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Article

Evaluated some Efflux-Pump gene Expression under ciprofloxacin stress in Pseudomonas aeruginosa

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic bacteria; adaptation in different environments makes them very aggressive and life-threatening pathogens for humans, especially patients who suffer from weak immunity. This study aimed to evaluate the gene expression of each mexA and mexB gene in Pseudomonas aeruginosa isolates, which were isolated from clinical samples; screening of efflux pumps in multidrug P. aeruginosa phenotypically was done by the Cather wheel method. Study genes expression of efflux pump genes under ciprofloxacin antibiotic stress and Et. Br were done in different concentrations of cip and EtBr. One hundred and ten (110) isolates of Pseudomonas spp. were collected from different hospitals in Baghdad city, (50) isolates of Pseudomonas aeruginosa identified and confirmed were done. The results appeared according to the type of clinical sources the fifty-isolate distributed as 35 (70 %) burns swab samples, 10 (20 %) from wounds swabs, and 5 (10 %) from ear discharge. The results of mexA Ct values ranged from 32.1 to 36.79 with an average of 34.337, and the results of mexB Ct values ranged from 14.74 to 19.96 with an average of 16.791 compared to the rpsL (Ct = 11.14) reference gene. The results showed that the mean of Ct of gene expression was noticed when put under stress of Et.Br. + CIP and CIP in mexA were 37.195 and 34.702, respectively, compared to control 34.337; on the other hand, the gene expression between both treatments was over-expressed in the treatment of CIP 32 compared to CIP+Et.Br. The Cather wheel method expression results were noticed when stressed, Et.Br. + CIP and CIP in mexB gene expression were 19.626 and 18.872, respectively, compared to control 16.791. In conclusion, compared to the control, both treatments did not induce the expression of mexB genes; on the other hand, the gene expression between both treatments was expressed in the treatment of CIP 32 compared to CIP+Et.Br.

Keywords: Pseudomonas aeruginosa, mexA, mexB, Cather Wheel.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic bacteria; adaptation in different types of environments make them very aggressive and life-threatening pathogens for human, especially patients who suffer from weak immunity (e.g., Immuno-compromised), in addition to their ability to cause diseases in hospitals [1]. Moreover,

infections that are caused by multidrug-resistant (MDR) strains of Pseudomonas spp. It became more common, especially with limited therapy, considered a big problems and may be due to death, as well as extensively drug-resistant (XDR) strains [2, 3]. Properties like low outer membrane permeability, the expression of efflux pumps, and the synthesis of antibiotic-inactivating enzymes all contribute to intrinsic resistance, especially the acquired resistance that may occur as a result of mutational alterations or horizontal transfer of resistance genes by mobile genetic elements (MGEs) such as integrons, transposons, or plasmids, this acquired resistance leads to the emergence of new strains that are more resistant to antibiotics [3.] "Permeability barrier" refers to the ability of the bacterial cell envelope to prevent antibiotics from entering the cell; on the other hand, it is becoming increasingly clear that the action of efflux pumps, whether working alone or in cooperation with decreased expression of porins, is a part of this "barrier [4]. As a result, restricting the amount of medication that can enter bacterial cells is an essential component. Genes that code for efflux pumps can be located on plasmids (for example, transmissible elements) or chromosomes. Genes encoding efflux pumps located on plasmids can be acquired by any bacteria in which the plasmid can replicate. However, they are not present in all members of a particular bacterial species [5]. Efflux pumps (EPs) are considered membrane transporter proteins that have a vital role in the intrinsic and acquired antibiotic resistance mechanisms of P. aeruginosa [6]. This organism is inherently resistant to various structurally unrelated antimicrobial medicines due to the poor permeability of its outer membrane and the constitutive synthesis of multiple EPs with broad substrate specificity [7, 8]. The most known systems of efflux are MexAB-DprM, MexCD-OprJ, MexEF-OprN, and MexXYOprM; these systems can efflux several antibiotics, which is found especially in P. aeruginosa due to multidrug resistance. Each pump is targeted to a different set of antimicrobial agent substrates. (e EP genes are found in all strains but are not substantially expressed), a mutation in a regulatory gene, such as mexR, which regulates the mexAB-OprM genes, can cause increased expression [9, 10]. This study aimed to evaluate gene expression of the mexA and mexB genes in Pseudomonas aeruginosa isolates under ciprofloxacin stress.

MATERIALS AND METHODS

Isolation and Identification of Pseudomonas aeruginosa

One hundred and ten (110) isolates of Pseudomonas spp. were obtained during this study, collected from different hospitals in Baghdad city. All samples were cultivated on different media (Blood agar, MacConkey agar medium, cetrimide agar). Morphological and chemical (VITEK system 2 compacts) were done for identification.

Determination of ciprofloxacin MIC

Using Mueller-Hinton 160 microliter broth as the diluent, double serial dilutions of ciprofloxacin and gentamicin were generated in a microtiter plate starting with a stock concentration of 10 mg/1 ml. The dilutions ranged from 1 to 1024 g/ml. Except for the wells that served as the negative controls, each well was seeded with 10 microliter of a bacterial suspension that was 20 microliter in volume and was comparable to the McFarland standard no.0.5 (1.5 108 CFU/ml). Microtiter plates were kept in an incubator at 37 degrees Celsius for between 18 and 20 hours. Following the incubation period, 20 microliter of resazurin dye was added to each of the wells, and the plates were then returned to the incubator for another 2 hours to check for any color shifts. The Minimum Inhibitory Concentrations were found by visually determining, in broth micro dilutions, the lowest concentrations of the extracts at which the color changed from blue to pink in the resazurin broth assay. This was done to find the Minimum Inhibitory Concentrations (MICs) [11].

