How to Cite:
Mohammed Saleh, M. A. A.- jabber, & Naji, H. F. (2022). Detection of blatem, blactx-m, and blashv genes in clinical isolates of multidrug-resistant pseudomonas aeruginosa. *International Journal of Health Sciences*, *6*(S7), 3239–3253. https://doi.org/10.53730/ijhs.v6nS7.12451

Detection of bla_{tem}, $bla_{ctx\text{-}m}$, and bla_{shv} genes in clinical isolates of multidrug-resistant pseudomonas aeruginosa

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Abstract---Objectives: Extended spectrum β-lactamase (ESBLs), which are produced by multidrug-resistant (MDR) *Pseudomonas aeruginosa*, are a critical problem that demands efficient infection management strategies to break their spread. The prevalence of clinical isolates varies widely around the world and changes fast over time. The objectives of this research are to determine the spread of ESBLs (*bla*$_{TEM}$, *bla*$_{CTX\ M}$, and *bla*$_{SHV}$) genes among the isolates of *P. aeruginosa.* and to find the antibiotic susceptibility among these isolates which reflect the rate and severity of pathogenicity of these bacteria in Iraq. Methods: one hundred and fifty clinical specimens were collected from wound, burn and diabetic foot swabs of patients hospitalized in Babylon and Baghdad hospitals. *P. aeruginosa* isolates were identified based on their morphological features (culturally and microscopically), biochemical tests, Vitek 2 compact, PCR-sequencing for the *P. aeruginosa*-specific gene and the antibiotic susceptibility tests were also performed. Results: Forty-six isolates of *P. aeruginosa* were isolated and identified, these isolates were resistant to tobramycin, piperacillin, cefepime, imipenem, ofloxacin, aztreonam and netilmicin, (100%). The lowest resistance of *P. aeruginosa* isolates was to ciprofloxacin (43 %). PCR experiments showed that *P. aeruginosa* have *bla*$_{TEM}$ 27/46 (58.69%), *bla*$_{SHV}$ 29/46 (63%), and *bla*$_{CTX\text{-}M}$ 31/46 (67.39%). Conclusion: The development of antimicrobial resistance in *P. aeruginosa* is a significant issue affecting people worldwide and is an unintended consequence of current medical practices. Thus, there is a need for studying how to control multidrug-resistant *P. aeruginosa* to decrease the rate and the severity of infections.

Keywords---*P. aeruginosa*, ESBLs *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX\text{-}M}$ genes, MDR.

Introduction

P. aeruginosa is a prevalent Gram-negative opportunistic bacterium in burn and wound units, it is consider one of the most common nosocomial pathogens (Crone *et al.,* 2020; Suwaidan and Naji, 2020). Burn victims with (MDR) *P. aeruginosa* infections are difficult to treat (Safaei *et al.*, 2017; Salimi *et al.*, 2010). The growing incidence of MDR strains is connected to longer hospitalization and increased mortality and morbidity (Chauhan *et al.*, 2012). *P. aeruginosa* can develop antibiotic resistance through site-targeted drug modification, outer membrane modification, β-lactamases production, and efflux pumps. Antibiotic resistance is rising due to the frequent use of antibiotics in burn units, a lack of alternatives, and their high prices (Ali *et al.*, 2020). β-lactamases-resistant *P. aeruginosa*, includes ESBL, AmpC β-lactamases, and metalo β-lactamases, have risen due to transposable genetic elements that promote resistance. β-lactamases are A, B, D, and C. MBLs (metallo β-lactamases) are a class B mechanism, unlike A, C, and D. (de Almeida *et al.*, 2017). Some microorganisms, like *P. aeruginosa*, produce ESBLs, which hydrolyze antimicrobial medicines and cause resistance (Nasser *et al.*, 2020). *SHV*, *CTX-M*, and *TEM* are *SHV*-family ESBL genes discovered in *P. aeruginosa*. *TEM*-1 was the first plasmid-mediated β-lactamases found in the early 1960s (Datta and Kontomichalou, 1965). *TEM*-1 is the most studied Gram-negative class A enzyme. Gram-negative bacilli's most common β-lactam resistance mechanism is spreading (Eiamphungporn *et al.*, 2018; Seyedjavadi *et al.*, 2016). *TEM* genes are resistant to most medications, including penicillin and cephaloridine (bla_{TEM}). Amino acid changes have led to new, more potent antibiotic resistance (ESBL) (Peymani *et al.*, 2017). *Klebsiella pneumonia* evolved *SHV*-type β-lactamases as chromosomally encoded enzymes Livermore, (1995). The first ESBL found in 1985 was *SHV*-2, which differed from *SHV*-1 by a single amino acid change from glycine to serine at position 238. (Huletsky *et al.*, 1993). In the late 1980s, *CTX-M* type-lactamase enzymes emerged. *CTX-M* was first used in a German publication (MMasoud *et al.*, 2022). *CTX-M*-1, *CTX-M*-2, *CTX-M*-8, *CTX-M*-9, and *CTX-M*-25 have different amino acid identities (Bonnet, 2004). Hence, this study was taken to focus on the detection of some antibiotic resistant genes in clinical isolate of MDR *P. aeruginosa*.

I. Materials and Methods

Collection of Samples

A total of 150 samples from wound, diabetic foot and burn swabs of patients were collected. These patients hospitalized in Babylon hospitals (Al-Hillah Teaching Hospital and Imam AL-Sadiq Teaching Hospital) and also in Baghdad hospitals (Burns Specialized Hospital, Martyr Ghazi Hariri Hospital, Baghdad Teaching Hospital, and National Center for Educational Laboratories) of both genders with different ages. The specimens were collected during the period from September 2021 to January 2022 Table 1.

