



Assessment of Efflux Pump Activity and Screened the Prevalence of Associated Genes Mex A and B among MDR *Pseudomonas aeruginosa* Isolates

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Abstract: Efflux pumps play a critical role in the biology of *Pseudomonas aeruginosa*. These pumps are membrane-bound transport systems that actively expel various substances, including antibiotics, toxic compounds, and metabolic byproducts, out of the bacterial cell. The aim of research to detect the presence of efflux pump genes Mex A and Mex B in *Pseudomonas aeruginosa* isolated from different sources. One hundred samples were taken from male and female patients within four age groups (1-9, 10-29, 30-50 and > 50). The study revealed that 50% of samples were positive to *P. aeruginosa* with no significant variation among gender and age groups but there was a little higher rate in the young people group than older people group. The isolates subjected to measure the biofilm production ability and results revealed there were 39 (78%) out of 50 isolates confirmed as *P. aeruginosa* strong biofilm formation while the rest isolates showed weak and moderate production of biofilm. The antibiotic sensitivity test was done using the disc diffusion method for all fifty strains which shows very high resistance to selected antibiotics, especially Piperacillin and Ticarcillin-clavulanate, the most antibiotic-effective bacterial growth was Piperacillin-tazobactam. Phenotypic detection of efflux pump activity was done using the ethidium bromide cartwheel method and revealed that 32 (17 isolates positive for all concentration; 15 isolates positive against different concentration) (64%) of examined isolates were positive to efflux pump in all concentrations of ethidium bromide dye. While 18 (36%) isolates were inactive for the efflux pump in all concentrations. Ten efflux pump positive isolates were selected for molecular detection. The result of the molecular study using conventional PCR detection for Mex A and Mex B genes shows that all ten isolates have efflux pump genes.

Key words: *Pseudomonas aeruginosa*, efflux pump, MexAB, cartwheel, MDR

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Introduction

Pseudomonas aeruginosa is a gram-negative, aerobic bacteria that can be found in several environments, including soil, plants, and mammalian tissues. *P. aeruginosa* can survive on medical equipment and other surfaces by utilizing its crucial binding elements, such as flagella, pili, and biofilms. It is now a significant contributor to antibiotic resistance and nosocomial

infections (1). It frequently corresponds to healthcare-associated infections, including ventilator-associated pneumonia (VAP), intensive care unit infections, central line-associated bloodstream infections, surgical site infections, urinary tract infections, burn infections, keratitis, and otitis media. The elevated death rate among persons diagnosed with these conditions is ascribed to *P. aeruginosa*'s capacity to

acclimatize to environmental alterations and swiftly acquire resistance to pharmaceuticals (2).

The increasing number of nosocomial infections related to multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains of *P. aeruginosa* poses a considerable challenge in antimicrobial therapy. (3). *P. aeruginosa* infections are difficult to treat because of both acquired and intrinsic resistance to many kinds of therapeutic antibiotics. The causes of intrinsic resistance include reduced outer membrane permeability, inducible β -lactamase synthesis, and multidrug efflux mechanisms (4) (5), *P. aeruginosa* has many multidrug efflux systems (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM), which are recognised as critical factors in multidrug resistance among the majority of clinical isolates (6). MexAB-OprM is considered the most critical efflux pump facilitating antibiotic resistance in *P. aeruginosa* due to its ability to transport a diverse range of drugs. MexAB-OprM is associated with resistance to fluoroquinolones, chloramphenicol, and certain β -lactams. (7) (8).

Material and Methods

Sample collection

This prospective study was conducted between December 2023 to April 2024 and included 100 clinical samples collected from patients with burn (n = 38), urine (n = 28), sputum (n = 12), ear swabs (n = 8) and wound infections (n = 14). From Both male and female patients 44 (44.00%), and 56 (56.00%) respectively, with ages ranging between 1 year to 75 years who attended various hospitals in Baghdad Governorate.

