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ORIGINAL ARTICLE

Prevalence and molecular analysis of antibiotic resistance of *Pseudomonas aeruginosa* isolated from clinical and environmental specimens in Basra, Iraq

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ABSTRACT

Background and Objectives: The steady increase in the spread of multidrug-resistant *Pseudomonas aeruginosa* (MDR) has become a major threat to the global health systems, including Iraq. This study aimed to investigate the prevalence and the molecular basis of antibiotic resistance in *Pseudomonas aeruginosa* isolated from clinical and environmental samples.
Materials and Methods: *Pseudomonas aeruginosa* strains were identified by standard microbiological procedures followed by PCR confirmation. Antibiotic susceptibility testing, for 16 antimicrobials, was conducted according to the Clinical and Laboratory Standard Institute (CLSI) standardized by disk diffusion and VITEK 2 methods. Detection of beta-lactamases (ESBLs, AmpC and carbapenemase) activities and related encoding genes was performed by using phenotypic methods and PCR technique respectively.
Results: A total of 81 clinical specimens and 14 environmental samples were positive for *P. aeruginosa.* Antimicrobial susceptibility test showed high rates of resistance to antipseudomonal cephalosporines (74.74 to 98.95%), aztreonam (82.11%), antipseudomonal carbapenems (68.4%), piperacillin/tazobactam (69.5%) ciprofloxacin (71.6%), and aminoglycosides (69%), with emergence of resistance to colistin (7.4%) among tested *P. aeruginosa.* Among the tested isolates, 69 (72.63%) strains were MDR, of which 63 (91.3%) strains were extremely drug resistance (XDR). Most of the isolated strains harbored one or more of ESBL genes (bla_{SHV-2a}, $bla_{CTX-M-28}$, bla_{VEB-2}, $bla_{OXA-677}$, bla_{PER}) with predominant $bla_{OXA-677}$, but none of the MBLs (GIM, SIM, SPM, IMP) and AmpC (FOX) genes were detected.
Conclusion: The results highlighted a high prevalence rate of MDR and XDR and emergence of colistin resistance *P. aeruginosa* at Basra hospitals, Iraq.

Keywords: *Pseudomonas aeruginosa*; Multidrug resistant; Extremely drug resistant; Colistin

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen which has a foremost role in developing bacterial infections, especially in immunocompromised and seriously ill patients (1). It flourishes best in humid environment and can survive even with minimal growth factors (2). Hence, *P. aeruginosa* colonizes moist health care settings, medical equipment and hospitalized patients, for long periods of time, causing life-threatening infections such as ventilator-associated pneumonia, sepsis, soft tissue,

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wounds and urinary tract infections as well as frequent exacerbations in people with cystic fibrosis (2, 3). In recent years, the rate of multi drug-resistant *P. aeruginosa* (MDR) has intensely increased and has now become a major challenge to global public health which is linked to high morbidity and death. This is because of the ability of this microorganism to acquire resistance to the most effective antibiotics (4). Several studies evaluated the risk factors for MDR strains and their association with mortality (5-7). The results showed an increase in mortality associated with MDR stains (OR 4.89) compared to susceptible infections (6).

Production of beta-lactamase enzymes is one of the main mechanisms of intrinsic resistance in *P. aeruginosa*. These enzymes can be categorized into 4 types including (A, B, C, and D) classes based on their amino acid sequences (8). These enzymes hydrolyze β-lactams but are different in their active sites. A, C, and D classes possess serine in their active site, whereas class B β-lactamases are metalloenzymes which needs zinc ions for β-lactam hydrolysis (9). *P. aeruginosa* producing class C β-lactamase exhibited resistance to antipseudomonal cephalosporins (10). Many *P. aeruginosa* strains produce extended-spectrum-β-lactamases (ESBLs) which mediated resistance to β-lactam antibiotics, including cefotaxime, ceftriaxone, and ceftazidime, and aztreonam. The majority ESBLs are categorized in class A, except OXA-type ESBLs are in enzyme class D (11). *P. aeruginosa* strains producing only ESBLs are susceptible to cephamycins and carbapenems. The genes encoding the most Ambler class A beta-lactamase enzymes are OXA, TEM, SHV, PER-1, CTX group 1, CTX group 2, CTX group 9, and VEB-1 (12). Strains producing TEM and SHV type ESBLs showed susceptibility to cefepime and to piperacillin/tazobactam but this susceptibility is diminished by increasing the inoculum to 10^7 organisms (13). Whereas strains producing CTX-M and OXA-type ESBLs are resistant to cefepime with no inoculum effect (13).

