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Molecular detection of *bla*$_{OXA-10}$(OXA-10) type Beta-lactamase encoding gene among extended spectrum Beta-lactamase isolates of *Pseudomonas aeruginosa*

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Abstract

Background and objective: *Pseudomonas aeruginosa* is an opportunistic pathogen and inherently resistant to many antibiotics and can mutate to even more resistant strains during therapy. Resistance to the antibiotics in this group of bacteria increased due to the activity of β-lactamase genes and one of the most important groups of genes, *bla*OXA gene producing enzymes. The current study aimed to determine the prevalence of Ambler class D β-lactamases, including OXA-10 gene among *P. aeruginosa* isolated from patients in Erbil, Kurdistan.

Methods: Different clinical specimens were taken from patients with clinical symptoms of infection during one year. Identification was carried out on all isolates by Vitek2 system. Antibiotic susceptibility for antimicrobial agents was performed according to the clinical and laboratory standards institute (CLSI) guidelines. Production of Ambler class D β-lactamases was confirmed by polymerase chain reaction technique.

Results: A total of 100 isolates of *P. aeruginosa*, 57 isolates (57%) had shown resistance to six or more than six antibiotics, and 15 isolates showed resistance to one antibiotic. Also, none of the resistant isolates were showed complete resistance to all antibiotics. Out of 89 *P. aeruginosa*, 38.2% of isolates possessed *the bla*OXA-10 gene.

Conclusion: The results revealed the occurrence of extended-spectrum β- lactamases producing *Pseudomonas aeruginos*us, and proper infection control practices are crucial to avert the spreading of ESBL-producing isolates in hospitals.

Keywords: *bla*$_{OXA-10}$; Beta-lactamase; Drug resistance; Erbil; *Pseudomonas aeruginosa.*

Introduction

Antibiotic resistance is a daunting phenomenon with a growing impact on patient safety, particularly for nosocomial infections.[1] The nosocomial strains of *P. aeruginosa* are frequently resistant to a broad range of commercially available antibiotics. Their prevalence appears to be increasing worldwide, especially as a cause of ventilator-associated pneumonia and in patients with severe burn injuries.[2] Critically ill patients are prone to colonization and infection by antibiotic-resistant bacteria because of the frequent exposure of these patients to antibiotics. This dangerous array increased the need for broad-spectrum antibiotics, reduced antimicrobial efficacy, and increased antibiotic resistant strains.[3] These infec-tions are complicated to treat due to the occurrence of multidrug-resistant (MDR) organisms, which include resistance to beta-lactams, aminoglycosides, fluoroquinolones, and carbapenems.[4] Carbapenems were subsequently introduced as the last resorts of antibiotics due to their high potency and the exceptional broad spectrum of antimicrobial activity, but the carbapenemases, are capable of causing resistance to a wide variety of β-lactam antibiotics, including carbapenems.[5] *P. aeruginosa* can become resistant to the carbapenems through a number of mechanisms mainly due to the production of OXA-type carbapenemases (class D)

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according to the Ambler classification.[6,7] Currently, the number of OXA-type carbapenemases has increased,[8] and narrow-spectrum Class D *bla*$_{OXA-10}$ gene are often detected in *P. aeruginosa* and other gram-negative bacteria.[9] The current study's objective was to isolate and identify *P. aeruginosa* from patients with nosocomial infections at Erbil's main hospitals, delineate the molecular characteristics and antibiotic resistance of infections caused by *P. aeruginosa* producing class D *bla*OXA-10 genes in multidrug-resistance isolates.

Methods

Patient's specimens

Hundred *P. aeruginosa isolates* that were consecutively collected from different specimens, comprising urine, blood, sputum, and swabs from an infected wound and burn patients, were taken from three different hospitals in Erbil, Kurdistan region: Rozhawa Emergency Hospital (Burn unit), Rizgari Teaching Hospital, and Hawler Teaching Hospital from September 2013 to October 2014. The patients who were on antibiotics were excluded. This study was achieved according to the ethical committing at Hawler Medical University, and all persons had given their acceptance to share in the study.

