Iraqi Journal of Biotechnology, 2025, Vol. 24, No. 3, 156-165

Synergistic Effect of Curcumin and Tobramycinon the *mexX* Geneexpression in Resistant *Pseudomonas aeruginosa* isolates

[1]Iftekhar Matlob Kafi, [2]Ghusoon Ali Abdulhasan

[1,2] Department of Biology, College of Sciences, University of Baghdad

Received: July 9, 2024 / Accepted: July 31, 2024 / Published: November 3, 2025

Abstract: till now there are few studies that evaluate the effect of curcuminon efflux pump gene expression, the aim of this study to investigating thecurcumin's roleas efflux pump inhibitor against the 93 isolates of *P. aeruginosa*collected as identedby expert from laboratoryof Iraqi hospital/Baghdad during the period from September2023to December 2023.16S rRNA was employed to identify bacteria. For 40 isolates resistant to tobramycin, the results of agar dilution showed theminimum inhibitory concentrations (MIC) values ranged between 32-2048 µg/ml.Using tobramycin with curcumin significantly enhanced the sensitivity ($p \leq 0.05$) of *P.aeruginosa* isolates to tobramycin. DNA extractions were conducted on all 40 *P. aeruginosa* isolates to confirm the *mexX* presence. After the treatment of bacterial cells with tobramycin, the antibiotic upregulated the *mexX*gene expression in *P. aeruginosa* but downregulated the expression of other isolates. The tobramycin with curcumin combination of upregulated *mexX*expression in *P. aeruginosa* and downregulated the expression of others.Current findings show that the combination ofcurcumin and antibiotics inhibits*P. aeruginosa* more effectively than antibiotics alone.

Keywords*: Pseudomonas aeruginosa*, Tobramycin, Curcumin, MDR, *MexX*.

Corresponding author: (Email: iftkharmatlop@gmail.com).

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium that is widespread and can cause nosocomial infections, as well as fatal infections in immunocompromised individuals, including those with cancer, post-surgery, severe burns, or a human immunodeficiency virus (HIV) infection. *P. aeruginosa* is a bacterium that has the potential to develop resistance to the majority of commonly used antibiotics(1).Nevertheless, the treatment of *P. aeruginosa* infections has become a significant challenge as a result of the bacterium's capacity to resist a significant number of the currently available antibiotics(2). A critical mechanism of drug resistance in bacteria is the overexpression of multidrug efflux pumps.Resistance-nodulation-cell immunocompetent hosts are capable of effectively preventing infection through the immune system. The resistance-nodulation-division (RND) family is clinically relevant to multidrug efflux systems in Gram-negative bacteria (3).*P. aeruginosa* continues to be problematic from a treatment perspective,as it is armed with potent virulence factors. The *MexXY* plays a crucial role in the intrinsic resistance of *P. aeruginosa* to

aminoglycosides(4).The overexpression of *MexXY* efflux pump is counted the widespread resistance mechanism (5).In bacteria, the efflux pump mechanisms can be inhibited by many compounds to keep the intracellular concentration of antibiotics that repress the pathogen activity(6). The compounds are known as efflux pump inhibitors. These inhibitors may be synthetic or natural. The inhibitors derived from plants have an effective role against many efflux pump families(7). Curcumin is a phenolic compound that is obtained from the rhizomes of the *Curcuma longa* plantandhas been shown to possess direct broad-spectrum antibacterial activities against Gram-negative and Gram-positive bacteria(8).The function of curcumin as efflux pump inhibition is observed by decreasing MIC values for numerous antimicrobial agents versus *P. aeruginosa* multidrug-resistant strains(3, 9, 10).

Little attention has been paid to evaluate the effect of curcumin on efflux pump gene expression.Therefore, the aim of this study is toinvestigate curcumin'srole as an efflux pump inhibitor that increases some aminoglycoside antibiotics activity against the resistant isolates of *P. aeruginosa*.

