Original Article

Molecular Study of Some β-Lactamase Genes among Multidrug Resistant Pseudomonas aeruginosa Clinical Isolates

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Abstract

Background: *Pseudomonas aeruginosa* possesses a wide variety of antimicrobial defense mechanisms, including many chromosomal determinants and intricate regulatory pathways involved in both intrinsic and adaptive resistance. **Objectives:** The aim of the present study was to investigate the distribution of β-lactamase genes among multidrug resistance *P. aeruginosa* isolated from clinical samples collected from different hospitals. **Materials and Methods:** from July to October of 2022, 194 cotton swape from clinical samples, including burns, urine, diabetic foot, gunfire bombs, and wounds, were gathered from participants of varying gender and age. *Pseudomonas aeruginosa* was isolated by culturing it on Cetrimide agar and using microscopy and biochemical tests. The diagnosis was confirmed by using molecular methods through the use of polymerase chain reaction (PCR) to amplify the 16sRNA gene. The molecular identification, by using PCR amplification of the three genes $bla_{oxa}145$, $bla_{oxa}181$, and *per-1*. **Results:** The results of the molecular study of the three mentioned genes obtained in this study were as follows, number of samples that were positive to the presence of $bla_{oxa}145$ 34/36(94.44%), for $bla_{oxa}181$ 21/36(58.33%) and *per-1* 31/36(86.11%). A number of samples that were positive to *per-1* only was 2(5.55%), 5 (13.89%) samples were harboring only $bla_{oxa}145$, samples that had both $bla_{oxa}145$ *and per-1* were 8 (22.22%), 21 (58.33%) of samples contained all the three genes $bla_{oxa}145$, $bla_{oxa}181$ and *per-1*; and only two (5.55%) of samples were having none of the resistant genes under study. **Conclusion:** The results of this study show the widespread of the bla_{oxa} family that is, resistant to β-lactamase antibiotics, and this poses a threat to public health.

Keywords: $bla_{oxa}145$, $bla_{oxa}181$ and *per-1*, MDR, *P. aeruginosa*

Introduction

Pseudomonas aeruginosa is considered an opportunistic infection when seen in people with impaired immune systems. It is the leading cause of hospital-acquired infections, such as urinary tract infections, surgical site infections, pneumonia, bloodstream infections, and potentially lethal sepsis.[1,2] Due to the fact that it has been related to devastating infections in burn patients and persons with cystic fibrosis, it is one of the most significant ESKAPE viruses from a medical and epidemiological standpoint. *Pseudomonas aeruginosa* has been classified as a "critical priority pathogen" by the World Health Organization because it has been demonstrated to be resistant to many drugs, including a wide range of different types of medication.[3,4] Multidrug-resistant (MDR) strains of *P. aeruginosa* have emerged as a direct result of the organism's increasing resistance to many antibiotics, which has been attributed to the overuse of these drugs. Infections caused by *P. aeruginosa* are notoriously deadly.[5]

Class A β-lactamase genes are the most widely distributed,[6] and they may be found on plasmids, integrons, or the chromosomes of Gram-negative bacteria. The β-lactamases belonging to the extended-spectrum β-lactamases (ESBLs) family are extremely common and important in *P. aeruginosa* clinical isolates.[7] This includes enzymes like pseudomonas extended resistant (PER), Guiana

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Table 1: PCR primers and their conditions used in this study (designed by this study)

Gene		Primer (5′-3′)	Product size (bp)	Annealing Temp (°C)	Cycle no.	Reference
16sRNA	F	TGCCTGGTAGTGGGGGATAA	505	66	35	Shaebth (2018)
	R	GGATGCAGTTCCCAGGTTGA				
Per-1	F	ACTGTAGGCGTTGCAGTGTG	198	61	35	Designed
	R	CGGAGCCCAGGTATTCTGTA				
bla $_{Oxa}$-145	F	AAGCCGTCAATGGTGTTTTC	204	60	30	Designed
	R	CCCATTGTTTCATGGCTCTT				
bla $_{OXA}$-181	F	CAGAAGCAGAAGGAGGTGGA	205	63	30	Designed
	R	GTGGGGTTGTTTGGCATGAT				

