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Genotyping, Pandrug Resistance, Extensively Drug-Resistant, and Multi Drug-Resistance Detection of *Pseudomonas aeruginosa* Isolated From Patients in the West of Iran

Samaneh Rouhi[1,2], Rashid Ramazanzadeh[2,3*], Bijan Nouri[4]

Abstract

Objectives: Resistance to multiple antibiotic classes is called multidrug resistance (MDR), extremely drug resistance (XDR), and pandrug resistance (PDR). In addition, the genotyping survey of resistant bacteria is a prominent factor in epidemiological surveying. Therefore, the present study aimed to conduct an epidemiological survey in order to detect XDR, MDR, and PDR of *Pseudomonas aeruginosa* isolated from clinical specimens in Kurdistan province, Iran.

Materials and Methods: During (December) 2015-(August) 2017, a total of 134 strains of *P. aeruginosa* were detected by the polymerase chain reaction and were tested for antibiotic resistance, MDR, XDR, and PDR. The related specimens of the patients with nosocomial infections were subjected to BOX-PCR analysis. Finally, the data were analyzed using Stata software with frequency determination, Fisher exact test, and Logistic regression ($P \leq 0.05$). BOX-PCR analysis was performed by GelJ version 1.13 software.

Results: Based on the results, the lowest and highest resistance and susceptibility rates in 134 isolates of *P. aeruginosa* were related to cefpodoxime (93.28%), imipenem (27.61%), colistin (69.40%), and cefpodoxime (6.71%), respectively. In addition, MDR and XDR isolates were observed in 97.76% and 15.67% of *P. aeruginosa* isolates, respectively. However, PDR was detected in none of the isolates while BOX-PCR demonstrated four main clusters with 56 unique patterns in nosocomial infection isolates. Further, a significant relationship was observed between MDR and XDR isolates and nosocomial infection ($P \leq 0.05$). Eventually, there was a significant association between ward, the type of specimens, and hospitals with nosocomial infection ($P \leq 0.05$).

Conclusions: In general, a high frequency of antibiotic resistance was observed in this study whereas no genetic correlation was observed between *P. aeruginosa* strains. In sum, selecting the most effective antibiotics and devising an efficient way for controlling the antibiotic resistance should be taken into consideration.

Keywords: Genotyping, Pandrug Resistance, Extensively Drug resistance, Multidrug resistance, *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is an important bacterium since it has high intrinsic and acquired resistance against different classes of antimicrobial drugs including beta-lactams, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins (1,2). Specific outer membrane proteins (i.e., efflux transport systems), the production of beta-lactamases and carbapenemases, mutations in chromosomal resistance genes, or the acquisition of resistance genes from other bacteria are considered the mechanisms of resistance in *P. aeruginosa* (3). Some of *P. aeruginosa* strains are resistant to multiple classes of antibiotics, which are called multidrug resistance (MDR), extremely drug resistance (XDR), and pandrug resistant (PDR) strains (4,5). According to the Clinical Laboratory Standards Institute (CLSI), MDR is defined as the acquired non-susceptibility to at least one agent in three or more antimicrobial categories; In addition, XDR is referred to as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories) and finally, PDR is defined as non-susceptibility to all agents in all antimicrobial categories (6). Further, an epidemiological survey of *P. aeruginosa* indicates that this microorganism is commonly found in different environments such as soil, water, plants, hospital environment, sinks, floors, baths, soap dishes, as well as animals and humans (1,7). Different genotyping methods are used for epidemiological research of these bacteria in nosocomial infection. Specifying the clones and determining the correlation between such clones are important to epidemiological studies of *P. aeruginosa* infections in different regions (2). Furthermore, different methods are applied for bacterial genotyping such as pulsed-field gel electrophoresis, multiple-locus variable number tandem repeat analysis, and multilocus sequence typing. Additionally, repetitive element-based polymerase chain reaction (rep-PCR) like repetitive extragenic

