Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.125

Molecular Detection of OXA-143 beta-lactamase gene in P. aeruginosa

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Abstract. Pseudomonas aeruginosa, an opportunistic-bacteria that causes many clinical and hospital infections, struggles to be treated due to its drug resistance. This bacterium resists beta-lactams by producing broad-spectrum beta-lactamases. This study will determine antibiotic resistance and identify the blaOXA-143 gene in clinical P. aeruginosa isolates. This study isolated P. aeruginosa from patients using McConkey agar. Gram staining, oxidase, catalase, MRVP, motility in SIM medium, and fluorescent pigment synthesis on cetrimide agar identified and validated P. aeruginosa isolates. DNA was extracted from isolates using the kit. Primers were created and confirmed using NCBI. PCR was used to detect blaOXA-143. The 16S rRNA gene was PCR-analyzed to confirm the isolates and test the PCR test. P. aeruginosa was isolated from 100 samples: blood (36%), urine (30%), wound (20%), and trachea (14%). Penicillin had 100% resistance in a study of 100 P. aeruginosa isolates, followed by cefixime at 86%. Ipenem, meropenem, calcitin, and cefotaxime had resistance rates of 42%, 40%, 68%, and 50%, respectively. The lowest Cefepim resistance was 34%. In this investigation, 47% of isolates were multidrug-resistant. All isolates were verified by 16SrRNA detection. None of the isolates had the blaOXA-143 gene.

Highlights:

1. P. aeruginosa shows high resistance to multiple antibiotics.
2. 47% isolates were multidrug-resistant, confirmed via 16S rRNA.
3. blaOXA-143 gene was not detected in any isolates.

Keywords: P. aeruginosa, beta lactamase, Oxacillinase, blaOXA143

Introduction

Hospital-acquired infections are defined as infections that manifest after a patient's admission to a hospital, which were absent at the time of admission, and exhibit symptoms within 72 hours of admission [1]. WHO reports indicate that millions of individuals in both developing and developed nations are infected with these diseases annually. This rate is notably high for Europe, impacting approximately seven out of every 100 hospitalized patients. This rate is significantly elevated in intensive care units (ICUs). The high infection rate in ICUs is attributable to patients' compromised conditions and the frequent utilization of invasive devices, including central catheters,

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urinary catheters, and ventilators. Approximately 37,000 patients in Europe succumb to these infections annually [2]. Pseudomonas aeruginosa is very adaptable and can develop resistance mechanisms to antimicrobial treatment; it is a leading cause of nosocomial infections, particularly in patients with impaired immune systems [3]. When treating severe infections produced by this bacterium, beta-lactam antibiotic drugs are typically prescribed. In contrast, P. aeruginosa clinical isolates are known to produce beta-lactamases, including cephalosporinases and carbapenemases [4]. Worldwide, hospital-acquired infections (HAIs) caused by multidrug-resistant (MDR) Pseudomonas aeruginosa are widespread. Opportunistic infections caused by Pseudomonas aeruginosa are common in immunocompromised individuals and can manifest in a variety of ways, including UTIs, respiratory tract infections, bacteremia, and burn infections. Patients suffering from serious burns are at increased risk of death [5].

The capacity to manufacture extended-spectrum β-lactamases is one factor that contributes to the emergence of resistance in P. aeruginosa. One enzyme that can break down the chemical structure of penicillins and cephalosporins is β-lactamase. The β-lactam ring of several β-lactams can be broken down by extended-spectrum β-lactamases (ESBLs) [6,7]. Multiple genes, including as blaBES, blaVEB, blaPER, blaOXA, blaCTX-M, blaSHV, and blaTEM, are responsible for producing ESBLs. It is possible that the genes responsible for ESBL synthesis are disseminated more widely among the bacterial population and between various species and genera by horizontal gene transfer, which can occur through conjugation, transformation, or transduction [8].

Methods

Sample Collection

One hundred fifty clinical samples were retrieved for this investigation from four internal departments of Musa bin Jafar Quchan Hospital: burn emergency, intensive care unit, surgery, and general medicine. Patients in the hospital had their urine, blood, trachea, and wounds sampled. Patients in this research ranged in age from twenty to forty years old while they were hospitalized. In strict adherence to all applicable health regulations, the samples were sent to the university's laboratory for additional analysis. The next step was to cultivate the clinical samples using nutrient agar and MacConkey agar. The gram staining, oxidase test, catalase, MRVP, motility examination at 42 ° C,

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and study of fluorescent pigment synthesis in cetrimide culture media were used for the identification and confirmation of P. aeruginosa isolates.