Materials and methods

Identification of bacterial isolates

Ninety three isolates collectedor provided as identified by expert from laboratories of Iraqi hospitals/Baghdad,all isolated were culturedon the culture media including (MacConkey agarand cetrimide agar in sterile conditions),after the growth of bacteria, the isolates were identified by manual biochemical tests included (catalase and oxidase)tests, then confirmation identification was done by a polymerasechain reaction (PCR)technique to amplify the*16S rRNA* by usingspecies-specific primer.

DNA extraction

The DNA of *P. aeruginosa* isolates was extracted using the Genomic DNA Mini Kit (Presto™ Mini gDNA Bacteria Kit/ Taiwan) according to the manufacturer's instructions. The purity and concentration of DNAmeasurement by usingnanodrop (ACT geneNas-99/China). DNA was extracted and stored at -20ºC until it was required.

Detection16S rRNAand*mexX* gene of*P. aeruginosa*isolates by PCR

Primer selection:

Theprimers were used for detection of (16S rRNA and *mexX*)genewere described as in(Table 1).

Table (1): The primers used in this study.

Gene Name	Sequence (5´–3´)	Product size (bp)	References
16S rRNA	F:5′-GGGGGATCTTCGGACCTCA-3' R:5′-TCCTTAGAGTGCCCACCCG-3'	956	Spilker *et al.*; 2004
mexX	F:5′-TGCTGTTCCAGATCGACCCT-3' R:5′-TGTACTCGCGTTCGCTGATG-3'	372	This study
Fbp	F:5′-CCTACCTGTTGGTCTTCGACCCG-3′ R:5′-GCTGATGTTGTCGTGGGTGAGG-3′	215	Chakravary*et al.* ,2017

PCR master mix

Optimization of PCR master mix for amplification of 16S rRNAand *mexX* gene (Table 2).

Table (2): component of PCR reaction in this study.

component	Volume (25µL)
master mix	12.5 µL
Forward Primer	1 µl
Reverse Primer	1 µl
DNA	2 µL
Nuclease free water	8.5 µL

PCR program

Optimization of PCR program for amplification of 16S rRNA and *mexX* gene in (Table 3 and4).

Table (3): PCR program for detection16S rRNA.

Steps	Temperature (°C)	Time	Cycle
Initial Denaturation	94	2 mins	1
Denaturation	94	25 sec.	25Cycle
Annealing	56	40 sec.	
Extension	72	40 sec.	
Final extension	72	5 mins.	1

Table (4): PCR program for detection *mexX* gene.

Steps	Temperature (°C)	Time	Cycle
Initial Denaturation	95	3 mins	1
Denaturation	95	40 sec.	30Cycle
Annealing	60	20 sec.	
Extension	72	30 sec.	
Final extension	72	5 mins.	1

A conventional PCR was implemented to amplify the 16S rRNA(11) and *mexX*. After gel electrophoresis, *P. aeruginosa* isolates were identified by the presence of 956 bp bands as well as amplification of*mexX*372 bp.

Antibiotic susceptibility test

The antibioticsusceptibility test was done according to Clinical Laboratory Standard Institute (CLSI,2023) (12), by using disc diffusion method, the bacteria suspension transfer by sterile cotton swab, it was spread on Muller-Hinton Agar medium, after that the antimicrobial disc of [Amikacin (30 µg/disc), Tobramycin (30 µg/disc), Netilmicin (30 µg/disc) and Streptomycin (300 µg/disc)].Were placed on the agar by a sterile forceps, the plate inverted and incubated at 37°C ± 1 for 18-24 hours.

Curcumin extraction

The turmeric rhizomes were desiccated in an oven at 105°C for 3 hours, and Soxhlet was used to extract curcumin. The desiccated rhizomes were triturated in a mortar and screened through an 80-mesh sieve to produce a uniform powder with a particle size of 0.18 mm. The ground turmeric powder (25g) was weighed, embedded in a thimble, and inserted into the Soxhlet. The extraction solvent, acetone, was gradually added to the Soxhlet. After extraction, the experiment was conducted at 56°C for 8 hours. A rotary evaporator operating under vacuum at 35°C, separated the extract from the acetone. The residue (oleoresin) was accurately measured and desiccated for subsequent applications (3).