Bacterial isolation and Identification:

Bacterial isolates identified as *P. aeruginosa* through colonial morphology on culture media and some biochemical identification and confirmed by VITEK 2 (bioMerieuxInc, France) automated system (100 isolates) were stored at - 20ºC in trypticase soy broth supplemented with 15% glycerol.[10] Identification of the *P. aeruginosa* was carried out using the conventional method and confirmed by the VITEK 2 automated system (bioMerieuxInc, France).[11]

Antimicrobial susceptibility testing

Antibiotic sensitivity testing was performed through agents was performed and interpreted according to the clinical and laboratory standards institute (CLSI) criteria for ampicillin(10µg), amoxicillin-clavulanic acid(30µg), piperacillin-tazobactam(100/10µg), cefuroxime(30µg), cefoxitin(30µg), cefotaxime(30µg), ceftazidime(30µg), cefepime(30µg), imipenem(10µg), meropenem(10µg), gentamicin(10µg), tobramycin(10µg), ciprofloxacin(30µg), norfloxacin(10µg), nitrofurantoin(300µg), colistin(10µg), and trimethoprim(5µg).[11]

Molecular assay

The polymerase chain reaction was performed on multi-drug resistant *P. aeruginosa* isolates to detect the extended-spectrum β-lactamases. Specific primers were used to detect ESBL-encoding genes*(bla*$_{OXA-10}$ gene).[12] Specific primers were selected to detect the genes encoding class D and their extended-spectrum derivatives; OXA-10was used for PCR amplification. The PCR master mix for OXA-10 was prepared according to the standard protocol, to perform gel electrophoresis method (Table 1).[13]

Table 1: Primers used for amplification of ESBLs genes in the study of *P. aeruginosa.*

Target gene	Primers	Primer sequence	Product size (bp)	References
OXA-10	OXA-10A	5'-GTC TTT CGA AGT ACG GCA TTA-3'	699	Jiang *et al.,* 2006
	OXA-10B	5'-ATT TTC TTA GCG GCA ACT TAC-3'		

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Results

Identification of *Pseudomonas aeruginosa*

The Gram-negative non-lactose fermenter isolates, oxidase-positive, grown at 42ºC, and some have the ability to produce a blue-green pigment on Mueller-Hinton agar were identified as *P. aeruginosa* and confirmed by VITEK 2 (bioMérieux).

Antibiotic resistance in *P. aeruginosa* isolates

The susceptibility test of the isolates was interpreted. All isolates were resistant to at least two or more anti-pseudomonal antibiotic groups. The resistance rate of *P. aeruginosa* to ciprofloxacin, cefepime, tobramycin, piperacillin/tazobactam, and gentamicin were 51%, 59%, 61%, 63%, and 73%, respectively. All *P. aeruginosa* isolates 100% showed resistance to cefotaxime, cefuroxime, and cefoxitin. Out of the 100 isolates, 83 were resistant to at least one carbapenem, 51% and 60% were resistant to meropenem, imipenem, respectively. The results are presented in Table 2. Colistin was the most effective drug with all *P. aeruginosa* isolates were found to be 100% susceptible to colistin. Multi-resistant isolates were selected for ESBL detection by PCR.

Table 2: The susceptibility of *P. aeruginosa* isolates to antibiotics.

Antibiotics	Ampicillin	Amoxicillin/Clavulanic acid	Amoxicillin/Clavulanic acid	Cefuroxime	Cefuroxime Axetil	Cefoxitin	Cefotaxime	Ceftazidime	Cefepime	Imipenem	Meropenem	Gentamicin	Tobramycin	Ciprofloxacin	Norfloxacin	Nitrofurantoin	Colistin	Trimethoprim
% of resistant isolates	100	100	64	100	100	100	100	63	59	60	51	73	61	51	55	99	0	100
% of Intermediate isolates	0	0	35	0	0	0	0	4	8	13	10	2	0	6	0	0	0	0
% of sensitive isolates	0	0	1	0	0	0	0	33	33	27	39	25	39	43	45	1	100	0

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The results of this study showed that 57 samples of *P. aeruginosa* (57%) are resistant to six or more than six antibiotics, and 15 isolates showed resistance to one antibiotic. Also, none of the resistant isolates were showed complete resistance to all antibiotics, as shown in Figure 1.