DNA extraction

Prior to conducting polymerase chain reaction (PCR), the DNA template has to be retrieved from the bacterium using the Synaclone DNA extraction kit as directed.

Primer Design

The accuracy of the PCR test and the strains of Pseudomonas aeruginosa were confirmed by the use of 16SrRNA gene amplification in P. aeruginosa. The primers used in this work may be found in the table below, along with their oligonucleotide sequences.

Table 1: Primer sequences

Primer	Sequence	Product size
16SrRNA F	5-TGCCTGGTAGTGGGGGATAA-3	505bp
16SrRNA R	3-GGATGCAGTTCCCAGGTTGA-5	
OXA-143 F	5-TGGCACTTTCAGCAGTTCCT-3	150bp
OXA-143 R	3-AATCTTGAGGGGGCCAACC-5	

Polymerase Chain Reaction

PCR reaction was carried out on the DNA template that was prepared by utilizing primers that were generated and diluted to a concentration of 10 pmol. The following is an example of the thermocycler program that is used for 16srRNA primers:

Table 2: The thermocycler program used for 16srRNA primers is as follows:

Step	Temperature, °C	Time	Cycle
Initial denaturation	95	5 min	1
Denaturation	95	30 sec	30
Annealing	58	30 sec	
Extension	72	1 min	
Final extension	72	10 min	1

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Table 3: The thermocycler program used for OXA-143 beta-lactamase gene primers is as follows:

Step	Temperature, °C	Time	Cycle
Initial denaturation	95	5 min	1
Denaturation	95	1 min	30
Annealing	58	30 sec	
Extension	72	1 min	
Final extension	72	3 min	1

Statistically Analysis

The data collected in this study was subjected to statistical analysis using SPSS version 26, with Chi-Square tests performed at a significance level of less than 0.05 [9].

Result and Discussion

Prevalence of S. aeruginosa in Different Clinical Sample

The frequency analysis results indicated that from a total of 150 clinical samples collected at Musa Bin Jafar Hospital in Quchan city, 100 samples (66.66%) of P. aeruginosa were successfully isolated. The peak incidence of P. aeruginosa infection was observed in the age group of 35-40 years, accounting for 60% of cases. The occurrence of P. aeruginosa was documented in blood samples (36%), urine (30%), wound (20%), and trachea (14%). Consequently, the current findings indicate that the highest incidence of infection attributed to P. aeruginosa was recorded in the ICU ward and in blood samples, with a frequency of 36% (46 patients).

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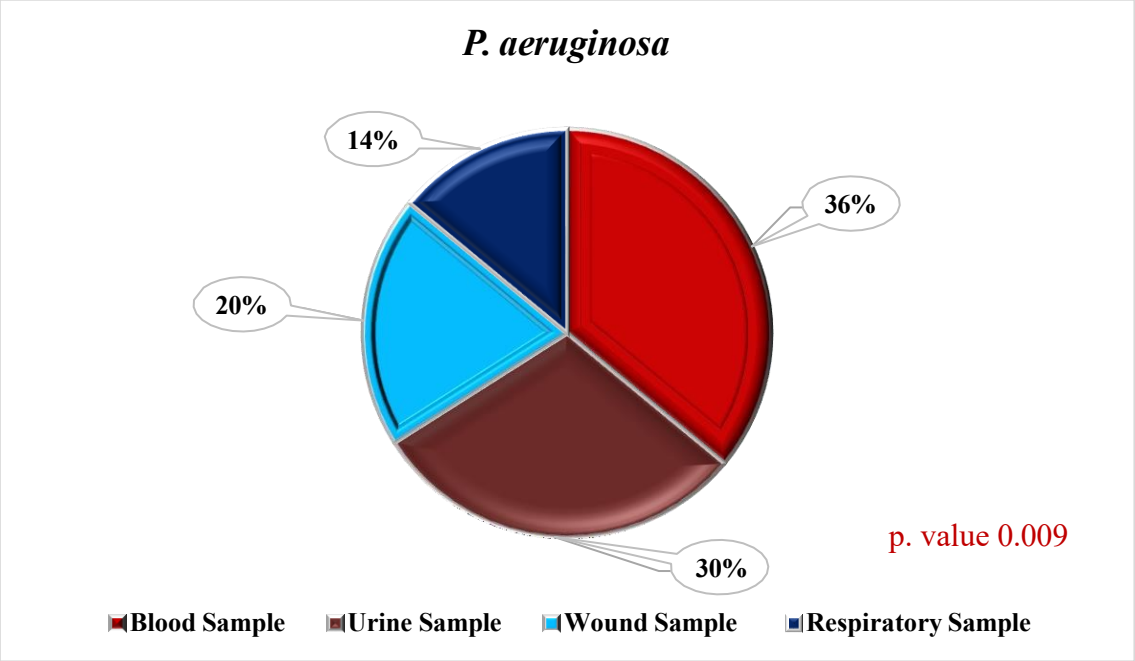