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[1]Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran. [2]Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran. [3]Microbiology Department, Kurdistan University of Medical Sciences, Sanandaj, Iran. [4]Social Determinants of Health Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran.
*Corresponding Author: Rashid Ramazanzadeh, Tel: +989143104424, Email: atrop_t51@yahoo.com

palindromic (REP) sequence, the enterobacterial repetitive intergenic consensus (ERIC) sequence, and the BOX elements are utilized for typing purposes (8-10). Different studies confirmed the antibiotic resistance and genotyping of *P. aeruginosa* using REP sequence. For example, Saderi and Owlia found that among 88 *P. aeruginosa* strains isolated from clinical specimens of the patients, 54.5% and 33% of the isolates were determined as MDR and XDR, respectively (4). Considering ERIC-PCR, Savari et al demonstrated a similarity between *P. aeruginosa* clinical isolates in the hospitals (11). In another study, Wolska et al surveyed the epidemiological discrimination of clinical *P. aeruginosa* isolates by BOX-PCR fingerprinting technique and concluded that isolates had no genetic relationship (7). Therefore, epidemiological survey and resistant strains detection are crucial for controlling the spread of these bacteria and better planning for the antibiotic administration. Accordingly, the current study sought to investigate XDR, MDR, and PDR of *P. aeruginosa* isolated from patients with nosocomial and non-nosocomial infections and to perform the relevant epidemiological survey in Kurdistan province, Iran.

Materials and Methods

Study Population

This descriptive cross-sectional study was performed during (December) 2015-(August) 2017 with the approval of the Ethics Committee of Kurdistan University of Medical Sciences under the ethical code of MUK.REC. 1394/337. A number of 146 *Pseudomonas* spp. isolates were collected from clinical specimens of 49 women and 97 men (each clinical specimen was related to one patient). Included criteria were all the patients with *P. aeruginosa* nosocomial and non-nosocomial infections who referred to the following tertiary referral hospital from December 2015 to August 2017; Toohid, Besat, and Kowsar (in Sanandaj), Imam Hossein (in Bijar), Imam Khomeini (in Saqqez), and Fajr (in Marivan) Hospitals. Clinical specimens which were not identified in molecular and phenotypic tests as *P. aeruginosa* strains were excluded from the study. In addition, patients with nosocomial *P. aeruginosa* infections were detected according to national nosocomial infections surveillance system, surveillance of disease control and prevention, as well as nursing diagnoses for infection management in the hospitals, namely, the nosocomial infections which occurred after the first 48 hours of hospital admission (12).

Pseudomonas aeruginosa Detection Using Phenotypic Tests

All 146 clinical isolates of *Pseudomonas* spp. were tested based on general phenotypic methods for *P. aeruginosa* detection using phenotypic tests.

The isolates were routinely cultured on blood agar, MacConkey, eosin-methylene blue agar, and Müller-Hinton agar (MHA) and then, incubated at 37°C for 24 hours. Further, growth in an aerobic environment, gram-stain, oxidase, and catalase tests (Pattan Teb, Iran), as well as movement test in sulfur-indole-motility agar, indole, methyl red, Voges-Proskauer, and Simmons' citrate tests were performed on these isolates. Finally, urease, OF (Oxidative/Fermentative), arginine dehydrogenase, lysine decarboxylase, and ornithine decarboxylase tests were conducted on such isolates as well; it is noteworthy that all the mediums were previously manufactured by Merck Company (13).

DNA Extraction of *Pseudomonas* spp.

At first, single and pure colonies of the overnight culture of this bacterium on MHA were dissolved in 500 µL sterile deionized water in 1.5 mL tubes. Based on the lysis procedure, 15 µL of 20% sodium dodecyl sulfate (Sinaclon, Iran) and 20 µL of proteinase K (Sinaclon, Iran) were added to the tubes. Then, a small amount of powdered glass, along with 500 µL of ethylenedinitrilotetraacetic acid (10 mM Tris, 1mM EDTA, pH 8.0) was added to tubes as well. After centrifugation (7000 rpm, 5 minutes), 20 µL of the supernatant was used for PCR and 3 µL of DNA in a concentration of <1000 ng/µL was employed as the DNA template.

