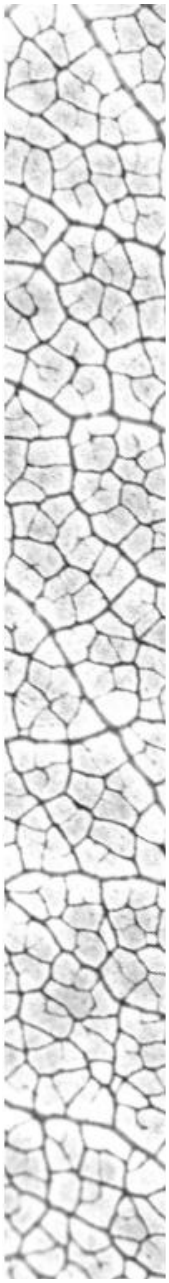

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bla$_{OXA}$ genotyping of multidrug resistant *Pseudomonas aeruginosa* isolated from clinical specimens

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Abstract

Background: Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen. It is a ubiquitous bacterium that is found and isolated from various environments including plants, animals, soil and humans. This bacterium accounts for 10-15% of the nosocomial infections worldwide and is considered the third most-common organism associated with hospital-acquired infections such as urinary catheter-associated infections, ventilator-associated pneumonia as well as blood, burn and wound infections**,** particularly involving wound infections in immunocompromised patients. **Methodology:** One hundred and sixty samples were collected from clinical specimens from the Babylon hospitals during the period of July-2019 to November-2019. These samples included 40 injury samples, 55 diabete-infected foot samples and 65 burn samples. Blood agar (Himedia) and MacConkey agar (Himedia) were used to isolated this bacterium, using the streaking technique and identified depending on their morphological properties (cultural and microscopical), biochemical tests, and then confirmed by PCR-sequencing for universal 16S rRNA gene. An antibiotic susceptibility tests were performed using clinical and laboratory standards institute guideline (2019). **Results:** The results revealed that only 30 isolates were P. aeruginosa. The highest resistant percentages toward the antibiotics were found with ceftazidime and cefotaxime (96.7 %), while the lowest resistant percentages were found with colistin and polymyxin B (40 %). The ability of these isolates to produce OXA β-lactamase was investigated using PCR, it was found that 26.6% of the isolates belong to OXA-I, 40% of the isolates belong to OXA-III and no one of these isolates belong to OXA-II. **Conclusion:** The rates of OXA-type β-lactamases producing P. aeruginosa isolates from the clinical specimens were notable, therefore, the management and the treatment strategies should be revised and the proper use of the infection-control measures is needed to reduce the spread of the resistant genes in the isolates of P. aeruginosa.

Keywords: P. aeruginosa, bla$_{OXA}$, diabete-infected foot, burn, injury, multi-drug resistance

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INTRODUCTION

One of the important characteristics of P. aeruginosa, is their intrinsic resistance to different antibiotics and their ability to acquire exchange of genetic material with other bacterial species, such as Klebsiella pneumoniae and Escherichia coli[1]. P. aeruginosa develops resistance against almost all antibiotics by several mechanisms like, multi-drug resistance (MDR) efflux pumps, resistant genes, biofilm formation, permeability of the low outer membrane and the production of shattered antibiotic enzymes, such as cephalosproinases and β-lactamases (Ali, Al-Kenanei, & Bdaiwi, 2020). It could potentially become resistance to all classes of antibiotics used to treat Gram-negative associated wound infections including β-lactams, aminoglycosides and fluoroquinolones.(Hocquet,, Plésiat, Dehecq, Mariotte, Talon, & Bertrand2010). The spread of multidrug resistant pathogenic bacteria is a major cause of concern all over the world being a nosocomial infection, β-lactamase producing P. aeruginosa is one amongst these towards which the human population is more vulnerable. Characterized by their ability to hydrolyze β-lactams, β-lactamase produced by P. aeruginosa is multi-resistant to a wide range of antimicrobials, such as penicillins, cephalosporins, cephamycins and carbapenems (Nasser, Gayen, & Kharat, 2020). The rate of wound infections caused by β-lactamase producing P. aeruginosa in Iraq has increased and several studies have identified their prevalence in the region. Several publications have appeared in recent years documenting the prevalence of antimicrobial resistance, also new surveillance data released on Jan 29th, 2018 by the World Health Organization (WHO) reveals