P. aeruginosa strains producing AmpC-β-lactamases are resistant to β-lactams antibiotics including cephamycins, monobactams, and extended-spectrum cephalosporins, but they differ from other ESBLs producers by their resistance to ESBLs inhibitors such as clavulanate and their ability to hydolyze cephamycins (cefoxitin and cefotetan) (2). Furthermore, increased production of (AmpC- βeta–lactamases) by certain *P. aeruginosa* isolates due to AmpC mutations generating carbapenem resistance (2).

As a result of multi-drug resistant of Gram-negative, carbapenems are used as drugs of choice for the treatment of infections caused by these organisms. Unfortunately, some carbapenemases producing *P. aeruginosa* currently exhibited resistance to carbapenems (14). Carbapenemases are members of molecular classes (A, B, and D) β-lactamases. Currently, an increasing prevalence of extended cephalosporins and carbapenems resistance has been reported, particularly for *P. aeruginosa* clinical isolates in numerous countries (15). In Iraq, in spite of all efforts carried out in other parts of the country (16, 17), yet we need more data to characterize the antibiotic profile of *P. aeruginosa*. This study aimed to determine the prevalence, pattern, and the molecular bases of antibiotics resistance among *P. aeruginosa* isolated from clinical and environmental samples in Basra province.

MATERIALS AND METHODS

Samples collection. A total of 250 (160 clinical) and (90 environmental) samples were collected from different hospitals in Basra province (Al-Fayhaa Teaching Hospital / Al-Fayhaa Burn Centre, Basrah Children's Specialty Hospital, Basrah Hospital for Women and Children and Al Sader Teaching Hospital) from 1st November 2021 to 28th February 2022. The clinical specimens included 106 swabs from patients with burns, 16 swabs from wounds, 6 swabs from abscess and ulcers, 5 skin swabs from patients in ICUs, 19 vaginal swabs, and 8 urine samples from patients with urinary tract infections. The environmental samples were collected from the surrounding of the same hospitals such as patient's bed, nursing room, newborn incubator, ventilation holes, treatment drums, surgical instruments tables, and ward sinks). All samples were investigated for detection of *P. aeruginosa*. The proposal on which the study is based was approved by the Health Research and Ethical Committee of Health authority and the management boards of the above hospitals. Written consent was obtained from all participants.

Isolation and identification of *Pseudomonas aeruginosa*. All of the specimens were investigated for the presence of *Pseudomonas* genus, and *P. aeru-*

ginosa according to standard procedures (18). Briefly, the collected swabs were placed in tubes containing 5 ml of selective media cetrimide agar and incubated at 37°C for 24 hours, then the samples were subcultured on Pseudomonas chromogenic agar and incubated for 24 hours then subjected to microscopic examination and different biochemical tests (18).

Molecular detection of *P. aeruginosa*. Polymerase chain reaction was used to confirm the identification of *Pseudomonas* genus, and *P. aeruginosa* using genus and species-specific primers (OprI for genus and OprL for *P. aeruginosa*) (Table 1). Total bacterial genome was prepared using commercial DNA extraction kit (Promega / USA).

PCR assays were performed in a 25 µl volume containing 12.5 µl master mix (Bioneer master mix), (1 µl) from each primer, (2 µl) bacterial DNA and (8.5) Nuclease -free water. After initial denaturation for 5 min at 94°C for 1 cycle, 30 cycles were carried out: (30 s) at 94°C, (30 s) at 55°C and (1) min at 72°C. The final cycle was followed by 72°C incubation for (10 min). All PCR products were separated on (1%) agarose gels, stained with ethidium bromide visualised by a UV light transilluminator.