Detection of Efflux Pump-Mediated Resistance under Ciprofloxacin and Ciprofloxacin+Ethidium Bromide stress.

Ciprofloxacin and EtBr stain were used to determine whether or not an efflux pump mechanism was present in MHA plates utilizing the EtBr-agar cartwheel (Et-BrCW) method. EtBrCW was performed by applying EtBr stain of varying concentrations (five, ten, fifteen, twenty, and twenty-five µL /mL) to MHA plates containing ciprofloxacin of varying concentrations (thirty-two, sixty-four, and one hundred twenty-eight µL /mL), which were prepared on the same day as the experiment and shielded from light. Following this, the plates were swabbed with A gel documentation device to take photographs of the cultures after placing them on an ultraviolet transilluminator. It was determined what concentration of EtBr was required to produce the least amount of fluorescence in the bacterial mass [12].

Gene Expression of mexX, oprD and oprM genes

mexA and mexB primers that were used in the study [13] with their sequences, mexA (Foreword) 5′-ACCTACGAGGCCGACTACCAGA-3′ and (Reverse) 5′-TTGGTCACCAGGGCGCCTTC-3′ (with 179 bp), mexB (Foreword) 5′-GTGTTCGGCTCGCAGTACTC-3′ and (Reverse) 5′-AACCGTCGGGATTGAC-CTTG-3′ (with 240 bp). They were normalized with the rpsL housekeeping gene.

1. Extraction of RNA

   By using TransZol Up Plus Kit (TRANS/China), RNA was extracted:

2. Removing of Genomic-DNA

   Using *EasyScript*® One-Step gDNA Removal and cDNA Synthesis Super-Mix (TRANS/China) kit, genomic DNA was removed.

3. First-Strand cDNA Synthesis

   The reaction component (and volume) of this step is summarized as follows: Total RNA/mRNA (0.1 ng-5 µg/10 pg-500 ng), Anchored Oligo dT 18 Primer 0.5 µg/µl (1 µl), Random Primer 0.1 µg/µl (1 µl), 2 × ES Reaction Mix (10 µl), EasyScript® RT/RI Enzyme Mix (1 µl), gDNA Remover (1 µl), RNase-free Water (Complete to 20 µl).

4. Assessment of RNA Concentration and Purity

The NanoDrop was used to assess the concentration of isolated RNA to detect the samples' goodness for further assessment in RT-qPCR. The RNA concentration was detected. The concentration of RNA in the samples was in the range of 82-108 ng/µl. In the case of RNA purity, the NanoDrop 2000c (Thermo Fisher Scientific, USA) was used, in which the absorbance of the sample is read at two wavelengths (260 and 280nm). An A260/A280 ratio of approximately 2.0 was suggestive that the RNA sample was pure. In the present study, the expression of the target genes (mexA and mexB), considered essential resistance genes in P. aeruginosa, was done for isolates. All the tested genes, isolate number. The quantitative Real-Time PCR technique was performed for quantification detection and gene expression analysis of antibiotics resistance efflux pump genes and normalized by housekeeping (rpsL) gene in resistant Pseudomonas aeruginosa. Genotypic expression using RT-PCR technique to detect mexA and mexB genes among P. aeruginosa isolates by extraction of RNA. At present, many studies have explained the role of efflux pumps in P. aeruginosa resistance. The current study examination was meant to explain quality articulation of efflux qualities in antibiotics resistance by RT-PCR in broad medication obstruction P. aeruginosa segregated from Clinical isolates in Baghdad city hospitals of Iraq. The efflux pump has been recognized as one of the significant complexes involved in resistance to most of the classes of antibiotics [5]. It has been reported that the prevalence of efflux pump overexpression in clinical P. aeruginosa strains ranged from 14-75% [12].

5. Gene Expression by rtPCR

Analysis of gene expression by using RT-PCR was performed for RNA samples. The reaction components (and volume) for RT-PCR were 2×EasyTaq® PCR

SuperMix (10 µl), cDNA (2 µl), Primers F+R (2 µl), Nuclease-free Water (6 µl). The thermal Cycle of RT-PCR of the studied genes was: for mexB gene Denaturation 94°C for 10 sec, Annealing 58°C for 15 sec, Extension 72°C for 20 sec., for mexA gene Denaturation 94°C for 5 sec., Annealing 68°C for 15 sec., Extension 72°C for 20 sec.

Statistics Analysis

The statistical analysis was done by using the LSD test, and way ANOVA at P value < 0.05 was performed by using GraphPad Prism 7 Statistics software.

RESULTS

Distribution of P. aeruginosa According to Clinical Source

The fifty isolates of P. aeruginosa were identified and confirmed. These isolates distributed belonged to clinical sources as 35 (70 %) swab samples, 10 (20 %) from wound swabs, and 5 (10 %) from ear discharge. Figure 1 shows the Distribution of P. aeruginosa burns isolates according to clinical sources.