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Table 1. PCR primers and their conditions used in this study

Primer	Sequence (5-3)	Product(bp)	Condition	Ref.
16S-F	AGAGTTTGATCCTGGCTCA	1489	95C:30 sec. 53C:30 sec. 72C:150 Sec.	MacFaddin, 2000). (Loy, et al. 2002).
16S-R	GGTTACCTTGTTACGACTT			
OXA-IF	TCTTTCGAGTACGGCATTAGC	760	95C:30 sec. 60C:30 sec. 72C:90 Sec.	(Loy, et al. 2002).
OXA-IR	CCAATGATGCCCTCACTTTCC			
OXA-IIF	GCCAAAGGCACGATAGTTGT	700	95C:30 sec. 60C:30 sec. 72C:90 Sec.	(Loy, et al. 2002).
OXA-IIR	GCGTCCGAGTTGACTGCCGG			
OXA-IIIF	AGCCGTTAAAATTAAGCCC	908	95C:30 sec. 60C:30 sec. 72C:90 Sec.	(Lin, et al. 2012).
OXA-IIIR	CTTGATTGAAGGGTTGGGCG			

widespread and in some cases, excessive levels of antimicrobial resistance across the globe in the most common bacterial infections caused by bacteria, among them P. aeruginosa (El-Shouny, et al. 2018). The growing rates of antimicrobial resistance among this bacterium is a major issue worldwide and the most common mechanisms of this resistance are the production of β-lactamases, including four Ambler classification classes A, D, B, and C. β-lactamases are encoded genes that are a part of the bacterial chromosome or by genes acquired by transfer of mobile genetic elements (Bonomo, 2017). The OXA type β-lactamases (belong to molecular class D and functional group 2d) are widespread and have been mostly identified in clinical isolates of P. aeruginosa, which commonly confer resistance to cephalosporins and carbapenems (Bush, & Jacoby, 2010). Five distinct groups of oxacillinases have been described, OXA group I includes OXA-5, OXA-7, OXA-10, its derivatives (OXA-11, OXA-14, OXA-16, OXA-17), and OXA-13, its derivatives (OXA-19, OXA-28), OXA group II includes OXA-2, OXA-3, OXA-15 and OXA-20, OXA group III includes OXA-1, OXA-4, OXA-30 and OXA-31, OXA group IV is defined by OXA-9, and OXA group V currently consists of a single enzyme, Beta-lactamase LCR-1. In addition, OXA-18 does not belong to any of these groups and has very low amino acid identity with other oxacillinases (Bert, Branger, & Lambert-Zechovsky, 2002). Therefore, current study aims to investigate bla_{OXA} genotypes among MDR-P. aeruginosa isolated from different clinical samples in Babylon hospitals

MATERIAL AND METHODS

Collection of Samples

One hundred and sixty samples were collected from the Babylon hospitals, the specimens were divided into three groups, the first group included forty swabs obtained from different injuries, the second group included fifty-five swabs obtained from diabetes-infected foot and the third group included sixty five swabs obtained from burnes. These samples were collected within the period of July 2019 to November 2019.

Table 2. Preparation of PCR reaction mixture (Promega, USA)

PCR	Volume (µL)
Master Mix	12.5
DNA template	3
Forward primer	1.5
Reverse primer	1.5
Nuclease free water	6.5
Total	25

Bacterial Isolation and Identification.

P. aeruginosa were isolated on blood agar (Himedia), and MacConkey agar (Himedia) using streaking technique and identified depending on their morphological properties (cultural, microscopical) and biochemical tests (MacFaddin, 2000). and then confirmed by PCR-sequencing for universal 16S rRNA gene.

DNA Extraction

For molecular identification of *P. aeruginosa*, the whole genomic DNA was extracted using IntronBio Kit (Korea)

PCR Primers and Conditions

The primers of PCR amplification of specific genes used in this study were synthesized by Macrogen (Korea), these primers and their reactions are demonstrated in **Table 1**.

Preparation of PCR Reaction Mixture

The reaction mixture was prepared according to the manufacturer's instruction (Promega, USA), as shown in **Table 2**.