Table 4.4 Distribution of isolated bacteria with their percentages in the collected specimens

Specimen type	Positive for *P. aeruginosa*		Negative for *P. aeruginosa*		Total		P value
	No.	%	No.		No.	%	
Injuries	17	21	65	79	82	100	0.103
diabetic foot	6	46	7	54	13	100	
Burns	23	42	32	58	55	100	
Total	46	31	104	69	150	100	

Isolation and Identification

All specimens were cultivated on blood agar, MacConkey agar (Himedia), and cetrimide agar (Himedia) using a sterile loop and incubated at 37 C° for 24 hr (Al-Ahmadi and Roodsari., 2016). Pure colonies in glycerol-containing nutrient broth (Himedia) at 4 C°. (Jawetz *et al.*, 2019). The shape, size, texture, and colony organization of bacteria grown on MacConkey (Himedia), blood (Himedia), cetrimide (Himedia), and nutrient agar (Himedia) were studied. A single colony was Gram-stained and studied under a 100x oil-emersion light microscope (Al-Ahmadi and Roodsari, 2016). The isolates were identified depending on morphological properties (for cells and colonies) and biochemical testes as described by MacFaddin, (2000).

DNA Extraction

Whole genomic DNA was extracted according to manufacturer standard for *P. aeruginosa* molecular identification (Favorgen, Taiwan).

PCR primers and Conditions

PCR cycling thermal program parameters were used in this reaction for detection of *P. aeruginosa* specific, bla_{TEM}, bla_{CTX-M}, and bla_{SHV} genes. The Macrogen (Korea) manufactured of PCR primers used in this work are shown in Table 1.

Table 1. PCR primers and their conditions used in this study, Macrogen (Korea).

Primer		Sequence (5----------›3)	Amplicon size (bp)	Conditions (D,A and E)	Cycle No.	Source
TEM	F	GAGTATTCAACATT CCGTGTC	861	94˚C/1 min 57˚C/1 min 72˚C/2 min	35	(Bokaeian *et al*., 2015)
	R	TAATCAGTGAGGCACCTATCTC				
SHV	F	AAGATCCACTATCGCCAGCAG	231	94˚C/30sec 64˚C/1 min	35	
	R	ATTCAGTTCCGTTTCCCAGCGG				

				72˚C/2 min		
CTX-m1	F	GACGATGTCACTGGCTGAGC	499	94˚C/60sec 57˚C/60sec 72˚C/60sec	35	
	R	AGCCGCCGACGCTAATACA				
P.aeru	F	GGGGGATCTTCGGACCTCA	956	95˚C/60sec 61˚C/45sec 72˚C/60sec	35	(Spilker et al., 2004)
	R	TCCTTAGAGTGCCCACCCG				

Abbreviations: D, denaturation; A, annealing; E, extension; F, forward primer; R, Reverse primer.

Preparation of Reaction Mixture

The mixture reaction was performed in 12 µl of PCR Pre Mix (Bioneer, South Korea), the total volume of the reaction was 25 µl consisting of 2 µl from each primer forward and reverse, 3 µl of DNA, and the volume completed up to 6 µl with free nucleases deionized water according to the company's instructions and reaction buffer mixed, negative control contains all the above contents without DNA template was also used. The amplification reactions were performed in an automated thermos cycler apparatus (Clever Scientific, UK). as demonstrated in Table 2.

Table 2. Preparation of PCR Mixture

Contents of the mixing reaction	**Volume**
Master Mix	**12 µl**
DNA Template	**3 µl**
Forward primer (10 pmol/µl)	**2 µl**
Reverse primer (10 pmol/µl)	**2 µl**
Nuclease free water	**6 µl**
Total volume	**25 µl**

Agarose Gel Electrophoresis

PCR products were run on 1% agarose gel dyed with 5ml Red Safe for 1 hour at 75 volts. The gel well was loaded with 5 L of amplification products and 1 L of loading dye. The 100-1500 bp DNA marker (Promega, USA) was employed to measure amplified gene electrophoresis fragments. The DNA bands were imaged by gel documentation system (Biometra-Germany) (Lin *et al.*, 2012).

Antibiotic Susceptibility Test

The phenotypic identification of ESBL was done using the disk diffusion test. Antibiotics administered included ceftazidime (30 g), tobramycin (10 g), amikacin (30 g), netilmicin (30g), ciprofloxacin (5 g), tetracycline (5 g), amoxicillin (30 g), cefotaxime (30 g), imipenem (10 g), and aztreonam (30 g), meropenem (10g), Pip (10g). *P. aeruginosa* was determined to be resistant or sensitive based on the zone of inhibition according to the criteria of the Clinical and Laboratory Standard Institute CLSI (2021) (Wi *et al.*, 2017).

II. Results and Discussion

Isolation and Identification

The present study was conducted on 46 (named PA1 to PA2) screened isolates of *P. aeruginosa*, obtained from one hundred and fifty clinical specimens such as burns, injuries and feet of diabetics who had contracted the infection, where 23 (50%) were isolated from burns, 17 (37 %) from injuries, and 6 (13 %) feet of diabetics.

