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Evaluation of Some Efflux Pump Genes in *Pseudomonas aeruginosa* and their Relation to Antibiotic Resistance

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Abstract

In this study, all 100 samples were collected from people suffering from burns, wounds, ear infections, blood, sputum samples, and urine from both genders. The specimens were collected from medical city hospitals during the period between September 2022 and January 2023. The results of culture and biochemical tests showed that 50 isolates were P. aeruginosa. The VITEK2 compact system confirmed the identity of 35 isolates. A VITEK2 compact system tested 35 strains of *Pseudomonas aeruginosa* for drug susceptibility. These strains were resistant to Cefotaxime 25 (71.43%), Ceftazidime 25 (71.43%), Cefepime 25 (71.43%), Imipenem 22 (62.86%), Meropenem 23 (65.71%), 22 (62.86%), Gentamicin 22 (62.86%), Ciprofloxacin 18 (51.43%), and Norfloxacin 21 (60%). The VITEK2 compact system used regular PCR to identify the efflux pump genes (*mexT* and *mexF*) in 35 isolates. The results indicated that *mexT* was positive in 20 isolates (57.1%), *mexF* was positive in 18 isolates (51.4%), and *mexF* was negative in 17 isolates (48.6%).

Keywords: *Pseudomonas aeruginosa*, *mexT*, *mexF* efflux gene, antibiotic resistance.

1. Introduction

Pesudomonas aeruginosa is a Gram-negative, motile, heterotrophic rod-shaped bacterium. It is a facultative aerobe that grows through aerobic and anaerobic respiration. *Pesudomonas aeruginosa* grows well at (37 °C), but it can stay alive in a wide range of temperatures from (4 to 42 °C), and is a lactose non-fermentor in MacConkey agar [1, 2]. This leads to the development of diseases in animals, plants, and humans. Opportunistic bacteria significantly contribute to mortality and morbidity in individuals with immunodeficiencies and cystic fibrosis (CF) [3, 4]. Because of its high inherent resistance to antibiotics, *Pseudomonas aeruginosa* remains a major cause of infections in Western society. This intrinsic resistance has been shown to result from the interaction of secondary resistance mechanisms like energy-dependent multidrug efflux, periplasmic lactamase, and unusually low outer-membrane permeability [5]. Given the level of natural resistance that exists, mutational resistance to the majority of antibiotic classes can easily develop [6, 7]. *mexT* is a transcriptional regulator that plays an important role in the multidrug resistance of *Pseudomonas aeruginosa*. Researchers have found that mexT manages the activity of several efflux pump genes, such as MexEF-OprN, MexAB-OprM, and MexXY-OprM. *mexT*

is also involved in the regulation of other genes involved in virulence and adaptation, such as those encoding quorum sensing and biofilm formation [8, 9]. *mexF* is a main constituent of theMexEF-OprN efflux pumps and plays an important role in multidrug resistance, virulence, and adaptation in *Pesudomonas aeruginosa* [10, 11]. The goal is to investigate the rule of genes responsible for antibiotic pump efflux, mexF and mexT, and their relationship to antibiotic resistance.

2. Materials and Methods

2.1. Collection of specimens

It was put one hundred clinical specimens of urine, burn swabs, wound swabs, sputum, blood, and ear swabs. It was then inoculated on MacConkey agar and incubated for (24 hr).(at 37 C°). The pale non lactose fermenter colonies were chosen, and one colony was inoculated on Cetrimide medium for biochemical assays [12, 13].

2.2. Bacterial Identification

2.2.1. Identification of *Pseudomonas aeruginosa* by biochemical Tests

2.2.1.1 Oxidase Test

Put the oxidase reagent (thetetra-methyl-p-phenylenediamine dihydrochloride) on paper, and the bacterial inoculum was obtained with a cotton-tipped swab. When the color changes to purple, microorganisms are delayed oxidase-positive [14].

2.2.1.2. Catalase test

In a petri dish, insert a microscope slide. Collect a small number of organisms. Place 1 drop of 3% H_2O_2 on the organism. The formation of bubbles indicates a catalase-positive reaction, whereas no bubble formation indicates a catalase-negative reaction [15, 16].

2.2.1.3. Identification of *P. aeruginosa* isolates and Antimicrobial Sensitivity by VITEK2 Compact System

The VITEK 2 method (BioMe'rieux) is a novel automatic method for detecting bacterial and selection tests using fluorescence-based technologies:

Bacterial isolates are grown in nutrient agar using the streaking technique and incubated for 24 hours at 37 °C.