extended-spectrum-lactamases (GES), and Vietnam extended-spectrum β-lactamase (VEB). Isolates from Turkey and other Mediterranean nations are particularly rich in PER-lactamase,[8,9] an enzyme that hydrolyzes most penicillins and cephalosporins. Ceftazidime, aztreonam, and cefepime resistance are all facilitated by the VEB enzyme, which is well inhibited by clavulanate and avibactam.[10] While the clavulanate, tazobactam, avibactam, relebactam, and vaborbactam carbapenemase inhibitors are effective against *P. aeruginosa* isolates harboring GES family members, GES-1 is superior than cefotaxime in hydrolyzing ceftazidime.[11] Transposons often transport the genes for these three enzymes, allowing them to be exchanged across Gram-negative bacteria.[12] The emergence of resistance to -lactams is mostly attributable to class A enzymes. Many articles have been written about these enzymes and what they do for Gram-negative bacteria. However, further research is desperately needed to determine why these enzymes are resistant to lactams that are used in the clinic. Therefore, we sought to assess the incidence of PER, VEB, and GES-lactamase-producing genes in clinical isolates of *P. aeruginosa* in this part of Iran because their presence is so crucial to the evolution of resistance to lactams.[13]

Material and Methods

Sample collection

From July to October of 2022, 194 cotton swape from clinical samples, including burns, urine, diabetic foot, gunfire bombs, and wounds, were gathered from participants of varying gender and age.

Bacteria isolation and diagnosis

Pseudomonas aeruginosa was isolated by culturing it on Cetrimide agar and using microscopy and biochemical tests. The diagnosis was confirmed by using molecular methods through the use of polymerase chain reaction (PCR) to amplify the 16sRNA gene.

Primer design and PCR conditions

In this study, four primers, 16sRNA was used as a diagnostic gene, and the rest of the primers for detection of antibiotic resistance genes (Per-1,. bla Oxa-145, bla OXA-181) [Table 1]. The PCR reaction mixture in Table 2.

Table 2: PCR reaction mixture

No.	Contents	Volume (µL)
1.	Master mix	12.5
2.	Template DNA	3
3.	Forward primer (10 pmol/µL)	1.5
4.	Reverse primer (10 pmol/µL)	1.5
5.	Nuclease-free water	4
6.	Stain	2.5
7.	Total volume	25

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before a sample was taken. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee according to document number 7/17/1336 (including the number and date on February 21, 2022) to get this approval.

Results

Out of 194 samples, only 36 (18.55%) samples gave *P. aeruginosa* bacteria. These bacteria were isolated on a Cetrimide agar medium and diagnosed through well-known biochemical tests. The diagnosis was confirmed by using the PCR technique to amplify a 16sRNA gene, and all 36 samples were positive, as shown in Figure 1.

For the purpose of investigating antibiotic resistance genes to find out their relationship to multidrug resistance phenomena, three genes were studied in this paper *bla$_{oxa}$145*, *bla$_{oxa}$181,* and *per-1*; The results of the presence of these genes within the samples that were isolated during this study are shown in Table 3.

In this study, it was observed that there were high prevalence rates for the three genes under study (*per-1* and *bla$_{oxa}$145*, *bla$_{oxa}$181*), as the percentage of *per-1* was 31/36 (86.11%), while for the *bla$_{oxa}$145*gene the highest was 34/36 (94.44%), while the *bla$_{oxa}$181* gene was the lowest with a ratio of 21/36 (58.33%), but it is still a high percentage and poses a danger in multidrug resistance [Table 4].

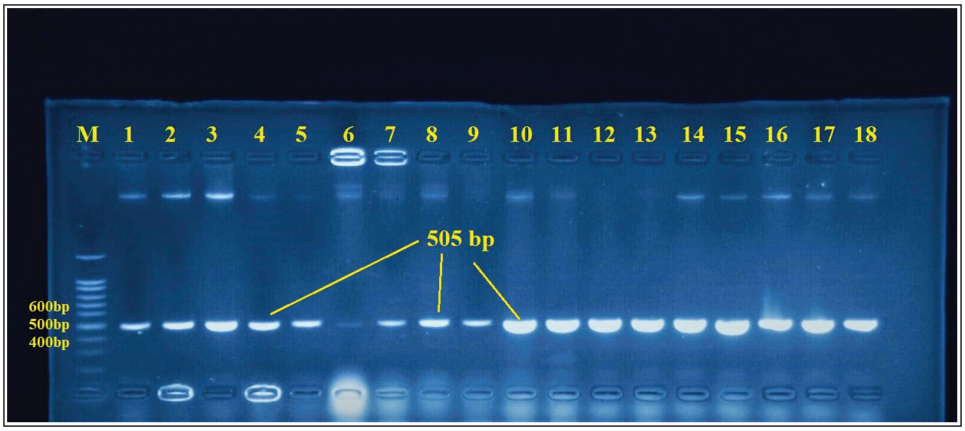

Figure 1: Agarose gel electrophoresis for amplified (505 bp) *P. aeruginosa*-specific gene 16S rRNA of DM patients. Bands were fractionated by electrophoresis on a 1.5% gel (80 min., 85 V/cm). (DNA ladder marker [100–1500] bp); isolates: 1–36 positive results

