(RESEARCH ARTICLE)

Molecular analysis of *pseudomonas aeruginosa* isolated from clinical and environmental sources at Uniosun teaching hospital Osogbo

Adeyemo Rashidat Abolore [1, 3, *], Itunuoluwa Oyelayo [1, 2], Adeola Ayanyinka [1, 2], Olawumi Ayobami Otutu [1, 2], Titilayo Adenike Olayinka [1, 2], Olowe Rita Ayanbolade [1], Olayinka Opaleye [1, 2], Olusola Ojurongbe [1, 2] and Olugbenga Olowe [1, 2]

[1] Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria.
[2] Humboldt Research Hub, Center for Emerging and Re-emerging Infectious Diseases, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Oyo State, Nigeria.
[3] Medical Laboratory Science department, Fountain University, Osogbo, Osun state, Nigeria.

Abstract

Background: *Pseudomonas aeruginosa* is a significant public health concern due to its ubiquity and ability to cause severe infections, particularly in immunocompromised individuals. Its intrinsic resistance and propensity to develop multidrug resistance exacerbate treatment challenges. This study investigated antibiotic resistance patterns and the presence of extended-spectrum β-lactamase (ESBL) genes in *P. aeruginosa* isolates from clinical and environmental sources.

Objectives: The study aimed to determine antibiotic resistance patterns, multidrug resistance prevalence, and the occurrence of ESBL genes (*bla*CTX-M, *bla*SHV, and *bla*TEM) in *P. aeruginosa* isolates.

Methods: A total of 150 swab samples from clinical and environmental sources were collected from UNIOSUN Teaching Hospital, Osogbo, between July and December 2022. Isolation and identification of *P. aeruginosa* were performed using cetrimide agar and standard diagnostic tests. Antibiotic resistance was evaluated using the disc diffusion method, and ESBL gene detection was conducted using PCR. Data were analyzed and presented as tables and percentages.

Results: Among 100 *P. aeruginosa* isolates, 66.7% were multidrug-resistant. Clinical isolates exhibited the highest resistance to ciprofloxacin, ticarcillin, and aztreonam (82%) and the lowest resistance to imipenem (22%). Environmental isolates showed similar trends, with aztreonam and ticarcillin resistance at 76% and the lowest resistance to imipenem (38%). ESBL genes *bla*TEM and *bla*SHV were detected in 5.4% of isolates, while no isolate was positive for *bla*CTX-M.

Conclusions: The high prevalence of multidrug-resistant *P. aeruginosa* and β-lactamase-producing strains highlights the urgent need for targeted antibiotic development and strict hygiene protocols in healthcare settings.

Keywords: *Pseudomonas Aeruginosa*; Multidrug Resistance; Antimicrobial Resistance; Extended-Spectrum B-Lactamase; Healthcare-Associated Infections.

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* Corresponding author: Adeyemo Rashidat Abolore

1. Introduction

Pseudomonas aeruginosa is a non-fermentative, Gram-negative bacterium that is common throughout the environment, including in healthcare facilities where 10–20% of nosocomial infections are caused by it. These infections manifest as septicemia in the intensive care unit, wound infections, and burns (Vala et al., 2014; Tofas, 2017). *P. aeruginosa* is very adaptable, and can become resistant to broad-spectrum antibiotics, which makes it one of the world's most common causes of MDR infections (Olowe, 2012). These strains of antibiotic resistance add to the public health problem worldwide — especially in high-risk groups, including burn victims where MDR *P. aeruginosa* causes most mortality and morbidity (Khosravi and Mihani, 2008).

Multidrug resistance in *P aeruginosa* is often correlated with the activity of -lactamases such as extended-spectrum -lactamases (ESBLs) and metallo-lactamases (MBLs), which shut down key antibiotics such as -lactams and carbapenems (Tofas, 2017). These enzymes are often encoded by genes like blaCTX-M, blaSHV, and blaTEM, all of which appear on mobile genetic material, allowing them to travel between the health care and environment (Bradford, 2001; Abderahman et al., 2021). Since ESBL-producing *P. aeruginosa* were first identified in Europe in the 1980s, and MBL-producing strains in Japan in 1991, their prevalence has rocketed globally (Bradford, 2001).