The identification of these isolates was accomplished through the use of culture and microscopic exams, biochemical tests, the Vitek 2 compact, and finally, confirmation was achieved via PCR-sequencing for a *P. aeruginosa* unique gene. MacConkey agar colonies were pale because they couldn't ferment lactose sugar. It smelled like fermented grapes. On blood agar, the colonies were black and most had a translucent halo, indicating they could hemolysis red blood cells (Jawetz, *et al.* 2019), on Nutrient agar, *P. aeruginosa* colonies were recognized based on pigments and odor generation (grape-like odor), with greenish colonies (DeBritto *et al.*, 2020). while the colonies of *P. aeruginosa* appeared on chromogenic agar in greenish blue color as depicted in Figure (1).

The Gram stain films of these isolating cultures revealed single or bi-arrangement. Gram-negative bacilli. All *P. aeruginosa* isolates have shown a positive result in biochemical tests for oxidase, catalase tests, and pigment (blue water-soluble pigment pyocyanin, yellow-green pyoverdine,) (Barbhaiya and Rao, 1985; El-Fouly *et al.*, 2015), and the urease test. IMVIC tests have yielded negative results, although isolates have shown positive results for citrate testing, despite negative results for other tests (C). Because Kligler iron agar is strictly aerobic, Gram's stain negative, alkaline, and does not affect the bottom, H2S is positive with gas production (Behbahani *et al.*, 2019)**.** Results obtained were compared with specifications in 21st edition of Bergey's Manual of Systematic Bacteriology.

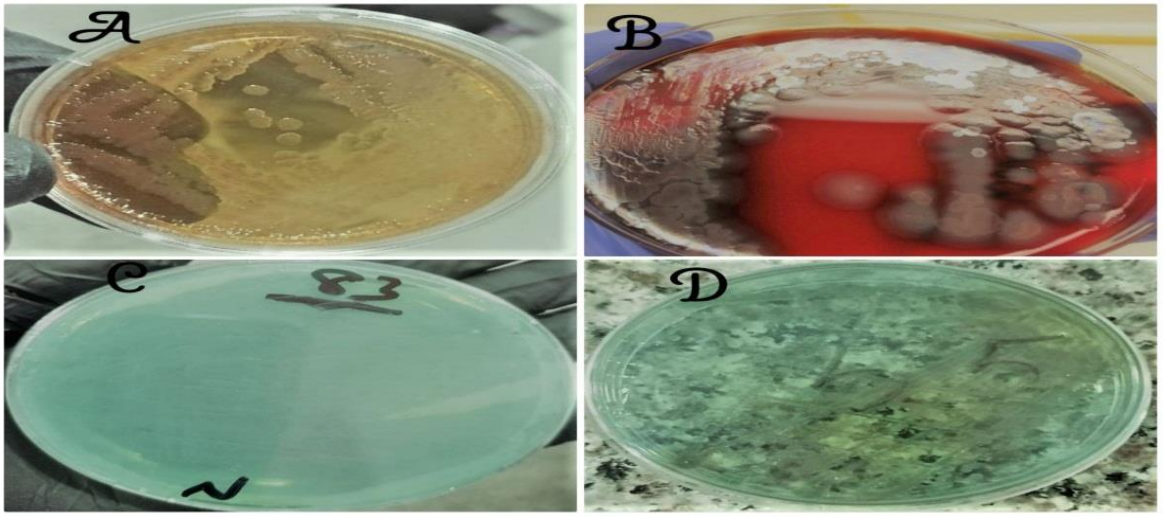
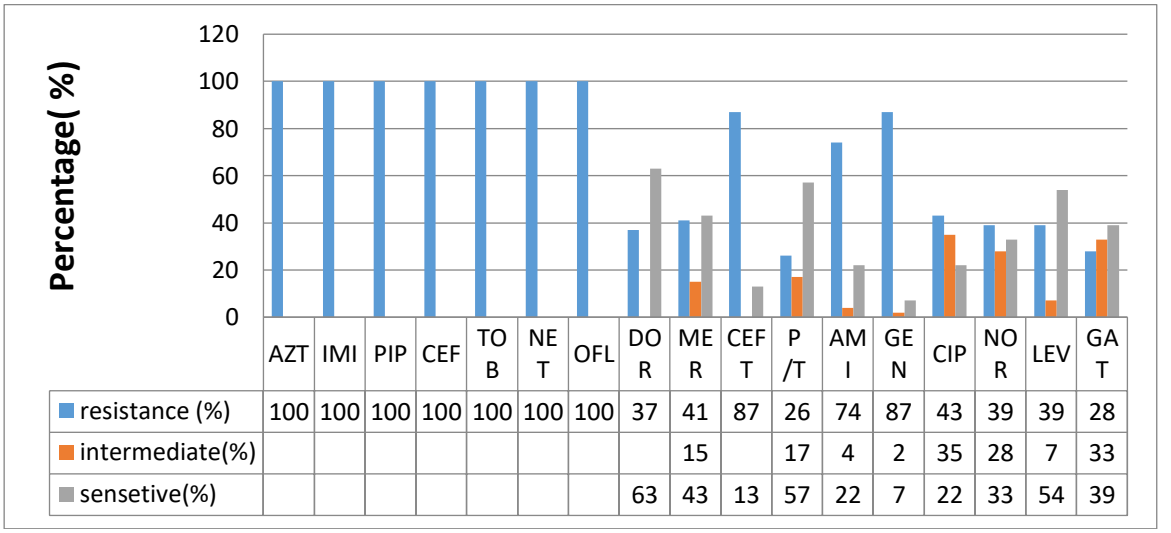


Figure 1. *P. aeruginosa* colonies on (A) MaCconky agar and (B) Blood agar, (C) Nutrient agar and (D) chromogenic agar.