Antibiotic susceptibility test. The Kirby-Bauer disc diffusion technique was used to evaluate anti-

Table 1. Primers used in this study

No.	Primers	Sequence 5′ — 3′	Amplicon size (bp)	Reference
1	OprI	F 5′- ATGAACAACGTTCTGAAATTC TCTGCT-3′ R 5′- CTTGCGGCTGGCTTTTTCCAG-3′	249 bp	(27)
2	OprL	F 5′- ATGGAAATGCTGAAATTCGGC-3′ R 5′- CTTCTTCAGCTCGACGCGACG-3′	504 bp	(27)
3	TEM	R 5′- ATAAAATTCTTGAAGAC-3′ F 5′- TTACCAATGCTTAATCA-3′	1,075 bp	(23)
4	SHV	F 5′- TGGTTATGCGTTATATTCGCC-3′ R 5′- GCTTAGCGTTGCCAGTGCT-3′	867 bp	(23)
5	PRE	F 5′- AATTTGGGCTTAGGGCAGAA-3′ R 5′- ATGAATGTCATTATAAAAGC-3′	933 bp	(28)
6	VEB	F 5′- CGACTTCCATTTCCCGATGC-3′ R 5′- GGACTCTGCAACAAATACGC-3′	642 bp	(23)
7	CTX-M13U	F 5′- GGTTAAAAAATCACTGCGTC-3′ R 5′- TTGGTGACGATTTTAGCCGC-3′	863 bp	(23)
8	CTX-M9U	F 5′- ATGGTGACAAAGAGAGTGCA-3′ R 5′- CCCTTCGGCGATGATTCTC-3′	870 bp	(23)
9	CTX-M25U	F 5′- ATGATGACTCAGAGCATTCG-3′ R 5′- TGGGTTACGATTTTCGCCGC-3′	865 bp	(23)
10	OXA	F 5′- GTCTTTCG(A)AGTACGGCATTA-3′ R 5′- ATTTTCTTAGCGGCAACTTAC-3′	699 bp	(23)
11	FOXM	F 5′- AACATGGGGTATCAGGGAGATG-3′ R 5′- CAAAGCGCGTAACCGGATTGG-3′	190 bp	(24)
12	VIM	F 5′- GATGGTGTT TGG TCG CAT A-3′ R 5′- CGA ATG CGC AGC ACC AG-3′	390 bp	(25)
13	IMP	F 5′- GGAATAGAGTGGCTTAATTCTC-3′ R 5'- CCAAACCACTACGTTATCT-3′	188 bp	(25)
14	GIM	F 5'- TCG ACA CAC CTT GGT CTG AA -3′ R 5'- AAC TTC CAA CTT TGC CAT GC-3′	477 bp	(25)
15	SPM	F 5'- AAA ATC TGG GTA CGC AAA CG-3′ R 5'- ACA TTA TCC GCT GGA ACA GG-3′	271 bp	(25)
16	SIM	F 5′- TAC AAG GGA TTC GGC ATC G-3′ R 5′- TAA TGG CCT GTT CCC ATG TG-3′	570 bp	(25)

biotic susceptibility on Mueller-Hinton agar (Oxoid Limited, Hampshire, England) in accordance with the Clinical and Laboratory Standard Institute guidelines (19). The following drugs were used to determine the antibiogram of the *P. aeruginosa*: cefotaxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), amikacin (30 µg), ampicillin / sulbactam (30 µg), piperacillin (100 µg), piperacillin / tazobactam (100+10 µg), ciprofloxacin (5 µg) gentamicin (10 µg) colistin (10 µg), ceftazidime (30 µg) ciprofloxacin (5 µg), and colistin (10 µg) all were bought from TMMEDIA, India. Cefepime (30 µg), aztreonam (30 µg), and amoxicillin / clavulanic acid (20+10 µg) were bought from Liofilchem, Italy.

VITEK 2 system using (AST- GN30) was used to confirm the results of the antibiotic susceptibility test and to determine the MIC values of the tested antibiotics.

Phenotypic detection of ESBL production. The double-disk synergy test (DDST) was performed to detect ESBL as described by Tzelepi et al. 2000 (20). Briefly, disks of ceftazidime, cefotaxime, and aztreonam (30 µg each) were placed (30 or 20) mm (center to center) from an amoxicillin 20 µg/ clavulanic acid 10 µg disk. The presence of ESBL was indicated by an increase in zones of inhibition towards amoxicillin–clavulanic acid antibiotic disks.

Phenotypic detection of AmpC production. Phenotypic detection of AmpC was carried out as defined by Black et al. 2005 (21). Briefly, cefoxitin 30 µg (FOX) was placed on Mueller-Hinton agar's surface medium was inoculated with a susceptible *E. coli* lawn using the standard disk diffusion technique. Impregnate two blank discs with (20 µl) of a 1:1 solution of saline and 100X_EDTA were placed on the flank of the FOX disk. Colonies of tested strain were applied on blank disks. Following incubation for 24 hours at 35°C, Plates were checked for signs of enzymatic inactivation of cefoxitin, such as an indentation or flattening of the zone of inhibition (positive result), or for the lack of distortion, such as no discernible inactivation of cefoxitin (negative result).

