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Original Article

Genotypic Investigation of Antibiotic Resistance: Presence of BlaOXA-48 Gene in Clinical Isolates of *Pseudomonas aeruginosa*

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Abstract

Background: Identifying antimicrobial resistance patterns and antibiotic resistance genes in clinical isolates of *Pseudomonas aeruginosa* is an important strategy for effectively combating infections. This study aimed to identify the antimicrobial resistance patterns and the presence of *blaOXA-48* beta-lactamase gene in *P. aeruginosa* isolated from clinical samples.

Methods: We collected 100 isolates of *P. aeruginosa* from different clinical samples, including urine, tracheal tube, blood, and burn wounds. The antimicrobial resistance patterns of the isolates were investigated using the disk diffusion method. Extended-spectrum beta-lactamase (ESBL) strains were analyzed phenotypically by the combined disk method. Furthermore, the presence of the *blaOXA-48* gene in the isolates was assessed genotypically via molecular techniques.

Results: The highest resistance of the isolates was detected for ceftazidime (89%) and ciprofloxacin (83%), whereas the highest sensitivity was detected for colistin (18%). The phenotypic method identified 68 (68%) ESBL-producing isolates among 100 isolates. In addition, *blaOXA-48* gene was detected genotypically in 33 (48.5%) isolates among 68 ESBL-producer isolates. Moreover, a significant association was found between the presence of the *blaOXA-48* gene and *P. aeruginosa* isolates' resistance to cefepime, imipenem, and gentamicin ($P<0.05$).

Conclusion: The study highlighted the significant prevalence of antimicrobial resistance in *P. aeruginosa*, particularly against ceftazidime and ciprofloxacin, although colistin remains largely effective. The association between the *blaOXA-48* gene and resistance to key antibiotics such as cefepime, imipenem, and gentamicin underscores the need for continuous monitoring and strategic antimicrobial stewardship to mitigate the spread of resistant strains.

Keywords: *Pseudomonas aeruginosa*, Antibiotic resistance, Extended-spectrum beta-lactamase, *blaOXA-48* gene

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Introduction

Pseudomonas aeruginosa is a pathogenic and opportunistic bacterium, which is a major factor in the death of patients with weakened immune systems. Its intrinsic resistance to antimicrobial substances worsens the treatment of infections caused by this bacterium (1,2). Lipopolysaccharides, pili, and polar flagellum in this opportunistic pathogen facilitate its adherence to the host cell membrane and play an important role in its pathogenicity (3). Beta-lactams are effective drugs for treating *P. aeruginosa*, but over time, some bacteria show resistance to this antibiotic by producing beta-lactamase enzymes (4).

Hospital infections are one of the major medical problems in both developed and developing countries, leading to the spread of infectious diseases in society (5). In recent years, there has been an increasing focus on hospital infections because they cause thousands of deaths worldwide (6). *P. aeruginosa* is one of the most common causes of hospital-acquired infections, which can induce septicemia, pneumonia, meningitis, and other fatal diseases (7). *P. aeruginosa* has an innate resistance to a wide range of antimicrobial and disinfectant substances such as ammonium compounds, hexachlorophene, soaps, and iodine solutions (8). Furthermore, most antibiotic-resistant strains can produce a wide spectrum of beta-lactamase enzymes (8).

The emergence of resistance in this bacterium is caused by several mechanisms, including the activation of efflux

pump, the production of beta-lactamase enzymes, and changes in the outer membrane containing group B carbapenemases (metallo-beta-lactamases) such as Verona integron metallo-beta-lactamase (VIM) and imipenemase (IMP) enzymes (9,10). Metallo-beta-lactamases, which need a cofactor such as zinc for their activity, can hydrolyze not only carbapenems but also all beta-lactam antibiotics (11). The resistance of *P. aeruginosa* strains to second- and third-generation (broad-spectrum) cephalosporins and monobactams may be due to the production of extended-spectrum beta-lactamase (ESBL) enzymes (12,13). These enzymes are encoded by different genes located on the chromosome or plasmid and open the beta-lactam ring, thereby inactivating the antibiotic (14).

Given the importance of *P. aeruginosa* in hospital infections and the lack of knowledge about the frequency of ESBLs in clinical samples, this study was conducted to determine the phenotypic and genotypic prevalence of ESBL-producing *P. aeruginosa* strains in different clinical samples from Asadabadi hospital, Tabriz, Iran.