The Antibiotic Susceptibility Testing

It was shown in Figure 2, as observed, Forty-six identified *P. aeruginosa* isolates (PA1 to PA46) were evaluated against 19 common antibiotics. The majority of isolates exhibited antibiotic resistance., particularly β-lactamase antibiotics. All 46 isolates of *P. aeruginosa* test were resistant to tobramycin, piperacillin, cefepime, imipenem, ofloxacin, aztreonam, and netilmicin. The isolate PA26 was resistant to all antibiotics except for meropenem and norfloxacin, however, isolate PA2 was responsive to the majority of antibiotics**.** Antibiotic such as the tobramycin, piperacillin, cefepime, imipenem, ofloxacin, aztreonam, and netilmicin all exhibited 100 percent resistance, whereas norfloxacin (39 %), piperacillin-tazobactam (26 %), levofloxacin (39 %), amikacin (74 %), meropenem (41 %), ciprofloxacin (43 %), doripenem (37 %)**.**



	AZT	IMI	PIP	CEF	TOB	NET	OFL	DOR	MER	CEFT	P/T	AMI	GEN	CIP	NOR	LEV	GAT
resistance (%)	100	100	100	100	100	100	100	37	41	87	26	74	87	43	39	39	28
intermediate(%)									15		17	4	2	35	28	7	33
sensetive(%)								63	43	13	57	22	7	22	33	54	39

Figure.2. Susceptibility patterns of *P. aeruginosa* to different antibiotics used in current study.

PCR Assay Results

As shown in Table 1, the β-lactamase genes varied among the isolates of *P. aeruginosa* (PA1 to PA46). the results of PCR amplification products of *β*-lactamases genes showed that 31 (67.9%) isolates carried *bla*$_{CTX-M}$ at 499 bp (Figure 3), 29 (63%) isolates at 231 bp (Figure 4) had bla$_{SHV}$, and 27 (58.69%) isolates at 861 bp (Figure 5) had *bla*$_{TEM.}$ There were 16 isolates (34.78%) possessed *bla*$_{TEM}$, bla$_{CTX-M}$ and bla$_{SHV}$ genes. the *bla*$_{CTX-M}$ and bla$_{SHV}$ genes were identified in 7 (15.21%) isolate, and also *bla*$_{CTX-M}$ and bla$_{TEM}$ genes were identified in 5 isolates (10.86%) whereas 7 isolates (PA10, PA39, PA41, PA42, PA43, PA44, PA45, and PA46) lacked of the three genes. the isolates PA5, PA23, and PA40 were found to have *CTX-M* gene, whereas the PA17 and PA29 isolates were found to possess *SHV* gen, on the other hand, the PA21 and PA31 isolates have *TEM* gene. the distribution of (*SHV, TEM and CTX-M*) Genes of *P. aeruginosa*. the distribution of (*SHV, TEM and CTX-M*) Genes of *P. aeruginosa* in current study show in table 2. PCR amplification revealed the presence and lack of β-lactamase genes in several isolated samples.

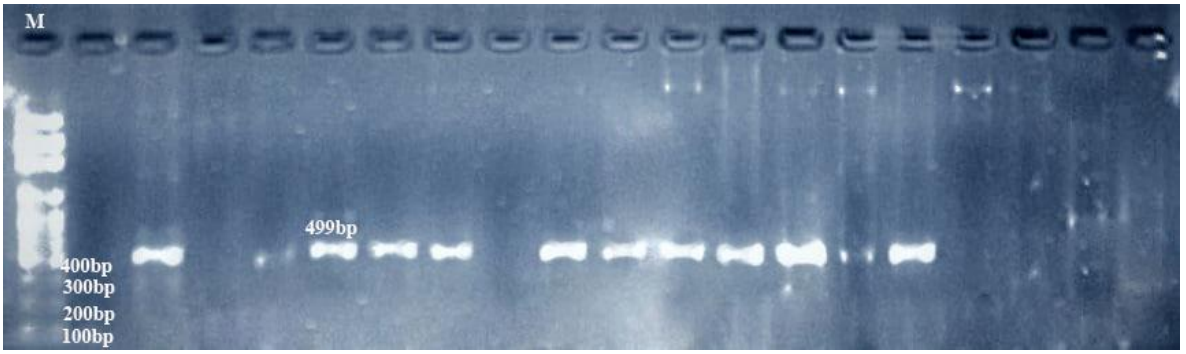


Figure 3: The presence and absences of bla$_{CTX-M}$ PCR product (499bp) in some isolated samples.

PCR products were separated by electrophoresis in an 2% agarose gel, at 75 V\Cm for 80 min. M: Marker DNA ladder size (100bp).

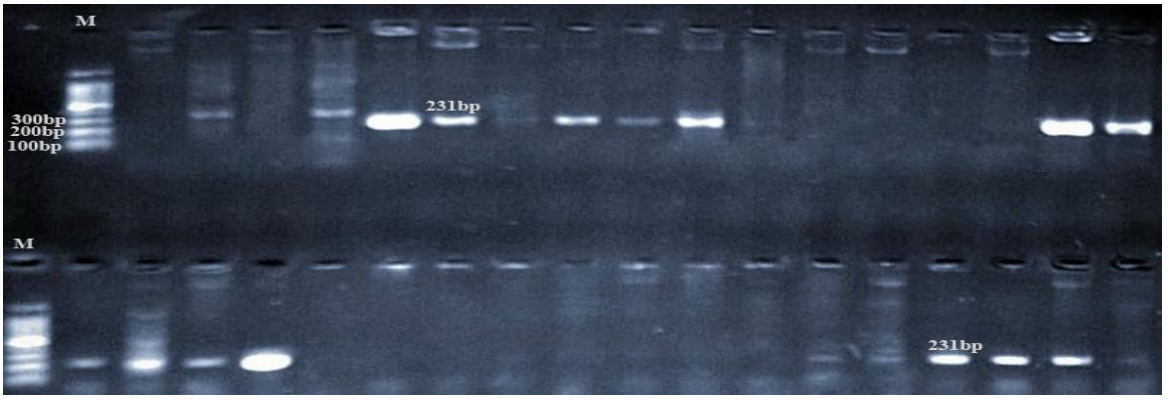


Figure 4: The presence and absences of bla$_{SHV}$ PCR product (231bp) in some isolated samples.