Phenotypic detection of MBL production. A mixed disc synergy test using imipenem and EDTA was applied to identify isolates that produced MBL. EDTA solution (0.5 M, pH 8) was made by dissolving (18.61g) EDTA in (100 ml) distilled water, correcting the pH to 8 with NaOH, and then autoclaved. The organisms that were tested were cultured on the surface of Muller Hinton agar plates. Imipenem (10 µg) or meropenem (10 µg) discs were put on the surface of agar plates, and one imipenem and one meropenem disc impregnated with a (5 µl) EDTA solution. Zones of inhibition surrounding EDTA discs were evaluated and compared with other disks after 16-18 hours of incubation at 35°C. Positive findings were defined as an increase in zone diameter of at least 7 mm around the imipenem - EDTA and meropenem - EDTA discs (22).

Detecting of ESBLs, AmpC, and MBL genes in *P. aeruginosa*. Using specific primer (Table 1), PCR was performed to detect ESBLs genes (TEM, SHV, PRE, VEB, CTX-M3, CTX-M9, CTX-M25, OXA), AmpC gene (FOX), and MBL genes (VIM, IMP, GIM, SPM, SIM). Standard PCR amplification experiments were carried out as described by Jiang et al. 2005 (23), Ejikeugwu et al. 2020 (24), and Ellinngton et al. 2007 (25) respectively. A (20 µl) of the PCR results from selected *P. aeruginosa* isolates were sent for DNA sequencing in accordance with the specifications of the Macrogen Company (Seoul, South Korea). The obtained sequences were examined and aligned using the Bio Edit application (26).

GenBank accession numbers. The DNA sequences of the bla_{SHV-2a} , $bla_{CTX-M-28}$, bla_{VEB-2} , $bla_{OXA-677}$ ESBL genes from the representative iso¬lates have been deposited in the GenBank database under accession numbers OP253964-OP253973 for isolates KZ1-KZ10, respectively.

Statistical analysis. The statistical analysis was performed using SPSS Version 22 (Package for Social Sciences).

RESULTS

Prevalence of *P. aeruginosa* in clinical and environmental specimens. A total of 101 (40.4%) samples were found positive for *Pseudomonas* strains of which 95 (94.05%) were *P. aeruginosa*. Out of 160 clinical specimens, 83 (51.9%) *Pseudomonas* strains were isolated and 81 (97.59%) of them were *P. aeruginosa*. Out of 90 environmental samples, 18 (20%) *Pseudomonas* strains were isolated and 14 (78%) of them were *P. aeruginosa*. All *Pseudomonas* isolates (101) that identified by routine microbial test were positive for genus specific PCR, of which 95 isolate were identified as *Pseudomonas aeruginosa* by species specific PCR. Six strains were identified as *Pseudomonas*, but not *P. aeruginosa*.

Antibiotic susceptibility and MICs. Table 2 shows the profile of antibiotics resistance respective MIC distributions for clinical isolates and environmental isolates (95 isolates) of *P. aeruginosa*. Among the antibiotics tested, *P. aeruginosa* isolates showed high resistance to 2nd, 3rd and 4th generation of cephalosporins and monobactam with MIC values exceeded the breakpoint MIC of these antibiotics. Also, there was more than 68% of the tested *P. aeruginosa* strains were resistant to carbapenems with high value of MICs (≥ 16 µg/ml). Most of tested strains (69 to 100%) were resistant to penicillins/ ß-lactamase inhibitors. The results explored an emergence of colistin resistant strains (7.4%) among tested *P. aeruginosa*. Most of the tested strains were multidrug resistant. According to the European center for disease prevention and control (ECDC) instructions, 69 (72.63%) of *P. aeruginosa* isolated strains were categorized as multi drug-resistant (MDR) strains of which 63 (91.3%) were extensively drug-resistant (XDR). Out of 63 XDR strains, 61 (96.82%) were isolated from burn samples, 1 (1.58%) strain isolated from abscess and 1 (1.58%) strain isolated from the environmental samples. MDR was defined as resistant to at least one agent in (≥3) antimicrobial categories, XDR was defined as resistant to at least one agent in (≥ 6) antimicrobial categories, and PDR was defined as resistant to all agents in all antimicrobial categories (29). No intermediate isolates were detected.