Table 3: Distribution of bla*OXA-145*, bla*OXA-181*, and PER-1 Genes of *P. aeruginosa*

Genes distribution	No.	%
Per-1 only	2	5.55
bla_{oxa}*145*	5	13.89
bla_{oxa}*145 and Per*-1	8	22.22
bla_{oxa}*145*, bla_{oxa}*181* and *per-1*	21	58.33
Total	36	100

Table 4: Distribution of gene group of *P. aeruginosa* isolates

No.	Genes	No.	%
1	*Per*-1	31	86.11
2	bla_{oxa}*145*	34	94.44
3	bla_{oxa}*181*	21	58.33

Figures 2–4 show the agarose gel electrophoresis results of PCR product for the three genes used in this study.

Discussion

ESBLs, Metallo-lactamases, and in rare cases, AmpC plasmid-lactamases are produced by bacteria that have developed resistance to β-lactam antibiotics, leading to acquired resistance.[14] Phylogenetic analysis of β-lactamases has revealed the presence of genes that can be transmitted through plasmids and integrons, such as blaVEB and blaPER in *P. aeruginosa*.[15] Class A β-lactamases have 100% similarity and are highly conserved, as was found in a study by Ambler.[16] These enzymes belong to class A2 of the Ambler classification and are primarily responsible for hydrolyzing cephalosporins like cephalothin, ceftazidime, and cefotaxime, as well as aztreonam and penicillins[15]

The result of bacteria that have *Per*-1 31 (86.11%) compatible with Haghighi and Goli study, which found that the two most frequent PER enzymes, PER-1 and PER-2, are only partially inhibited by avibactam compared to other class A-lactamases.[11] However, in our analysis, 93.54% of cefepime-resistant and 64.51% of imipenem-resistant isolates harbored the blaPER gene, while 70.27% of meropenem-resistant and 78.57% of doripenem-resistant isolates did so as well.[13]

OXA-145 belongs to the OXA-10 family of lactamases and has a wide spectrum of activity. The hydrolysis spectrum has shifted from penicillins to third-generation cephalosporins and monobactams due to the deletion of Leu-165. A Lys-73 that had been decarboxylated caused penicillin hydrolysis to be lost.[17] In a study conducted by Sezadehghani and his group, they found that among *P. aeruginosa* isolates, blaOXA-145 was found in 18.3%, blaOXA-224 in 22.0%, blaOXA-539 in 40.3%, and blaOXA-675 in 10.1%.[18]

Pseudomonas aeruginosa NRZ-49259 was discovered to have blaOXA-181 localized on a chromosome. There was a 3153 bp area on a 2.6 Mbp contig that was identical to the *Klebsiella pneumoniae* plasmid pKP3-A (GenBank accession number JN205800.1). This area included the ISEcp1 insertion sequence, blaOXA-181, truncated lysR- and er-like genes, and a repA gene that made up the Tn2013 transposon. However, NRZ-49259 lacked the whole 3′ end of the repA gene (744 bp) and the 5′ end of ISEcp1 (1008 bp). Intriguingly, a chromosomal position of blaOXA-181 exhibiting similarity to pKP3-A was earlier discovered for an English isolate of *P. aeruginosa*.[19] The high prevalence of MDR *P. aeruginosa* (100%) demonstrates the critical necessity for epidemiological surveillance, as it indicates an alarmingly high availability of class 1 integrons in our region.[20] The presence of blaOXA-181 was confirmed by PCRs targeting both common and unusual carbapenemase genes. WGS results verified the gene's chromosomal location. Although *P. aeruginosa* strain from England has been characterized as having the same genetic organization of blaOXA-181, the two isolates had very different sequence types (ST111/ST235).[21] Baban's research focuses on antimicrobial stewardship to

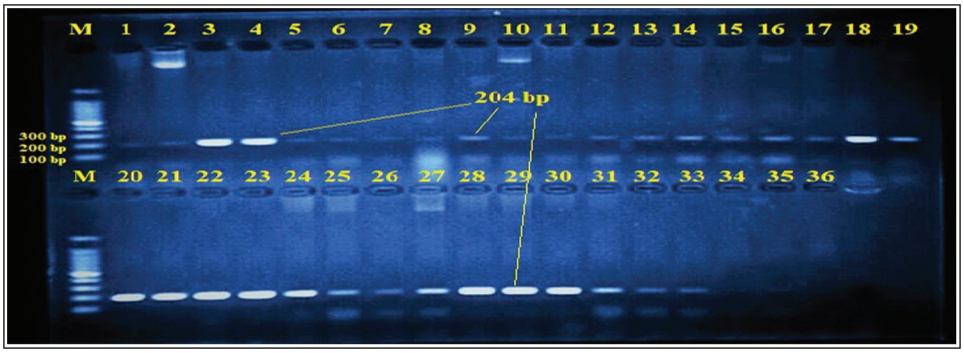

Figure 3: The absence of bla$_{OXA-145}$ PCR product (204 bp) in some isolated samples (PsA34 and PsA36). PCR products were separated by electrophoresis in a 1.5% agarose gel at 80 V/cm for 60 min. M: (DNA ladder marker [100–1500 bp])