PCR for *Pseudomonas aeruginosa* Strains Detection

In the next step, PCR was performed for *P. aeruginosa* detection: *gyr*B (Gyrase B) was used as the housekeeping gene in order to identify the specific *P. aeruginosa* strain forward (F-5′-CCTGACCATCCGTCGCCACAAC-3′) and reverse (R-5′-CGCAGCAGGATGCCGACGCC-3′); primers (222 bp, SinaClon, Iran) in the volumes of 25 µL (i.e., 7.5 µL deionized water, 3 µL DNA template in concentration of about <1000 ng/uL, 1 µL forward (F)-primer and 1 µL reverse (R)-primer in concentrations of 0.5 µM and 12.5 µL 2X Master mix) were used for PCR. *P. aeruginosa* strain ATCC 25922 (Pasteur Institute, Iran) and deionized water were used as positive and negative controls, respectively. Finally, 8 µL of the amplification product was loaded on 2% agarose gel with a ladder (Sinaclon, Iran) of 1500 bp (14).

The Detection of PDR, XDR, and MDR Isolates of *Pseudomonas aeruginosa*

To this end, a 0.5 McFarland standard was prepared using the *P. aeruginosa* which was cultured on blood agar and then, it was cultured on MHA. Antibiotic disks were placed 2-2.5 cm apart on MHA plates and they were incubated at 37°C for 24 hours. Next, the diameters of inhibition zones were measured, recorded, and then compared with the CLSI standard. Antibiotics disks (from Rosco Company, Denmark) utilized in the present study include antimetabolite; trimethoprim/sulfamethoxazole (SXT 25 µg), polypeptides; colistin (CO 10 µg), quinolone; nalidixic acid (NAL 30 µg) and ciprofloxacin (CIPR 5 µg), aminoglycoside; tobramycin (TOB 10 µg), gentamicin

(GEN 10 µg), tetracycline (TET 30 µg), amikacin (AMI 30 µg), beta-lactam; imipenem (IMI 10 µg), ceftriaxone (CTR 30 µg), amoxicillin (AMOXY 10 µg), ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg), cefpodoxime (CPD 10 µg) and cefepime (FEP 30 µg) (4,6,15).

BOX-PCR for Genotyping

BOX-PCR was performed for 56 strains related to nosocomial infection. Furthermore, DNA in BOX-PCR was extracted using a gram-negative DNA extraction kit (Sinaclon, Iran). Additionally, BOX primer sequences (5′-CTACGGCAAGGCGACGCTGACG-3′, Macrogen, South Korea) were utilized in BOX-PCR amplification for typing and distributing these repetitive sequences in *P. aeruginosa*. The amplification conditions included initial denaturation at 95°C for 3 minutes at one cycle, followed by 35 cycles; denaturation at 94°C for 3 minutes, annealing at 48°C for 1 minute, extension at 72°C for 2 minutes, and final extension at 72°C for 5 minutes at one cycle. Eventually, 8 µL of the amplification product was loaded on 2% agarose gel and the ladder 3000 bp (Sinaclon, Iran) was used as the marker (11).

Statistical Analysis

The Stata software, version 12, as well as frequency determination and Fisher exact test was employed to analyze the data. In addition, a logistic regression model was utilized to examine the effects of independent variables in the presence of each other ($P \leq 0.05$). Finally, to draw the dendrogram, banding patterns of BOX-PCR were analyzed using GelJ software, version 1.3 (https://sourceforge.net/projects/gelj/) with a dice tolerance of 2.0, unweighted pair group method with arithmatic mean (UPGMA) (that is a simple agglomerative [bottom-up] hierarchical clustering method) and a 95% similarity.