Determination of Minimal Inhibitory Concentration (MIC)

The agar dilution method was prepared according to (CLSI, 2023). The agar was made using Muller Hinton agar with serial dilutions of tobramycin (8-4096 µg/ml). The antibiotic two-fold dilutions were set between 4-2048 µg/ml, and bacterial inoculum (final concentration of 1×106 cell/ml) was prepared and used within 30 minutes of preparation. The plates were labeled for inoculation bacteria, and 5µL of inoculums were applied to the surface. The plates were incubated at 37°C ± 1 for 18-24 hours. This above procedure was repeated with tobramycin (8-4096 µg/ml) and curcumin (10,15,20,25,30,50,60,70,80,90,100 µg/ml)**.**The minimal inhibitory concentration (MIC) breakpoint for tobramycin or tobramycin and curcumin was determined based on the clinical and laboratory standards institute criteria (12).

Expression of *mexX*

For expression of *mexX* gene before and after treatment with tobramycin and curcumin, quantitative real-time PCR was used. Five isolates were chosen to determine the effect of tobramycin and tobramycin with curcumin. These isolates were selected based on their fold change of tobramycin MIC after adding curcumin. The RNA of treated and not-treated bacterial cells were extracted according to the manufacturer'sdirections using GENEzol TriRNA pure kit (Geneaid, Thailand). The primers used forexpression of *mexX* were the same used for conventional PCR. The housekeeping gene (*fbp*) primers were used (Table 1)(13). One-step RT-qPCR was used to convert RNA to cDNA and amplification of *mexX* gene. The component of reaction mentioned in table 5). The ABI PRISM 7500 sequence detection system (Applied Biosystems, USA) was employed to conduct the PCR reactions using program in (Table 6). The ΔΔCT calculation was employed to quantify the expression of the *mexX* gene, and the fold of gene expression was calculated as 2-ΔΔCT.

Table (5): component of RT-PCR reaction in this study.

component	Volume (20 µL)
master mix	10 µL
Luna RT enzyme mix	1 µl
Forward Primer	0.8 µl
Reverse Primer	0.8 µl
RNA	3 µL
Nuclease free water	4.4 µL

Table (6): PCR program for *mexX* gene expression.

Steps	Temperature °C	Time	Cycle
Reverse transcriptase activation	55	10 min.	1
Initial denaturation	95	1 min.	1
Denaturation	95	10 sec.	40
Extension	60	30 sec.	

Statistical analysis

All features appeared as frequencies and percentages, and the Pearson-Chi-square test was detected to reveal significant differences in percentages. P≤0.05 was measured as significant.Our

data were analyzed using SPSS v. 21.0 statistical software

Results and Discussion

In the present study, *P. aeruginosa*was most common bacteria isolates recorded 93 (53.14%) from 175 isolates collected from many specimen sources. While 82 (46.86%) of the total isolates were another bacterial genus.Results by Roshani-Asl *et al.* (2018) (14) indicate out of the 128 clinical samples, 75 (58.6%) were positive for *P. aeruginosa*. A recent study by Mohammad and Flayyih1 (15) isolated *Pseudomonas* spp. from wounds, burns, and eye swabsfrom hospitals in Baghdad.*P. aeruginosa* colonies on Blood agar produce mucoid-type colonies with a typical metallic sheen, and greenish-blue in color on cetrimide agar medium (16). The morphology of the colonies on Blood agar, and Cetrimide agar was initially utilized to identify bacterial isolates. Conventional PCR 16S rRNA was depended on confirm the identification of *P. aeruginosa*(11), as shown in Figure (1). A clear single PCR product band (956bp) identifies bacterial isolates as *P. aeruginosa*.