PCR products were separated by electrophoresis in an 2% agarose gel, at 75 V\Cm for 80 min. M: Marker DNA ladder size (100bp).

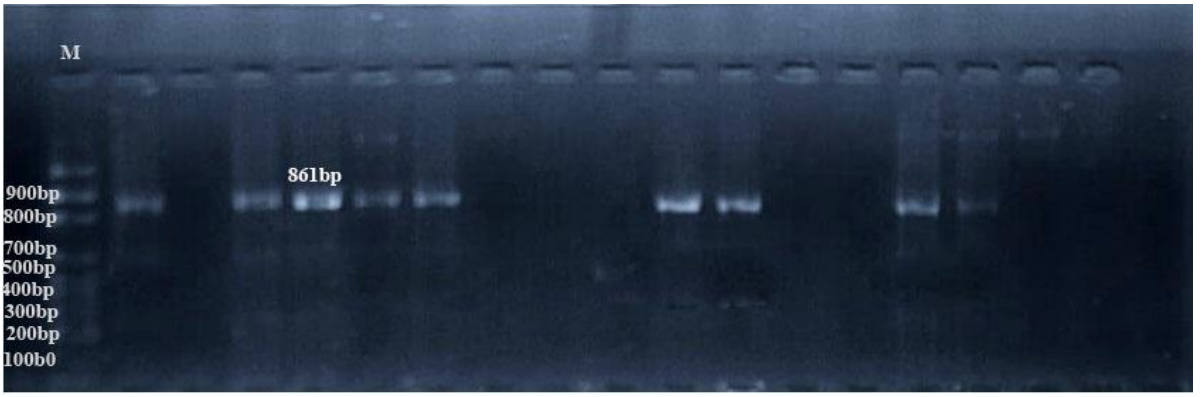


Figure 5: The presence and absences of bla*TEM* PCR product (861bp) in some isolated samples.

PCR products were separated by electrophoresis in an 2% agarose gel, at 75 V\Cm for 80 min. M: Marker DNA ladder size (100bp).

The Identification of the antibiotic-resistant isolates that carried bla*TEM*, bla*SHV*, and bla*CTX-M* genes in the current study, showed an association between antibiotic resistance and positive molecular detections for the bla*TEM*, bla*SHV*, and bla*CTX-M* genes, which were detected in *P. aeruginosa* isolated when ESBLs type β-lactamase were produced employing antibiotic resistance. Table 3 showed the highest resistance isolates of *P. aeruginosa* to different antibiotics. the isolate with bla*CTX-M* gene, followed by bla*SHV* and bla*TEM*, (NO.24) had the highest resistance rates to most antibiotics. On the other hand, the isolates that carried the three resistance genes (bla*TEM*, bla*SHV*, and *blaCTX-M*) revealed more excellent resistance to seven antibiotics with 100% percent as showed in Table 3.

Table 3: Distribution of gene group of *P. aeruginosa* isolates.

Isolate No.	SHV Gen	TEM Gen	CTX-M Gen	Genotype
PA1	-ve	+ve	+ve	*TEM and CTX-M*
PA2	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA3	-ve	+ve	+ve	*TEM and CTX-M*
PA4	+ve	+ve	-ve	*SHV and TEM*
PA5	-ve	-ve	+ve	*CTX-M*
PA6	+ve	+ve	-ve	*SHV and TEM*
PA7	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA8	-ve	+ve	+ve	*TEM and CTX-M*
PA9	+ve	-ve	+ve	*SHV and CTX-M*
PA10	-ve	-ve	-ve	*None*
PA11	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA12	**-ve**	+ve	+ve	*TEM and CTX-M*
PA13	+ve	-ve	+ve	*SHV and CTX-M*
PA14	+ve	-ve	+ve	*SHV and CTX-M*
PA15	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA16	+ve	+ve	+ve	*SHV,TEM and CTX-M*

PA17	+ve	-ve	-ve	*SHV*
PA18	+ve	-ve	+ve	*SHV and CTX-M*
PA19	+ve	-ve	+ve	*SHV and CTX-M*
PA20	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA21	-ve	+ve	-ve	*TEM*
PA22	+ve	-ve	+ve	*SHV and CTX-M*
PA23	-ve	-ve	+ve	*CTX-M*
PA24	-ve	+ve	+ve	*TEM and CTX-M*
PA25	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA26	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA27	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA28	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA29	+ve	-ve	-ve	*SHV*
PA30	+ve	+ve	-ve	*SHV and TEM*
PA31	-ve	+ve	-ve	*TEM*
PA32	+ve	-ve	+ve	*SHV and CTX-M*
PA33	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA34	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA35	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA36	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA37	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA38	+ve	+ve	+ve	*SHV,TEM and CTX-M*
PA39	-ve	-ve	-ve	None
PA40	-ve	-ve	+ve	*CTX-M*
PA41	+ve	-ve	-ve	None
PA42	-ve	-ve	-ve	None
PA43	-ve	-ve	-ve	None
PA44	-ve	-ve	-ve	None
PA45	-ve	-ve	-ve	None
PA46	-ve	-ve	-ve	None

Abbreviation: +ve: gene possessing., -ve: gene lacking

Table 4. Distribution of bla*SHV*, bla*TEM* and bla*CTX-M* Genes of *P. aeruginosa*.