P. aeruginosa pathogenesis is influenced by virulence agents: exotoxins, elastases and proteases that damage host tissues and disrupt the immune system (Klockgether and Tümmler, 2017). Its environmental ubiquitousness and genetic adaptability are also factors in the widespread resistance to antibiotics (Turkina and Vikstrom, 2019). Researchers have described different resistance levels across *P. aeruginosa* strains. In clinical isolates, for example, resistance to imipenem, ciprofloxacin and aztreonam reached as high as 63%, 83% and 88%, respectively (Mirsalehian et al., 2011; Zarie et al., 2018). Resistance has been also found among environmental strains, which present a double challenge to infection control and public health (Nicolas et al., 2022).

While this issue is globally significant, there is limited data on MDR *P. aeruginosa* and prevalence of ESBL genes locally, especially in Nigeria. The proposed explanation for this study is that MDR *P. aeruginosa* is common in the clinical and environmental environment and that ESBL genes play a key role in its resistance. It is imperative to be aware of these dynamics in the development of effective control methods, such as targeted antibiotic treatments and more efficient infections prevention in healthcare settings.

The aim of this work was to investigate the pattern of antibiotic resistance, multidrug resistance and ESBL genes (blaCTX-M, blaSHV, and blaTEM) in clinical and environmental isolates of *P. aeruginosa.* By unravelling these resistance mechanisms, this work will hopefully shed light on the role that antimicrobial stewardship programs can play in mitigating the emerging antibiotic-resistant threat. This research is vital for managing the increasing prevalence of MDR *P. aeruginosa* infections, facilitating treatment efficacy, and ensuring public safety.

2. Material and methods

2.1. Specimen Collection

A total of 150 samples were collected from patients and environmental sources at UNIOSUN Teaching Hospital, Osogbo, between July and December 2022. Fifty clinical *Pseudomonas aeruginosa* isolates were obtained from wound, urine, ear, sputum, catheter tip, vaginal, and throat swabs. Additionally, 50 environmental isolates were collected from drainages, water, dumping sites, food materials, water pipes, wall surfaces, and wash-hand basins.

2.2. Bacterial Identification

The collected samples were inoculated in asparagine broth and incubated at 37°C for 24 hours. Sub-cultures from the broth were performed on cetrimide agar and further incubated at 37°C for 24 hours. Identification of isolates was based on Gram staining, colonial morphology, and biochemical tests, including oxidase and motility tests. Pure bacterial colonies were stored in Tryptic Soy Broth (TSB) (Merck, Germany) mixed with 20% glycerol at -20°C for subsequent analysis.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using the disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. The antibiotic disks used (Mast Co. Ltd, UK) included cefepime (30 µg), ceftazidime (30 µg), ticarcillin (75 µg), aztreonam (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), and amikacin

(30 μg). Results were interpreted as sensitive, intermediate, or resistant according to CLSI guidelines. Isolates resistant to three or more classes of antibiotics were classified as multidrug-resistant (MDR).

2.4. ESBL Production

Sixteen clinical and 21 environmental isolates resistant to cefepime and ceftazidime were screened for ESBL production using the disk diffusion method as per CLSI guidelines. Cefepime disks were placed on Mueller-Hinton agar seeded with standardized bacterial suspensions and incubated at 37°C for 18–24 hours. Isolates with inhibition zones >27 mm were considered potential ESBL producers. Confirmation was performed using a cefotaxime-clavulanic acid combination disk, with a ≥5 mm increase in inhibition zone diameter indicating ESBL production.

2.5. DNA Extraction

Genomic DNA was extracted from *P. aeruginosa* isolates using a Qiagen commercial DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.6. Polymerase Chain Reaction (PCR) for ESBL Gene Detection

The presence of ESBL genes (*bla*TEM, *bla*SHV, and *bla*CTX-M) was determined using conventional PCR. Primer sequences specific to these genes are listed in Table 1. PCR reactions were carried out in a final volume of 24 μL, containing 2 μL of bacterial DNA, 12 μL of 2X PCR pre-mixture (GeneON, Germany), 5 pmol of each primer, and deionized water. Amplifications were performed in an Eppendorf Nexus gradient Mastercycler under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 32 cycles of denaturation (30 seconds at 94°C), annealing (30 seconds at 52°C or 60°C), and extension (1 minute at 72°C), with a final extension at 72°C for 7 minutes. PCR products were visualized using a 1.2% agarose gel stained with ethidium bromide. Amplicon sizes were confirmed using a molecular weight marker (GeneRuler, ThermoFisher Scientific, MA).

2.7. Statistical Analysis

All data were analyzed using Microsoft Excel 2016.