Figure 1. Prevalence of P. aeruginosa isolates in different clinical samples

Antibiotics Susceptibility

The disk diffusion method was employed to assess the antibiotic resistance and sensitivity of the isolated samples. The disks utilized included meropenem, imipenem, cefepime, penicillin, cefixime, cefotaxime, and calcitin, with the diameters of the growth inhibition zones interpreted according to the CLSI standard.

Figure 2: Formation of a halo of ingrowth in the disk diffusion test

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In a study of 100 isolates of P. aeruginosa, the highest level of resistance recorded was to penicillin at 100%, followed by cefixime at 86%. The resistance of the strains to various antibiotics was observed as follows: 68% exhibited resistance to calcitriol, 40% to meropenem, 42% to imipenem, 50% to cefotaxime, and 97% to calcitriol. The lowest resistance observed was to cefepime, occurring at a frequency of 34%. The findings are presented in Table 4.

Table 4: Frequency of P. aeruginosa isolates to the antibiotics

Antibiotics Percentage of resistance in clinical samples

Antibiotics	**Percentage of resistance in clinical samples**			
	Blood	**Urine**	**Wound**	**Respiratory**
Cefixime	43	27	13	15
Imipenem	46	31	12	8
Meropenem	41	23	15	19
Cefepime	38	22	17	22
Cefotaxime	39	28	14	17
Penicillin	100	100	100	100
Colistin	45	36	11	5

PCR Results

The amplification of the 16SrRNA gene was utilized to verify the strains of P. aeruginosa and to ensure the precision of the PCR reaction. The results validated the identified strains, and furthermore, the accuracy of the PCR for the blaOXA-143 gene was also substantiated. Figure 3 demonstrates the presence of a 505 bp band, which signifies the existence of the 16SrRNA gene.

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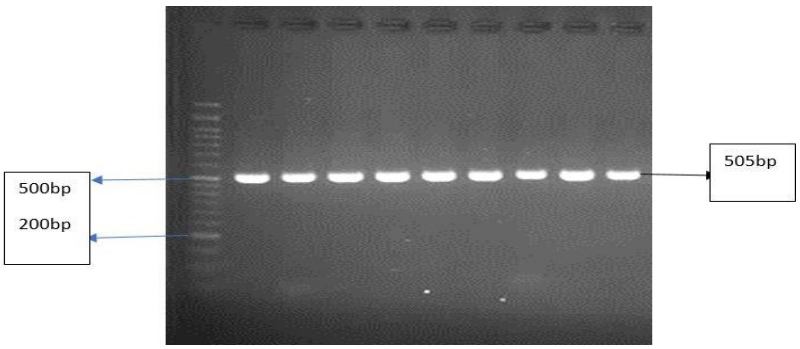

Figure 3: PCR results of the 16SrRNA gene in P. aeruginosa isolates. Observation of a 505 bp band in the first well from the left, indicating a 50 bp leader, with subsequent wells containing PCR products.

The presence of the blaOXA-143 gene, associated with antibiotic resistance mechanisms in Pseudomonas aeruginosa, was investigated using PCR. The electrophoresis results of the gene are presented in Figure 4.16. It is evident that none of the examined strains possessed the blaOXA-143 gene.