Results

Sample Information

Totally, 133 (91.09%) and 134 (91.78%) *P. aeruginosa* isolates were detected in the phenotypic and the PCR tests, respectively. The isolates resulted from PCR test included 13 (9.70%) outpatients, 119 (88.80%) in-patients, and 2 (1.49%) cases of a diseased patient. However, 12 samples (8.21%) were identified in none of the phenotypic test and PCR as *P. aeruginosa* isolates. The studied isolates belonged to 87 (64.92%) men and 47 (35.07%) women. Further, nosocomial infection was diagnosed in 56 (41.79%) in-patients including 14 (25%) women and 42 men (75%). Based on the results related to the antimicrobial susceptibility pattern of 134 isolates of *P. aeruginosa*, the highest and lowest resistance rates were related to beta-lactam categories, cefpodoxime (93.28%), and Imipenem (27.61%), respectively. Furthermore, the highest and lowest susceptibility rates in isolates belonged to polypeptide categories including colistin (69.40%), as well as beta-lactam categories, namely, Cefpodoxime (6.71%), respectively (Table 1).

The Antimicrobial Susceptibility, MDR, XDR, and PDR Patterns of *Pseudomonas aeruginosa* Isolates

The *P. aeruginosa* antimicrobial categories and agents were used to define MDR, XDR, and PDR as follows: polypeptide; CO, aminoglycoside; TOB, GEN, AMI, and beta-lactam; IMI, FEP, CIPR, and CAZ (6). Additionally, MDR isolates were observed among 131 (97.76%) *P. aeruginosa* isolates. Finally, 21 (15.67%) *P. aeruginosa* isolates were detected as XDR while no PDR isolates were

Table 1. The Antimicrobial Susceptibility Pattern of 134 *Pseudomonas aeruginosa* Isolates From Clinical Specimens

Antimicrobial Categories	Antimicrobial Agents	Number of isolates (%)		
		Resistant	Intermediate	Susceptible
Antimetabolite	SXT	114 (85.07)	3 (2.23)	17 (12.68)
Polypeptide	CO	41(30.59)	-	93 (69.40)
Quinolone	NAL	113 (84.32)	3 (2.23)	18 (13.43)
	CIPR	87 (64.92)	13 (9.70)	34 (25.37)
Aminoglycoside	TOB	77 (12.68)	4 (2.98)	53 (39.55)
	GEN	86 (64.17)	6 (4.47)	42 (31.34)
	TET	122 (91.04)	2 (1.49)	10 (7.46)
	AMI	52 (38.80)	6 (4.47)	76 (56.71)
Beta-Lactam	IMI	37 (27.61)	7 (5.22)	90 (67.16)
	CTR	100 (74.62)	20 (14.92)	14 (10.44)
	AMOXY	122 (91.04)	2 (1.49)	10 (7.46)
	CAZ	89 (66.41)	5 (3.73)	40 (29.85)
	CTX	109 (81.34)	3 (2.23)	22 (16.41)
	CPD	125 (93.28)	-	9 (6.71)
	FEP	103 (76.86)	6 (4.47)	25 (18.65)

SXT: Trimethoprim/sulfamethoxazole; CO: Colistin; NAL: Nalidixic acid: CIPR: Ciprofloxacin; TOB: Tobramycin; GEN: Gentamicin; TET: Tetracycline; AMI: Amikacin; IMI: Imipenem; CTR: Ceftriaxone; AMOXY: Amoxicillin; CAZ: Ceftazidime; CTX: Cefotaxime; CPD: Cefpodoxime; FEP: Cefepime.

found in this study.