Laboratory tests

Identification of bacterial isolates

Isolates were examined macroscopically after gram staining. The bacterial isolates were diagnosed by observing their ability to grow on the diagnostic media represented by the medium blood agar, MacConkey agar, and cetrimide the changes caused by the growing colonies on these media were observed and their phenotypic characteristics were studied in terms of the shape, size, smell, and color of the growing colonies (9).

Biochemical Tests

Biochemical tests, including Oxidase test, Urease test, Motility test, Kligler test, and IMVC tests, were employed for the diagnosis of clinical samples and then validated using VITEK 2 compact system.

Antibiotic susceptibility testing (Disc diffusion method)

Employing the Kirby-Bauer technique, a sensitivity assessment for bacterial isolates (11). The inhibition zone was quantified in millimeters, and the findings were analyzed according to the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. The antibiotics evaluated included: Piperacillin (100 μ g), Cefotaxime (30 μ g), Cefepime (30 μ g), Ticarcillin-clavulanate (75/10 μ g), Piperacillin-tazobactam (100/10 μ g), Aztreonam (30 μ g), Imipenem (10 μ g), Meropenem (10 μ g), Gentamicin (10 μ g), Amikacin (30 μ g), Levofloxacin (5 μ g), and Ciprofloxacin (5 μ g).

Assessment of biofilm formation (Microtiter plate method)

The microtiter plate method utilizes crystal violet staining and optical density (OD) measurement. Biofilm intensity was estimated by measuring absorbance at 630 nm using an ELISA reader. The absorbance readings indicated the intensity of biofilm

thickness produced by the examined isolates on the microtiter well surface. The results were classified into three categories: strong biofilm producer, weak biofilm producer, and moderate biofilm producer (12).

Morphological Detection of Efflux Pump activity

The EtBr-agar cartwheel conducted for the morphological detection of efflux pumps involved preparing dilutions of all bacterial isolates using sterile physiological saline and measuring turbidity with the McFarland Standard instrument. This study was conducted on bacterial isolates exhibiting antibiotic resistance characteristics by employing the cartwheel agar-EtBr method, utilizing tryptic soy agar medium and ethidium bromide dye at varying doses as specified in (13).

Genetic identification

DNA was extracted from a culture of *P. aeruginosa* using the Presto™ Mini gDNA Bacterium Kit. The visualization

of PCR products was accomplished using electrophoresis on a 1.2% agarose gel stained with Ethidium bromide. The primers employed in this study were designed using *P. aeruginosa* NCBI as a reliable reference. A lyophilized form of these primers was supplied by Macrogen Company. The genes of amplicon size and their respective genetic sequences utilized in this study are displayed in Table 1. This study utilized the PCR technique to identify the presence of the 16SrRNA (housekeeping gene) and the efflux pump genes MexA and MexB in 10 isolates. The PCR reaction was performed with a final volume of 25 µl. Each reaction comprised 12.5µl of master mix, 1.5µl of forward primer, 1.5µl of reverse primer, 4.5µl of nuclease-free water, and 5µl of template DNA. Agarose gel electrophoresis was employed to assess amplified PCR results. An investigation of a Neogen/USA DNA marker was conducted for each gene.

Table (1): Primers used in this study.

Target gene	Primer name	Forward 5' → 3'	Reverse 5' → 3'	Product Size bp
16SrRNA	PA SS	GGGGGATCTTCGGACCTCA	TCCTTAGAGTGCCACCCG	956
MEX A	MEX A	GACCCTGAATACCGAGCTGC	GGTCGATCTGGTAGAGCTGC	142
MEX B	MEX B	CTGTCGATCCTCAGTCTGCC	CTGTTCTGAAGGTCACGTGA	215

Result and discussion

The isolation and identification revealed gram-negative bacilli, these bacilli gave: beta-hemolysis on blood agar, non-lactose fermenter on MacConkey agar (9), and gave a greenish appearance in cetrimide agar (14) Figure (1) A, B,C respectively.