Agarose Gel Electrophoresis

The amplification products of PCR were run on horizontal agarose gel (1%) stained with ethidium bromide for 1 hour at 80 volts. 5 µL of amplification products plus 1 µL of loading dye were loaded in the well of the gel. The DNA marker 100-1500 bp (Promega, USA) was used to detect the size of the electrophoresis fragment of amplified genes. The DNA bands were photographed by a gel documentation system (Biometra-Germany) (Lin, et al. 2012; Obinna-Echem, & Torporo, 2018).

Antibiotics Susceptibility

The disk diffusion of susceptibility tests were performed according to (CLSI 2019) guidelines (Weinstein, 2019). The antibiotic discs (MAST diagnosis, England) used in this study included:

Table 3. Distribution of isolated bacteria with their percentages in the collected samples

Sample type	Sample No.	P. aeruginosa		Other bacteria		No growth	
		No.	%	No.	%	No.	%
Injuries	40	12	7.5	23	14.4	5	3.1
diabetic foot	55	6	3.8	20	12.5	29	18.1
Burns	65	12	7.5	29	18.1	24	15
Total	160	30	18.8	72	45 %	58	36.2

Table 4. The antibiotics susceptibility of P. aeruginosa isolates

Isolate Name	PIP	CAZ	CEF	AT	IMP	MEM	CS	PB	GM	TOB	AK	NET	CIP	OFX	TCC	CTX	CRO	LEV
PA1	R	R	R	R	S	R	S	S	R	R	R	R	R	R	R	R	R	R
PA2	R	R	R	R	R	R	S	S	R	I	R	R	R	R	R	R	R	I
PA3	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
PA4	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
PA5	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
PA6	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R
PA7	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
PA8	R	R	R	R	S	R	S	S	R	R	R	R	R	R	R	R	R	R
PA9	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
PA10	R	R	R	R	R	R	S	S	R	R	R	R	R	S	R	R	R	S
PA11	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
PA12	R	R	S	R	R	R	S	S	S	S	R	R	R	S	R	R	R	R
PA13	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
PA14	R	R	S	S	R	R	S	S	R	R	R	R	R	S	R	R	R	S
PA15	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
PA16	R	R	R	R	S	R	S	S	R	R	R	R	R	R	R	R	R	R
PA17	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	R	S
PA18	R	R	R	R	S	S	R	R	R	S	S	R	S	R	R	R	R	S
PA19	R	R	S	R	S	S	R	R	R	R	S	S	I	R	R	R	R	S
PA20	R	R	S	R	S	S	R	R	R	R	S	R	R	S	R	R	R	S
PA21	I	R	R	R	S	S	S	S	R	R	S	R	R	S	R	R	I	R
PA22	I	R	R	R	S	S	R	R	R	R	S	S	S	S	R	R	R	R
PA23	R	R	S	S	S	R	R	R	R	R	R	R	R	S	R	R	R	S
PA24	I	R	S	S	S	S	R	R	R	R	R	I	S	S	I	R	R	S
PA25	S	R	S	S	S	S	R	R	R	R	S	S	S	S	S	R	S	S
PA26	R	R	R	R	S	I	S	S	I	S	R	R	R	R	R	R	R	R
PA27	R	I	S	S	S	S	R	R	R	S	S	S	I	S	S	R	R	S
PA28	I	R	R	I	S	S	R	R	R	R	S	S	I	S	I	R	R	I
PA29	R	R	S	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
PA30	R	R	R	R	R	R	S	S	R	R	R	R	R	S	R	R	R	S

imipenem (10 µg), colistin (10 µg), amikacin (30 µg) cefepime (30 µg) ceftazidime (30 µg), tobramycin (30 µg), gentamicin (30 µg), ciprofloxacin (5 µg), polymyxin B (300 units), ofloxacin (5 µg), aztreonam (30 µg), netilmicin (30 µg), ticarcillin-clavulanate (75/10µg), cefotaxime (30 µg), ceftriaxone (30 µg), azithromycin (30 µg), levofloxacin (5 µg)and piperacillin (100 µg). All tests were done on Muller Hinton Agar (Merck, Germany).