The distribution of antibiotics resistance among the tested isolates according to the type of clinical and environmental samples is shown in Table 3. The highest rate of resistance was observed in *P. aeruginosa* isolated from the burn samples.

Phenotypic detection of ESBLs, AMPC and carbapenemase production. Based on antibiotics profile, definite *P. aeruginosa* were selected for phenotypic tests. The results showed that out of 95 tested strains, 29 (29.58%) isolates were ESBLs producers of which 22.1% were clinical isolates and 8.4% were environmental strains. Among 65 tested strains, 65 (100) strains were MBLs producers of which 64 (98.46%) were clinical isolates and one strain (1.53%) was environmental isolate. For detection of AmpC, 19 strains were tested, the results showed 5 clinical isolates and one environmental isolate were positive.

Table 2. Antibiotic susceptibility profile of isolated *Pseudomonas aeruginosa* strains

Class		Antibiotics	Percentage of resistant isolates	Percentage of sensitive isolates	MIC µg/ml
Cephalosporins		(2G) Cefoxitin	97.90%	2.1%	ND*
		(3G) Ceftriaxone	74.74%	25.26%	ND
		(3G) Cefotaxime	76%	24%	≥ 64
		(3G) Ceftazidime	98.95%	1.05%	≥ 64
		(4G) Cefepime	75.80%	24.20%	≥ 64
penicillins/	Ampicillin group	Amoxicillin/clavulanic acid	88.4%	11.6%	ND
ß-lactamase		Ampicillin/sulbactam	100%	0	ND
inhibitors	Piperacillin group	*Piperacillin*	72.6%	27.4%	ND
		Piperacillin /tazobactam	69.5%	30.5%	≥ 128
Monobactams		Aztreonam	82.11%	17.89%	ND
Carbapenems		Imipenem	68.40%	31.60%	≥ 16
		Meropenem	68.40%	31.60%	≥ 16
Lipopeptides		Colistin	7.40%	92.60%	ND
Aminoglycosides		Gentamicin	68.40%	31.60%	≥ 16
		Amikacin	69.48%	30.52%	≥ 64
Fluoroquinolones		Ciprofloxacin	71.60%	28.40%	≥ 4

ND* not determined

Table 3. Distribution of antibiotics resistance among the type of clinical and environmental samples

Antibiotics	No. (%) of resistant isolates (n=95)								
	Burn n=(66)	Wound n=(3)	ICU pa-tients n=(2)	UTI n=(2)	Abscess & ulcer n=(4)	Vagina n=(4)	Hospital environment n=(14)	P value*	Odds ratio (95% C.I.⩾)
Cefoxitin	64 (96.96)	3 (100)	2 (100)	2 (100)	4 (100)	4 (100)	14 (100)	1.000	1.33 (0.14-12.37)
Ceftriaxone	59 (89.39)	0	0	0	4 (100)	1 (25)	7 (5)	0.0001	-
Cefotaxime	62 (93.93)	0	0	0	2 (50)	2 (50)	6 (4.3)	0.0001	-
Ceftazidime	66 (100)	3 (100)	2 (100)	2 (100)	4 (100)	4 (100)	13 (92.85)	0.308	0.33 (0.03-4.04)
Cefepime	58 (87.87)	1 (33.33)	1 (50)	1 (50)	2 (50)	2 (50)	7 (50)	0.001	1.00 (0.11-9.23)
Amoxicillin/clavulanic acid	60 (90.90)	2 (66.66)	1 (50)	2 (100)	4 (100)	3 (75)	12 (85.7)	0.292	0.50 (0.03-7.54)
Ampicillin/sulbactam	66 (100)	3 (100)	2 (100)	2 (100)	4 (100)	4(100)	14 (100)	-	-
Piperacillin	63 (95.45)	0	0	0	2 (50)	1 (25)	3 (21.4)	0.0001	-
Piperacillin/Tazobactam	63 (95.45)	0	0	0	1 (25)	1(25)	1 (7.1)	0.0001	-
Aztreonam	59 (89.39)	3 (100)	1 (50)	0	4 (100)	4 (100)	7 (50)	0.002	1.22 (0.09-16.43)
Imipenem	63 (95.45)	0	0	0	1 (25)	0	1 (7.1)	0.0001	4.33 (0.21-90.85)
Meropenem	63 (95.45)	0	0	0	1 (25)	0	1 (7.1)	0.0001	-
Colistin	4 (6.06)	1 (33.33)	0	1 (50)	0	1 (25)	0	0.093	-
Gentamicin	63 (95.45)	0	0	0	1 (25)	0	1 (7.1)	0.0001	-
Amikacin	63 (95.45)	0	0	0	1 (25)	0	2 (14.3)	0.0001	-
Ciprofloxacin	64 (96.96)	0	0	0	1 (25)	0	3 (21.4)	0.0001	-