The isolates were subjected to biochemical tests and the results showed positive for (Oxidase, Motility, and Simmon's citrate tests) and negative for (Indole, -Methyl-red, -Voges-Proskauer, Urea and Kligler iron tests).

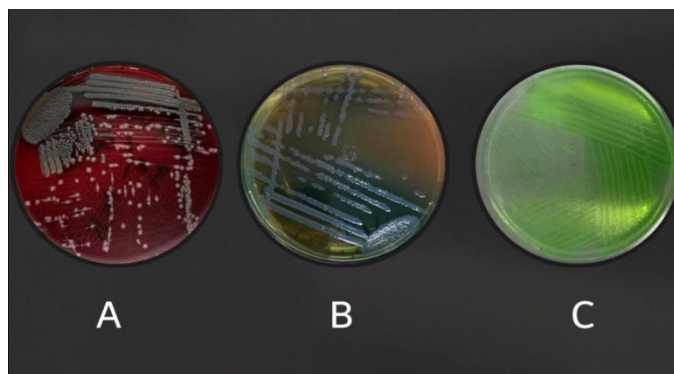


Figure (1) *P. aeruginosa* growing on enrichment media (A) : Blood agar, selective (B: MacConkey agar, and differential (C): Cetrimide agar at 37 C 24 H.

The result of the isolation of *P.aeruginosa* shows a percentage of total 50% positive isolate, isolate distributed in the samples source as burn 19, urine 14, wound 7, ear swab 4,

and sputum 6, out of 100 samples used this percentage recorded in both male and female with no significant variation (Table 2).

Table (2): Distribution of *P. aeruginosa* according to gender and the source of the sample

Sample Source	Gender			
	Male No(%)	Female No(%)	P-value No	Total No(%)
Burn	6 (27.27)	13 (46.43)	0.098 NS	19 (38.00)
Urine	4 (18.18)	10 (35.71)	0.177 NS	14 (28.00)
Wound	5 (22.73)	2 (7.14)	0.367 NS	7 (14.00)
Ear Swab	3 (13.64)	1 (3.57)	0.502 NS	4 (8.00)
Sputum	4 (18.18)	2 (7.14)	0.381 NS	6 (12.00)
Total	22 (44.00)	28 (56.00)	0.396 NS	50 (00)
P-value	0.169 NS	0.0074 **	---	0.0067 **

** (P<0.01), NS: Non-Significant.

This result was in agreement with many studies that show a nearly close percentage of positive isolate with no significant variation between males and females like Zwaïd and Al-Dahmashi (16) and Gawad and Gharbi (17), also the higher percentage of isolation of *P.aeruginosa* in burns than other sources and this may be due to losing skin barrier by skin damage leading to a moist and warm environment that enhances more bacterial growth and colonization more over burns impairs

immune system leading to evading opportunistic pseudomonas to grow and form biofilms(18).

Other studies that disagree with the prevalence of *P.aeruginosa* which recorded a higher prevalence of infection with a higher percentage in males than females like Mirzaei *et al* (19); Mohamed *et al* (20) ; Ghanem *et al* (21), may be related to the geographic, climatic, hygienic and social factor (21).

Table (3): Distribution of *Pseudomonas aeruginosa* according to age group and its relationship with gender.

Gender	Age group					Total
	1-9	10-29	30-50	>50	P-value	
Male	8 (36.36%)	7 (31.82%)	5 (22.73%)	2 (9.09%)	0.281 NS	22 (44.0%)
Female	10 (35.71%)	6 (21.43%)	8 (28.58%)	4 (14.29%)	0.414 NS	28 (56.0%)
Total	18 (36.00%)	13 (26.00%)	13 (26.00%)	6 (12.00%)	0.0376 *	50
P-value	0.872 NS	0.894 NS	0.417 NS	0.602 NS	---	0.396 NS

* (P<0.05), NS: Non-Significant.