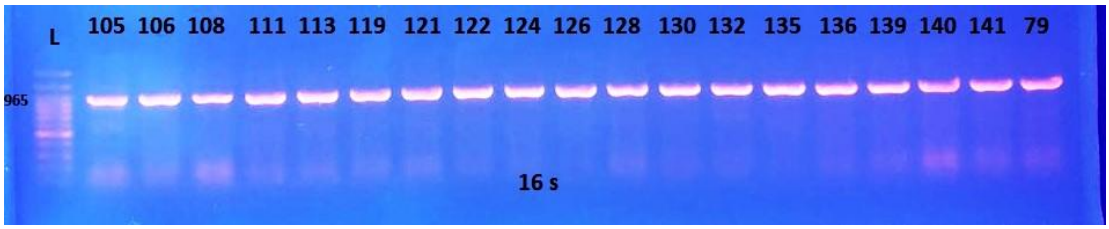

Figure (1): Agarose gel electrophoresis of PCR of the 16S RNA (956 bp) of *P. aeruginosa* isolates. Agarose 1.5%, 100 V for 75 minutes and visualized on a UV transilluminator after being stained with a safe red dye. L: Leader that(100-1200bp).

16S rRNA results are specific for the detection of *P. aeruginosa*isolates, Additionally, current outcomes showed most of *P. aeruginosa*isolated from theburn (30.1%), urine (30.1%), wound (18.3%), sputum (14.0%) and ear swab (7.5%) with significantly different ($p<0.001$).The highest isolation percentage in burn may be due to the moist and warm environment that favors the growth of *P. aeruginosa,* whichthrives in these circumstances. another study indicates results nearly to the study which *P. aeruginosa* isolated fromburn (30%), urine (5.46%), wound (10%), and ear swab(2.73%) (17).

A total of 93 clinical isolates of *P. aeruginosa* were tested for their susceptibility to four antimicrobial agents. Data for the present study showed *P. aeruginosa*scored the highest resistance toward Netilmicin (50.5%), followed by Amikacin (45.2%), compared to other antibiotics (43.0%) to tobramycin and streptomycin with significant difference ($p<0.05$) (Figure 2).A comparison between the current study's findings and some previously recorded*P. aeruginosa* resistance against aminoglycosides (Amikacin, Gentamicin and tobramycin) was (70%) (18).The results of another study showed resistance (70 %) of isolates to amikacin andtobramycin antibiotic, which belong to aminoglycosides class, from Forty-six isolates of P. aeruginosa bacteria (19).

The findings of this investigation demonstrated that curcumin was capable of decreasing the MIC value of tobramycin in isolates of *P. aeruginosa* that were obtained from a diverse array of hospitals. Additionally, curcumin can be employed as a native compound to improve the effective treatment of tobramycin-resistant isolates.