Gene	Isolate. No	%
SHV	2	4.35
TEM	2	4.35
CTX-M	3	6.5
SHV and TEM	3	6.52
SHV and CTX-M	7	15
TEM and CTX-M	5	10.9
SHV,TEM and CTX-M	16	34.7
None	8	17.

Table 5 Pattern of highest resistance isolates of *P. aeruginosa* that carried antibiotic resistances genes (SHV, TEM and CTX-M) to different antibiotics

Antibiotic	SHV		TEM		CTX-M		SHV and TEM		SHV and CTX-M		TEM and CTX-M		SHV, TEM, and CTX-M		None	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Aztreonam	2	100	2	1OO	3	100	3	100	7	100	5	100	16	100	8	100
Imipinem	2	100	2	1OO	3	100	3	100	7	100	5	100	16	100	8	100
Piperacillin	2	100	2	1OO	3	100	3	100	7	100	5	100	16	100	8	100
Cefepime	2	100	2	1OO	3	100	3	100	7	100	5	100	16	100	8	100
Tobramycin	2	100	2	1OO	3	100	3	100	7	100	5	100	16	100	8	100
Netilmicin	2	100	2	1OO	3	100	3	100	7	100	5	100	16	100	8	100
Ofloxacin	2	100	2	1OO	3	100	3	100	7	100	5	100	16	100	8	100
Doripenem	1	50	0	0	1	33	2	66	2	28	0	0	6	37	5	62
Meropenem	0	0	1	50	1	33	2	66	3	42	2	40	6	37	4	50
Ceftazidim	2	100	2	100	3	100	2	66	5	71	4	80	13	81	8	100
Piper./Tazo.	2	100	1	50	1	33	2	66	1	14	0	0	2	12	2	25
Amikacin	2	100	1	50	2	66	2	66	7	100	5	100	11	68	4	50
Gentamicin	2	100	2	100	3	100	2	66	7	100	4	80	14	87	8	100
Ciprofloxacin	0	0	0	0	0	0	1	33	5	71	3	60	6	37	4	50
Norfloxacin	1	50	1	50	1	33	1	33	0	0	3	60	8	50	3	37
Levofloxacin	0	0	1	50	2	66	1	33	4	57	1	20	4	25	5	62
Gatifloxacin	0	0	1	50	0	0	2	66	2	28	1	20	5	31	2	25

Antimicrobial resistance has emerged as a major threat to the successful delivery of healthcare globally, and Gram-negative bacilli are among the most significant causes of severe nosocomial and community-onset bacterial infections in humans (Pitout, 2008).

In many hospital and community settings, *Pseudomonas aeruginosa* (*P. aeruginosa*) is a prevalent nosocomial infection that causes serious infections. The ESBL-producing isolates of *P. aeruginosa* strains are linked to higher rates of morbidity, mortality, and medical expenses. A public health emergency is the quick spread of antibiotic resistance among these strains (Adekunle *et al.*,2021).

The antimicrobial resistance profiles of the samples were compared after identification verification testing. The isolates of *P. aeruginosa* exhibited high levels of resistance to several antibiotics, particularly beta-lactams. The majority of identified isolates were resistant to at least six different types of antibiotics.

Shirehjini *et al.* (2017) found significant levels of antibiotic resistance in *P. aeruginosa* strains and discovered that 34.2% of the bacteria had a *TEM* gene. According to Peymani *et al.* (2017) the most often found gene in *P. aeruginosa* strains was blaTEM-1 (26.7%), which was followed by $bla_{CTX\text{-}M}$-15 (17.3%), bla_{SHV}-1 (6.7%), and bla_{SHV}-12 (4%).

By using the PCR technique, Bahrami *et al.* (2018) looked into the presence of the beta-lactamase genes bla_{SHV}, bla_{TEM} and $bla_{CTX\text{-}M}$, in 96 clinical isolates of *P. aeruginosa* in Bandar Abbas. There were 23 isolates with $bla_{CTX\text{-}M}$, 23 isolates with bla_{SHV}, 26 isolates with bla_{TEM}, 55 isolates with $bla_{OXA\text{-}48}$, and 12 isolates with $bla_{OXA\text{-}48}$, respectively, with prevalence rates of 23.95 %, 23.08 %, 57.29 %, and 12.5%. (19). These results are similar in some aspect to those mentioned in this study.Nosocomial bacterial infections' patterns of resistance can change significantly over time and from one country to another (Prashanth and Badrinath, 2004).

In our study, all the isolates displayed high resistant to Imipinem (100%) and also displayed unusually high level of Meropenem resistance (41%) isolates (Table 3). These findings are consistent with the study in Saudi Arabia, it has been shown that as little as 10% of Gram-negative organisms isolated from tertiary care hospitals are imipenem sensitive (Al Johani *et al.*,2010). In routine allergy tablet diffusion assays, Klebsiella pneumonia and Escherichia coli isolates were shown to be imipenem-susceptible, according to Galani *et al.* (2008).

Since it is frequently plasmid-mediated, the carbapenem resistance caused by the production of β-lactamases has the potential to spread quickly. In order to start effective infection control measures and stop their unchecked spread in clinical settings, early identification of β-lactamases is required. By using PCR amplification, the existence of β-lactamases in Egyptian hospitals was verified (Zowawi et al.,2013).