RESULTS

A total of thirty isolates of P. aeruginosa were isolated from 160 clinical specimens. Of these, 12 isolates were isolated from injuries, 12 isolates were isolated from burns and 6 isolates were isolated from diabetes-infected foot. These isolates were identified using cultural and microscopic examinations, biochemical tests and then confirmed by PCR-sequencing for universal 16S rRNA gene. The colonies on Macconkey agar were a pale color due to their inability to ferment the lactose sugar present in this culture medium and had a smell similar to the smell of fermented grapes, on blood agar, its their colonies were in a dark color, and most of them were surrounded by a transparent halo, which indicates their ability to hemolysis (Jawetz, et al. 2019). Light microscopic observations of Gram stain films of these isolates showed bacilli cells were single or biarrangement and Gram-negative bacteria. P. aeruginosa isolates have given in biochemical tests; a positive result for oxidase and catalase tests, while have given negative results to the urease test. IMViC tests, of isolates showed negative results for indol, methyl red (MR) and Voges-Proskauer (VP) tests, but they gave a positive result of citrate test (C). In Kligler iron agar have given alkaline slant and did not change the bottom, H2S negative without gas production due to the fact that it is strictly aerobic and negative to Gram's stain (Bert, Branger, & Lambert-Zechovsky, 2002). The details of distribution and percentages of the isolates were summarized in **Table 3**.

Thirty isolates (PA1 to PA30) of P. aeruginosa were tested against 18 common antibiotics. It was found that most isolates were resistant to the antibiotics, especially to β-lactam antibiotics. P. aeruginosa isolate PA15 was resistance to all antibiotics used in this study except for aztreonam, the isolates PA2, PA3, PA4, PA5, PA7, PA9, PA11 and PA13 were resistance to all antibiotics except for colistin and polymyxin B, whereas the isolates PA25 of P. aeruginosa were sensitive to most antibiotics. **Table 4** shows the pattern of antibiotic resistance of

