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REVIEW ARTICLE

Expression of aph(3')-IIb and Aminoglycoside Efflux Pump Regulatory Genes in Clinical Extensive Drug Resistant *Pseudomonas aeruginosa.*

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Abstract:

Background:A remarkable level of aminoglycosides resistance expressed by Pseudomonas aeruginosa is well known. One of the most important mechanisms of aminoglycoside resistance is over expression of efflux pumps and aminoglycoside modifying enzymes.

Aim of Study: To evaluate the expression of aph(3')-IIb and MexXY-OprM Pump Regulatory Genes in XDR Pseudomonas aeruginosa in Al-Diwaniyah city.

Methods: During the period from November 2021 to August 2022, 50 clinical P. aeruginosa isolated from hospitalized patients admitted to Al-Diwaniyah Burn Center and Al-Diwaniyah Teaching Hospital including 25(31.3%) burns, 15(22.7%) wounds, and 10(18.5%) diabetic foot ulcers. The vitek2 compact system was employed for identification and antibiotic susceptibility test of P. aeruginosa. The isolates were submitted to PCR and qRT-PCR for aph(3')-IIb, mexZ, and parR detection and gene expression.

Results: Out of 50 P. aeruginosa, 22(44%) XDR and 9(18%) MDR isolates were detected. All XDR isolates were resistance to all tested antibiotics except one. Aminoglycoside resistance was exhibited in 100% of XDR isolates phenotypically. All isolates (100%) harbored ph(3')-IIb, mexZ, and parR genes. The qRT-PCR results showed significant overexpression of aph(3')-IIb and parR, while mexZ revealed significant low expression in XDR P. aeruginosa compared with susceptible P. aeruginosa isolates.

Conclusion: High frequencies of XDR P. aeruginosa from various clinical sources, which further restricts the range of available treatments. The predominant mechanisms of aminoglycosides resistance exhibited by XDR P. aeruginosa in this region are overexpression of efflux pumps, and aminoglycoside modifying enzyme, aph(3')-IIb.

Keyword: XDR P. aeruginosa, aminoglycoside modifying enzymes, aph (3')-IIb, MexXY- OprM Pump, Aminoglycoside resistance.

Introduction

Pseudomonas aeruginosa, a metabolically adaptable bacterium, is known to cause a variety of opportunistic infections and is acknowledged as one of the significant causes of severe hospital-acquired infections (1).

Pseudomonas aeruginosa is an opportunistic pathogen that can cause an abundance of acute and chronic life-threatening infections in immune compromised patients. These infections are associated with a elevated mortality and morbidity incidence (2). Due of P.aeruginosa's capacity to withstand several of the currently available antibiotics, treating infections caused by this bacterium has become extremely difficult.

A several antibiotics like aminoglycosides, quinolones, and lactams that P. aeruginosa is resistant to. Intrinsic, acquired, and adaptive resistance are the three main mechanisms that P. aeruginosa uses to counter antibiotic attack (3).

Aminoglycosides as broad-spectrum antibiotics have a vital aspect in clinical settings , where they are used to treat life-threatening Gram-negative bacterial infections acquired in the hospital (4).

Pseudomonas aeruginosa infections are commonly treated with aminoglycosides. Resistance to aminoglycosides is often linked to the presence of genes encoding aminoglycoside-modifying enzymes (AMEs), which can be transmitted between organisms by horizontal gene transfer. Inducing active antibiotic efflux into the extracellular space is an established mechanism of adaptive resistance (5).

There are four efflux pump systems in P. aeruginosa that are of great interest to researchers: MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM. Each of these systems is

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named after a mexicillin resistance gene (6).

It has been demonstrated that the MexXY-OprM efflux pump plays a key part in the development of resistance to fluoroquinolones and aminoglycosides, both of which are important antibiotic classes in the treatment of serious infections caused by P. aeruginosa (7).

MexZ, a repressor protein, regulates the expression of mexXY genes (8). Increased expression of the MexXY/OprM efflux system and decreased sensitivity to AGs have been linked to mutations in mexZ found often in clinical isolates (9).