In Table (3) the distribution of *P.aeruginosa* with regarded to age group was recorded. As a general view there is no significant variation between the four age groups that examined but a little highest prevalence was shown in the young patients than those of older than 50 years this result was in agreement with Ahmed *et al* (22) but disagree with Ekrem and Rokan (23) who study prevalence in Sulaymaniyah City and this may be associated with the activity of person and involvement of various clinical hygiene practices like reported by Okan *et al* (24).

Biofilm production was examined in this study by the microtiter plate method and the results obtained were

divided into three groups: weak, moderate and strong this was summarized in Table (4), the higher one is the strong producer biofilm with significant variation at ($P < 0.01$) with moderate and weak producer strains, this variation in biofilm formation between isolate may be related to many factors, eg: Isolate the capacity to develop biofilm, distinguishing the principal number of adhering cells due to variations in the quality and amount of quorum sensing signaling molecules (autoinducers) produced by the isolate, the strong, moderate, and weak biofilm was divided on the biofilm thickness according to Al-Sheikhly *et al.* (25)

Table (4): Distribution of *Pseudomonas aeruginosa* sample according to results of biofilm.

Biofilm formation	No.	Percentage (%)
Strong	39	78.00
Moderate	4	8.00
Weak	7	14.00
Total	50	100%
P-value	---	0.0001 **
** ($P < 0.01$).		

Table (5): Antibiotic sensitivity of *Pseudomonas aeruginosa* towards selected antibiotics.

Antibiotic	Symbol	Resistant	Intermediate	Sensitive	P-value
Piperacillin	PIP (PRL)	44 (88%)	4 (8%)	2 (4%)	0.0001 **
Piperacillin-tazobactam	PTZ	10 (20%)	8 (16%)	32 (64%)	0.0001 **
Ticarcillin-clavulanate	TCC	46 (92%)	4 (8%)	0 (0%)	0.0001 **
Amikacin	AK	23 (46%)	0 (0%)	27 (54%)	0.0001 **
Gentamicin	GEN	26 (52%)	1 (2%)	23 (46%)	0.0001 **
Aztreonam	AT	24 (48%)	5 (10%)	21 (42%)	0.0001 **
Cefepime	CPM	28 (56%)	0 (0%)	22 (44%)	0.0001 **
Ceftazidime	CAZ	29 (58%)	2 (4%)	19 (38%)	0.0001 **
Ciprofloxacin	CIP	27 (54%)	2 (4%)	21 (42%)	0.0001 **
Levofloxacin	LE	29 (58%)	2 (4%)	19 (38%)	0.0001 **
Imipenem	IPM	25 (50%)	1 (2%)	24 (48%)	0.0001 **
Meropenem	MEM	26 (52%)	0 (0%)	24 (48%)	0.0001 **
	P-value	0.0001 **	0.0089 **	0.0001 **	---
** ($P < 0.01$).					

The antibiotic sensitivity of *P.aeruginosa* to selected antibiotics was shown in Table (5). As a general result of our isolate appears to be resistant to approximately all antibiotics used except (TZP) which was effective against (64%) of the isolate, more than one antibiotic shows closely percentage

sensitive and resist to *P.aeruginosa*, the result recorded were compared with other researchers in and out Iraq and we conclude that all studies were different among each other, this major differences in sensitivity and resistance to antibiotic may related to clinical specimen examined, the social factor of

population and exposure to antimicrobial agents like reported by Ahmed Hasan *et al* (26).

All *P. aeruginosa* isolates in this study submitted the ethidium bromide cartwheel method to explain efflux pump activity, with results depending upon the stain concentration as a reference for phenotypic detection. The findings indicate that there are (18) inactive strains (negative for efflux pump activity) across all concentrations of ethidium bromide.

On another hand there is (17) isolates positive for efflux pump in all concentrations.

Five strains show positive efflux pump at the concentration of (0.125, 0.25, 0.5, 1,0), and there is (10) isolates were positive at the concentration of (0.125, 0.25, 0.5, 1,0, 1.5, 2.0), this result was shown at the Table (6).

The negative result exhibits fluorescence under UV light due to the retention of ethidium bromide within the cells, whereas the positive result does not glow as it cannot keep ethidium bromide.