Table 1 Primers Used for ESBL Gene Amplification

Target Gene	Primers	Sequence (5'-3')	Amplicon Size	Annealing Temp	Reference
*bla*TEM	Forward	GAGTATTCAACATTTTCGT	857 bp	52°C	Van et al. (2008)
	Reverse	ACCAATGCTTAATCAGTGA			
*bla*SHV	Forward	TCGCCTGTGTATTATCTCCC	768 bp	52°C	Van et al. (2008)
	Reverse	CGCAGATAAATAACCACAATG			
*bla*CTX-M	Forward	CGATGTGCAGTACCAGTAA	585 bp	60°C	Ali et al. (2024)
	Reverse	TTAGTGACCAGAATAAGCGG			

3. Results

Pseudomonas aeruginosa isolates were recovered from both clinical and environmental sources, with wound swabs (44%) being the most frequent clinical source (Table 2). Among environmental samples, drainages constituted the largest source, accounting for 40% of isolates (Table 3). The overall distribution of clinical and environmental isolates is shown in Tables 2 and 3.

Table 2 Frequency of Specific Sites of *P. aeruginosa* (Clinical Sources)

Specimen	No. of Samples Collected	Positive for *P. aeruginosa*	% of Total
Wound swab	30	22	44
Urine	13	9	18
Catheter tip	10	7	14
Vaginal swab	8	3	6
Ear swab	7	5	10
Sputum	8	3	6
Throat swab	6	1	2
Total	82	50	100

3.1. Antibiotic Resistance Patterns

Both clinical and environmental *P. aeruginosa* isolates exhibited the highest resistance to aztreonam and ticarcillin, with resistance rates of 82% and 76%, respectively. Imipenem demonstrated the lowest resistance rates, at 22% in clinical isolates and 38% in environmental isolates (Table 4). Resistance patterns for other antibiotics, including cefepime, ceftazidime, amikacin, and ciprofloxacin, were also observed, with ciprofloxacin resistance notably high in clinical isolates (82%).

Table 3 Frequency of Specific Sites of *P. aeruginosa* (Environmental Sources)

Specimen	No. of Samples Collected	Positive for *P. aeruginosa*	% of Total
Drainages	25	20	40
Dumping sites	20	17	34
Food materials	6	2	4
Water pipes	7	5	10
Wall surfaces	5	3	6
Wash-hand basins	5	3	6
Total	68	50	100

3.2. Multidrug Resistance

Table 4 Antibiotics Resistance Pattern of Clinical and Environmental *P. aeruginosa* Isolates

Drug Class	Drug	Clinical Isolates n (%)	Environmental Isolates n (%)
Beta-lactams	Imipenem	11 (22)	19 (38)
	Aztreonam	41 (82)	38 (76)
	Ticarcillin	41 (82)	38 (76)
Cephalosporins	Cefepime	21 (42)	26 (52)
	Ceftazidime	32 (64)	31 (62)
Aminoglycosides	Amikacin	20 (40)	24 (48)
Fluoroquinolones	Ciprofloxacin	41 (82)	20 (40)

Multidrug resistance (MDR) was observed in 72% of clinical isolates and 56% of environmental isolates (Table 5). The majority of MDR isolates exhibited resistance to five or more antibiotic classes, with 36.11% of clinical isolates and 39.29% of environmental isolates resistant to five classes of antibiotics.

3.3. ESBL Gene Detection

Among *P. aeruginosa* isolates resistant to cefepime and ceftazidime, 32.4% were positive for ESBL genes (*bla*-TEM or *bla*-SHV). Specifically, *bla*-TEM was identified in 33.3% of environmental isolates and 6.3% of clinical isolates, while *bla*-SHV was detected in 9.5% of environmental isolates and 12.5% of clinical isolates. None of the isolates tested positive for *bla*-CTX-M. Overall, 5.4% of isolates carried both *bla*-TEM and *bla*-SHV genes (Table 6).