BOX-PCR Results

As shown in Figure 1, BOX-PCR fingerprinting with 60% of similarity represents 4 main clusters (i.e., C1-C4) with 56 unique patterns. In addition, C1 is the predominant cluster with 15 patterns containing the most amount of isolates or predominant *P. aeruginosa* isolates 15/56 (26.78%). Further, gel electrophoresis of BOX-PCR products demonstrates 2-11 bands between 100–1000 bp, which is displayed in Figure 2. Furthermore, nearly 5-10 bands exist for the *P. aeruginosa* isolates. Additionally, based on the results of Table 2, the highest distribution rates of *P. aeruginosa* strains, isolated from nosocomial infection, are observed in intensive care unit (n = 39, 69.64%), followed by neurology (n = 6, 10.71%), men's surgery (n = 4, 7.14%), burn (n = 2, 3.57%), and emergency, men's internal, oncology, operating, and women heart (each with 1 isolate, 1.78%). In addition, higher rates of *P. aeruginosa* strains are isolated from tracheal (n = 29, 51.78%), urine/culture (n = 12, 21.42%), wound (n = 5, 8.92%), as well as blood/culture, lung secretions, and pleural fluid (each with 3, 5.35%) strains and sputum with 1 (1.78%) strain.

The Results of Statistical Analysis

There was a significant relationship between MDR and XDR isolates and nosocomial infection ($P \leq 0.05$). Further, a significant relationship was observed between the wards (ICU) and the type of specimens (i.e., tracheal and lung secretion) and hospitals (Toohid and Besat) with nosocomial infection ($P \leq 0.05$).

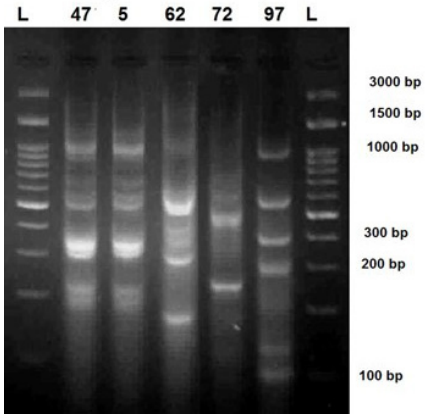

Figure 2. The Agarose Gel Electrophoresis of BOX-PCR Fingerprinting Patterns From the Genomic DNA of P. aeruginosa Strains Isolated From Nosocomial Infections in Different Hospitals
Note. L: Ladder 3000 bp; 47-97 numbers: Number of strains.

Discussion

Resistant *P. aeruginosa* is viewed as an important cause of the nosocomial infections of hospitalized patients. The widespread outbreak of infectious diseases by *P. aeruginosa* is reported in different parts of the world with a mortality rate of 23% (16,17). Furthermore, the prevalence of MDR and XDR of *P. aeruginosa* isolates is increasing, causing serious problems in hospital settings, in particular, ICU ward (2,18). In the current study, 134