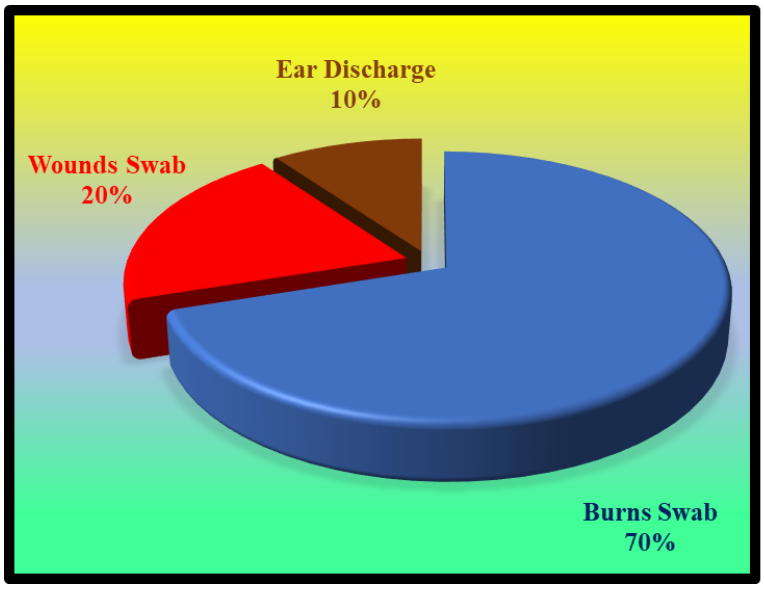

Figure 1. Distribution of P. aeruginosa isolates according to clinical sources

Determination of the ciprofloxacin MIC

The broth microdilution method was used to determine the MIC of ciprofloxacin in a 96-well microtiter plate. No isolates that were sensitive or resistant to CIP were noticed. The susceptibility of the P. aeruginosa isolates (no.1 to no.18 isolates) against ciprofloxacin was tested by determining the MIC using a microtiter plate. MIC for ciprofloxacin ranged from 0.25 to 256 mg/L. According to the established breakpoint values CLSI (2021) recommended, the P. aeruginosa isolates with MIC ≥ 2 mg/L are considered ciprofloxacin resistant. Nearly all tested isolates were resistant to ciprofloxacin by MIC test (MIC ≥ 2 and ≥ 16 mg/L, respectively). The results showed that the concentration of MIC ranged from 8 to 256 µg/ml. The MIC of ciprofloxacin for different isolates was which can inhibit growth. (Figure 2 shows the MIC of ciprofloxacin).

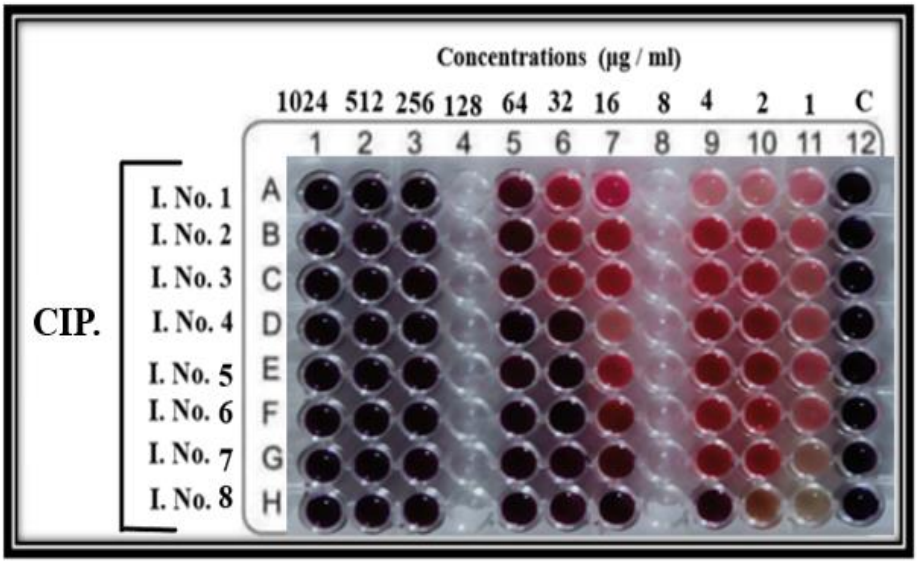

Figure 2. The MIC of ciprofloxacin against P. aeruginosa.

