *Pseudomonas aeruginosa*: Opportunistic Pathogen and Lab Rat. *Microbiol*. (Reading England), **2020**, *166(1)*, 30–33. http://dx.doi.org/10.1099/mic.0.000860
6. Rossi, E.; La Rosa, R.; Bartell, J.A.; Marvig, R.L.; Haagensen, J.A.J.; Sommer, L. M.; Molin, S.; Johansen, H.K. *Pseudomonas aeruginosa* adaptation and evolution in patients with cystic

fibrosis. *Nature reviews. Microbiology,* **2021**, *19*(5), 331–342. https://doi.org/10.1038/s41579-020-00477-5

7. Al-Saeedi, R., H., A.; Raheema, R.H. Molecular detection of some virulence genes In *Pseudomonas aeruginosa* clinical isolates. *Indian Journal of Public Health Research & Development,* **2019**, *10(4),* 769-77.
8. Seleem, N.M., Abd El Latif, H. K., Shaldam, M. A.; El-Ganiny, A. Drugs with new lease of life as quorum sensing inhibitors: for combating MDR Acinetobacter baumannii infections. *European Journal of Clinical Microbiology & Infectious Diseases,* **2020**, *39*, 1687–1702. http://dx.doi.org/10.1007/s10096-020-03882-z
9. Wang, M.; Zhao, L.; Wu, H.; Zhao, C.; Gong, Q.; Yu, W. Cladodionen is a potential quorum sensing inhibitor against *Pseudomonas aeruginosa*. *Mar. Drugs,* **2020**, *18*, E205. http://dx.doi.org/10.3390/md18040205.
10. Foca, M.; Jakob , K.; Whittier, S.; Latta, P.D.; Factor, S.; Rubenstein, D.; Saiman, L. Endemic *Pseudomonas aeruginosa* infection in a neonatal intensive care unit. *New England Journal of Medicine,* **2000**, *343(10),* 695-700.
11. Jodrá, V. M. ; Díaz-Agero Pérez , C. ; Sainz de Los Terreros Soler, L.; Saa Requejo, C.M.; Dacosta Ballesteros, D.; Quality Control Indicator Working Group. Results of the Spanish national nosocomial infection surveillance network (VICONOS) for surgery patients from January 1997 through December 2003. *A American Journal of Infection Control,* **2006**, *34(3),* 134–41. http://dx.doi.org/10.1016/j.ajic.2005.10.004
12. Krishnamoorthy, G.; Weeks, J.W.; Zhang, Z.; Chandler, C.E.; Xue, H.; Schweizer, H.P.; Ernst, R.K.; Zgurskaya, H.I. Efflux Pumps of *Burkholderia thailandensis* Control the Permeability Barrier of the Outer Membrane. *Antimicrob. Agents Chemother*, **2019**, *63*, e00956-19. http://dx.doi.org/10.1128/AAC.00956-19
13. Kumar, S.; Lekshmi, M.; Parvathi, A.; Ojha, M.; Wenzel, N.; Varela, M.F. Functional and Structural Roles of the Major Facilitator Superfamily Bacterial Multidrug Efflux Pumps. *Microorganisms*, **2020**, *8*, 266. http://dx.doi.org/10.3390/microorganisms8020266
14. Al-Saadi Z.H.; Abdullah. R.M. Phenotypic and molecular detection of *Escherichia coli* efflux pumps from UTI patients. *Biochemical & Cellular Archives,* **2015**, *19(Supplement 1)*, 2371-2376. DOI: 10.35124/bca.2019.19.S1.2371.
15. Venter, H.; Mowla, R.; Ohene-Agyei, T.; Ma, S. RND-type drug efflux pumps from Gram-negative bacteria: molecular mechanism and inhibition. *Frontiers in microbiology*, **2015**, *6*, 377. http://dx.doi.org/10.3389/fmicb.2015.00377.
16. Zhi Li, X.; Elkins, C.A.; Zgurskaya, H.I. *Efflux-Mediated Antimicrobial Resistance in Bacteria Mechanisms, Regulation and Clinical Implications*. International Publishing. Switzerland pp. 848, 2016.
17. Shao, X.; Xie, Y.; Zhang, Y.; Liu, J.; Ding, Y.; Wu, M.; Wang, X.; Deng, X. Novel therapeutic strategies for treating Pseudomonas aeruginosa infection. *Expert Opinion On Drug Discovery*, **2020**, *15(12)*, 1403–1423. https://doi.org/10.1080/17460441.2020.1803274 .
18. Levinson, W. Review of Medical Microbiology and Immunology. 14th ed. McGraw-Hill education, Inc. **2016**, 821
19. Hemraj, V.; Diksha, S.; Avneet, G. A Review on Commonly Used Biochemical Test for Bacteria. *IJLS,* **2013**, *1(1),* 1-7.
20. Baron, E.J.; Finegold, S. M.; Peterson, I.L.R. Bailey and Scotts Diagnostic Microbiology. 9th ed. Mosby Company. Missouri, **2007**.
21. CLSI. Performance standards for antimicrobial susceptibility testing. 28th ed. Clinical and Laboratory Standards Institute, Wayne, PA2018 (CLSI supplement M100), **2018**.
22. MacFaddin J. F. Biochemical tests for Identification of Medical Bacteria. 4th ed., Waverly press, Inc., Baltimore, U.S.A, **2004**.