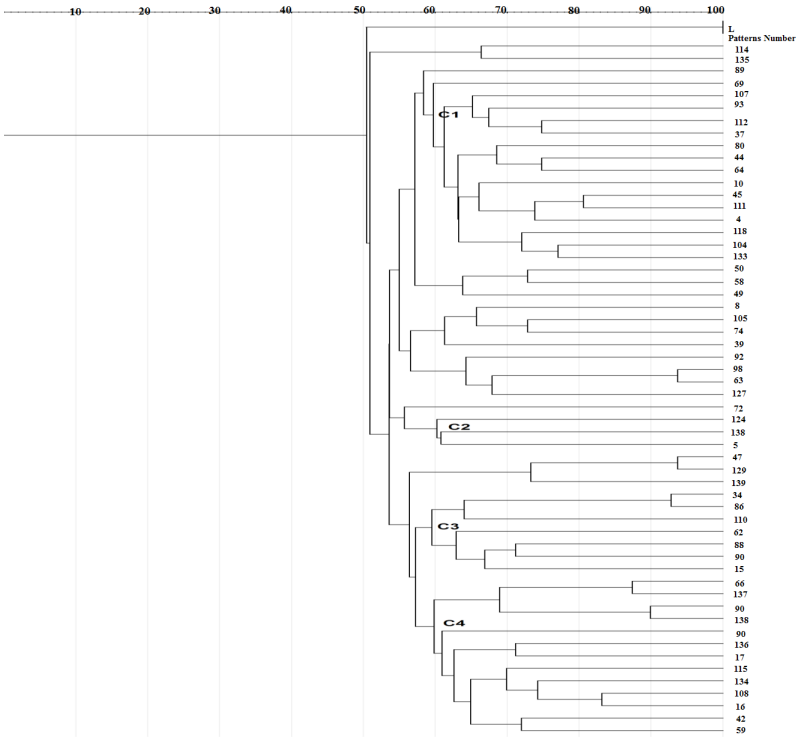

Figure 1. The Dendrogram Analysis Related to the Genomic Similarity of *Pseudomonas aeruginosa* Strains in Nosocomial Infection
Note. C: Clusters C1–C4 with 56 patterns; L: Ladder.

Table 2. The Characterization of the Total of 56 *Pseudomonas aeruginosa* Strains Isolated From Nosocomial Infections

Patterns Number of Nosocomial Infection *P. aeruginosa*	XDR	MDR	Sample Type	Ward	Hospital
S4	No	Yes	Tracheal	ICU	Toohid
S107	Yes	Yes	U/C	Neurology	Toohid
S108	No	Yes	B/C	Emergency	Toohid
S104	No	Yes	Tracheal	ICU	Toohid
S110	No	Yes	Tracheal	ICU	Toohid
S97	No	Yes	Tracheal	ICU	Toohid
S105	Yes	Yes	B/C	Men's Surgery	Toohid
S8	No	Yes	Tracheal	ICU	Besat
S98	No	Yes	Pleural Fluid	Men's Surgery	Toohid
S5	No	Yes	Tracheal	ICU	Toohid
S15	No	Yes	Tracheal	ICU	Besat
S16	No	Yes	Tracheal	Neurology	Besat
S17	No	Yes	Tracheal	ICU	Besat
S18	No	Yes	Sputum	ICU	Besat
S50	No	Yes	Tracheal	ICU	Besat
S58	No	Yes	Tracheal	ICU	Besat
S34	No	Yes	Tracheal	ICU	Besat
S37	No	Yes	Tracheal	ICU	Besat
S49	No	Yes	Tracheal	ICU	Besat
S59	No	Yes	U/C	Men's Surgery	Toohid
S47	No	Yes	Wound	ICU	Toohid
S44	No	Yes	U/C	Neurology	Toohid
S45	Yes	Yes	U/C	Oncology	Toohid
S42	No	Yes	Tracheal	ICU	Besat
S39	Yes	Yes	Pleural Fluid	Operating	Besat
S62	No	Yes	U/C	Neurology	Besat
S63	Yes	Yes	Tracheal	ICU	Besat
S66	No	Yes	U/C	Men's Surgery	Toohid
S69	No	Yes	Tracheal	ICU	Besat
S72	No	Yes	Tracheal	ICU	Besat
S74	No	Yes	Wound	Burn	Toohid
S80	Yes	Yes	Pleural Fluid	ICU	Toohid
S86	No	Yes	Tracheal	ICU	Besat
S88	Yes	Yes	Tracheal	ICU	Besat
S96	No	Yes	U/C	ICU	Toohid
S89	No	Yes	Wound	ICU	Toohid
S90	Yes	Yes	U/C	ICU	Toohid
S92	No	Yes	Wound	Burn	Toohid
S93	Yes	Yes	Tracheal	ICU	Besat
S111	No	Yes	B/C	ICU	Toohid
S112	No	Yes	U/C	Neurology	Toohid
S114	No	Yes	Tracheal	ICU	Toohid
S115	Yes	Yes	Lung Secretions	ICU	Toohid
S118	No	Yes	Lung Secretions	ICU	Toohid
S123	Yes	Yes	Tracheal	ICU	Besat
S124	Yes	Yes	Tracheal	ICU	Toohid
S127	No	Yes	Tracheal	ICU	Toohid
S129	No	Yes	U/C	Men's Internal	Fajr
S133	No	Yes	U/C	ICU	Toohid
S134	No	Yes	U/C	ICU	Toohid
S135	No	Yes	Tracheal	ICU	Besat
S136	No	Yes	Tracheal	ICU	Besat
S137	No	Yes	Tracheal	ICU	Besat
S138	No	Yes	Lung Secretions	Neurology	Toohid
S139	No	Yes	Wound	Women Heart	Toohid
S64	No	Yes	Tracheal	ICU	Besat