Concern regarding the spread of aminoglycoside and other antimicrobial resistance genes in P. aeruginosa clinical isolates through horizontal gene transfer has expanded in recent years (10). The aminoglycoside-modifying enzymes (AMEs) that are encoded by acquired resistance genes inactivate aminoglycoside antibiotics by catalyzing modifications at the hydroxy or amino groups of the 2-deoxy streptamine sugar moieties. These modifications can be phosphorylation via aminoglycoside phosphoryltransferases (Aph), acetylation via aminoglycoside acetyltransferases (Aac), or adenylation via aminoglycoside nucleotidyltransferases/adenylyltransferase (Ant) (11). Because the genes for these modifying enzymes are frequently found on mobile genetic elements like plasmids, transposons, integrons, insertion sequences, phages, and integrative and conjugative elements they have the capacity to spread between and among species (12).

Acquisition of an AME was strongly correlated to overexpression of MexXY aminoglycoside efflux pump (13).

Materials and Methods

A total of 200 clinical samples were obtained from patients, including 80 burns, 66 wounds, and 54 diabetic feet ulcers.. Between November 2021 and August 2022, patients were admitted to Al-Diwaniyah Teaching Hospital, Feminine and Children Teaching Hospital, and Al-Diwaniyah Burn Center. Pseudomonas aeruginosa isolates identified by Viteck2 compact system (Biomerieux, France ,Cardtype:GN,ID-N222).

The Vitek2 compact (Biomerieux, France, Card type: GN, AST-N222) was used to assess the antibiotic susceptibility of P. aeruginosa isolates. Each isolate was tested against 14 antibiotic agents from 7 different antibiotic classes. All results were interpreted and all isolates were categorized as susceptible, intermediate, or resistant to each antibiotic tested in accordance with Clinical and Laboratory Standards Institute 2021 criteria (14).

The multiple antibiotic resistance (MAR) index for the P. aeruginosa isolates was determined using the findings of the susceptibility testing in order to evaluate antibiotic resistance trends and the development of new resistant isolates. Each isolate's MAR index was calculated by dividing the total number of antibiotics tested by the number of antibiotics to which the isolate was resistant (15). If the MAR index is more than 0.2, then it is confirmed that this antibiotic was widely used in the setting where this isolate firstly emerged.

As recommended by the manufacturer guideline for the Genomic DNA purification kit (Geneaid, USA), genomic DNA was isolated from P. aeruginosa isolates. Each isolate's DNA purity and concentration were assessed using a Nanodrop device (THERMO, USA).

Polymerase chain reaction (PCR) employed to screen of the presence of the aminoglycosides modifying enzyme gene: aph(3')-IIb and MexXY efflux pump regulatory genes : mexZ and parR. The PCR thermocycling conditions and specific primers were showed in Table (1) and Table (2), respectively.

Table (1) The PCR thermocycling conditions protocol

Gene	PCR Amplicon (bp)	Annealing Temp./time
aph(3')-IIb	813	56 °C / 30 sec.
mexZ	883	56 °C / 30 sec.
ParR	881	56 °C / 30 sec.

Table (2): Primers

Gene		Primer Sequence	Amplicon size(bp)	Reference
aph(3')-IIb	F	ATGCATGATGCAGCCACCTCCAT	813	(16)
	R	CCTACTCTAGAAGAACTCGTCCA		
mexZ	F	TATGATCTGCGGCGCCTTTC	883	(17)
	R	TTCGGAACAAGGCGTCTGCA		
parR	F	ATCTCGAACGAGTCGCTGGAG	881	
	R	GTAGAACGCGTCGATGACATGG		
16S rRNA	F	AGAGTTTGATCMTGGCTCAG	355	(18)
	R	GCTGCCTCCCGTAGGAGT		

Total RNA were extracted from XDR and susceptible P. aeruginosa isolates by using (TransZol Up Plus RNA kit). Nanodrop was used to assess the extracted RNA, measuring RNA concentration (ng/L) and RNA purity by detecting the absorbance at 260/280 nm. The cDNA synthesis was carried out in accordance with manufacturer's instructions utilizing the extracted RNA samples and the SolGent DiaStarTM RT Kit to synthesis cDNA from mRNA transcripts. The Go TaqR qPCR Master Mix Kit was used to prepare qRT-PCR master mix (Table 3) and RT-PCR thermocycler conditions (Table 4).