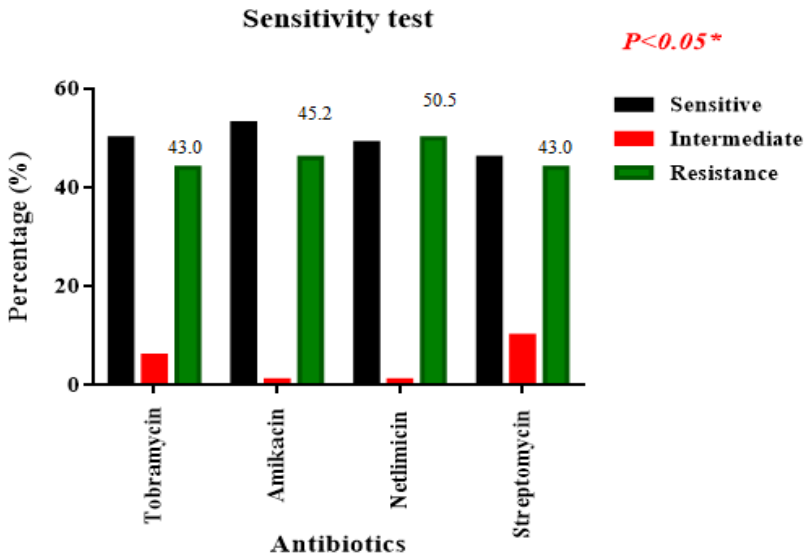

Figure (2): Antimicrobial susceptibility test for 39*P. aeruginosa*isolates. The results of the disc diffusion test were evaluated for four antimicrobial agents,includingAmikacin (30 µg/disc), Tobramycin (30 µg/disc), Netilmicin (30 µg/disc), Streptomycin (300 µg/disc).

After MIC had been conducted for 40 isolates resistant to tobramycin, the results showed the MIC values ranged between 32-2048 µg/ml. The MIC values 2048 and 1024 µg/ml scored the highest percentage and (55%) were significant ($p<0.05$) compared to other concentrations (27.5% for 512 µg/ml, 10% for 256 and 128 µg/ml, 5% for 64 µg/ml and 2.5% for 32 µg/ml).

The MIC of thetobramycin combination with curcumin was determined. There was a significant enhancement in the sensitivity ($p\leq 0.05$) of *P.aeruginosa isolates* to tobramycin upon the use of70µg/ml of curcumin (Figure 3), at the same timeother concentrations caused lower reduction in MIC or inhibited the bacteria without antibiotics. The highest percentage of isolates (22.5%) observed two-fold changes in MIC followed by four-fold (12.5%), seven-fold (10%), three-fold (7.5%), while other folds ranged between 2.5-5%.

At 70 µg/ml of curcumin, none of the isolates were susceptible to curcumin alone, indicating its inhibitor activity of the efflux pump. Additionally, the concentration 1024 scored highest percentage (97.5%), followed by 2048 (95.0%), 512 (65.0%), 256 (60.0%) and 128 (50.0%).

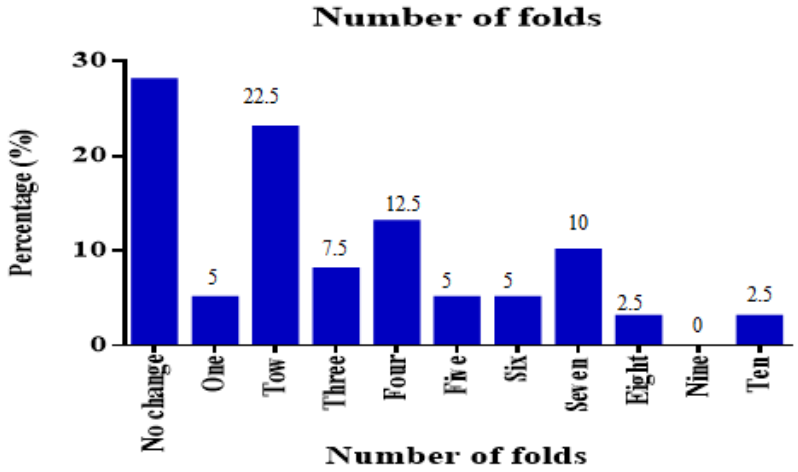

Figure (3): Fold change of tobramycin MIC after adding 70 µg/ml Curcumin.

These results agreed with other studiesthat show significant differences between the effects of gentamicin and ciprofloxacin when combined with curcumin on *P. aeruginosa*isolates(3, 10). The combination of antibiotics with curcumin an efflux pump inhibitor can be used as an effective strategy for the resistance in bacterial isolates and restore the infectivity of antibiotic to increase bacterial cell death (20). Ballard *et al*. demonstrated that the MICs of antibiotics can be reduced by combining natural curcumin (21).In this study, A clear single PCR product bands (372 bp) detection *mexX* genewere confirmed with gel electrophoresis, as shown in (Figure 4). These results were similar to the results of Mohi-Aldeen *et al* in (2018) illustrated that all isolates (100%) have *mexX*(22).