1. We filled the testing tube with 3.0 ml of sterile saline.
2. We used a stick or sterile swab to convert sufficient pure cultural colonies and suspend isolation colonies into normal saline.
3. McFarland adjusted the turbidity (0.5–0.63) and used the Densi ChekTM turbidity meter.
4. We obtained the results after 4–6 hours.

2.3. Molecular Assay

2.3.1. DNA Extraction from Bacteria

The DNA of the isolates that showed high antibiotic resistance was extracted according to the protocol of the Easy Pure® Genomic DNA Kit Transgene® (China).

2.3.2. Detection of *mexT* and *mexF* in *a Pseudomonas aeruginosa* isolate

Amplification of the tested gene was performed by conventional PCR, and the primer sequence was designed using 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), 1.5 µl of "forward primer" of mexF (GATCGGAGGCATCGTTTCGTT) and 1.5µl of "reverse primer" of mexF (GCGAGGACATGTACAGCATCC) (10 pmol/µl) from each primer, Green master mix (17.5 µl), 5µl DNA, (4.5µl) nuclease free water polymerase (NEB® England), to give a final volume

(25 µl). The adjustments to the cycling program for the mexF gene were: Initial Denaturation (94 ºC) for 5 minutes, denaturation (94 ºC) for 30 seconds, annealing (56ºC) for 45 seconds, extension (72 ºC) for 45 seconds, and final extension (72 ºC) for 7 minutes. The cycling program for the mexF gene included initial denaturation at 94C for 5 minutes, denaturation at 94C for 30 seconds, annealing at 55C for 45 seconds, extension at 72C for 45 seconds, and final extension at 72C for 7 minutes.

2.4. Statistical Analysis

Statistical inference for the Social Science SPSS (version 21, GraphPad Software, San Diego, California, USA) program was used for data entry and analysis. In this cross-sectional study, the odds ratio (OR) was estimated to define the association between the presence of a bacterial gene and antibiotic resistance. An ANOVA test was used to test the significance level for different laboratory parameters among the study groups.

3. Results

The results of this study show that the source of these isolates was burn swab n = 20 (10%), urine culture n = 3 (1.5%), wound swab n = 9 (4.5%), sputum n = 9 (4.5%), ear swab n = 4 (2%), and blood n = 5 (2.5%), with a significant difference (P<0.05).

The VITEK2 compact system confirmed the identity of only 35 isolates, revealing their resistance percentages: Cefotaxime Resistant Isolate 25 (71.43%), Ceftazidime Resistant Isolate 25 (71.43%), Cefepime Resistant Isolate 25 (71.43%), Imipenem Resistant Isolate 22 (62.86%), Meropenem Resistant Isolate 23 (65.71%), Amikacin Resistant Isolate 22 (62.86%), Gentamicin Resistant Isolate 22 (62.86%), Ciprofloxacin Resistant Isolate 18 (51.43%), and Norfloxacin Resistant Isolate 21 (6 as shows the result in **Table 1**.

Table 1. Number and percentage of *Pseudomonas aeruginosa* isolate according to Antibiotics Resistant**.**

Antibiotic	Resistant Isolate No.%	Sensitive Isolate No. %	p-value
Cefotaxime	25 (71.43%)	10 (28.57%)	0.011*
Ceftazidime	25 (71.43%)	10 (28.57%)	0.011*
Cefepime	25 (71.43%)	10 (28.57%)	0.011*
Imipenem	22 (62.86%)	13 (37.14%)	0.128 NS
Meropenem	23 (65.71%)	12 (34.29%)	0.086 NS
Amikacin	22 (62.86%)	13 (37.14%)	0.128 NS
Gentamicin	22 (62.86%)	13 (37.14%)	0.128 NS
Ciprofloxacin	18 (51.43%)	17 (48.57%)	0.866 NS
Norfloxacin	21 (60%)	14 (40%)	0.237 NS
p-value	0.984 NS	0.907 NS 0.733 NS	-

While using conventional PCR, the result showed that 20 (57.1%) of the isolates were carrying the mex T gene, and 15 (42.9%) were not carrying the Mex T gene. Conversely, we detected the Mex F gene in 18 (51.4%) of the isolates, while we did not detect it in 17 (48.6%). All isolates under study showed a band of 185 bp for the mext gene and 182 for the mexf gene (Figures 1 and 2).