Detection of ESBL genes. All 95 *P. aeruginosa* isolates were screened for the common genes encoding ESBL using specific primers (Table 1). Most of the isolated strains harbored one or more of ESBL genes (*bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{VEB}$, *bla*$_{OXA}$, *bla*$_{PER}$), but none of the MBLs (GIM, SIM, SPM, IMP) and AmpC (FOX) genes were detected. Basic Local Alignment Search Tool (BLAST) analysis of DNA sequences for PCR products showed 99%-100% identity with *bla*$_{OXA-677}$ (ID: NG_062272.1); *bla*$_{OXA-677}$ (ID: MH780098.1); *bla*$_{SHV-2a}$ (ID: AF074954.1); *bla*$_{CTX-M-28}$ (ID: KY792758.1); *bla*$_{VEB-2}$ (ID: AY027870.1).

Out of 95 tested isolates, 84 strains (88.42%) were positive for at least one gene (Table 4). Of the 14 genes, the most abundant was the *bla*$_{OXA-677}$ gene (56 isolates, 58.94%), followed by the *bla*$_{SHV-2a}$ (25 isolates, 26.31%), *bla*$_{CTX-M-28}$ (18 isolates, 18.94%), and PER (4 isolates, 4.21%), *bla*$_{VEB-2}$ (4 isolates, 4.21%). None of the studied isolates tested positive for the TEM, CTX-M9, CTX-M25, FOX, GIM, SIM, SPM and IMP genes. The most common combination found in the isolates was the *bla*$_{OXA-677}$ and *bla*$_{CTX-M-28}$ genes (17.89%) (Table 4), Tested strains that harbored different ESBL genes showed different antibiotic susceptibility patten (Table 5). Table 6 represents the relation between phenotype and genotypes tests with significant differences ($P < 0.05$). The results showed that all the strains (39 isolates) that harbored *bla*$_{OXA-677}$ gene exhibited positive phenotypic test for MBLs, and 14 (66.66%) of those harbored *bla*$_{SHV-2a}$ gene were ESBLs producer (Table 6). None of the environmental strains showed positive results for *bla*$_{OXA-677}$ gene, but 11 (78.6%) strains of them harbored *bla*$_{SHV-2a}$ gene and one strain (7%) was positive for each *bla*$_{PER}$ and

Table 4. Incidence of genes encoding beta lactamase enzymes and a combination of these genes in the isolated *P. aeruginosa* strains

Gene	Isolates (%)	Gene combination	Isolates (%)
TEM	0 (0%)	*bla*$_{SHV-2a}$	21 (22.1)
bla$_{SHV-2a}$	25 (26.31)	*bla*$_{CTX-M-28}$	1 (1.05)
bla$_{CTX-M-28}$	18 (18.94)	*bla*$_{OXA-677}$	39 (41.05)
bla$_{CTX-M9}$	0	PER	1 (1.05)
bla$_{CTX-M25}$	0	SHV-2a + PER	1 (1.05)
bla$_{OXA-677}$	56 (58.94)	SHV-2a + VEB-2	2 (2.1)
bla$_{PER}$	4 (4.21)	PER + VEB-2	1 (1.05)
bla$_{VEB-2}$	4 (4.21)	OXA-677 + CTX-M-28	17 (17.89)
VIB	0	SHV-2a + PER + VEB-2	1 (1.05)
GIM	0		
SIM	0		
SPM	0		
IMP	0		

bla$_{VEB-2}$ gene (data not shown).

Eleven strains were negative in PCR for all tested genes and showed positive results for phenotypic tests ESBLs (2 isolates), AmpC (1 isolate), MBLs (6 isolates) and 2 strains were negative to phenotypic tests.