Detection of Efflux Pump-Expression in the presence of Cipro only and expression in the presence of Cipro + Et. Br

The EtBr-agar cartwheel (EtBrCW) method is a practical methodology to assess efflux activity in large collections of clinical isolates of different bacterial species [4,12]. This method allows the comparison of different isolates based on their capacity to extrude EtBr. The isolates are streaked in solid media containing increasing concentrations of EtBr and the fluorescence emitted, which is inversely proportional to their capacity to extrude the compound, is compared to the fluorescence of control strains. Using this approach to test a collection of 32, 64, and 128 µg/ml ciprofloxacin-resistant P. aeruginosa. The current study could discriminate ten isolates into three distinct groups: a group of isolates at the highest 128 µg/ml ciprofloxacin concentration tested with five concentrations of Et.Br. 5, 10, 15, 20, 25 µg/ml; a group of isolates at the highest 64 µg/ml ciprofloxacin concentration tested with same concentrations of Et.Br.; and a third a group of isolates at the highest 32 µg/ml ciprofloxacin concentration tested with same concentrations of Et.Br. The detection of the Efflux pump mechanism was performed using the EtBr-agar cartwheel (EtBrCW) method in MHA plates by using ciprofloxacin (with 3 concentrations 32, 64, 128) and EtBr stain (five concentrations with each CIP concentration). The EtBr-agar cartwheel screening method showed efflux activity in 10 MDR strains. Table 1 shows the results of EtBr-agar cartwheel methods, and Figure 3 shows the fluorescence of strains because EtBr, which the MexAB pumps out and MexXY-OprM, becomes fluorescent when it enters the cells and binds to nucleic acid [14].

Isolate No.	Conc. of CIP (32)	Control	Conc. of EtBr 5	Conc. of EtBr 10	Conc. of EtBr 15	Conc. of EtBr 15	Conc. of EtBr 20	Conc. of EtBr 25
1	+ ve	Grow	- ve	- ve	+ ve	+ ve	+ ve	+ ve
2	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
3	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
4	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
5	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
6	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
7	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
8	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
9	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
10	+ ve	Grow	- ve	+ ve	+ ve	+ ve	+ ve	+ ve

Table 1. Efflux Activity at Varying Concentrations of Ethidium Bromide and Ciprofloxacin (32).

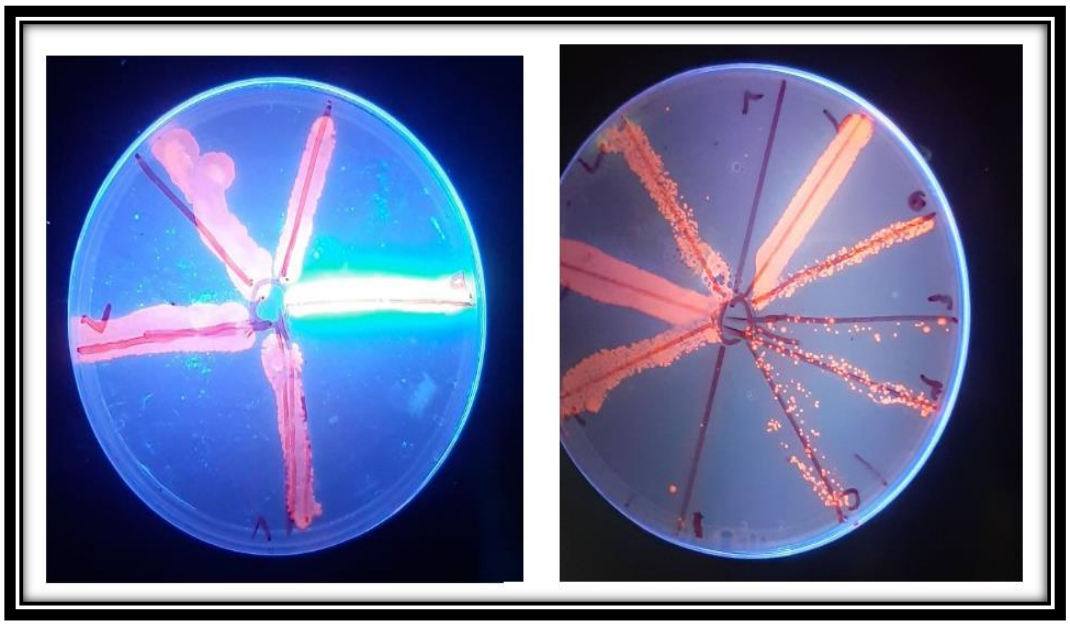

Figure 3. Characterization of reference and clinical isolates according to their efflux capacity.

Fluorescence of P. aeruginosa strains on agar plates containing increasing concentrations of EtBr. Cultures were swabbed in TSA plates containing increasing concentrations of EtBr. Following overnight incubation at 37˚C for 18 hours, fluorescence was detected under UV light.

Results of Efflux Pump Expression

Results of Housekeeping Gene Expression

Analysis of RNA expression using techniques like real-time PCR has traditionally used reference or housekeeping genes to control for error between samples [15]. The current results show an amplification curve indicating the occurrence of P. aeruginosa rpsL housekeeping gene was obtained in samples. Figure 4 (A and B) represents the Ct curve of the rpsL gene. RpsL Ct results ranged from 10.03 to 12.13, averaging 11.14.