As a percentage our study recorded (18) isolates (36%) were negative for efflux pump activity and (32) isolate (64%) were positive for efflux pump activity this result was disagree with AL-Mhesin, W.A.H. *et al.* (27) who recorded (42%) of *P. aeruginosa* isolates to have efflux pump activity, and also highly disagreed with study in Egypt that proven all isolates of *P. aeruginosa* were 100% production efflux pump (28), this disagreement may related to source of sample collected because these two studies limited only with urine and wound.

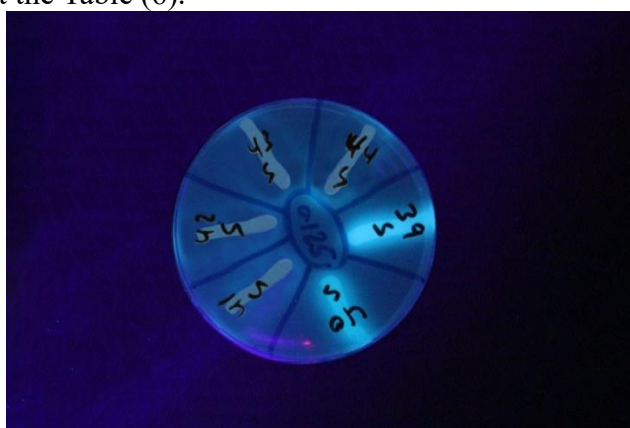


Figure (2) Some of positive (non-fluorescence) and negative (fluorescence) strains using ETBR dye.

Table (6): Results of phenotypic analysis of efflux pumps in *P. aeruginosa* utilizing varying doses of ethidium bromide dye in tryptic soy agar.

No. of isolates	%	Ethidium bromide concentration							
		0.125	0.25	0.5	1.0	1.5	2.0	2.5	5.0
5	64%	+	+	+	+	-	-	-	-
10		+	+	+	+	+	+	-	-
17		+	+	+	+	+	+	+	+
18	36%	-	-	-	-	-	-	-	-

The result of the molecular study using PCR supports our previous diagnosis of *P. aeruginosa* using 16sRNA. On detection for Mex A and Mex B genes in ten selected strains that diagnose these efflux pump genes in all

the ten strains selected. The expression levels of both genes were recorded at 100%, indicating a high expression rate in our isolate. This elevated expression of the two genes contributes to the development of antibiotic resistance in

our isolate and underscores the influence of efflux pumps on the reclassification of clinical strains as susceptible, intermediate, or resistant, corroborating the findings of Horna *et al.*(29), this isolate show that isolate used in detection Mex A and Mex B genes gave higher resistance to

antibiotic and this was consistent with Vitkauskiene *et al* (30) and with Ghanbarzadeh *et al* (31) , also this result was closely agree with Othman *et al* (32) how reported how high resistance of *P. aeruginosa* to some antibiotic .