23. Pourahmad, M.; Javadi, A.; Kamran, A.; Ataei, B.; Yaran, M.; Jharomi, A.S. A clinical debate concerning aminoglycoside resistance genes among *Pseudomonas aeruginosa* strains. *Middle East Journal of Family Medicine*, **2018**, *7(10)*, 172. http://dx.doi.org/10.5742/MEWFM.2018.93327
24. Al-Dahmoshi, H.O.M. *Genotypic and Phenotypic Investigation of Alginate Biofilm Formation among Pseudomonas aeruginosa Isolated from Burn Victims in Babylon, Iraq.* Ph.D. thesis, Babylon University, Science Faculty-Biology Department, Iraq, **2013**.
25. Todar, K. *Pseudomonas aeruginosa*. Textbook of Bacteriology. *Science Journal*, **2011**, *304*,.14219.
26. Leboffe, M.J. and Pierce, B.E. A photographic Atlas for the Microbiology Laboratory. 4th ed. Morton Publishing Company, **2011**, 96.
27. Adekunle, O.C.; Mustapha, A., Odewale, G.; Ojedele, R.O. Detection of antibiotic resistance genes among multiple drug resistant *Pseudomonas aeruginosa* strains isolated from clinical sources in selected health institutions in Kwara State. *Research Journal of Health Sciences*, **2022**, *10(1)*, 40-48. http://dx.doi.org/10.4314/rejhs.v10i1.5.
28. Jalil, M. B.; Abdul-Hussein, Z. R.; Al-Hmudi, H. A. Isolation and identification of multi drug resistant biofilm producer with burn wound infection in Basra province/ Iraq. *IJDR*, **2017**, *7(11)*, 1-5.
29. Al-Shwaikh, R.M.A.; Alornaaouti, A.F. Detection of *tox A* gene in *Pseudomonas aeruginosa* that isolates from different clinical cases by using PCR . *Ibn Al-Haitham Journal for Pure and Applied Science*, **2017**, *Special Issue*, 26-30. http://dx.doi.org/10.30526/2017.IHSCICONF.1767
30. Al-Azzawi , S.N.; Abdullah R.M. Study of the resistance of *P. aeruginosa* isolated from wounds and burns for some disinfects and antiseptic from some Baghdad hospitals. *Journal of Pharmaceutical Sciences and Research*, **2018**, *10(6)*, 1481-1484.
31. Obaid, S.A.; Al-Shwaikh, R.M. Evaluation the Efficacy of Bacteriophage against *Pseudomonas aeruginosa* Isolated from Wound and Burn Infections. *Pakistan Journal of Medical & Health Sciences* **2022**, *16(04)*, APR 2022. http://dx.doi.org/10.53350/pjmhs22164440
32. Al-Arnaouti, A.F.M. *Study of genotyping and some virulence factors for Pseudomonas aeruginosa bacteria*. MSc. thesis, College of Education for Pure Sciences, Ibn Al-Haytham, University of Baghdad, **2015**. 123