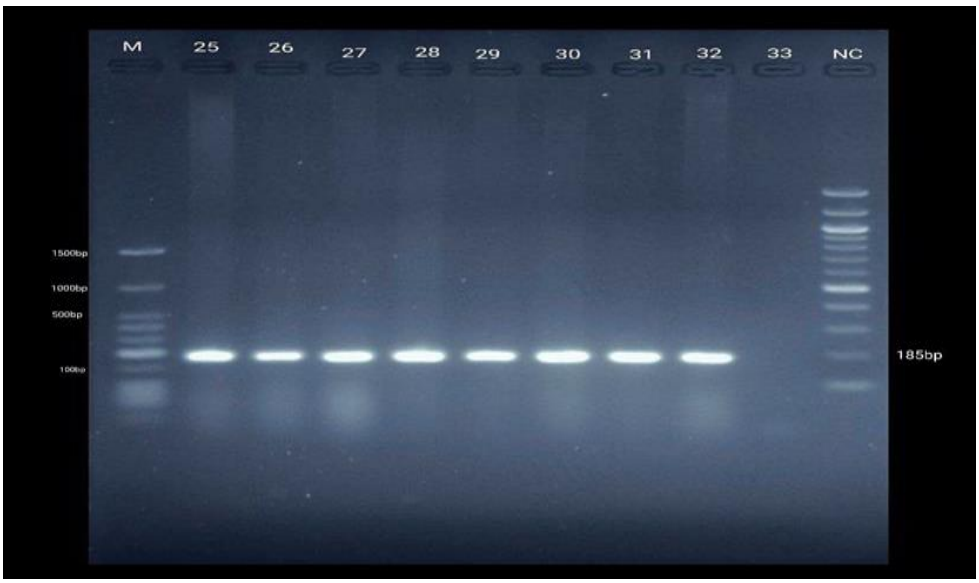

Figure 1. Amplified PCR product of *mexT* Gene (185bp) of *P.aeruginosa*. 1.5% agarose gel electrophoresis stained with red save die (10mg/ml). 100v/m Amp for 75min.TBE buffer (1x). M:100bp DNA marker. Lanes:26-33**.**

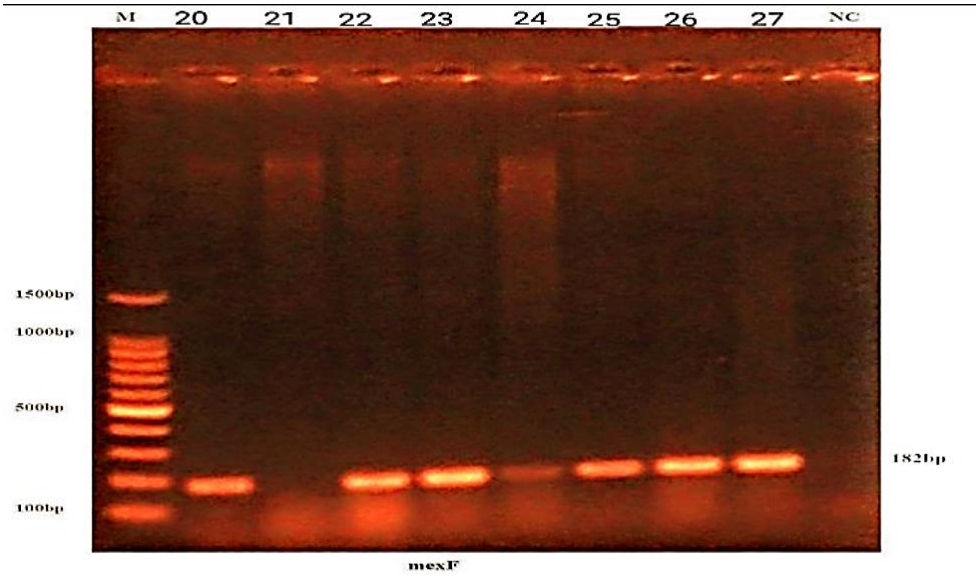

Figure 2. Amplified PCR product of *mexF* (182bp) Gene (of *P.aeruginosa*. 1.5% agarose gel electrophoresis stained with red save die (10mg/ml). 100v/m Amp for 75min.TBE buffer (1x). M:100bp DNA marker. Lanes:20-27 .