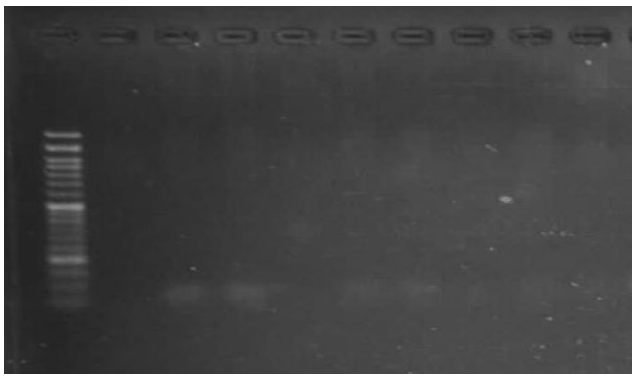

Figure 4: PCR findings of the blaOXA-143 gene in P. aeruginosa. The first well on the left side is a 50 bp DNA ladder, while the second well is a negative control. The following wells show: PCR results from germ samples used in therapeutic settings

Discussion

One mechanism by which Pseudomonas aeruginosa evades beta-lactam drugs is by producing extended-spectrum beta-lactamases. The transmission of ESBL-producing genes across P. aeruginosa strains might complicate the treatment of hospital-acquired

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infections, which are commonly caused by P. aeruginosa. So, it's crucial to regularly study this microorganism for antibiotic resistance.

Blood (36%), urine (30%), wounds (20%), and trachea (14%), among other clinical samples, were used to isolate P. aeruginosa in the research. Thus, the present investigation found that P. aeruginosa infection occurred most frequently in the intensive care unit and in blood samples (36% of the total, 46 patients). Mardaneh et al. [10] and Aslani et al. [11] found that nosocomial infections are prevalent in wounds and urinary tracts, and bacteria in wound infections are highly resistant to drugs. We found the same thing as Peymani et al. [12] in 2014, who also found that blood samples included the greatest percentage of P. aeruginosa isolates.

Among the antibiotics tested, cefixime had the greatest resistance rate at 86%, followed by calcitriol at 68%; nevertheless, as a fourth-generation cephalosporin, cefepime had the lowest resistance rate of 34%. Our findings are lower than those of the research by Komijani et al. [13], which found that 54% of Pseudomonas aeruginosa isolates exhibited resistance to cefepime.

The following antibiotic-resistant strains were found in the clinical samples used for this investigation: 50% were resistant to cefotaxime, 100% to penicillin, 40% to meropenem, and 42% to imipenem. Very concerning is the high occurrence of antibiotic resistance, particularly to calcitriol (68%). In terms of penicillin resistance, the current study is in agreement with a previous one by Ullah et al. [14] that found that meropenem had the lowest rate of resistance at 66.5% and ampicillin the highest rate at 34.94%. Similarly, Moniri and Tavajjohi [15] discovered that 13% of the isolates were resistant to all antibiotics tested, and that over 30% of the isolates were multidrug-resistant. Additionally, ciprofloxacin had the lowest resistance rate at 11.9% and piperacillin the highest at 36.8%.

Nearly half of the P. aeruginosa strains found in this investigation were multidrug-resistant. Differences in sample size, geographic region, and bacterial origin may account for the conflicting findings between our study and others. Our investigation found that a significant number of isolates were multidrug-resistant, which raises concerns about the possibility of treatment failure.

Infections produced by cephalosporinase-producing strains of P. aeruginosa that are sensitive to carbapenems are most effectively treated with these medications

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because carbapenem molecules are more resistant to hydrolysis by a wide number of serine β-lactamases. Acquisition of carbapenem resistance is a process that occurs in tandem with intrinsic processes, including changes in the OprD purine level, increased expression of efflux pumps, chromosomal AmpC β-lactamase synthesis, and mutations in specific targets [16].

Like other class D beta-lactamases that hydrolyze carbapenems, OXA-143 hydrolyzes penicillins and carbapenems, but it does not hydrolyze extended-spectrum cephalosporins to a substantial extent [17]. Pinheiro et al. found that 12 Acinetobacter baumannii isolates (60%) had the bla-KPC2 and bla-oxa143 genes out of 51 Pseudomonas aeruginosa and 48 Acinetobacter baumannii isolates. Nineteen (45%) of the twenty P. aeruginosa isolates tested showed evidence of the bla-KPC2 and bla-oxa143 genes. In addition, every single strain of P. aeruginosa exhibited resistance to at least one of the following antibiotics: gentamicin, cefepime, piperacillin/tazobactam, tigecycline, and trimethoprim/sulfamethoxazole. Contrarily, all Acinetobacter baumannii isolates were resistant to tigecycline, meropenem, imipenem, and piperacillin/tazobactam. Patients' clinical results showed that 58.9% of those infected with these microbes passed away [18].

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