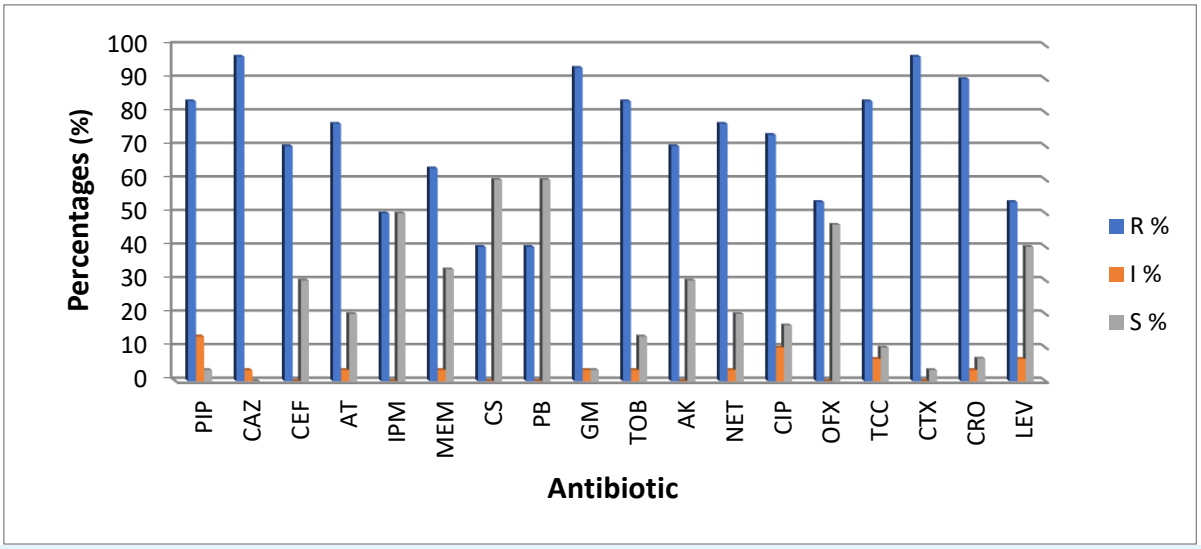

Fig. 1. The antibiotics susceptibility percentages of all P. aeruginosa isolates

Table 5. The pattern of the gene groups of P. aeruginosa isolates

Isolate No.	OXA- I	OXA- II	OXA- III	Genotype
PA1	+ve	-ve	-ve	OXA-I
PA2	-ve	-ve	-ve	Non
PA3	-ve	-ve	-ve	Non
PA4	-ve	-ve	+ve	OXA-III
PA5	+ve	-ve	-ve	OXA-I
PA6	-ve	-ve	+ve	OXA-III
PA7	-ve	-ve	+ve	OXA-III
PA8	+ve	-ve	-ve	OXA-I
PA9	-ve	-ve	-ve	Non
PA10	-ve	-ve	+ve	OXA-III
PA11	+ve	-ve	+ve	OXA-I & OXA III
PA12	+ve	-ve	-ve	OXA-I
PA13	-ve	-ve	-ve	Non
PA14	+ve	-ve	+ve	OXA-I & OXA III
PA15	+ve	-ve	+ve	OXA-I & OXA III
PA16	-ve	-ve	-ve	Non
PA17	+ve	-ve	+ve	OXA-I & OXA III
PA18	-ve	-ve	+ve	OXA-III
PA19	-ve	-ve	-ve	Non
PA20	-ve	-ve	+ve	OXA-III
PA21	-ve	-ve	-ve	Non
PA22	-ve	-ve	-ve	Non
PA23	-ve	-ve	+ve	OXA-III
PA24	-ve	-ve	-ve	Non
PA25	-ve	-ve	-ve	Non
PA26	-ve	-ve	-ve	Non
PA27	-ve	-ve	-ve	Non
PA28	-ve	-ve	-ve	Non
PA29	-ve	-ve	+ve	OXA-III
PA30	-ve	-ve	-ve	Non

Abbreviations: +ve : Positive. , -ve : Negative.

bacterial isolates in this study. The antibiotics susceptibility test results showed that the percentages of a resistance toward these antibiotics were as follow: piperacillin 83.4%, ceftazidime 96.7%, cefepime 70 %, Aztreonam 76.7 %, imipenem 50%, meropenem 63.4%, colistin 40 %, polymyxin B 40%, gentamicin 93.4%, tobramycin 83.4%, amikacin 70 %, netilmicin 76.7%, ciprofloxacin 73.4%, ofloxacin 53.4 %, ticarillin-clavulanate 83.4%, cefotaxime 96.7%, ceftriaxone 90%, and levofloxacin 53.4%, in addition, some isolates revealed intermediate resistance towards some

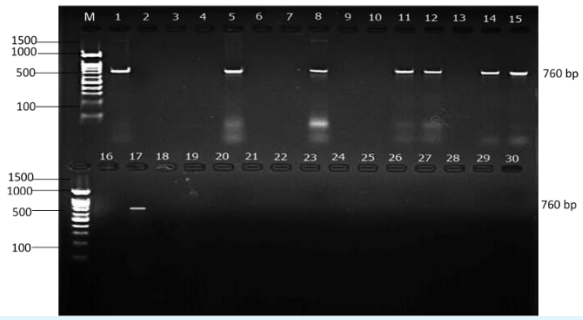

Fig. 2. Gel electrophoresis of PCR product for detection of bla OXA-I gene (760bp) using 1% agarose for 60 min. at 80 V\Cm. Lane M: Marker DNA ladder size (100-1500bp). Lanes 1, 5, 8, 11, 12, 14, 15 and 17 positive for bla OXA-I (760 bp)

antibiotics. The antibiotics susceptibility percentages of all P. aeruginosa results are shown in **Fig. 1**.

Abbreviations : R, resistance; S, sensitive; I, intermediate; PIP, piperaillin; CAZ, ceftazidime; CEF, cefepime; AT, aztreonam; IMP, imipenem; MEM, meropenem; CS, colistin; PB, polymyxin; GM, gentamicin; TOB, tobramycin; Ak, amikacin; NET, netilmicin; CIP, ciprofloxacin; CRO, ceftriaxone; TCC, ticarillin-clavulanate;CTX, cefotaxime; OFX, ofloxacin; LEV, levofloxacin.