Furthermore, a recent study similar to our findings found that out of fifty-four *P. aeruginosa* isolates, 48 (88.8%) gave positive results for efflux system *MexX* (23).

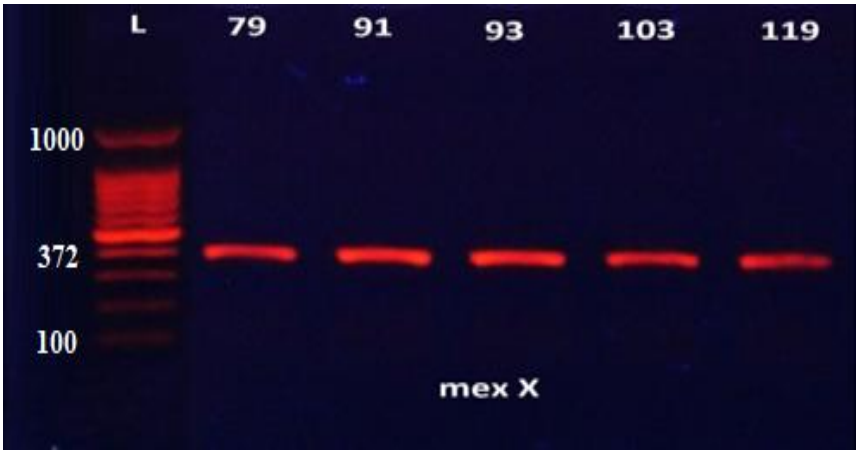

Figure (4): Agarose gel electrophoresis of PCR of the *mexX*gene (372 bp) of *P. aeruginosa* isolates. Agarose 1.5%, 100 V for 75 minutes, stained with safe red dye and visualized on a UV transilluminator. L: Lader (100-100bp).

One-step RT-qPCR was done usingthe SYBR green method to determine the effect of sub-MIC of tobramycin only and tobramycin

combination with curcumin on gene expression of *mexX*genein five isolates of *P. aeruginosa*whichhad four-fold change. The MIC of tobramycin for-four isolates was 2048 µg/ml and 512 µg/ml for the resistant isolate.

After treatment of bacterial cells with tobramycin only, the antibiotic upregulated the gene expression of *mexX*gene in *P. aeruginosa* isolates (79, 103, and 119), but downregulated the gene expression of other two isolates (91 and 93), as illustrated in (Table 7). The upregulation of *mexX*gene leads to increase in gene expression resulting from specific mutation in their regulatory genes especially after treatment with excessive and incorrect use of antibiotics, while downregulation of gene expression maybelong to because of specific mutation in *mexX*gene.Themutations that responsible for increasing expression levels of *mexX*gene, was happened in *mexZ* gene, lead to high levelsof*mexXY*,which resulted in resistance to aminoglycoside (MIC elevated 2-8 fold) (24).

Furthermore, the combination of tobramycin antibiotic with curcumin upregulated the gene expression of *mexX*gene in *P. aeruginosa* isolates (91, and 119), and the downregulated the gene expression significantly ($p \leq 0.001$) of rest isolates (79, 93, and 103).Whiledownregulation of *mexX*gene of (103)was the most effect.Downregulation of *mexX*genein two isolates (79 and 103) involved in efflux pumps, indicates the enhancement effect of tobramycin in the presence of curcumin which may repair the damage caused by using antibiotics(Table 7).Given that, upregulated expression of *mexX* with combination of tobramycin antibiotic and curcumin, it could be suggested that this geneis govern by different regulation mechanisms.Curcumin-encapsulated in nanoparticles resulting in downregulation of *mexX* and *oprM* genes in *P. aeruginosa*when treated with curcumin and ciprofloxacin compared to cells treated with ciprofloxacin alone (25).