Table (3) qRT-PCR Master Mix for target genes and housekeeping genes

No.	qPCR master mix	Volum
1	cDNA template (10ng)	5 µL
2	Forward primer (10pmol)	1 µL
3	Reverse primer (10pmol)	1 µL
4	qPCR master mix	10 µL
5	CXR dye	0.3 µL
6	Nuclease free water	2.7 µL
Total		20 µL

Table (4) qRT-PCR thermocycler conditions

qPCR step	Temperature	Time	cycle
Initial denaturation	95 °C	10 min.	1
Denaturation	95 °C	20 sec.	40
Annealing	58 °C	30 sec.	
Extension	58 °C	30 sec.	
Melting	65-95 °C		1

Using the Livak approach, which was presented by (19) as the following equations, the data analysis of qRT-PCR for target genes and housekeeping genes were collected:

$$\triangle CT\ (Test) = CT\ (target\ gene,\ test) - CT\ (HKG,\ test)$$

$$\triangle CT\ (Control) = CT\ (target\ gene,\ control) - CT\ (HKG,\ control)$$

$$\triangle\triangle CT = \triangle CT\ (Test) - \triangle CT\ (Control)$$

$$Fold\ change\ (target/HKG) = 2^{-CT}\ \triangle\triangle CT$$

Statistical Analysis

The Statistical Package for Social Sciences version 23 for Windows software, and Microsoft Excel 2010 were used to conduct the statistical study.

Results

Of the 200 clinical samples (80 burns, 66 wounds, and 54 diabetic foot ulcers) from hospitalized patients, 136 resulted in positive bacterial cultures, and out of these just 50 (36.8%) P.aeruginosa isolates have been identified in 25 (43.1%) burns, 15 (37.5%) wounds, and 10 (26.3%) DFU (Figure 1).

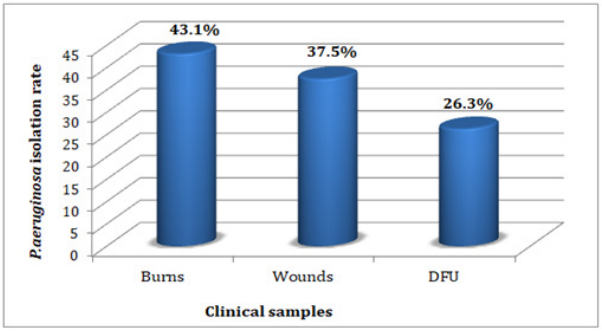

Figure (1):Distribution rate of 50 Pseudomonas aeruginosa isolates from various clinical samples

Antibiotic susceptibility testing on the collected isolates revealed variable patterns of resistance (Figure 2) and (Table 5). In order to describe patterns of multiple drug resistance, isolates were classified as MDR or XDR, according to (20) 9/50 (18.0%) isolates of P. aeruginosa were determined to meet the criteria for MDR P. aeruginosa. Additionally, 22/50 (44.0%) of the isolates were classified as XDR (isolates non-susceptible to at least one drug in all but two or fewer anti-pseudomonal antimicrobial categories (Figure3). More significantly, 21/50 (42.0%) of them were resistant to all 14 antibiotics that were examined in the current study, which included 7 categories.

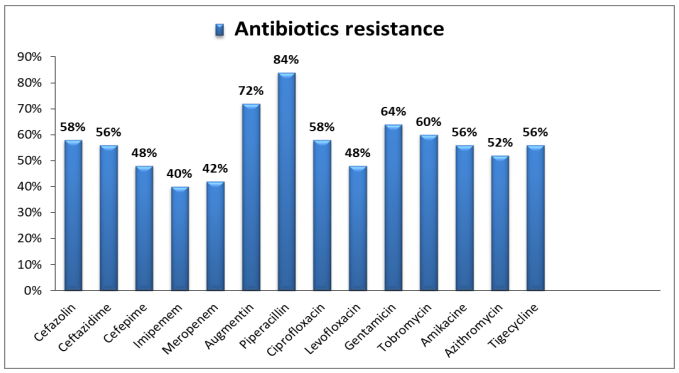

Figure (2):Antibiotics susceptibility patterns of 50 Pseudomonas aeruginosa isolates by Vitek2 compact.