Detection of OXA beta-lactamase genes of P. aeruginosa isolates

PCR detecting sequences of the genes of OXA group were positive, eight (26.7 %) isolates carry this gene, OXA group-I, twelve (40%) isolates carry OXA group-III, four isolates carry both OXA group-I and OXA group-III, while OXA group-II did not appear in any isolates as shown in **Table 5**. The agarose gel electrophoresis of amplification products of PCR showed that the isolates PA1, PA5, PA8, PA11, PA12, PA14, PA15 and PA17 were specific to OXA group-I, 700bp (**Fig. 2**) and 12 isolates PA4, PA6, PA7, PA10,

Table 6. The Pattern of blaOXA-I and blaOXA-III of antibiotics of P. aeruginosa

Antibiotics	bla_{OXA-I}		$bla_{OXA-III}$		bla_{OXA-1} +$bla_{OXA-III}$	
	No.	%	No.	%	No.	%
Piperacillin	8	100	12	100	4	100
Ceftazidime	8	100	12	100	4	100
Cefepime	6	75	8	66.6	3	75
Aztreonam	6	75	9	75	2	50
Imipenem	6	75	8	66.7	4	100
Meropenem	8	100	9	75	4	100
Colistin	2	25	6	50	2	50
Polymtxin	2	25	6	50	2	50
Gentamicin	7	87.5	12	100	4	100
Tobramycin	7	87.5	11	91.6	4	100
Amikacin	7	87.5	9	75	3	75
Netilmicin	7	87.5	11	91.6	3	75
Ciprofloxacin	7	87.5	10	83.3	3	75
Ofloxacin	5	62.5	6	50	2	50
Ticarcillin-clavulanate	8	100	11	91.6	4	100
Cefotaxime	8	100	11	91.6	4	100
Ceftriaxone	8	100	11	91.6	4	100
Levofloxacin	6	75	5	41.6	2	50

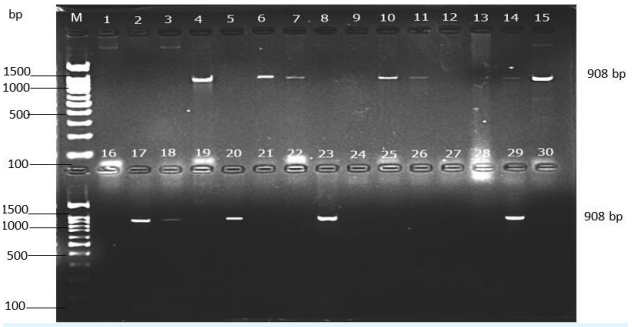

Fig. 3. Gel electrophoresis of PCR product for detection of blaOXA-III gene (760bp) using 1% agarose for 60 min. at 80 V\Cm. Lana M: Marker DNA ladder size (100-1500bp). Lanes 4, 6, 7, 10, 11, 12, 14, 15, 17, 20, 23 and 29 positive for blaOXA-III (908bp)

PA11, PA14, PA15, PA17, PA18, PA20, PA23 and PA29 were specific to OXA group-III, 908 bp (**Fig. 3**).

Detection of bla_{OXA-I} and $bla_{OXA-III}$ genes of antibiotics

In the current study, OXA-type β-lactamases production using antibiotics resistance and molecular detection of bla_{OXA-I}, and $bla_{OXA-III}$ genes in P. aeruginosa isolates revealed that there is a harmony between results of antibiotic resistance and positive molecular detection of these genes. The highest resistance rate to most antibiotics was observed in the isolates of OXA group I then group III genes, as shown in **Table 6**.

The comparison of the antibiotics resistance between OXA-type β-lactamase isolates producing and OXA-type β-lactamase isolates non-producing for P. aeruginosa are summarized in **Table 7**.

Table 7. Comparison of the antibiotics resistance of P. aeruginosa percetages between OXA-type β-lactamases producers and non-producers

Antibiotic	Isotates of OXA-type β-lactamases producing (%)	Isotates of OXA-type β-lactamases non-producing (%)
Piperacillin	100	71.4
Ceftazidime	100	92.8
Cefepime	68.7	71.4
Aztreonam	81.2	71.4
Imipenem	50	50
Meropenem	81.2	42.8
Colistin	37.5	42.8
Polymtxin	37.5	42.8
Gentamicin	93.7	100
Tobramycin	50	50
Amikacin	81.2	57.1
Netilmicin	93.7	64.2
Ciprofloxacin	93.7	78.5
Ofloxacin	50	50
Ticarcillin-clavulanate	93.7	85.7
Cefotaxime	93.7	100
Ceftriaxone	50	50
Levofloxacin	56.2	64.2