Table (7): effect of antibiotic and curcumin on gene expression of *mexX*

mexX			
Isolate code	**Fold of gene expression after treatment with antibiotic only**	**Fold of gene expression after treatment with antibiotic combination with curcumin**	**P value**
79	1.64718	0.01217	p<0.001***
91	0.05207	1.99078	p<0.001***
93	0.02194	0.00465	p<0.001***
103	38.31932	0.00002	p<0.001***
119	2.37841	2.46229	**p>0.05^{NS}**
control	1.00000	1.00000	1.00
P value	p<0.001***	p<0.001***	

Conclusion

The curcumin is natural and effected compound is able to downregulate of *mexX* gene of MDR *P. aeruginosa* isolates from different clinical sources. Additionally, curcumin can be employed as a native compound to improve the effective treatment of tobramycin-resistant isolates.

References

1. Coșeriu, R.L.; C. Vintilă; M. Pribac; A.D. Mare; C.N. Ciurea; R.O. Togănel; A. Cighir; A. Simion, and A. Man. (2023).

Antibacterial Effect of 16 Essential Oils and Modulation of mex Efflux Pumps Gene Expression on Multidrug-Resistant *Pseudomonas aeruginosa* Clinical Isolates: Is Cinnamon a Good Fighter? *Antibiotics*, 12(1),163

2. Pang, Z.; R. Raudonis; B.R. Glick; T.-J. Lin, and Z. Cheng. (2019). Antibiotic resistance in *Pseudomonas aeruginosa:* mechanisms and alternative therapeutic strategies. *Biotechnology Advances*, 37(1), 177-192.
3. Sulaiman, S.D. and G.A. Abdulhasan. (2020). Curcumin as Efflux Pump Inhibitor Agent for Enhancement Treatment Against Multidrug Resistant *Pseudomonas aeruginosa.*Isolates. *Iraqi Journal of Science*, 61(1), 59-67.
4. Aires Julio, R.; T. Köhler; H. Nikaido, and P. Plésiat. (1999). Involvement of an Active Efflux System in the Natural Resistance of *Pseudomonas aeruginosa* to Aminoglycosides. *Antimicrobial Agents and Chemotherapy*, 43(11), 2624-2628.
5. Armstrong, E.S. and G.H. Miller. (2010). Combating evolution with intelligent design: the neoglycoside ACHN-490. *Current Opinion in Microbiology*, 13(5), 565-573.
6. Mosolygó, T.; A. Kincses; A. Csonka; Á.S. Tönki; K. Witek; C. Sanmartín; M.A. Marć; J. Handzlik; K. Kieć-Kononowicz; E. Domínguez-Álvarez, and G. Spengler. (2019). Selenocompounds as Novel Antibacterial Agents and Bacterial Efflux Pump Inhibitors. *Molecules*, 24(8).
7. Kumar, D. and S. Jandaik. (2016). A Review on Efflux Pump Inhibitors of Gram-Positive and Gram-Negative Bacteria from Plant Sources. *International Journal of Current Microbiology and Applied Sciences*, 5, 837-855.
8. Dai, C.; J. Lin; H. Li; Z. Shen; Y. Wang; T. Velkov, and J. Shen. (2022). The Natural Product Curcumin as an Antibacterial Agent: Current Achievements and Problems. *Antioxidants*, 11(3), 459
9. Negi, N.; P. Prakash; M.L. Gupta, and T.M. Mohapatra. (2014). Possible Role of Curcumin as an Efflux Pump Inhibitor in Multi Drug Resistant Clinical Isolates of *Pseudomonas aeruginosa*. *J Clin Diagn Res*, 8(10), Dc04-7.
10. Eshra, K. A. and Shalaby, M. M. (2017). Efflux pump inhibition effect of curcumin and phenylalanine arginyl β-naphthylamide (PAβN) against multidrug resistant Pseudomonas aeruginosa isolated from burn infections in Tanta University Hospitals. *The Egyptian Journal of Medical Microbiology (EJMM)*, *26*(1).
11. Spilker, T.; Coenye, T.; Vandamme, P. and LiPuma, J. J. (2004). PCR-based assay for differentiation of Pseudomonas aeruginosa from other Pseudomonas species recovered from cystic fibrosis patients. *Journal of clinical microbiology*, *42*(5), 2074-2079.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 33th edition. Clinical and Laboratory Standards Institute. 2023: Berwyn (PA).
13. Chakravarty, S.; Melton, C. N.; Bailin, A.; Yahr, T. L. and Anderson, G. G. (2017). Pseudomonas aeruginosa magnesium transporter MgtE inhibits type III secretion system gene expression by stimulating rsmYZ transcription. *Journal of bacteriology*, *199*(23), 10-1128.
14. Roshani-Asl, P.; Rashidi, N.; Shokoohizadeh, L. and Zarei, J. (2018). Relationship among antibiotic resistance, biofilm formation and lasB gene in Pseudomonas aeruginosa isolated from burn patients. *Clin Lab*, *64*(9), 1477-1484.
15. Mohammad, A. J. and Flayyih, M. T. (2021). Antibacterial Activity of Thuja orientalis and Green Tea in Pseudomonas areogenosa infection. *Int. J. Agricult. Stat. Sci. Vol*, *17*(2), 837-843.
16. Albermani, S. S. and Al-Jumaili, E. F. A. (2021). Screening of Production Pyocin S5 Isolate from Multidrug Resistant Pseudomonas aeruginosa. *Iraqi journal of biotechnology*, *1*(20).
17. Nikbin, V. S.; Aslani, M. M.; Sharafi, Z.; Hashemipour, M.; Shahcheraghi, F. and Ebrahimipour, G. H. (2012). Molecular identification and detection of virulence genes among Pseudomonas aeruginosa isolated from different infectious origins. *Iranian journal of microbiology*, *4*(3), 118.
18. Alhashimy, S. K.; Ismaeel, Z. A. L. and Abdulrazaq, M. A. (2021). Investigate Pseudomonas aeruginosa resistant to antibiotics and studying the antibacterial effect of Rosemary oil at different concentrations. *Journal of Education and Scientific Studies*, *3*(17).
19. AL-Fridawy, R. A. K.; 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*, *19*(2).