DISCUSSION

The emergence of multidrug-resistant *Pseudomonas aeruginosa* has become a major threat to the global health systems (2). This study aimed to investigate the prevalence and the molecular basis of antibiotic resistance of *P. aeruginosa* isolated from clinical and environmental samples.

In the present study, *P. aeruginosa* was the most predominant species in the isolated strains. *P. aeruginosa* isolates represented 97.6% of the tested clinical *Pseudomonas* isolates. It also represented 78% from the tested environmental *Pseudomonas* isolates. The results showed that the prevalence of *P. aeruginosa* isolated from clinical samples was higher than that reported in other local and regional studies (16, 21, 30). On the other hand, the prevalence of *P. aeruginosa* isolated from environmental samples was more or less than that reported by other studies (30, 31). These results point to the importance of implementing hygienic strategies and prevention methods in hospital settings to minimize the spread of *P. aeruginosa* in hospital wards.

In this study, the susceptibility of ninety-five *P. aeruginosa* strains against 16 agents from 7 antimicrobial* classes were investigated. The results showed high prevalence of multidrug-resistant (MDR) and extensively drug resistant (XDR) strains (66.31%), which is higher than that reported by other studies in Iran (16.5-41%) and Iraq (12.4%) (16, 32, 33), but less than that stated by other studies in Brazil (71.4%) and Egypt (70%) (34, 35). It was reported that the rate of multidrug-resistant rates in other geographical areas ranged between 15% and 30% (31). The present results revealed that 92.8% of the MDR/XDR strains were isolated from burn specimens. Our finding is consistent with other studies which stated that the majority of the MDR *P. aeruginosa* strains were isolated from burn samples (34). Worldwide increasing of MDR *P. aeruginosa* could be attributed to improper using of antibiotics in hospital and community in addition to accumulate a variety of resistance mechanisms (31). The pres-

Table 5. The relation between the presence of ESBLs genes and antibiotics resistance

Gene	Isolate	NO. (%) of Antibiotic resistance															
	n=95 (%)	CX	CTR	CTX	CAZ	FEP	AUG	A/S	PI	PIT	AT	IMI	MRP	CL	GEN	AK	CIP
bla$_{SHV-2a}$	21 (22.1)	19 (90)	4 (19)	5 (23)	21 (100)	9 (42)	16 (76)	21 (100)	3 (14)	0	11 (52)	0	0	3 (14)	0	0	1 (4%)
PER	1 (1.05)	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100%)
SHV + PER	1 (1.05)	1 (100)	1 (100)	0	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)	0	0	0	0	1 (100)	1 (100)
SHV + VEB	2 (2.1)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)	0	1 (50)	0	0	0	0	0	1 (50)
PER + VEB	1 (1.05)	1 (100)	1 (100)	0	1 (100)	0	0	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)
SHV + PER + VEB	1 (1.05)	1 (100)	1 (100)	0	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)	0	0	0	0	0	0
bla$_{CTX-M-28}$	1 (1.05)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	a0	1 (100)	1 (100)	1 (100)
bla$_{OXA-677}$	39 (41.05)	39 (100)	38 (97)	39 (100)	39 (100)	37 (94)	38 (97)	39 (100)	38 (97)	39 (100)	39 (100)	39 (100)	39 (100)	1 (2)	39 (100)	39 (100)	39 (100)
OXA-677 + CTX-M-28	17 (17.89)	17 (100)	16 (94)	17 (100)	17 (100)	15 (88)	15 (88)	17 (100)	17 (100)	17 (100)	17 (100)	17 (100)	17 (100)	2 (11)	17 (100)	17 (100)	17 (100)
P value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 6. The relation between the production of ESBLs, AmpC, and carbapenemase and the presence of the encoding genes.

Gene type	No. of isolates positive by PCR	No. of isolates positive by Phenotypic tests					
		ESBLs	P-value	AmpC	P-value	MBLs	P-value
bla_{SHV-2a}	21	14		0		0	
$bla_{OXA-677}$	39	3		1		39	
$bla_{CTX-M-28}$ + $bla_{OXA-677}$	17	4	0.006	3	0.03	17	0.003
bla_{SHV-2a} + bla_{VEB-2}	2	2		1		0	
bla_{SHV-2a} + bla_{PER}	1	0		1		0	
$bla_{CTX-M-28}$	1	0		1		1	
PER	1	1	_	1	_	1	_
bla_{PER} + bla_{VEB-2}	1	1		1		1	
bla_{SHV-2a} +bla_{PER} + ƚla_{VEB-2}	1	1		1		0	
Total	84	26		10		59	