Table 5 Multidrug-Resistance Patterns of *P. aeruginosa* Isolates

No. of Drugs Resistant	No. of Environmental Isolates (n=28)	Resistance (%)	No. of Clinical Isolates (n=36)	Resistance (%)
3	1	3.57	5	13.89
4	2	7.14	9	25.00
5	11	39.29	13	36.11
6	11	39.27	5	13.89
7	3	10.71	4	11.11

Table 6 Clinical and Environmental *P. aeruginosa* Isolates Tested for ESBL Genes

ESBL Genes	Environmental Positive n (%)	Clinical Positive n (%)
bla-TEM	7 (33.3%)	1 (6.3%)
bla-SHV	2 (9.5%)	2 (12.5%)
bla-CTX-M	0 (0%)	0 (0%)

4. Discussion

This study analysed clinical and environmental isolates of *P. aeruginosa*, recovering 100 isolates from 150 samples, which produced a prevalence of 66.7%. The antibiotic resistance was measured in four different classes of antibiotics: -lactams (imipenem, aztreonam, ticarcillin), cephalosporins (cefepime, ceftazidime), aminoglycosides (amikacin) and fluoroquinolones (ciprofloxacin). Clinical isolates exhibited resistance to imipenem, aztreonam, ticarcillin, cefepime, ceftazidime, amikacin, and ciprofloxacin, respectively, at rates of 22.4%, 82%, 42%, 44%, 82%, 82% and 82% respectively. Environmental isolates had similar resistance profiles, exhibiting highest resistance for aztreonam (76%) and ticarcillin (76%) and lowest resistance for imipenem (38%).

These observations agree with Sorkh et al. (2017), which identified resistance for imipenem as 60%, meropenem as 88%, ciprofloxacin as 84%, ceftazidime as 44%, and ticarcillin as 84%. Similarly, Mahmoud et al. (2013) found high resistance, 91.2% for ceftazidime, 98.2% for cefepime, and 82.5% for aztreonam. In contrast, Noriko et al. (2008) found lower resistance: 37.5% for imipenem, 18.3% for meropenem, 12.1% for both aztreonam and ciprofloxacin. Similarly, Zarie et al. (2018) observed imipenem and meropenem resistance of 47% and 55%, respectively. These differences in resistance levels might be influenced by geographic differences in antimicrobial prescriptions, infection control practices and resistance-type spread.

Multidrug-resistant (MDR) *P aeruginosa* (resistant to three or more classes of antibiotics) was found in 72% of clinical isolates and 56% of environmental isolates in this study. These findings agree with Zarie et al. (2018), with MDR levels of 45% and 37.5% for clinical and environmental isolates, respectively. Biswal et al. (2014) and Mahmoud et al. (2013) identified MDR rates of 36.2% and 52% for clinical isolates, respectively. Conversely, Noriko et al. (2008) reported an MDR rate of just 1.6% (most likely a reflection of the disparity in Japanese health care and antibiotic administration).

The MDR rates observed in this study might be attributed to the virulence of *P. aeruginosa*, and to the widespread, poorly managed use of antibiotics across the study area.

Extended-spectrum -lactamase (ESBL) production was observed in 32.4% of sampled *P aeruginosa* isolates, and the genes bla-TEM and bla-SHV were found in 18.8% of clinical isolates and 42.8% of environmental isolates. In fact, 5.4% of isolates tested positive for both bla-TEM and bla-SHV. None of the isolates were positive for bla-CTX-M. This result is consistent with Mahmoud et al. (2013), who reported that 45.6% of clinical isolates resulting in ESBLs were MDR. However, Abdelrahman et al. (2021) found elevated ESBL levels, including bla-CTX-M, in clinical isolates. bla-CTX-M did not appear in this study, which might reflect different detection protocols (multiplex PCR) or regional variations in strains of P. aeruginosa.

Since little literature exists on ESBL-producing *P aeruginosa* isolated in the environment, this research was extremely valuable. The results point to the prevalence of ESBL-producing *P. aeruginosa* in both the clinical and the environmental setting, which are key factors for MDR in this study. These findings point to the need for greater antibiotic stewardship and rigorous infection control to prevent the spread of drug-resistant *P. aeruginosa*.

5. Conclusion

This study highlights the high prevalence of *Pseudomonas aeruginosa* in both clinical and environmental sources, with significant resistance to multiple classes of antibiotics, particularly β-lactams and cephalosporins. The high rates of multidrug resistance and the detection of ESBL genes (*bla*-TEM and *bla*-SHV) underscore the critical need for stringent antibiotic stewardship and infection control measures in healthcare settings. The absence of *bla*-CTX-M in this study highlights regional variability in resistance gene distribution, necessitating further research to understand these dynamics. The findings emphasize the urgent need for targeted interventions to combat antimicrobial resistance and improve public health outcomes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

This study was conducted following ethical guidelines and was approved by the Osun State Health Research Ethical Committee.

Statement of informed consent

The present research work does not involve any studies performed on animals or humans by the authors. Data were collected and handled in compliance with ethical standards.

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