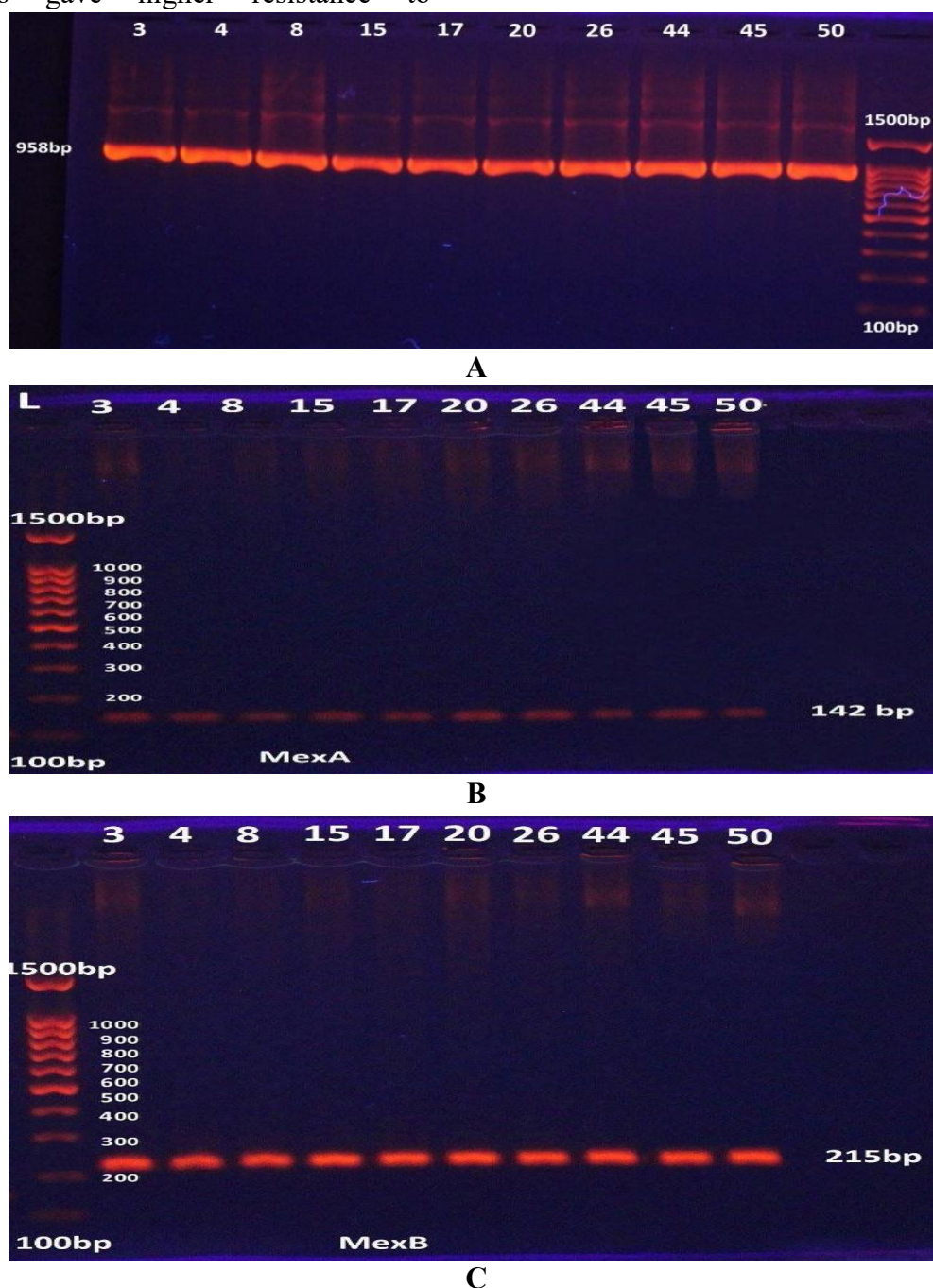


Figure (3): Agarose gel electrophoresis of PCR products of *Pseudomonas aeruginosa* isolates. (A) : PCR products of 16sRNA . (B) : PCR products of Mex A. (C): PCR products of Mex B gene. Lane (L) : DNA Ladder; Lanes (3-50) samples of *P. aeruginosa* isolates (1 % agarose gel at 70V for 1 hr).

Conclusion

The present investigation revealed a significant incidence of *P. aeruginosa* across several clinical samples, particularly in burn cases, with these bacteria exhibiting considerable resistance to numerous medicines, notably Piperacillin. *P. aeruginosa* shows a high ability to produce a strong biofilm that supports its resistance to antibacterial drugs. Phenotypic detection for efflux pump activity on all concentration of ethidium bromide dye revealed 64% positive for active efflux pump and 32% were negative. The *P. aeruginosa* show detection of Mex A and Mex B genes in all isolates used in this study.