Table (5) Antibiotic Susceptibility patterns of MDR and XDR P. aeruginosa from different clinical sources

Isolate No.	Source of isolate	Antibiotic Susceptibility patterns	Resistance Type
Pa1	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa2	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa3	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa4	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa5	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa6	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa7	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa8	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa9	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa10	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa11	Burn	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa12	Burn	CFZ, CAZ, CEF, AUG, CIP, LEV, GEN, AK	MDR
Pa14	Burn	CAZ, AUG, PI, GEN, TOB	MDR
Pa15	Burn	AUG, GEN, AK, AZM	MDR
Pa18	Burn	CFZ, IPM, AUG, PI, CIP, GEN	MDR
Pa20	Burn	CFZ, PI, GEN, TOB, AK, TGC	MDR
Pa24	Burn	CFZ, CAZ, AUG, PI, GEN, TOB, AK, AZM	MDR
Pa26	Wound	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa27	Wound	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa28	Wound	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa29	Wound	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa30	Wound	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa31	Wound	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa34	Wound	PI, CIP, GEN, TOB, AZM	MDR
Pa38	Wound	CFZ, CAZ, AUG, PI, CIP, GEN, TOB	MDR
Pa41	DFU	CFZ, CAZ, CEF, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa42	DFU	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa43	DFU	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa44	DFU	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa45	DFU	CFZ, CAZ, CEF, IPM, MER, AUG, PI, CIP, LEV, GEN, TOB, AK, AZM, TGC	XDR
Pa46	DFU	AUG, PI, CIP, GEN, TOB, AK	MDR

CFZ: Cefazolin, CAZ: Ceftazidime, CEF: Cefepime, IMP: Imipemem, MER: Meropenem, AUG: Augmentin, PI: Piperacillin, CIP: Ciprofloxacin, LEV: Levofloxacin, GEN: Gentamicin, TOB: Tobramycin, AK: Amikacin, AZM: Azithromycin , TGC: Tigecycline, Pa: Pseudomonas aeruginosa, XDR: Extensive drug resistant, MDR: Multi-drug resistant, DFU: Diabetic foot ulcer.

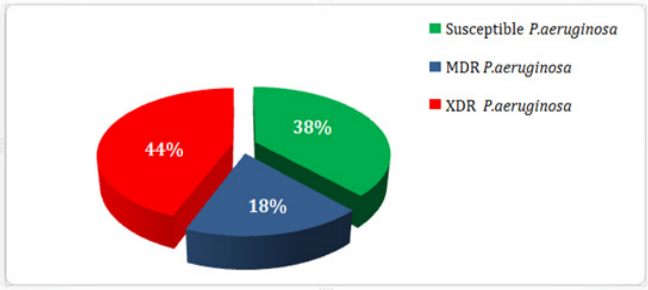

Figure (3) The distribution rates of antibiotics susceptibility patterns of Pseudomonas aeruginosa isolates from different clinical sources.

The findings of this study revealed that all 22 XDR P. aeruginosa isolates were 100% resistant to the aminoglycoside drugs tested gentamicin, amikacin, and tobramycin. Also, MDR isolates exhibited resistance at a rate of 100%, 66.6%, and 55.5% to gentamicin, amikacin, and tobramycin, respectively (Figure 4).

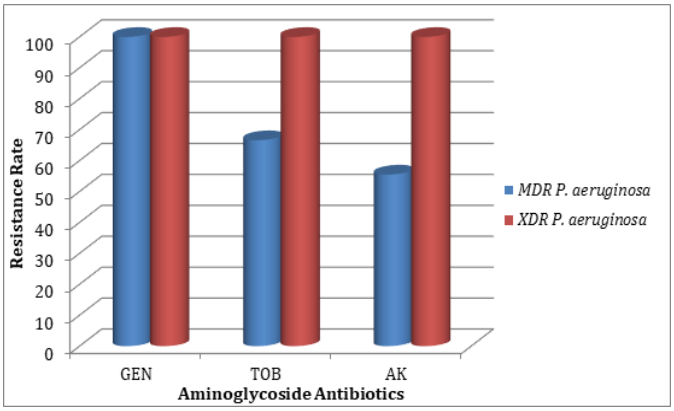

Figure (4) Aminoglycoside Resistance Rates in MDR and XDR P. aeruginosa isolates

The MAR index findings of current study determined considerable variances with the lowest MAR index being 0.28 and the highest MAR index being 1.0. The MAR indices that were greater than 0.2 were found in 100% (31/31) of the resistant isolates ranged from 0.28 to 1.0 with mean ± SD 0.830 ± 0.26, while MAR indices that were less than 0.2 were found in 0% (0/31) of the resistant isolates. This indicates that all resistant isolates are most likely to have originated from a high risk source (Table 6).