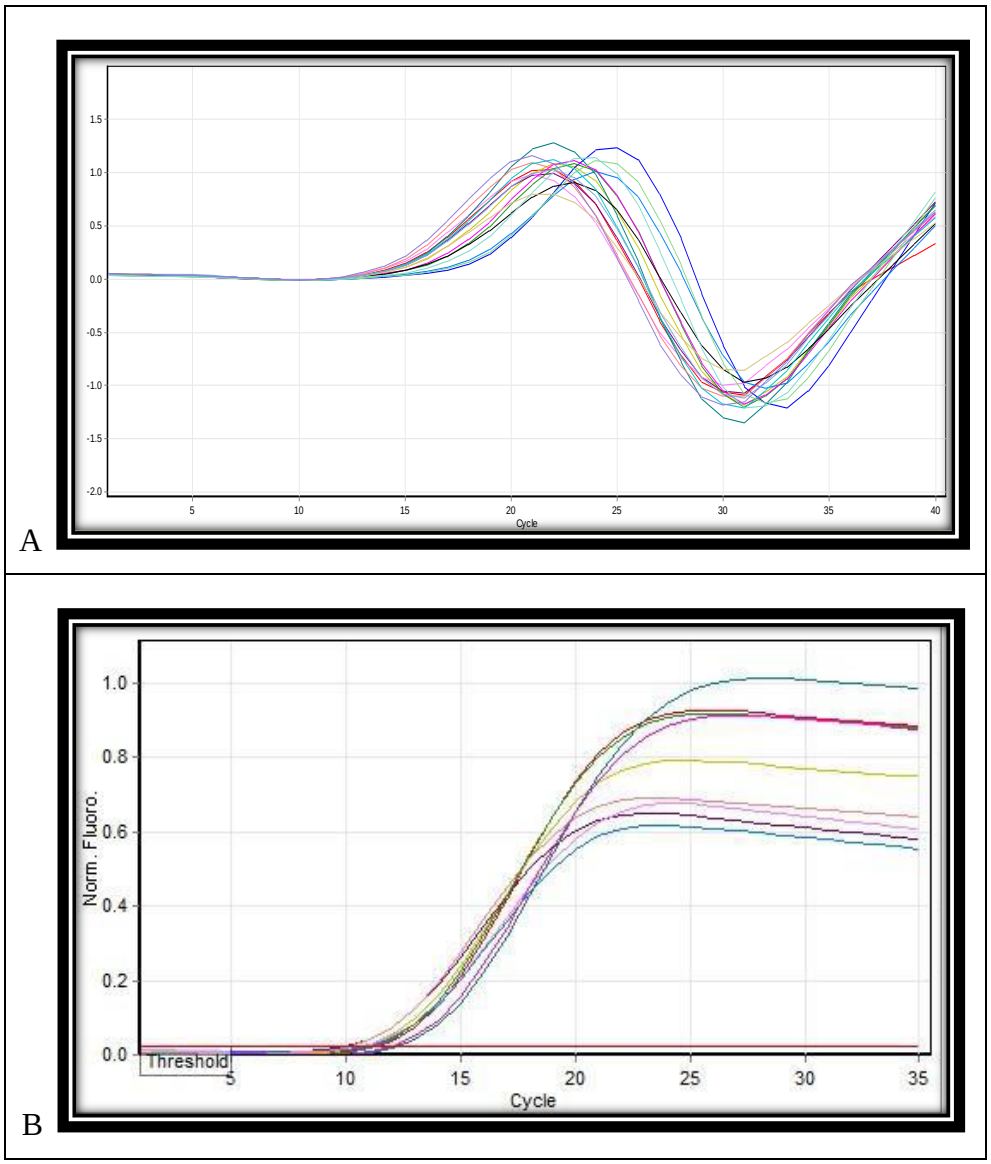

Figure 4. A: Comparative Quantitation Analysis of rpsl gene of P. aeruginosa. B: Amplification Curve of rpsl Gene

Housekeeping genes are usually chosen as internal controls to normalize real-time RT-PCR data [16]. One of the most commonly used housekeeping genes in the companion of gene expression data of P. aeruginosa is the rpsL gene. The results of our study are similar to Naser and Aubaid's, [17] studies which studied the expression of seven genes (mexA and mexB) by using RT-PCR when they applied the rpsL gene as a reference gene in P. aeruginosa, Friyah and Rasheed, [18] applied the rpsL gene as a reference gene in P. aeruginosa. The inherent assumption in using housekeeping genes in molecular studies is that their expression remains constant in the cells or tissue under investigation [19].

Results of mexA Gene Expression

Results of mean Ct values ranged from 32.1 to 36.79, with an average of 34.33, showing expression compared to the rpsL (Ct = 11.14) reference gene, as shown in Figures 5 and 6.

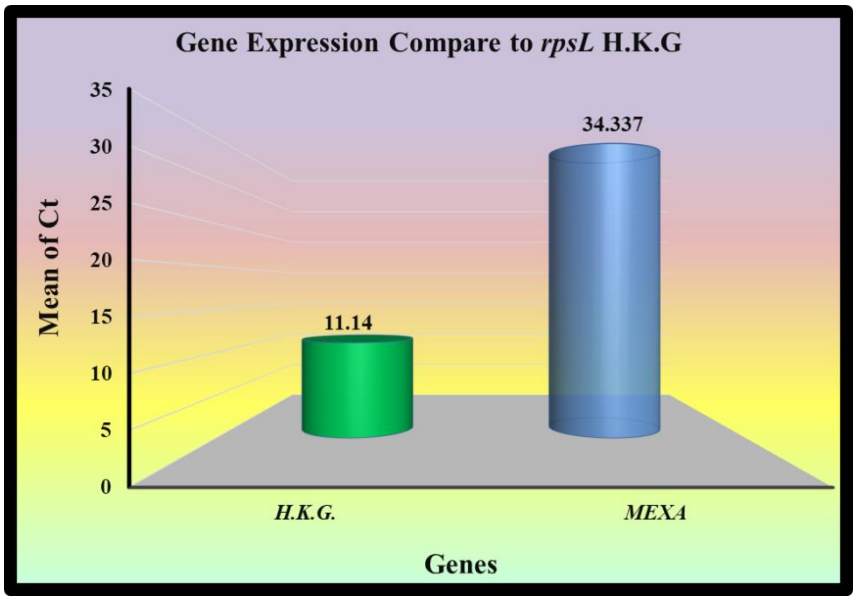

Figure 5. Mean of Ct Values of mexA Gene Compare to rpsL Reference Gene.