Materials and Methods

Collection of Isolates

This descriptive-analytic study involved collecting 100 clinical isolates of *P. aeruginosa* from various clinical samples, including urine, burn wound, tracheal tube, and blood, from patients referred to clinical laboratory in Asadabadi hospital, Tabriz, Iran, during 2020-2021. Biochemical analysis such as hemolysis, urea production, oxidase activity, H2S production, indole, Voges–Proskauer, catalase activity, citrate utilization, and gram staining were used for the primary identification of the isolates. The identified isolates were frozen in Tryptic Soy Broth (TSB) containing 15% glycerol at -70 ˚C for subsequent analysis.

Antibiotic Susceptibility Analysis

The antibiotic susceptibility of the isolates was evaluated using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) (15). This method involves placing various antibiotic-impregnated disks on agar plates previously inoculated with a bacterial suspension. The antibiotics diffuse radially outward through the agar, creating a concentration gradient. After 24 hours of incubation at 35 ± 1 ˚C, the diameters of the inhibition zones around each disk were measured visually. The antibiotic discs included cefepime (30 µg), imipenem (30 µg), tobramycin (5 µg), colistin (10 µg), ceftazidime (30 µg), amikacin (30 µg), piperacillin (100 µg), ciprofloxacin (5 µg), and gentamicin (10 µg). Moreover, the antibiotic susceptibility of *P. aeruginosa* ATCC 27853 was evaluated as a control.

Multidrug-Resistant Classification

Isolates demonstrating simultaneous resistance to at least three antibiotics were categorized as multidrug-resistant (MDR) (16).

Phenotypic Detection of Extended-Spectrum Beta-Lactamase-Producing Isolates

Phenotypic detection of the ESBL-producing isolates was conducted through the combination disc method. The antibiotic discs included cefotaxime (30 µg), ceftazidime (30 µg), cefotaxime-clavulanic acid (30 µg-10 µg), and ceftazidime-clavulanic acid (30 µg-10 µg). An isolate was considered ESBL-producing if the diameter of the growth inhibition produced by combined discs was greater than the diameter of the growth inhibition halo produced by the same discs alone (16).

Genotypic Detection of Extended-Spectrum Beta-Lactamase-Producing Isolates

The genomic DNA was extracted from the ESBL-producing *P. aeruginosa* isolates using a specific kit (Invitek Stratec Business, Canada) according to the manufacturer's instructions. Molecular detection of the *blaOXA-48* gene in DNA samples of the isolates was conducted by polymerase chain reaction (PCR) method in a 25 µL total volume (1 µL DNA sample, 1 µL each primer, 12.5 µL master mix, and 9.5 µL deionized water). The PCR experiment commenced with an initial denaturation cycle, which involved subjecting the sample to a temperature of 95 ˚C for 5 minutes, followed by 45 denaturation cycles at 94 ˚C, each lasting 30 seconds. Subsequently, the annealing procedure was carried out at 50 ˚C for 30 seconds, followed by an extension at 72 ˚C for 1 minute. Ultimately, a final extension cycle was executed at 72 ˚C for 5 minutes (16). The primer sequences employed in this study were as follows: forward 5′-GCTTGATCGCCCTCGATT-3′ and reverse 5′-GATTTGCTCCGTGGCCGAAA-3′. The amplified PCR product, with a length of 830 base pairs, was estimated by electrophoresis on a 2% agarose gel. *P. aeruginosa* ATCC 27853 was used as control.

Statistical Analysis

The raw data were analyzed by SPSS statistical software (version 16) and Excel software (version 2010). The association of antibiotic resistance with the presence of the *blaOXA-48* gene was analyzed using Fisher's exact test and the chi-square (χ^2) test. The statistical significance level was considered at P value < 0.05.

Results

Bacterial Isolates

This study thoroughly investigated 622 samples from various clinical sources, including urine, burn wounds, tracheal tubes, and blood. Of these, 100 clinical isolates of *P. aeruginosa* were successfully isolated and identified using biochemical analytical techniques. Out of 100 *P. aeruginosa* isolates, 18 (18%) were isolated from urine samples, 33 (33%) from burn wound samples, 26 (26%) from tracheal tube samples, and 23 (23%) from blood samples.

Antibiotic Resistance Patterns

The clinical isolates indicated a high rate of resistance to ceftazidime (89%), ciprofloxacin (83%), and tobramycin (81%) antibiotics. However, a high rate of sensitivity was observed to colistin (81%), amikacin (65%), and gentamicin (64%) antibiotics (Table 1).

Phenotypically Detected Extended-Spectrum Beta-Lactamase-Producing Isolates

Phenotypic analysis indicated that 68 (68%) out of the 100 clinical isolates of *P. aeruginosa* were ESBL-producing. Of these 68 ESBL-producing isolates, 14 were from urine samples, 23 were from burn wound samples, and 14 were from tracheal tube samples (Table 2).