Table (6): Multiple antibiotic resistance (MAR) indices of resistant P. aeruginosa isolated from different clinical sources

No. of antibiotics that an isolate is resistant to (n=14)	No. of resistant isolates (n=31)	MAR index	No. of isolates in clinical samples			X^2	P value
			Burn	Wound	DFU		
4	1	0.28	1(100)	0(0)	0(0)	3	0.22
5	2	0.35	1(50)	1(50)	0(0)	1.5	0.47
6	3	0.42	2(66.6)	0(0)	1(33.3)	3	0.22
7	1	0.5	0(0)	1(100)	0(0)	3	0.22
8	2	0.57	2(100)	0(0)	0(0)	6	0.05
12	1	0.85	0(0)	0(0)	1(100)	3	0.22
14	21	1	11	6	4	5.57	0.062
The proportion of isolates with MAR index ≥ 0.2 was 31/31 (100%)							
The mean MAR index for these isolates was (mean± SD) 0.830±0.26							
The proportion of isolates with MAR index less than 0.2 was 0/31(0%)							

All XDR and Susceptible P. aeruginosa isolates harbored aph(3')-IIb, parR, and mexZ genes as PCR revealed (Figure 5, 6, and 7).

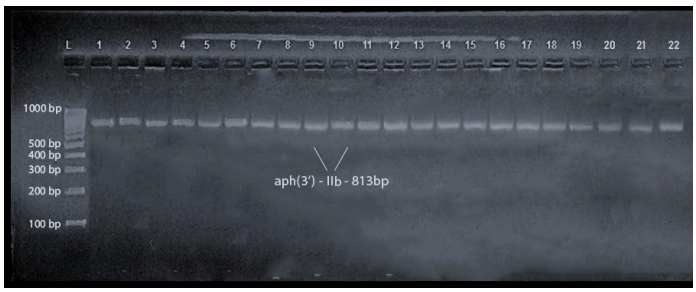

Figure (5): Ethidium bromide stained agarose gel electrophoresis (1.5%) of PCR amplified products of aminoglycosides resistance aph(3')-IIb gene for 1 hr. at 70 volt. Lane L: DNA marker (1500-100)bp. Lane(1-22): Extensive multidrug resistance Pseudomonas aeruginosa isolates that showed positive aph(3')-IIb gene at .813bp PCR product size

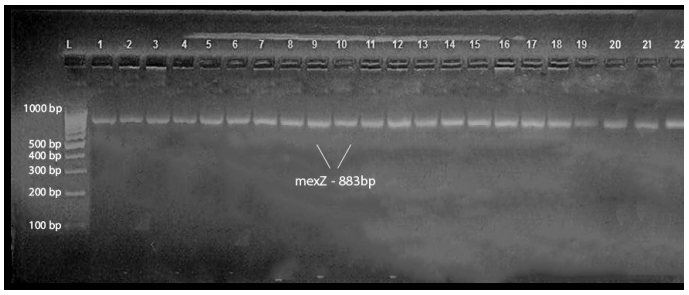

Figure (6): Ethidium bromide stained agarose gel electrophoresis (1.5%) of PCR amplified products of aminoglycosides

resistancemexZ gene for 1 hr. at 70 volt. Lane L: DNA marker (1500-100)bp. Lane(1-22): Extensive multidrug resistance Pseudomonas aeruginosa isolates that showed positive mexZ gene at 883bp PCR product size.

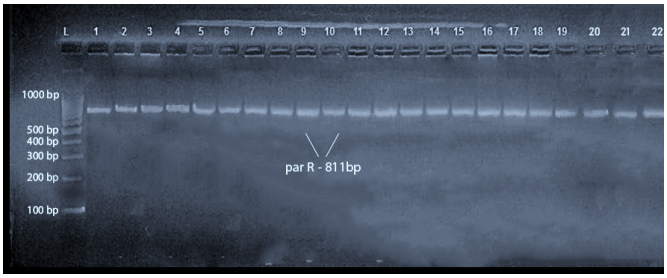

Figure (7): Ethidium bromide stained agarose gel electrophoresis (1.5%) of PCR amplified products of aminoglycosides resistance parR gene for 1 hr. at 70 volt. Lane L: DNA marker (1500-100) bp. Lane(1-22): Extensive multidrug resistance Pseudomonas aeruginosa isolates that showed positive parR gene at 881bp PCR product size.