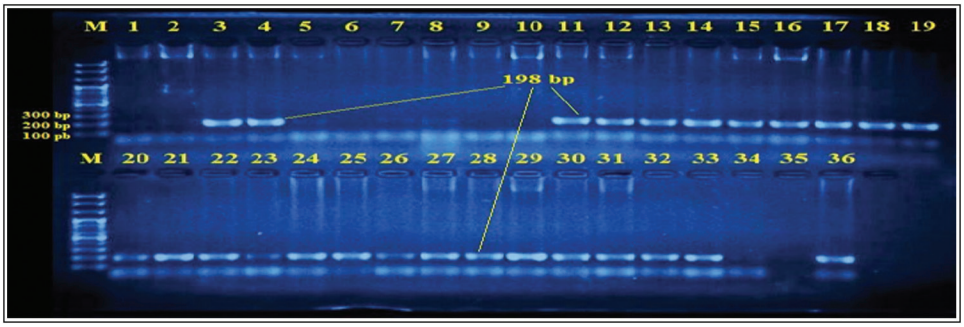

Figure 2: The absence of Per-1 PCR product (198 bp) in some isolated samples (PsA2, PsA6, PsA9, PsA10, and PsA35). PCR products were separated by electrophoresis in a 1.5% agarose gel at 80 V/cm for 60 min. M: (DNA ladder marker [100–1500 bp])

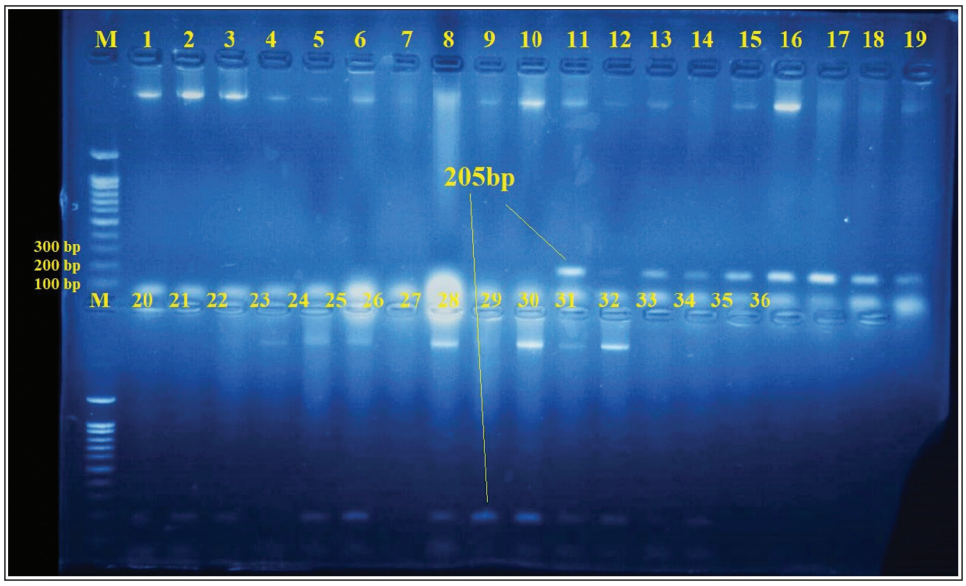

Figure 4: The absence of bla$_{OXA-181}$ PCR product (205 bp) in some isolated samples (PsA1, PsA2, PsA3, PsA4, PsA5, PsA6, PsA7, PsA8, PsA9, PsA10, PsA23, PsA26, PsA34, PsA35, and PsA36). PCR products were separated by electrophoresis in an 1.5% agarose gel at 80 V/cm for 60 min. M: (DNA ladder marker [100–1500 bp])

prevent the indiscriminate use of carbapenem antibiotics and early diagnosis of carbapenem-resistant isolates to prevent cross-transmission among critically ill patients. Active surveillance and strict infection prevention and control may stop carbapenemase resistance, according to Baban's findings.[22]

Conclusion

The results of this study show the widespread of the *bla*$_{oxa}$ family that is resistant to *β*-lactamase antibiotics, and this poses a threat to public health.

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Conflicts of interest

There are no conflicts of interest.

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