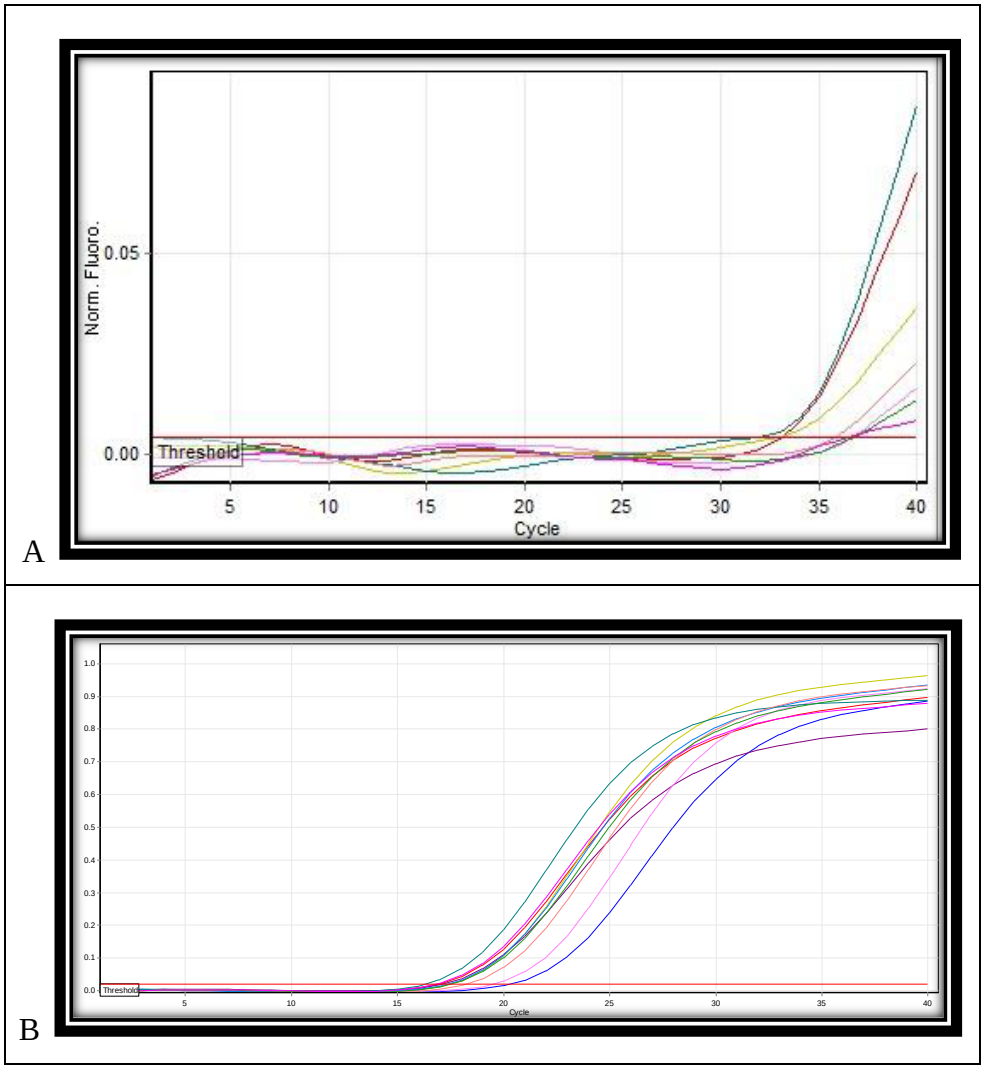

Figure 6. A: Comparative Quantitation Analysis of mexA gene of P. aeruginosa. B: Amplification Curve of mexA Gene

Results of mexB Gene Expression

Results of Gene Expression of Efflux Pump in the presence of Cipro only and expression in Cipro + Et. Br

The gene expression of three groups (first was non-treated control, which was the most effective isolates to CIP; second was treated with CIP 32 µg/mL + Et.Br. 25 µg/mL, and third CIP 64 µg/mL) was used to identify the effect of CIP and Et.Br. on gene expression of mexA and mix genes. The results show that the effectiveness of isolates was reduced compared to non-treated isolates. The minimum inhibitory concentrations (MICs) of antibiotics are typically at least twice as high for strains of a species that overexpress efflux pumps compared to strains of the same species that do not overexpress efflux pumps. This test is a useful tool for detecting efflux pump overexpression, which is one factor that contributes to multidrug cross-resistance in bacterial populations. Overexpression of efflux pumps was a potential contributing factor in developing antibiotic resistance in P. aeruginosa isolates [20].