The expression of aph(3')-IIb was significantly higher in the XDR P. aeruginosa isolates than in the control group consisting of antibiotic susceptible P. aeruginosa isolates (2.90±1.87and 1.049±0.40, P = 0.0, respectively). The expression of parR was significantly higher in the XDR P. aeruginosa isolates than in the control group consisting of antibiotic-susceptible P. aeruginosa isolates (5.376±3.27and 1.239±0.69, P = 0.0, respectively). While the expression of mexZ was significantly lower in the XDR P. aeruginosa isolates than in the control group consisting of antibiotic-susceptible P. aeruginosa isolates (0.280±0.98 and 1±0, P = 0.029, respectively). (Table 7), (Figure 8).

Table 7: Relative expression of Aminoglycoside resistance genes in XDR and susceptible P. aeruginosa

Gene	(Fold Change (Mean±SD		T-tese	P-value
	Susceptible P. aeruginosa	XDR P. aeruginosa		
aph(3')-IIb	1.049±0.40	2.90±1.87	4.426	*0
parR	1.239±0.69	5.376±3.27	4.410	*0
mexZ	1±0	0.280±0.98	2.289	*0.029

* Significant difference at P<0.05

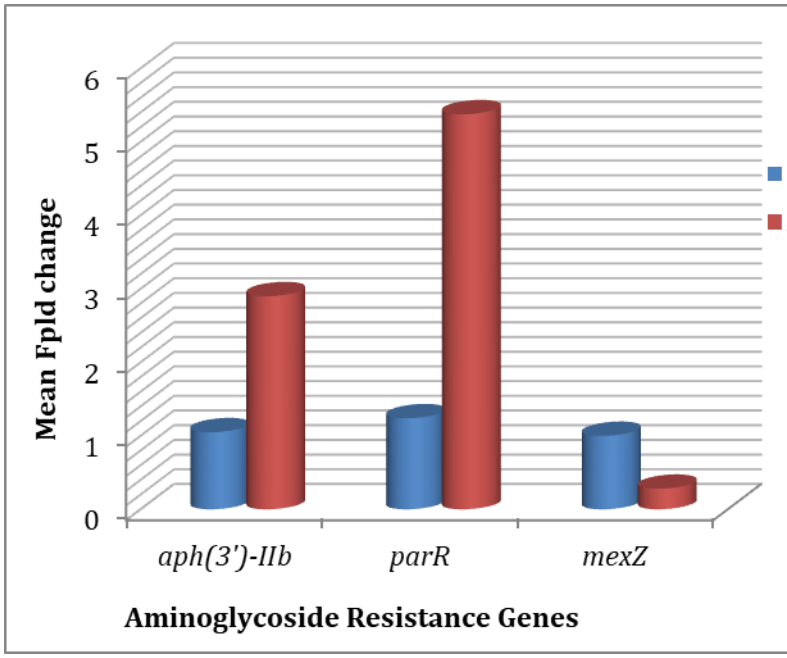

Figure 8: Fold change of aph(3')-IIb, parR, and mexZ expression

Discussion

Pseudomonas aeruginosa is a common Gram-negative opportunistic bacterium that emerged in healthcare-acquired infections and immune-compromised individuals . Most significantly, it has a tendency resistant to antibiotics via a diversity of resistance mechanisms (21). *Pseudomonas aeruginosa* is a major cause of morbidity and mortality among hospitalized patients It has the ability for spreading systemically from localized infections at various body sites causing sepsis which is the most significant cause of mortality, particularly in burned patients (1, 22).

The MAR index has been shown to be reliable, valid, and affordable tool for tracing the origins of antibiotic-resistant bacteria (23). *P. aeruginosa* isolates with a MAR score of 0.2 or above are thought to have originated from a high-risk source where several antibiotics are routinely used (24).

One of the most concerning results of this study is that 22(44.0%) of 50 *P. aeruginosa* isolates displayed an XDR phenotype. This worryingly high rate of XDR further reduces available treatment options. This study's finding may be attributed to the widespread misuse of antibiotics in Iraq. Recent reports of a high prevalence of XDR *P. aeruginosa* in Iraq (25, 26) and elsewhere around the world (27, 28, 29) provide cause for concern.