Detection of OXA-10 gene by Polymerase Chain Reaction

About 89 *P. aeruginosa* isolates were evaluated for *bla*$_{OXA-10}$. As a result, 38.2% (n=34) of the isolates had *bla*$_{OXA-10}$ gene (Figure 2).

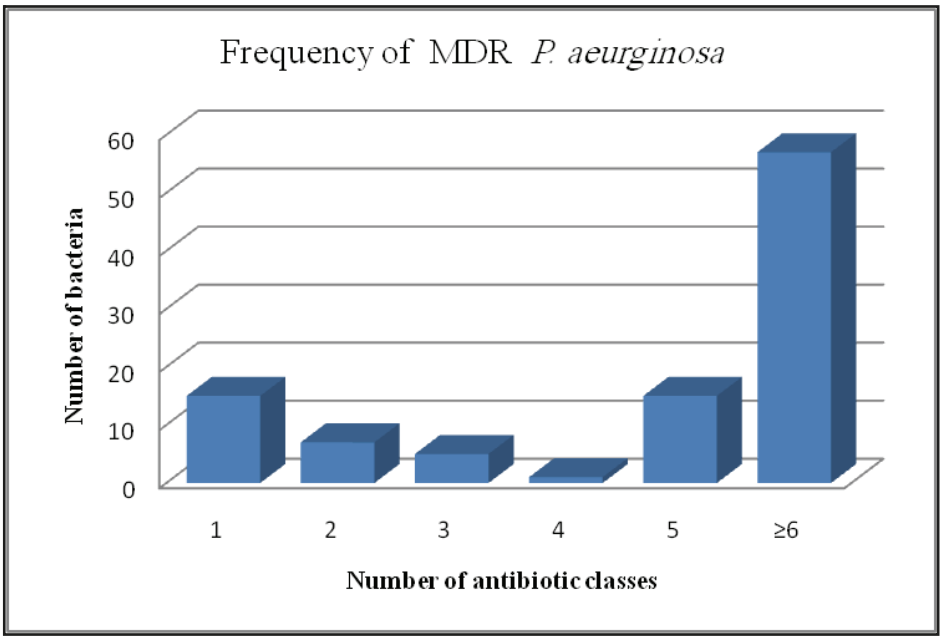

Figure 1: Analysis of drug resistance pattern against *P. aeruginosa* (n=100).

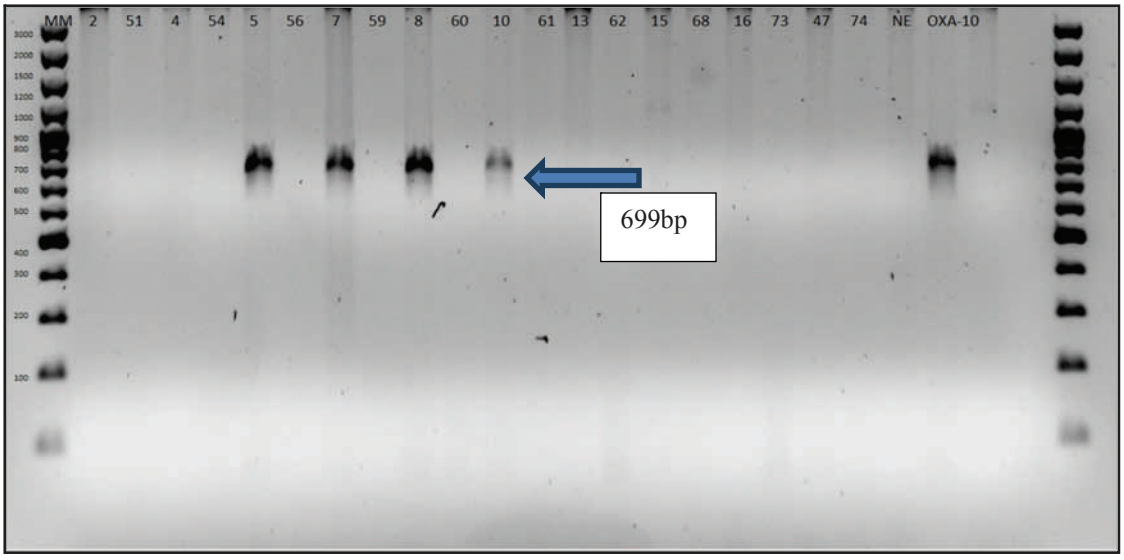

Figure 2: Gel picture of PCR amplification of *the bla*$_{OXA-10}$ gene in *P. aeruginosa* isolates. First and last Lane; 100bp DNA marker.2-74; representative isolates tested, NE, negative control, and OXA-10, positive control.