References

- Lodise, T. P. and Monique, R. B. (2019). *Pseudomonas aeruginosa*. MDR Gram-Negative Infections, 7-27.
- Al-Ameen, M. A. and Ghareeb, A.M. (2022). Prevalence of Colistin Resistance in *Pseudomonas aeruginosa* Isolated from Burn Patients in Sulaymaniyah City. Iraqi Journal of Biotechnology, 21(2): 713-722
- El Zowalaty, M. E.; Al Thani, A. A.; Webster, T. J.; El Zowalaty, A. E.; Schweizer, H. P. and Nasrallah, G. K. (2015). *Pseudomonas* Immunocompromised Patients: Relation to Initial Antibiotic Therapy and Survival Jpn. Journal of Infectious Diseases, 69: 91-96.
- Mohamad, S.M.; Rostami, S.; Zamanzad, B.; Gholipour, A. and Drees, F. (2017). Detection of exotoxins and antimicrobial susceptibility pattern in clinical *Pseudomonas aeruginosa* isolates. Avicenna Journal of Clinical Microbiology and Infection, 4 (4): 1-6.
- Al-Fridawy, R.A.; Al-Daraghi, W.A.H. and Alkhafaji, M.H. (2020). Isolation and Identification of Multidrug Resistance Among Clinical and Environmental *Pseudomonas aeruginosa* Isolates. Iraqi Journal of Biotechnology, Vol. 19, No. 2, 37-45.
- Poole, K. (2004). Efflux-mediated multi resistance in Gram-negative bacteria. J. Clin. Microbiol. Infect. 10:12-26.
- Pesingi, P. V.; Singh, B. R.; Pesingi, P. K.; Bhardwaj, M.; Singh, S. V.; Kumawat, M.; Sinha, D. K. and Gandham, R. K. (2019). MexAB-OprM efflux pump of *Pseudomonas aeruginosa* offers resistance to carvacrol: A herbal antimicrobial agent. Frontiers in Microbiology, 10, 2664.
- Eshra, K.A. and Shalaby, M.M. (2017). Efflux Pump Inhibition Effect of Curcumin and Phenylalanine Arginyl β -Naphthylamide (PA β N) against Multidrug Resistant *Pseudomonas Aeruginosa* Isolated from Burn Infections in Tanta University Hospitals. The Egyptian Journal of Medical Microbiology 26, 113-119.
- Levinson, W. (2016) Review of Medical Microbiology and Immunology. 14th ed. McGraw-Hill Education, Inc, 821.
- Baron, E.J.; Finegold, S. M.; Peterson, I.L.R. Bailey, and Scotts (2007) Diagnostic Microbiology. 9th ed. Mosby Company. Missouri.
- Smit, J. (2021). Performance Standards for Antimicrobial Susceptibility Testing. 31th ed. CLSI supplement M100.
- Atshan, S. S.; Nor Shamsudin, M.; Sekawi, Z.; Than Thian Lung, L.; Hamat, R. A.; Karunanidhi, A.; *et al.* (2012). Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus*. Journal of Biomedicine and Biotechnology. 2012: 1-12.
- Martins, M.; Mccusker, M. P.; Viveiros, M.; Couto, I.; Fanning, S.; Pages, J. M. and Amaral, L. (2013). A Simple Method for Assessment of MDR Bacteria for OverExpressed Efflux Pumps. Open Microbiology Journal. 7: 72-82.
- MacFaddin, J. F. (2004) Biochemical tests for Identification of Medical Bacteria. 4th ed., Waverly press, Inc., Baltimore, U.S.A.
- Todar, K. (2011) *Pseudomonas aeruginosa*. Textbook of Bacteriology. Science Journal, 304, 14219.
- Zwaid, A.J.A.A. and Al-Dahmoshi, H.O.M. (2022). Molecular detection of mexXY-oprM, mexPQ-opmE Efflux pumps in multi-drug-resistant *Pseudomonas aeruginosa* isolated from teaching hospitals in Babylon province, Iraq. Journal of Applied and Natural Science 14, 426-432.
- 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, Vol. 21, No. 2, 215-224.