Results of mexA Gene

Figure 9 shows that the mean of Ct of gene expression was noticed when treated with Et.Br. + CIP and CIP in mexA were 37.195 and 34.702, respectively, compared to control 34.337. This may explain why both treatments, compared to the control, did not induce the expression of mexA genes; on the other hand, the gene expression between both treatments was expressed in the treatment of CIP 32 compared to CIP+Et.Br., table 2 shows the Ct values and folding values of treated and control isolates.

Groups	Means Ct of mexA	Means Ct of rpsl	ΔCt (Means Ct of mexA - Means Ct of rpsl)	2-ΔCt	experimental group/ Control group	Fold of gene expression
Group 3 Eth	36.695	11.140	25.555	0.2 E-7	0.2 E-7/1.0 E-7	0.201
Group 2 Cip.	34.702	11.120	23.582	0.8 E-7	0.8 E-7/1.0 E-7	0.787
Group 1 control	34.337	11.100	23.237	1.0 E-7	1.0 E-7/1.0 E-7	1.000

Table 2. Folding Value of Gene expression for gene mexA Δct.

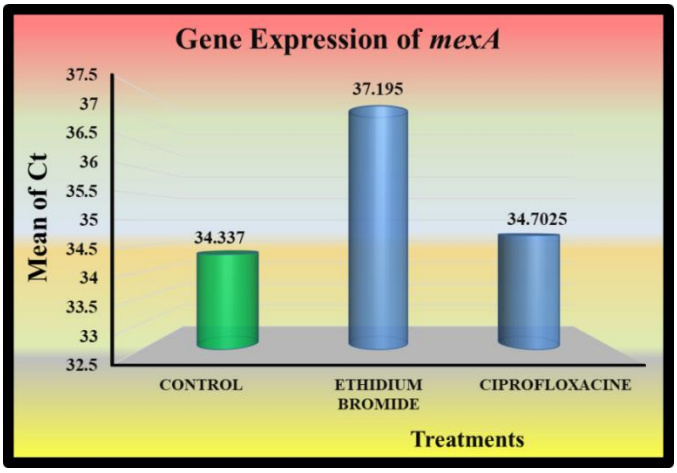

Figure 9. Results of mexA Gene Expression in the presence of Cipro only and in the presence of Cipro + Et.Br.

Results of mexB Gene

Figure 10 showed that expression was noticed when treated with Et.Br. + CIP and CIP in mexB gene expression was 19.626 and 18.872, respectively, compared to control 16.791; this may explain why both treatments compared to control did not induce the expression of mexB genes; on the other hand, the gene expression between both treatments was expressed in treatment of CIP 32 compare to CIP+Et.Br., table 3 shows the Ct values and folding values of treated and control isolates.

Groups	Means Ct of mexB	Means Ct of rpsl	ΔCt (Means Ct of mexB - Means Ct of rpsl)	2-ΔCt	experimental group/ Control group	Fold of gene expression
Group 3 Eth	19.626	11.140	8.486	0.0028	0.0028/0.0194	0.144
Group 2 Cip.	18.872	11.120	7.752	0.0046	0.0046/0.0194	0.240
Group 1 control	16.791	11.100	5.691	0.0194	0.0194/0.0194	1.000

Table 3. Folding Value of Gene expression for gene mexB Δct.

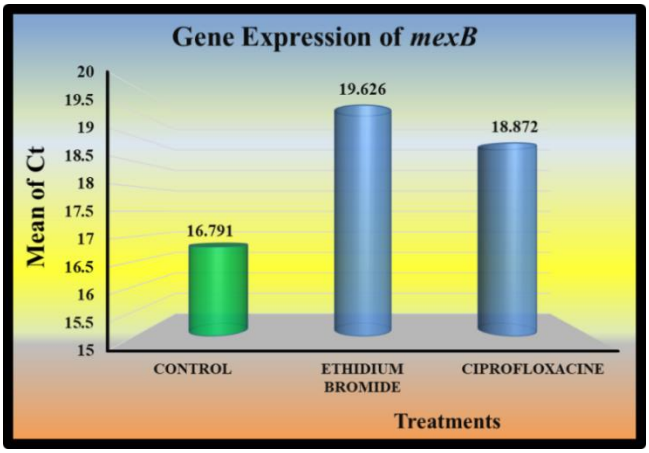

Figure 10. Results of mexB Gene Expression in the presence of Cipro only and in the presence of Cipro + Et.Br.