Genotypically Detected Extended-Spectrum Beta-Lactamase-Producing Isolates

Genotypic analysis indicated that 41 (60.30%) out of the 100 isolates carried the *blaOXA-48* gene and were considered ESBL-producing isolates (Table 3). The statistical analysis revealed a significant association between the presence of the *blaOXA-48* gene and *P. aeruginosa* isolates' resistance to cefepime, imipenem, and gentamicin (Table 4).

Discussion

The management of *P. aeruginosa* infections is difficult due to its inherent resistance to multiple antimicrobial agents through several mechanisms. These bacteria become resistant to beta-lactam antibiotics by producing beta-lactamase enzymes (17). Metallo-beta-lactamase enzymes, a type of beta-lactamases, cause resistance to most beta-lactam antibiotics (18). The rapid transmission and spread of *P. aeruginosa* strains capable of producing these enzymes have led to an increase in the number of hospital infections worldwide (19). *P. aeruginosa* is the third cause of hospital infections, following *Staphylococcus aureus* and *Escherichia coli* (20).

In the current study, 100 *P. aeruginosa* strains were isolated from urine, burn wounds, tracheal tubes, and blood samples, with 68 (68%) isolates identified as ESBL-producers. These strains indicated high resistance to antibiotics such as tobramycin (81%), ciprofloxacin (83%), and ceftazidime (89%), whereas the lowest resistance was related to gentamicin (18%). Moreover, the *blaOXA-48* gene was found in 41 (60.30%) of the ESBL-producing isolates.

Antibiotic resistance is an important global problem, thus numerous studies have been conducted on ESBL-producing *P. aeruginosa* isolates (21). In a study conducted by Fazeli et al in Isfahan, 94% of *P. aeruginosa* clinical isolates were resistant to imipenem, piperacillin, and ciprofloxacin antibiotics, with all strains showing resistance to ceftazidime and ticarcillin antibiotics (22). In a study on *P. aeruginosa* isolates from patients in the burn center of Tehran, Ranjbar et al reported that 97% of strains are resistant to imipenem, and 90% of strains

Table 1. Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* Isolates

Antibiotic	Abbreviation	Dose (µg)	Resistance Patterns		
			Sensitive	Semi-sensitive	Resistant
Ceftazidime	CAZ	30	5 (5%)	6 (6%)	89 (89%)
Ciprofloxacin	CIP	5	16 (16%)	1 (1%)	83 (83%)
Tobramycin	TOB	10	15 (15%)	4 (4%)	81 (81%)
Cefepime	CEP	30	21 (21%)	6 (6%)	73 (73%)
Piperacillin	PIP	100	36 (36%)	1 (1%)	63 (63%)
Imipenem	IPM	30	46 (46%)	5 (5%)	49 (49%)
Amikacin	AMK	30	65 (65%)	2 (2%)	33 (33%)
Gentamicin	GEN	10	64 (64%)	3 (3%)	33 (33%)
Colistin	CST	10	81 (81%)	1 (1%)	18 (18%)

Table 2. Frequency of ESBL-Producing Isolates Detected by Phenotypic Analysis

Clinical Sample	ESBL Positive	ESBL Negative	Total
Blood	1 (17%)	6 (6%)	23 (23%)
Urine	14 (14%)	4 (4%)	18 (18%)
Tracheal tube	14 (14%)	12 (12%)	26 (26%)
Burn wound	23 (23%)	10 (10%)	33 (33%)
Total	68 (68%)	32 (32%)	100 (100%)

Note. ESBL: Extended-spectrum beta-lactamase.

Table 3. Frequency of *blaOXA-48* in ESBL-producing *Pseudomonas aeruginosa* Isolates

Clinical Sample	Positive	Negative	Total
Blood	8 (11.75%)	9 (13.20%)	17 (25.00%)
Urine	8 (11.75%)	6 (8.80%)	14 (20.60%)
Tracheal Tube	11 (16.20%)	3 (4.40%)	14 (20.60%)
Burn Wound	6 (8.80%)	17 (25.00%)	23 (33.80%)
Total	33 (48.50%)	35 (51.50%)	68 (100%)

Note. ESBL: Extended-spectrum beta-lactamase.