ent results explored emergence of colistin resistance (7.4%) among XDR *P. aeruginosa* isolates. Colistin or polymyxin B are described as a last option used for the treatment of infections caused by (MDR and XDR) *P. aeruginosa* (31-33). In spite of the low percentage of *P. aeruginosa* isolates resistant to polymyxins, the detection of these organism is one of the critical concerns to address in order to enhance therapy of infections caused by MDR and XDR *P. aeruginosa* strains. Worryingly, the resistance to colistin was highly associated with high levels of resistance to other antimicrobials including carbapenems and monobactam. As far as we are aware, this is the first instance of colistin resistance among *P. aeruginosa* in Iraq, which represent a major health-care concern capable of restricting therapeutic options. However, susceptibly to colistin remains vastly high against *P. aeruginosa* approaching 100% in most countries in the area of the Middle East and North Africa (36). One limitation of the present study was the using of disk diffusion method to test the colistin susceptibility which has been removed from CLSI guideline since 2017. Further study is required by using more reliable method when be available to confirm our results.

The present results revealed that the prevalence of resistance to carbapenems was 68.40% which was higher than that reported in previous study in Iraq (12.4%) and other countries in the region including Jordan (21%), Egypt (62%), Saudi Arabia (30%), Oman (42%), Lebanon (30%), but relatively lower that reported in Bahrain (90%), Qatar (90.2), and Libya (87%) (16, 36). We found that most of the carbapenem-resistant isolates (97%) were isolated from burn samples and all of them were MBL producers. Furthermore, our results showed that the majority (89.39%) of MBL-producing carbapenem-resistant isolates were resistant to monobactam (aztreonam). These findings raise a major concern that requires Health Authorities to urgently work on finding rapid and accurate diagnostic procedures and regulating the dispensing of antibiotics, in addition to tightening microbiological control systems in hospitals. Also, the present results showed that none of the examined MBL genes (IMP, VIM, SPM, GIM, and SIM) were detected in any of the studied MBL-producing carbapenem-resistant isolates. This indicates that these microorganisms may have other type of MBL genes. On other hand, 56 (86.15%) carbapenem-resistant isolates harbored $bla_{OXA-677}$gene. These finding indicates the carbapenemase activity of OXA enzymes, which is consistent with that reported by other study (37). In addition, we found only one environmental carbapenem-resistant isolates, which also harbored $bla_{OXA-677}$gene. To the best of our knowledge, it is the first time we report the occurrence of $bla_{OXA-677}$gene with carbapenemase activity among *P. aeruginosa* in Iraq.

Regarding the resistance to third- and fourth-generation antipseudomonal cephalosporins observed in our study, they were typically high (74.74-98.95%) compared to previous studies in Iraq (41.2%) and many countries in the region including; (47.1%) Yemen, (66%) Libya, (68%) Egypt and (70%) Tunisia but relatively comparable to those reported in Qatar (96.6%) and Bahrain (86%) (16, 36).

Our results, also, showed that the resistance pattern of *P. aeruginosa* to aminoglycosides (kanamycin and gentamicin) and ciprofloxacin was higher than that reported in other studies in Iraq, Jordan, Saudi

Arabia, and Iran, but lower than that was found in Bahrain and Qatar (16, 36, 38).

In conclusion, the overall present findings indicated that the majority of the MDR/XDR *P. aeruginosa* were isolated from burn samples. Most of these microorganism harbored $bla_{OXA-677}$ gene with carbapenemase activity and low-level susceptibility to all examined antibiotics, with no rates that exceed 31.6%. Colistin presented high effectivity against tested *P. aeruginosa*. Unfortunately, colistin considered to be last resort to treat infections caused MDR/XDR *P. aeruginosa* and may be associated with marked side effects. These findings alarm the Health Authority to implement accurate infection control program and administration of appropriate antibiotic treatment procedures in our hospitals.

Although these data are important for understanding the antibiotic resistance profile of *P. aeruginosa* in Iraq, there are some limitations. The sources and number of clinical samples were insufficient to generalize the findings to the entire country. We did not examine all the agents in the all-antibiotic classes to determine the pan drug resistance (PDR) isolates. Also, further molecular studies are required to detect other MBL genes.

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