33. Peymani, A.; Naserpour-Farivar, T.; Zare, E.; Azarhoosh, K.H. Distribution of *blaTEM*, *blaSHV*, and *blaCTX-M* genes among ESBL producing *Pseudomonas aeruginosa* isolated from Qazvin and Tehran hospitals. *Iran. Journal of Preventive Medicine and Hygiene*, **2017**, *58(2)*, 155.
34. Al-Azzawi, S.N.A. Molecular study of the resistance of *Pseudomonas aeruginosa* isolated from wounds and burns treated with antiseptics. Master's thesis, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, **2018**.
35. Abdullah R.M. A Study the Effect of TiO_2 Nanoparticles Combination with Antibiotics and Plant extracts Against Some Gram Negative Bacteria. *Baghdad Science Journal*, **2016**, *13(3)*, 425-434. http://dx.doi.org/10.21123/bsj.2016.13.3.0425
36. Helmy, O.M.; Kashef, M.T. Different phenotypic and molecular mechanisms associated with multidrug resistance in Gram-negative clinical isolates from Egypt. *Infection and Drug Resistance*, **2016**, *10*, 479-498. http://dx.doi.org/10.2147/IDR.S147192.
37. Abbas, H.A.; El-Ganiny, A.M.; Kamel, H.A. Phenotypic and genotypic detection of antibiotic resistance of *Pseudomonas aeruginosa* isolated from urinary tract infections. *African Health Sciences*, **2018**, *18(1)*, 11-21. http://dx.doi.org/10.4314/ahs.v18i1.3.
38. Aghayan, S.S.; Kalalian Mogadam, H. Fazli, M. Darban-Sarokhalil, D. Khoramrooz, S.S.; Jabalameli, F.; Yaslianifard, S.; Mirzaii, M. **(2017)**. The Effects of Berberine and Palmatine on Efflux Pumps Inhibition with Different Gene Patterns in *Pseudomonas aeruginosa* Isolated from Burn Infections. *Avicenna J Med Biotechnol*, **2017**, *9(1)*, 2-7.
39. Murugan, N.; MalathiJ Therese, K.L.; Madhavan, H. N. Application of six multiplex PCR's among 200 clinical isolates of *Pseudomonas aeruginosa* for the detection of 20 drug resistance encoding genes. *The*

Kaohsiung Journal of Medical Sciences, **2018**, *34(2)*, 79–88,. http://dx.doi.org/10.1016/j.kjms.2017.09.010.

40. Abdallah A.L.; El Azawy, D.S.H. ; Mohammed, H.A. and El Maghraby, H. M. Expression of Mex AB-Opr M efflux pump system and meropenem resistance in *Pseudomonas aeruginosa* isolated from surgical intensive care unit. *Microbes and Infectoious Diseases*, **2021**, *2(4)*, 781-789. http://dx.doi.org/10.21608/mid.2021.92720.1187.
41. Tsutsumi, K. Yonehara, R.; Ishizaka-Ikeda, E.; Miyazaki, N.; Maeda, S.; Iwasaki, K.; Nakagawa, A.; Yamashita, E. Structures of the wild-type *MexAB–OprM* tripartite pump reveal its complex formation and drug efflux mechanism. *Nature communications*, **2019**, *10*, 1–10. http://dx.doi.org/10.1038/s41467-019-09463-9.