S: Sample; MDR: Multidrug resistance; XDR: Extremely drug resistance; ICU: Intensive care unit; UC: Urine/culture; BC: Blood/culture.

(91.78%) out of 146 *Pseudomonas* spp. were identified as *P. aeruginosa* using PCR. Additionally, the highest rates of resistance were found to be related to CPD (93.28%), AMOXY (91.04%), TET (91.04%), SXT (85.07%), NAL (84.32%), CTX (81.34%), FEP (76.86%), CTR (74.62%), CIPR (64.92%), CAZ (66.41%), GEN (64.17%), TOB (12.68%), AMI (38.80%), CO (30.59%), and IMI (27.61%) antibiotics, respectively.

Similarly, using the disk diffusion method, Shah et al (19) reported that antibiotic resistance rates of 254 *P. aeruginosa* isolates were 98.8%, 63.9%, 61.7%, 58.4%, 56.1%, 50%, 35.3%, 25.3%, and 10.4% to NAL, FEP, CTR, TOB, CAZ, CIPR, GEN, AMI, and IMI, respectively. The results of the above-mentioned study are highly similar to those of the current study. In both studies, isolates displayed the least resistance to metallo-β-lactamase (MBL). In another research by Siddiqua et al, 4489 different clinical specimens of *P. aeruginosa* isolates were tested by disk diffusion for antibiotic patterns. The isolates demonstrated resistance to FEP, CTR, CTX, and GEN in a range of 47% to 88%. In addition, CIPR, AMI, and MBL represented resistance rates of 76 to 87% although they were more effective antibiotics (20). These results are in line with the results of the antibiotic test in the present research. MBL indicated a further remarkable activity against *P. aeruginosa*. Further, carbapenem-hydrolyzing strains of *P. aeruginosa* were detected in different countries. Therefore, physicians need to be more careful in administering antibiotics which belong to this class (19). *P. aeruginosa* has different ways for resistance against the antibiotics. Antibiotic-resistant genes on mobile genetic elements spread among the bacteria and the antibiotic resistance is easily spread. Several resistant mechanisms exist against the antibiotics including beta-lactam antibiotics inactivation (by beta-lactamases), decreased permeability trough outer-membrane porins, and active efflux (21). Furthermore, the widespread use of antibiotics, the transmission of infection from patient to patient, the lack of or inappropriate infection control and hygiene, and the transfer of resistant genes in bacteria and patients can all affect the infection and resistance rate of antibiotics in different study regions (21,22). Miliani et al reported that *P. aeruginosa* was the third most common bacterium which was isolated from the patients with nosocomial infections and that high consumption rates of CAZ, MBL, CIPR, and AMI were the risk factors for resistance to this bacterium (22). In the current study, MDR and XDR *P. aeruginosa* isolates were found in rates of 97.76% and 15.67%, respectively. Using the disk diffusion method, Verma et al concluded that MDR *P. aeruginosa* isolates were identified in all 100 patients with nosocomial infections (23). Additionally, Adrizain et al found that 5 (0.2%) out of 299 patients, were infected with *P. aeruginosa*; and all of those 5 isolates were MDR; however, XDR and PDR were not detected (24). In addition, the current results indicate high levels of MDR which matches the results of Verma et al and Adrizain et al. In different studies, all of *P. aeruginosa* isolates were frequently found to be either MDR or XDR. Appropriate monitoring for MDR detection is necessary among *P. aeruginosa* isolates since the frequency of using antibiotics in clinical settings and in the animal industry is increasing. Further, the antibiotics are easily available and their unnecessary consumption causes the emergence of resistant strains (23,24). Nosocomial infections were found in 41.79% of the patients in the present research, most of which were isolated from ICU (69.64%) and tracheal specimens (51.78%). Esfahani et al reported that *P. aeruginosa* (13.9%), *Klebsiella* (11%), and *Escherichia coli* (6.4%) were the most prevalent bacterial infections in 1077 surveyed patients. Furthermore, according to him, ICU was the most common ward with nosocomial infections, which is similar to the results of the present study (25). All the isolates, from tracheal and mainly lung secretion specimens, were related to nosocomial infections. This type of infection is a serious threat to patients in high-risk wards such as ICU and those receiving mechanical ventilation. The incidence of ventilator-associated pneumonia is highlighted in different studies and its rate varies from 7% to 70% (26,27). Paling et al found that 226 (9.2%) out of 5093 patients, were colonized with *P. aeruginosa* in the ICU ward. This rate in ICU was less compared to the rate in the current study (28). *P. aeruginosa* is a major agent of bacteria which is the leading cause of healthcare-associated infections in ICU (29). Several risk factors increase infections in the ICU ward such as the use of catheters and other invasive equipment, certain groups of patients, those with trauma or burns, children, patients with high ages, and immunocompromised patients; and MDR strains are extensively emerged in this ward (30). The acquired resistance occurs due to the transmission of the resistant gene in bacteria in different geographical regions and this is a cause for great concern (31). Therefore, genotyping and the epidemiological survey of bacterial strains for detecting the origin and transmission patterns of bacterial strains is definitely necessary (32). BOX-PCR is a single primer which targets highly conserved repetitive elements in the genome of *P. aeruginosa*. Using BOX-PCR, Mehri et al observed 45 distinct profiles of *P. aeruginosa* genome (33). Additionally, Ahmed et al indicated the distribution of some genes between *P. aeruginosa* in different sources using BOX-PCR (34). *P. aeruginosa*, related to nosocomial infection, was genetically classified by BOX-PCR in the present study. In addition, 56 unique genetic patterns were detected in four clusters. The occurrence of genetic mutations in bacteria leads to the formation of different types and subtypes, resulting in different mutations and differences and thus unique patterns (35,36). Further, BOX-PCR is a very useful and rapid method with high discriminatory capabilities and is easily reproduced with a low cost while being a powerful method for genotyping and classifying the *P. aeruginosa* (7,34,35). Based on the