Table 2. Correlation between presence of genes with *Pseudomonas aeruginosa* (MDR) isolates

Gene	Antibiotic resistant		O. R^	P-value	(95%CI)
	Resistant	Sensitive			
mexT Positive	17 (68.0%)	3 (30.0%)	4.958	<0.001**	2.722-9.031
mexT Negative	8 (32.0%)	7 (70.0%)			
mexF gene Positive	14 (56.0%)	4 (40.0%)			
mexF Negative	11 (44.0%)	6 (60.0%)	1.909	0.024*	1.088-3.349

The result show that the relation between present of *mexT* gene and antibiotic resistance was (p <0.001**) that mean presence highly significant different and the relation between present of *mexF* gene and antibiotic resistance was (p<0.024*) that mean presence significant different, and Present correlation between presence of genes with Antibiotic resistance (**Table 2**).

4. Discussion

Pseudomonas aeruginosa isolates *Pseudomonas aeruginosa* isolates more frequently from burn specimens than from other sources, specifically from burn swabs (n = 20/10%), as patients with burns often lose their first line of defense and are more susceptible to nosocomial infections. Cetrimide agar, which is used to isolate *Pseudomonas aeruginosa*, appears on agar and produces fluorescein and pyocyanin [17, 18]. *Pesudomonas* on MacConkey agar appear as a pale colony because lactose is a non-fermentor. [19]. The oxidase test yielded a positive result when the bacterial colony changed to a blue-purple color within 10 seconds, while the catalase test showed bubble formation within 5–10 seconds [20, 21].

This result showed that there was a significant difference in resistance to the antibiotics Cefotaxime, Ceftazidime, and Cefepime, indicating that resistance to these antibiotics is high. These beta-lactam antibiotics and *Pseudomonas aeruginosa* contain beta-lactamase, making them resistant to these antibiotics. While other isolates for antibiotics Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, and Norfloxacin were non-significant, this means that resistance to these antibiotics is less than in the first group. The reason for this difference was the sensitivity of the number of isolates to antibiotics, as reported in the study [21, 22].

Another study showed the antibiotic resistance of *Pseudomonas aeruginosa* to gentamicin (65.95%) and imipenem (17.02%) [23, 24]. In another study presented by the scientist, the rate of resistance to imipenem and ceftazidime was 72%, in addition to 98% [25, 26]. Using a conventional PCR technique, molecular detection of two genes (*mexT* and *mexF*) in 35 isolates revealed no significant difference between the *mexT* and *mexF* genes in *P. aeruginosa* isolates. A different study found that an efflux pump gene was present in 88.8% of the 54 isolates that were used to find a gram-negative resistance mechanism [27, 28]. While all 39 Pseudomonas isolates tested positive for some efflux pump genes, only 23 of them (59%) showed efflux pump activity when assessed phenotypically.

Moreover, certain efflux pump genes were detected in 91% and 92% of the isolates, respectively [29]. Other studies found antibiotic resistance with the efflux pump gene in 14 sputum samples (23.3%) and 26 urine samples (43.3%), which accounted for the majority of isolates. Maximum resistance to antimicrobial agents was highest against cefepime (97%), ceftazidime (90%), gentamycin (87%), piperacillin (73%), and ciprofloxacin (60%). We found the least resistance to meropenem (63%), imipenem (60%), and piperacillin/tazobactam (43%). Multidrug resistance (MDR) comprised 12 (20%) isolates, and non-MDR comprised 10 (16.7%) isolates. 56.7% (34 strains) and 46.7% (28 strains) of all tested isolates had some efflux pump genes, respectively. Our findings show that all strains that carry some efflux pump genes also carry the mexa gene [30].

5. Conclusion

Pseudomonas aeruginosa isolates more frequently from burn specimens than from other sources, with a burn swab n = 20 (10%). In the molecular identification using *mexT* and *mexF* primers, 20 (57.1%) isolates were positive for the mexT gene, and *18* (51.4%) specimens were positive for the mexF gene. The research discovered a link between the genes mexT and mexF and Aeruginosa's resistance to antibiotics. The mexT gene had high significant differences ($p \leq 0.001$), and the mexF gene had significant differences ($p \leq 0.02$). *Pseudomonas aeruginosa* isolates are highly resistant to Cefotaxime 25 (71.43%), Ceftazidime 25 (71.43%), and Cefepime 25 (71.43%).

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The study was conducted in accordance with the ethical principles . It was carried out with patients verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by Baghdad University, College of Science a local ethics committee according to the document number CSEC\0922\0075 on 25/September/2022 to get this approval.

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