DISCUSSION

The fact that P. aeruginosa is capable of living in wet environments while requiring only a few nutrients and can resist antibacterial agents and disinfectants can be found in a wide variety of environments. Furthermore, because it can live in hospital environments and on medications, it risks patient care [21]. On the other hand, these abilities allow these bacteria to spread. The current results agreed with a local study conducted by Abed, [2], which concluded that isolates were distributed as follows: 46% from burns swab samples, 30% from wounds swab samples, and 14% from ear discharge. These results are also compatible with a local study conducted by Wtwt and Jarallah, [22]. According to Kareem [23], the development of mechanistic drug resistance in Gram-negative bacteria is greatly influenced by efflux systems. These systems pump dissolved compounds out of the cell, which enables bacteria to alter their internal environment by eliminating potentially harmful substances. These substances include metabolites, antimicrobial agents, and quorum-sensing signal molecules. According to the latest research results, multidrug-resistant Gram-negative bacteria in Iraq have developed a mechanism of drug resistance

mediated by efflux pumps. In order to pump antibiotics out of the cell, Gram-negative bacteria rely on protein assemblies composed of three parts. The tripartite complex is composed of three distinct proteins: an inner membrane protein (IMP) that is a member of the resistance nodulation cell division (RND) family, an outer-membrane protein (OMP), and a periplasmic membrane fusion protein (MFP) that connects the other two proteins. The IMP is inside the inner membrane, while the OMP is on the outer membrane. In therapeutically relevant multidrug-resistant Gram-negative bacteria, there is a significant incidence of drug resistance mediated by efflux transporters [24, 25]. Dorri et al. 26 concluded that mexA was detected in all isolates, and Abed 2 concluded that mexA was detected in 100% (20 isolates) of P. aeruginosa isolates. P. aeruginosa's overproduction of mexAB-oprM plays a crucial role in the generation of multidrug-resistant strains since periplasmic membrane fusion proteins (mexA, MFP, mexX, mexC, and mexF) are components of the RND-type efflux pump system, Antimicrobial drug resistance to quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, and beta-lactams has been linked to the MexAB-OprM efflux system [27]. The current study agreed with a local study by Dorri et al., [26], who concluded that mexB was detected in all isolates. Researchers have established that efflux-mediated resistance exists in many bacterial families. It was discovered before by several researchers that increased expression of an efflux mechanism is responsible for less antibiotic being accumulated in the organism. P. aeruginosa's Mex efflux pumps are particularly relevant because they have an unusually wide substrate specificity. Even though the genome of P. aeruginosa has been found to contain 12 potential members of this family of efflux systems [4,28]. Furthermore, the function of an efflux mechanism in fluoroquinolone-resistant clinical isolates has been described for multiple species of bacteria. This active efflux is caused by multidrug resistance (MDR) pumps that transport foreign compound molecules. Some examples of these chemicals include many antibiotics and antiseptics, as well as cationic dyes like ethidium bromide (EtBr) and acriflavine [29]. Gene expression of all CIP-treated isolates was not overexpressed; this may related to the presence of carbonyl cyanide 3-chlorophenylhydrazone (CCCP) as an efflux pump inhibitor, CCCP is a well-known proton motive force inhibitor efflux pump and it is usually be added to Mueller-Hinton agar while it is being prepared [30], the usage of CCCP as a screening agent in order to determine: first) the prevalence of efflux pump overexpression among multidrug-resistant isolates of P. aeruginosa; second) the contribution of efflux pump overexpression as the supposed mechanism for the multidrug cross-resistance between beta-lactams, fluoroquinolones, and aminoglycosides in P. aeruginosa; and third) the MIC reduction of beta-lactam, fluoroquinolones, and aminoglycosides in a stimulus way with an efflux pump inhibitor [20]. For ethidium efflux studies, the fluorescence intensity and CCCP did not impact the EtBr efflux in the strain [4]. This may explain why there was expression in the CIP 32 treatment compared to the CIP+Et.Br. treatment in all investigated genes (mexA, mexX, mexB, oprD and oprM). When a mutant strain of P. aeruginosa that lacked its principal multidrug efflux pump complex, MexAB-OprM, was cultured with 100 microM ethidium bromide, the fluorescence that was induced by its binding to DNA upon its entry into cells gradually decreased, as reported by Xu et al., [31]. The scientists concluded that the internal ethidium bromide "stimulated" either the breakdown of the ethidium bromide or its outflow through the assembly of undiscovered efflux pumps. Because these efflux pumps have a broad substrate specificity and can extrude many different antibiotic classes, such as B-lactams, quinolones, and aminoglycosides, it could be a reason to approve the hypothesis that the fluoroquinolones (FQ) resistance among P. aeruginosa isolated from clinical isolates, particularly from burn wound infections, could be in cooperation with resistance to other existing antipseudomonal agents through overexpression of [32]. The widespread usage of FQ

agents is probably one of the primary reasons that P. aeruginosa, isolated from burn wound infections, has developed resistance to FQ drugs. Because of this, extensive use of FQ drugs has a detrimental side effect that contributes to the susceptibility of P. aeruginosa to other antipseudomonal medicines through the FQ-selected up-regulation of multidrug efflux pumps [33]. Many different bacterial families have been reported to possess efflux-mediated resistance. Increased expression of an efflux mechanism is responsible for less antibiotic accumulation in the organism. P. aeruginosa's Mex efflux pumps are particularly relevant because of their unusually broad substrate specificity [2,4].

CONCLUSIONS

The MIC ciprofloxacin concentration ranged from 8 – 256 µg/ml, which can inhibit growth to isolates. The detection of the Efflux pump mechanism by using the EtBr-agar cartwheel showed that ciprofloxacin concentration 32 µg/mL and Ethidium Bromide concentration 25 µg/mL were the concentrations that showed fluorescence of P. aeruginosa strains in medium, overexpression was a notice in the expression of genes compared to control

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