results of the present study, diversity was observed among the clinical isolates of *P. aeruginosa* with high levels of MDR and XDR. These results suggest that managing and controlling nosocomial infections depend on adequate and appropriate antibiotic therapy. In sum, the strengths of this study were as follows:

- Studying the patients over 2 years,
- Including a large number of clinical specimens,
- Using valid criteria for diagnosing and determining bacterial resistance patterns according to CLSI standards,
- Performing an epidemiological survey based on PCR which was easy to use while being low cost.

However, in every human attempt, no doubt, there exist some limitations which need to be acknowledged.

Limitations

The limitations of this study included possible contaminations of the laboratory environment which may lead to false results and the lack of access to all patients with *P. aeruginosa* infection.

Conclusions

In general, BOX-PCR demonstrated a high level of genotypic heterogeneity in *P. aeruginosa* strains while there was no genetic correlation between such strains. Furthermore, considering the distribution of this bacterium in different hospitals and the antibiotic resistance of these strains, it is essential to study resistant strains, to administer antibiotics with more caution, and to determine resistant strains in laboratory tests.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Issues

The presents study was performed in accordance with the standards and regulations of the Ethical Committee of Kurdistan University of Medical Sciences.

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Supplementary Data

Supplementary file 1 contains Tables S1-S2.

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