Clinical XDR-PA isolates have complicated antibiotic resistance pathways. Several lines of evidence point to horizontal gene transfer and/or mutational resistance

as common causes of multidrug resistance (30). Conventional antibiotics are ineffective against P. aeruginosa infections due to the diversity of antibiotic resistance pathways, which in turn promotes to the emergence of diverse antibiotic resistant isolates (3).

In recent years, Iraq has been considered among the countries that reported high rates of antimicrobial resistance (31, 32, 33, 34). In addition, studies report a large increase (more than 50%) in antimicrobial resistance for P. aeruginosa across many Arab countries (35).

Aminoglycosides are frequent treatment of P. aeruginosa infections, nevertheless, this bacteria has developed an extensive repertoire of resistance mechanisms against this antibiotic category. Isolates possess aminoglycoside modifying enzymes (AMEs), which are classified into three basic families: phosphorylators (APH), acetylators (AAC), and adenylators (ANT). These enzymes are responsible for the inactivation of aminoglycosides (36).

High rates of resistance to aminoglycosides have been reported in the current study, with 56 percent of the isolates being resistant to amikacin, 58 percent to gentamicin, and 60 percent to tobramycin. Ciprofloxacin (with a 58% resistance rate) and levofloxacin (with a 48% resistance rate) are two examples of the fluoroquinolones antibacterial class. The findings of this study on the in vitro efficacy of amikacin against P. aeruginosa isolates are in line with those found by (37, 38). In contrast, (39) found that amikacin has a high susceptibility rate of 92.6% against P.aeruginosa, making it one of the most effective therapies for this pathogen.

In this study, it was found aph(3')-IIb, mexZ, and parR were 100% present in the XDR P. aeruginosa isolates. Some Iraqi studies in searched for the aph(3')-VI gene in the local isolates, and they obtained rates 87.5% and 25% (26, 40), while (41) did not record this gene in any of the obtained isolates.

Aminoglycoside 3'-phosphotransferases (APH(3')s) are most significant bacterial resistance enzymes to aminoglycoside antibiotics. The antibiotics' biological activity is destroyed as a result of these enzymes' transfer of the gamma-phosphoryl group of ATP to the 3'-hydroxyl of the drug (42). The presence of aph(3')-IIb on the P. aeruginosa transposon causes it to rapidly propagate in their population. As a result, the options of antibiotics that can cure P. aeruginosa infections become more limited. And as a regrettable consequence, it results in a health threat, particularly to the community that resides within hospitals.

All 22 of the extremely drug-resistant P. aeruginosa isolates and one of the susceptible ones included the mexZ gene, which functions as a repressor of the MexYX efflux pump. However, mexZ expression was significantly lower in XDR P. aeruginosa isolates than in antibiotic-susceptible isolates. Antibiotic resistance in these XDR P. aeruginosa isolates may result in part from their over-expression of an efflux pump, particularly MexXY-OprM. Since the 1980s (43). researchers have linked drug-efflux pumps and bacterial resistance. Overexpression of efflux pumps (especially the MexXY-OprM complex) is a major mechanism of aminoglycoside resistance. Therefore, it is possible that this efflux pump contributes to the development of multi-drug resistance in bacteria.

The ParR is a single component of the ParRS sensor, which consists of two individual parts. By controlling efflux components and porins, it imparts resistance to polycationic antibiotics such aminoglycoside and peptide antibiotics as well as naturally occurring polyamines.

The parR gene encodes a response regulator for the ParRS two-component system, which stimulates the MexXY efflux system and OprD porin loss upon induction or constitutive mexXY overexpression (44).

In general, these bacteria will employ a wide variety of strategies to circumvent the effects of any antibiotic. Studies conducted in the immediate area monitored these facts (45, 46).

In consequence of antibiotics resistance rise, the majority of the therapies that are now in use are no longer effective. Because of this, it is necessary to study the efficacy of drug combinations, alternative strategies or potential vaccinations to combat multi-drug resistant isolates .Fortunately, there are local studies that have dealt with this regard (34, 26, 47).

Conclusion

Antibiotic resistance is on the alarming rise, leading to an upward trend in extensively antibiotic resistant P. aeruginosa rates that necessitate urgent intervention.

Overexpression of the efflux pump MexXY and the presence of the aminoglycoside modifying enzyme Aminoglycoside 3'-phosphotransferases (APH(3') were revealed to be two of the key mechanisms responsible for aminoglycosides resistance in this study.

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