The bla_{TEM}, bla_{SHV}, $bla_{OXA\text{-}48}$ and $bla_{CTX\text{-}M}$ genotypes are prevalent in Asian countries (Bahrami *et al.*, 2018). In our study, a total of 38 isolates (82.6%) carried at least one of the ESBL genes (bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$), and 8 isolates (17.4%) carried no ESBL-producing genes. The most prevalent β-lactamase gene, according to the PCR results, was $bla_{CTX\text{-}M}$ that was detected in 31 isolates.

The frequency of the bla_{TEM} and bla_{SHV} genes were 100% and 66% respectively in the study of Bokaeian *et al.*, (2018). Their findings revealed that 6.89% of *P. aeruginosa* MDR isolates tested positive for ESBL.

The prevalence of the bla_{TEM} gene in study conducted by Mohammed *et al.* (2016) was 25%, which is lower than the comparable prevalence in our study.

Conclusion

The most crucial drugs for treating infections produced on by *P. aeruginosa* are carbapenems like imipenem and meropenem. Sadly, this study revealed a high level of imipenem resistance. Our findings also revealed many ESBL genes, including bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ Clinical isolates of *P. aeruginosa* expressed.

Therefore, it is best to refrain from prescribing and using antibiotics when they are not essential in order to stop the development of *P. aeruginosa* strains that are resistant to them.

Acknowledgments

The authors are grateful to the staff of Babylone hospitals (Al-Hillah Teaching Hospital, Mirgian and Imam AL-Sadiq Teaching Hospital) and Baghdad hospitals (Burns Specialized Hospital, Martyr Ghazi Hariri Hospital, Baghdad Teaching Hospital, and National Center for Educational Laboratories) Iraq for providing the clinical samples and required research facilities.

References

Al-Ahmadi, G. J., & Roodsari, R. Z. (2016). Fast and specific detection of Pseudomonas Aeruginosa from other pseudomonas species by PCR. *Annals of burns and fire disasters*, *29*(4), 264.

Adekunle, C., Mustapha, A., Odewale, G., & Richard, O. (2021). Detection of Antibiotic Resistance Genes Among Multiple Drug Resistant Pseudomonas Aeruginosa Isolated from Clinical Sources in Selected Health Institutions in Kwara State. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)*, *21*(6), 53-59.

Al Johani, S. M., Akhter, J., Balkhy, H., El-Saed, A., Younan, M., & Memish, Z. (2010). Prevalence of antimicrobial resistance among gram-negative isolates in an adult intensive care unit at a tertiary care center in Saudi Arabia. *Annals of Saudi medicine*, *30*(5), 364-369.

Ali, A. M., Al-Kenanei, K. A., & Bdaiwi, Q. O. (2020). Molecular study of some virulence genes of Pseudomonas aeruginosa isolated from different infections in hospitals of Baghdad. *Reviews in Medical Microbiology*, *31*(1), 26-41.

Bahrami, M., Mmohammadi-Sichani, M., & Karbasizadeh, V. (2018). Prevalence of SHV, TEM, CTX-M and OXA-48 β-Lactamase genes in clinical isolates of Pseudomonas aeruginosa in Bandar-Abbas, Iran. *Avicenna Journal of Clinical Microbiology and Infection*, *5*(4), 86-90.

Bahrami, M., Mmohammadi-Sichani, M., & Karbasizadeh, V. (2018). Prevalence of SHV, TEM, CTX-M and OXA-48 β-Lactamase genes in clinical isolates of Pseudomonas aeruginosa in Bandar-Abbas, Iran. *Avicenna Journal of Clinical Microbiology and Infection*, *5*(4), 86-90.

Barbhaiya, H., & Rao, K. (1985). Production of pyoverdine, the fluorescent pigment of Pseudomonas aeruginosa PAO1. *FEMS microbiology letters*, *27*(2), 233-235.

Behbahani, M. R., Keshavarzi, A., Pirbonyeh, N., Javanmardi, F., Khoob, F., & Emami, A. (2019). Plasmid-related β-lactamase genes in Pseudomonas aeruginosa isolates: a molecular study in burn patients. *Journal of Medical Microbiology*, *68*(12), 1740-1746.

Bonnet, R. (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrobial agents and chemotherapy*, *48*(1), 1-14.

Chauhan, A., Lebeaux, D., Ghigo, J.-M., & Beloin, C. (2012). Full and broad-spectrum in vivo eradication of catheter-associated biofilms using

gentamicin-EDTA antibiotic lock therapy. *Antimicrobial agents and chemotherapy*, *56*(12), 6310-6318.

Crone, S., Vives-Flórez, M., Kvich, L., Saunders, A. M., Malone, M., Nicolaisen, M. H., Martínez-García, E., Rojas-Acosta, C., Catalina Gomez-Puerto, M., & Calum, H. (2020). The environmental occurrence of Pseudomonas aeruginosa. *Apmis*, *128*(3), 220-231.

Datta, N., & Kontomichalou, P. (1965). Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. *Nature*, *208*, 239-241.

de Almeida, K. d. C. F., Calomino, M. A., Deutsch, G., de Castilho, S. R., de Paula, G. R., Esper, L. M. R., & Teixeira, L. A. (2017). Molecular characterization of multidrug-resistant (MDR) Pseudomonas aeruginosa isolated in a burn center. *Burns*, *43*(1), 137-143.

Eiamphungporn, W., Schaduangrat, N., Malik, A. A., & Nantasenamat, C. (2018). Tackling the antibiotic resistance caused by class A β-lactamases through the use of β-lactamase inhibitory protein. *International Journal of Molecular Sciences*, *19*(8), 2222.