20. 19Kourtesi, C.; Ball, A. R.; Huang, Y. Y.; Jachak, S. M.; Vera, D. M. A.; Khondkar, P.; ... and Tegos, G. P. (2013). Suppl 1: microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. *The open microbiology journal*, *7*, 34.
21. Ballard, E. and Coote, P. J. (2016). Enhancement of antibiotic efficacy against multi-drug resistant Pseudomonas aeruginosa infections via combination with curcumin and 1-(1-Naphthylmethyl)-piperazine. *Journal of Antimicrobial Agents*, 2: 116.
22. Mohi-Aldeen, Z. K. A.; Essa, M. A. and Al-Hasso, M. Z. (2018). Detection of Efflux Pumps Genes in Pseudomonasaeruginosa Isolates Isolated from Open Fracture Infections. *Kirkuk Journal of Science*, *13*(4), 155-169.
23. Friyah, S. H. and Rasheed, M. N. (2018). Molecular study of efflux MexX gene in Pseudomonas aeruginosa isolated from Iraqi patients. *Iraqi journal of biotechnology*, *17*(3).
24. Vogne, C.; Aires, J. R.; Bailly, C.; Hocquet, D. and Plésiat, P. (2004). Role of the multidrug efflux system MexXY in the emergence of moderate resistance to aminoglycosides among Pseudomonas aeruginosa isolates from patients with cystic fibrosis. *Antimicrobial agents and chemotherapy*, *48*(5), 1676-1680.
25. S. Rahbar Takrami, N. Ranji, and M. Sadeghizadeh, "Antibacterial effects of curcumin encapsulated in nanoparticles on clinical isolates of Pseudomonas aeruginosa through downregulation of efflux pumps," (in eng), *Mol Biol Rep*, vol. 46, no. 2, pp. 2395-2404, Apr 2019, doi: 10.1007/s11033-019-04700-2.