Table 4. Association Between Presence of *blaOXA-48* and Resistance in *Pseudomonas aeruginosa* Isolates

Antibiotic	Abbreviation	Resistant Isolates (N = 68)	Presence of *blaOXA-48*	*P* Value
Ceftazidime	CAZ	67 (67%)	41	0.077
Ciprofloxacin	CIP	60 (60%)	22	0.599
Tobramycin	TOB	61 (61%)	31	0.277
Cefepime	CEP	63 (63%)	51	0.011
Piperacillin	PIP	51 (51%)	23	0.111
Imipenem	IPM	52 (52%)	39	0.041
Amikacin	AMK	35 (35%)	11	0.327
Gentamicin	GEN	37 (37%)	27	0.018
Colistin	CST	22 (22%)	4	0.498

are resistant to amikacin (23). In another study, Salehi et al reported 86% resistance of *P. aeruginosa* to nalidixic acid and 79% resistance to ceftazidime (24). Mihani et al found a 71% resistance rate to ceftazidime (25). Previous studies reported a significant relationship between the use of antibacterial drugs (e.g., amikacin, ciprofloxacin, ceftazidime, and imipenem) and the spread of resistant *P. aeruginosa* strains (26, 27). Sadredinamin et al identified 95 imipenem- and meropenem-resistant strains from 100 *P. aeruginosa* isolates, with 81 isolates being ESBL-producing strains (28). Likewise, Fallah et al found that out of 100 *P. aeruginosa* strains isolated from clinical samples, 83 were resistant to imipenem, and 48 were ESBL-producers (29). These studies collectively indicate that antibiotic resistance in *P. aeruginosa* is pervasive and multifaceted, necessitating comprehensive strategies to address this issue. Potential solutions include the development of new antibiotics, the use of combination therapies to counteract resistance mechanisms, and the implementation of stringent antibiotic stewardship programs to reduce the misuse and overuse of existing antibiotics. Additionally, increased surveillance and molecular studies are essential for understanding the mechanisms of resistance and tracking the emergence and spread of resistant strains. By addressing these challenges through a multi-pronged approach, we can better manage and mitigate the impact of antibiotic-resistant *P. aeruginosa* on public health.

Due to the genetic nature of *P. aeruginosa*, it accepts all types of genes through plasmids and transposons, which may cause the rapid resistance of this bacterium to various antibiotics (30). Our results demonstrated that the frequency of the *blaOXA-48* gene in ESBL-producing *P. aeruginosa* isolates is 60.30%, with a significant association between the presence of this gene and resistance to cefepime, imipenem, and gentamicin. This gene has been detected in different geographical areas worldwide, including Turkey, India, Afghanistan, China, the Middle East, and African countries (31). Begum and Shamsuzzaman in Bangladesh detected the *OXA-48* gene in 20% of *P. aeruginosa* isolates (32). Mohamed et al in Sudan reported a 22.4% frequency of *OXA-48* in clinical isolates of *P. aeruginosa* (33). In another study in Sudan, Ali and Nagla found a 60% frequency of *OXA-48* in clinical isolates of *P. aeruginosa*, with a significant association between the presence of *blaOXA-48* and resistance to carbapenems (34). In a study by Al-Alaq et al in Iraq, the frequency of the *OXA-48* gene in 200 *P. aeruginosa* isolates obtained from the microbiology laboratory of the burn ward was 10% (35). Bahrami et al reported a 12.5% frequency of *blaOXA-48* in Isfahan, which is lower than that in our study (36). In Mashhad, Hashemi et al found that 46.34% of 82 *P. aeruginosa* isolates were resistant to imipenem, with the highest resistance rate in carbenicillin (69.5%). All imipenem-resistant *P. aeruginosa* isolates were confirmed by multiplex PCR using primers that targeted the *rpoD* gene. Additionally, in multiplex PCR, among these imipenem-resistant isolates, 23.6% carried the *blaOXA-48* gene (37). Interestingly, a common finding in previous studies is the increasing antibiotic resistance of *P. aeruginosa* isolates worldwide.

Limitations of the Study

To confirm isolates definitively, molecular tests are recommended. Although a series of biochemical assays, including Gram staining, urea synthesis, H2S generation, Voges–Proskauer, hemolysis, catalase, oxidase, and indole tests, were conducted, these tests are not specifically designed for the detection of *P. aeruginosa*. Therefore, alternative diagnostic tests should be employed to ensure accurate identification of *P. aeruginosa*. Additionally, although the study's focus on the *blaOXA-48* beta-lactamase gene and the antimicrobial resistance pattern provides valuable insights, the limited scope of the genetic analysis may overlook other relevant resistance mechanisms and genes present in the isolates. Future studies should consider comprehensive genomic analyses to capture a broader spectrum of resistance determinants.

Conclusion

This study revealed a high prevalence of ESBL-producing *P. aeruginosa* isolates in clinical samples. In addition, we assume that resistance of *P. aeruginosa* isolates to cefepime, imipenem, and gentamicin may be associated with the presence of the *blaOXA-48* gene. However, further research is needed to assess the prevalence of ESBL-producing *P. aeruginosa* in other regions of Iran and to explore the frequency of other beta-lactamase genes in clinical samples to combat infections caused by MDR of *P. aeruginosa*.

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