El-Fouly, M., Sharaf, A., Shahin, A., El-Bialy, H. A., & Omara, A. (2015). Biosynthesis of pyocyanin pigment by Pseudomonas aeruginosa. *Journal of Radiation Research and Applied Sciences*, *8*(1), 36-48.

Farzali Shirehjini, F., Amini, K., & Fatahi, H. (2017). Identification of blaCTX-M, blaSHV, and blaTEM genes in Pseudomonas aeruginosa strains isolated from human and animal samples using multiplex-PCR method. *Qom University of Medical Sciences Journal*, *10*(11), 51-60.

Galani, I., Rekatsina, P. D., Hatzaki, D., Plachouras, D., Souli, M., & Giamarellou, H. (2008). Evaluation of different laboratory tests for the detection of metallo-β-lactamase production in Enterobacteriaceae. *Journal of antimicrobial chemotherapy*, *61*(3), 548-553.

Huletsky, A., Knox, J., & Levesque, R. (1993). Role of Ser-238 and Lys-240 in the hydrolysis of third-generation cephalosporins by SHV-type beta-lactamases probed by site-directed mutagenesis and three-dimensional modeling. *Journal of biological chemistry*, *268*(5), 3690-3697.

Lin, S.-P., Liu, M.-F., Lin, C.-F., & Shi, Z.-Y. (2012). Phenotypic detection and polymerase chain reaction screening of extended-spectrum β-lactamases produced by Pseudomonas aeruginosa isolates. *Journal of Microbiology, Immunology and Infection*, *45*(3), 200-207.

Livermore, D. M. (1995). beta-Lactamases in laboratory and clinical resistance. *Clinical microbiology reviews*, *8*(4), 557-584.

MacFaddin, J. (2000). Biochemical tests for identification of medical bacteria, williams and wilkins. *Philadelphia, PA*, *113*.

MMasoud, S. M., Ibrahem, R. A., El-Baky, A., Rehab, M., El-Rahman, A., & Sherine, A. (2022). Prevalence of Blactx-M Gene Among MDR Escherichia Coli Isolated from Wound Infections in Minia University Hospital, Egypt. *Minia Journal of Medical Research*, 13-19.

Mohammed, H., Elsadek Fakhr, A., Al Johery, S. A. E., & Abdel Ghani Hassanein, W. (2016). Spread of TEM, VIM, SHV, and CTX-M β-lactamases in imipenem-resistant Gram-negative bacilli isolated from Egyptian hospitals. *International Journal of Microbiology*, *2016*.

Nasser, M., Gayen, S., & Kharat, A. S. (2020). Prevalence of β-lactamase and antibiotic-resistant Pseudomonas aeruginosa in the Arab region. *Journal of Global Antimicrobial Resistance*, *22*, 152-160.

Peymani, A., Naserpour-Farivar, T., Zare, E., & Azarhoosh, K. (2017). Distribution of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing P. aeruginosa isolated from Qazvin and Tehran hospitals, Iran. *Journal of preventive medicine and hygiene*, *58*(2), E155.

Peymani, A., Naserpour-Farivar, T., Zare, E., & Azarhoosh, K. H. (2017). Distribution of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing P. aeruginosa isolated from Qazvin and Tehran hospitals, Iran. *Journal of preventive medicine and hygiene*, *58*(2), E155.

Pitout, J. D. (2008). Multiresistant Enterobacteriaceae: new threat of an old problem. *Expert review of anti-infective therapy*, *6*(5), 657-669.

Prashanth, K., & Badrinath, S. (2004). In vitro susceptibility pattern of Acinetobacter species to commonly used cephalosporins, quinolones, and aminoglycosides. *Indian journal of medical microbiology*, *22*(2), 97-103.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, *6*(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949

Safaei, H. G., Moghim, S., Isfahani, B. N., Fazeli, H., Poursina, F., Yadegari, S., Nasirmoghadas, P., & Nodoushan, S. A. H. (2017). Distribution of the strains of multidrug-resistant, extensively drug-resistant, and pandrug-resistant Pseudomonas aeruginosa isolates from burn patients. *Advanced biomedical research*, *6*.

Salimi, H., Yakhchali, B., Owlia, P., & Lari, A. R. (2010). Molecular epidemiology and drug susceptibility of Pseudomonas aeruginosa strains isolated from burn patients. *Laboratory Medicine*, *41*(9), 540-544.

Seyedjavadi, S. S., Goudarzi, M., & Sabzehali, F. (2016). Relation between blaTEM, blaSHV and blaCTX-M genes and acute urinary tract infections. *Journal of Acute Disease*, *5*(1), 71-76.

Suwaidan, A. N., & Naji, H. F. (2020). bla OXA genotyping of multidrug resistant Pseudomonas aeruginosa isolated from clinical specimens. *EurAsian Journal of BioSciences*, *14*(2).

Wi, T., Lahra, M. M., Ndowa, F., Bala, M., Dillon, J.-A. R., Ramon-Pardo, P., Eremin, S. R., Bolan, G., & Unemo, M. (2017). Antimicrobial resistance in Neisseria gonorrhoeae: global surveillance and a call for international collaborative action. *PLoS medicine*, *14*(7), e1002344.

Zowawi, H. M., Balkhy, H. H., Walsh, T. R., & Paterson, D. L. (2013). β-Lactamase production in key gram-negative pathogen isolates from the Arabian Peninsula. *Clinical microbiology reviews*, *26*(3), 361-380.