DISCUSSION

The results of this study showed that there was a susceptibility of 30 isolates of *P. aeruginosa* isolated from clinical specimens to several locally available antibiotics, and also high levels of resistance to all antibiotics. It was found that the significant changes in resistance of *P.aeruginosa* to several antibiotics, 4 out of 18 antibiotics belong to the class cephalosporins (ceftazidime 96.7 %, cefepime 70 %, cefotaxime 96.7 % and ceftriaxone 90%) which function by disrupting the synthesis of the bacterial cell wall by inhibiting the formation of the peptidoglycan layer. Although their working is similar to penicillin (piperacillin 83.4 %) class of the antibiotics, they are widely known to be less susceptible to β-lactamases[14], 2 out of 18 antibiotics were carbapenems (imipenem 50 % and meropenem 63.4 %), 3 out of 18 antibiotics were fluoroquinolones (ciprofloxacin 73.4 %, ofloxacin 53.4% and levofloxacin 53.4%), 2 out of 18 antibiotics were polymyxins (colistin and polymyxin B 40 %), 4 out of 18 antibiotics were aminoglycosides (gentamicin 93.4 %, amikacin 70 %, tobramycin 83.4 % and netilmicin 76.7 %) and one out of 18 antibiotics was a monobactam (aztreonam 76.7 %). Carbapenems have a broader spectrum of action compared to cephalosporins and penicillins, although they also function by inhibiting bacterial cell wall synthesis. Fluoroquinolones, however, work by inhibiting cell division by affecting DNA gyrase required for bacterial DNA separation during the cell division process. Monobactam is similar to penicillin and acts by

inhibiting cell wall synthesis by blocking peptidoglycan cross-linking. With the exception of fluoroquinolones, aminoglycosides and polymyxins, all other types of the mentioned classes of antibiotics exhibit the beta-lactam structure against which the beta-lactamase producing *P. aeruginosa* have emerging resistance.(Naveed, et al. 2020). It was also shown that most of the isolates of *P. aeruginosa* were 100 % multi-drug resistant (3 different antibiotics resistance). The findings also showed that the prevalence of antibiotic resistance in *P. aeruginosa* isolates was very high relative to the results of other studies.(Hasan, 2019. Lafi, & Al-Ani, 2020. Shokoohizadeh, Alizade, Namordizadeh, & Karmostaji, 2020. Gajamer, et al. 2020). All isolates were resistant to all antibiotics, while the lowest resistance rate was seen for colistin and polymyxin B (**Table 4**). When the administration of a β-lactam, aminoglycoside, or quinolone is ineffective, the polymyxins, particularly colistin, remain as the antimicrobial drugs of last option (Mitra, Basu, Rath, & Sahu, 2020). The use of colistin and polymyxin B as a option of treatment for *P. aeruginosa* multidrug resistance in this study is a potential reason for the rise in other antibiotic-resistant isolates. In addition, prolonged hospitalization in burn patients, use of broad-spectrum medications at the start of hospitalization, ignorance of hygiene standards, lack of regular screening for extended-spectrum β-lactamases (ESBL)-producing strains of *P. aeruginosa* and colonization of environmental and multi-drug-resistant strains could be the possible reasons of the increasing resistance rate to expanded-spectrum cephalosporins, anti-*Pseudomonas* penicillin, carbapenems and quinolone in the *P. aeruginosa* isolated in the current study. These results showed a high level of resistance to cephalosporin, anti-*Pseudomonas* penicillin and carbapenems. β-Lactamases, enzymes open β-lactam ring and make antibiotics inactivated. These enzymes are coded by different genes located on chromosomes or plasmids. These enzymes, which are mostly ESBL, in Ambler classification are divided into four groups, A to D[20]. Various Ambler's class D ESBLs, such as OXA-type ESBLs have been identified and encountered most commonly in *P. aeruginosa.*(Bush, & Bradford, 2020). Based on these findings, the prevalence of OXA-type β-lactamases producing 66.7 % isolates as the most frequent and OXA-type β-lactamases non-producing of *P. aeruginosa*, among that 66.7 % was 40 % (12 isolates) showed the OXA-groupIII like the most frequent, OXA-group I as the least frequent with 26.7% (8 isolates) and the OXA group-II did not appear in any isolates as shown in **Table 5**. The results of the study of *ble*$_{OXA}$ genes compared with other results obtained in countries like Iran (Aghazadeh, et al. 2016) OXA-group I genes 56 %, OXA-group II 26 % and OXA-Group III 19 %, Egypt (Paramythiotou, & Routsi, 2016). the OXA-group II (60.7%) and in Iraq (Salim, & Alwan, 2017). the OXA group I 100 % was high compared to the results obtained in this study. The frequency of OXA-group I, II, and III reported in France[7] were 5%, 4%, and 4%, respectively, and was lower compared to the results obtained in this study. Some factors such as diversity of antibiotic use, geographic difference and different mechanisms of gene transfer such as horizontal gene transfer (including transposable elements) can be the cause of transmission of class D beta-lactamases genes among different bacterial strains may affect the diversity of gene, it is a global concern threatening all countries and communities (J Wolter, & D Lister, 2013).