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Discussion

The incidence of multidrug-resistant *P. aeruginosa* is increasing worldwide. This pathogen is intrinsically resistant to many antibacterial agents, such as most β-lactams.[14] Furthermore, it also has a remarkable capacity to develop or acquire new mechanisms of resistance to antibiotics. This may be related to the large size and the versatility of its genome and its distribution in aquatic habitats, which could constitute a reservoir for bacteria carrying other resistance genes.[15] The study results showed a high resistance rate to cephalosporins and a low resistance rate for ciprofloxacin. Most of the isolates were resistant to carbapenems and at least two or more anti-pseudomonal antibiotic groups and classified as multi-drug resistant *P. aeruginosa.* The rate of resistance in this study was higher than that recorded earlier by Tavajjohi.[16] According to a survey conducted byJaponi[17] in one of the burn hospitals in Shiraz, Iran,[18] reported all isolates were resistant to three or more antibiotic classes, the resistance rate of *P. aeruginosa* to different antibiotics as tobramycin and cefoxitin were (100%), imipenem, (98%), meropenem (94%), ceftazidime (92%) and piperacillin/ tazobactam (88%). Cephalosporins, a known anti-pseudomonas drug, especially the third-generation ceftazidime, has a high susceptibility pattern. According to this study, 63% of *P. aeruginosa* isolates from hospitals in Erbil were ceftazidime resistant. A study done in Iran showed 83.3% of *P. aeruginosa* isolates from major hospitals were ceftazidime resistant.[19] Since knowledge about antibiotic susceptibility can help choose the appropriate treatment agents and control nosocomial infections. The results of the current study agree with the results of other investigators who showed that colistin was the most effective antibiotic against MDR isolates.[17] Hence colistin remains the most consistently effective agent in vitro against *P. aeruginosa.*[20]The high prevalence of MDR *P. aeruginosa* may be related to the increasing numbers of immuno-compromised patients due to different diseases and contaminations of the environment. The inordinate accessibility of antibiotics of low quality and ineffective for treatment, as well as the consumption of drugs without a proper medical prescription, is associated with increased infections with these MDR bacteria.[21] The present study investigated the predominant β-lactamase coding genes such as OXA-10 through PCR showed that 38.2% of isolates had OXA-10 among the MDR *P. aeruginosa* isolates collected. In a study from Iran reported the prevalence of structural genes of *bla*$_{OXA-10}$ gene was 40 (64%) among 63 strain with multi-drug resistance,[14]Class D OXA β-lactamases were more frequently detected than class A in *P. aeruginosa*, which agrees with the study in Korea, that among the 252 isolates; 53 (21.0%) isolates harbored OXA-type enzyme similar current study *bla*$_{OXA-10}$ was the most prevalent.[22] The OXA-10 enzymes are frequently observed in *P. aeruginosa* and possess high-level hydrolytic activity against oxacillin, and methicillin and their activities are poorly or not inhibited by clavulanic acid.[23,24] The prevalence of ESBL among *P. aeruginosa* is variable from country to country and even among different institutions in the same country and continuously changing over time. This is due to uncontrolled country-specific differences in hygiene, diagnostic practices, community infection, and control policies that could provide different explanations for some of the observed variances.

Conclusion

The resistance rate to various antibacterial agents was remarkable in the present study. Since there are limited effective antibiotics against ESBLs producing *P. aeruginosa*, all isolates must be examined by antimicrobial susceptibility testing, which restricts their spreads and aids in managing treatment policy.

Competing interests

The authors declare no competing interests.

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