18. Brown, P.D. and Izudu, A. (2004). Antibiotic resistance in clinical isolates of *Pseudomonas aeruginosa* in Jamaica. Rev Panam Salud Publica, vol.16 n.2 .2004.
19. Mirzaei, B.; Bazgir, Z.N.; Goli, H.R.; Iranpour, F.; Mohammadi, F. and Babaei, R. (2020). Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran. BMC Research Notes. 13(1): 1-6.
20. Mohamed, F.; Askoura, M. and Shaker, G. (2019). Antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from different clinical sources. Zagazig Journal of Pharmaceutical Sciences. 28(2): 10-17.
21. Ghanem, S.M.; Abd El-Baky, R.M.; Abourehab, M.A.; Fadl, G.F. and Gamil, N.G. (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: 2371-2385.
22. Ahmed, F.Y.; Farghaly Aly, U.; Abd El-Baky, R.M. and Waly, N.G. (2020). Comparative study of antibacterial effects of titanium dioxide nanoparticles alone and in combination with antibiotics on MDR *Pseudomonas aeruginosa* strains. International Journal of Nanomedicine. 15: 3393-3404
23. Ekrem, K. and Rokan DK (2014). Antibiotic susceptibility patterns of "*Pseudomonas aeruginosa*" strains isolated from various clinical specimens. Sky J. Microbiol. Res. 2(2): 13-17.
24. Okon, K.; Agukwe, P.; Oladosu, W.; Balogun, S. and Uba, A. (2009). Antibiotic resistance pattern of "*Pseudomonas aeruginosa*" isolated from clinical specimens in a tertiary hospital in northeastern Nigeria. J. Microbiol. 8(2): 5-7.
25. Al-Sheikhly, M. A.; Musleh, L. N. and AlMathkhury, H. J. (2020). Gene Expression of *pelA* and *pslA* in *Pseudomonas aeruginosa* under Gentamicin Stress. Iraqi Journal of Science. 295-305.
26. Ahmed Hasan, S.; Mohamed Najati, A. and Sakran Abass, K. (2020) Prevalence and antibiotic resistance of "*pseudomonas aeruginosa*" isolated from clinical samples in Kirkuk City, Iraq. Eurasia J Biosci 14: 1821-1825.
27. AL-Mhesin, W.A.H. et al. (2024). Detecting the Efflux Pumps Gene (*mexB* and *oprM*) in MDR *Pseudomonas Aeruginosa* Isolated from Wound Infection. Ibn AL-Haitham Journal For Pure and Applied Sciences. 37, 3 (Jul. 2024), 50–60
28. Abbas, H.A.; El-Ganiny, A.M.; Kamel, H.A. (2018). Phenotypic and genotypic detection of antibiotic resistance of *Pseudomonas aeruginosa* isolated from urinary tract infections. African Health Sciences, 18(1), 11-21.
29. Horna, G.; López, M.; Guerra, H.; Saénz, Y. and Ruiz, J. (2018). Interplay between *MexAB-OprM* and *MexEF-OprN* in clinical isolates of *Pseudomonas aeruginosa*. Scientific Reports, 8(1), 16463.
30. Vitkauskienė, A.; Skrodenienė, E.; Dambrauskienė, A.; Macas, A. and Sakalauskas, R. (2010). *Pseudomonas aeruginosa* bacteremia: Resistance to antibiotics, risk factors, and patient mortality. Medicina, 46(7), 490–495.
31. Ghanbarzadeh Corehtash, Z.; Khorshidi, A.; Firoozeh, F.; Akbari, H. and Mahmoudi Aznavah, A. (2015). Biofilm formation and virulence factors among *Pseudomonas aeruginosa* isolated from burn patients. Jundishapur Journal of Microbiology, 8(10), e22345.
32. Othman, N.; Babakir-Mina, M.; Noori, C. K. and Rashid, P. Y. (2014). *Pseudomonas aeruginosa* infection in burn patients in Sulaimaniyah, Iraq: Risk factors and antibiotic resistance rates. Journal of Infection in Developing Countries, 8(11), 1498–1502.