The highest resistance rate to most antibiotics was observed in isolates of the OXA group I then group III genes (**Table 6**). It is reported that ESBLs in *Enterobacteriaceae* co-exist with mechanisms conferring resistance to other antimicrobial classes and as such these organisms become multidrug-resistant, hence limiting treatment options for these infections[18]. In the case of severe infections caused by OXA-type β-lactamases-producing bacteria, carbapenems are the antibiotics of first choice (Rahman, et al. 2015). However, several studies have reported emerging resistance to carbapenem antibiotics owing to the increased production of β-lactamases worldwide, which hydrolyze all β-lactam antibiotics including carbapenems. The result of this study showed that 66.7 % of OXA-type β-lactamases-producing, 26.7% of carbapenem-resistant *P. aeruginosa* isolates harboring OXA-group I gene (includes OXA-5, OXA-7 and OXA-10)[7]. It has been shown that the majority of the carbapenem resistance in *P. aeruginosa* is due to the production of carbapenemases, especially those belonging to the carbapenem-hydrolyzing OXA β-lactamases class D (CHDLs) which are encoded by the *bla* $_{OXA-10}$.(Alam, et al. 2018). Carbapenemases confer resistance not only to carbapenem antibiotics but also to penicillin and cephem antibiotics, therefore, the highest antibiotics resistance of the isolates may be because they contain these genes. This prevalence is equal to data from studies in Uganda where only 28.6% of carbapenemase-producers were detected among ESBL-producing *Enterobacteriaceae* (Okoche, et al. 2015). On the other hand, ceftazidime was ineffective among isolates of OXA-groups I and III, the OXA-group I (includes OXA-5, OXA-7 and OXA-10) confer greater resistance to ceftazidime than to cefepime(Aubert, Det al. 2001). in contrast, OXA-group III (includes OXA-1, OXA-4, OXA-30 and OXA-31) (MacFaddin, (2000). characteristically show decreased susceptibility to cefepime but remain susceptible to ceftazidime (Jia, et al. 2020). In this study, among the 8 isolates that showed resistance 75% to cefepime and resistance 100% to ceftazidime was carry OXA-group I gene, while among the12 isolates that showed resistance 66% to cefepime and resistance100% to ceftazidime was carry OXA-group III, as demonstrated by other investigations (Bush,

& Bradford, 2020, Salim, & Alwan, 2017). The OXA-type β-lactamases isolates producing were significantly more resistant than non-producing for *P. aeruginosa* to piperacillin, ceftazidime, aztreonam, meropenem, amikacin, netilmicin, ciprofloxacin and ticarcillin-clavulanate, **Table 7**. The OXA-group I, II and III genes determine resistance to carboxypenicillins and ureidopenicillins, the resistance to piperacillin resulting from the production of OXA-group II genes is lower than the resistance that develops when group I and group II oxacillinases are produced, the oxacillinases which predominantly occur in *P. aeruginosa* are responsible for the resistance to cefotaxime, ceftazidime, and aztreonam, called OXA-type ESBLs (Zhao, & Hu, 2010).

CONCLUSION

The rates of OXA-type β-lactamases producing *P. aeruginosa* isolates from clinical specimens were notable, therefore, the management and treatment strategies should be revised and the proper use of the infection-control measures is needed to reduce the spread of resistant genes in the isolates of *P. aeruginosa.*

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