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Antimicrobial Resistance Patterns, Resistance and Virulence Genes in *Pseudomonas aeruginosa* from Hospitalized Patients in Lagos, Nigeria

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Abstract

Background: *Pseudomonas aeruginosa* has been a major cause of healthcare-associated infections due to increasing antimicrobial resistance. This study investigates the prevalence, antimicrobial resistance and virulence determinants in *P. aeruginosa* from patients.
Materials and Methods: A total of 550 clinical samples were collected from patients attending public hospitals between August 2022 and July 2024 in Lagos. The samples were processed, and *Pseudomonas* isolates were identified. Furthermore, the isolates were subjected to antimicrobial susceptibility testing (AST) using standard protocols. Real-time Polymerase Chain Reaction was used with specific primers to detect resistance (*blaOXA-48, blaVIM*) and virulence (*oprL, toxA*) gene markers
Results: The prevalence of 6.7% (37/550) *Pseudomonas* species was recorded, consisting of 23 *P. aeruginosa*, 11 *P. alcaligenes*, and 3 *P. maltophilia*. A higher prevalence (5.2%) occurred in females than males, with the age group 31–49 years mostly implicated. There were no statistically significant associations between age or sex and infections ($p > 0.05$) recorded. *Pseudomonas aeruginosa* was 100% resistant to trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, colistin and tigecycline, 78.3% to meropenem, and 73.9% to ceftazidime. Eighteen distinct resistance patterns were observed. Carbapenemase production was detected in 73.9% of *P. aeruginosa*, extended-spectrum β-lactamase (ESβL) in 26.1%, and AmpC β-lactamase in 13%. Interestingly, 100% of *P. aeruginosa* expressed *oprL,* and 95.7% expressed *toxA,* while 13% of *P. aeruginosa* carried *blaOXA-48* and *blaVIM,* from septiceamia cases.
Conclusion: Multidrug-resistant *Pseudomonas aeruginosa* strains carrying *blaOXA-48* and *blaVIM* and virulence genes *oprL* and *toxA* are currently circulating in Lagos. A need for antimicrobial stewardship and molecular surveillance to mitigate the effects.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance, Hospital-acquired infections, antimicrobial susceptibility, multidrug-resistant (MDR)

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative, rod-shaped opportunistic pathogen commonly found in soil, water, humans, and animals. This organism causes bacteremia, septic shock, and pneumonia[1-2]. The cases of prolonged hospitalization have been attributed to the ability of the pathogen to develop resistance to multiple antibiotics. The global mortality estimate of *P. aeruginosa*-associated diseases caused by drug-resistant strains has been put at 559,00 deaths annually[3]. The organism accounts for approximately 7% of healthcare-associated infections and up to 25% of intensive care unit cases. In the United States alone, it is responsible for an estimated 51,000 healthcare-associated bloodstream infections annually, including 32,600 multidrug-resistant cases and 2,700 deaths[4-5]. *P. aeruginosa* spreads via contaminated medical devices and

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environmental reservoirs in healthcare settings[6]. High-risk groups include immunocompromised patients, those with chronic wounds, or users of invasive devices[7]. Outbreaks often link to contaminated water, person-to-person transmission through direct/indirect contact in dense settings like cystic fibrosis clinics, with strain-specific spread documented in vulnerable populations[8]. Globally endemic, *P. aeruginosa's* impact varies by region, including high-income nations such as the United States of America, European countries, and Japan. It primarily causes healthcare-associated infections (HAIs), ventilator-associated pneumonia (VAP), catheter-related bloodstream infections (CRBSI), and surgical site infections (SSI), accounting for 10–15% of acute care HAIs[9]. In low- and middle-income countries (LMICs), for example, India, Brazil, sub-Saharan Africa, including Nigeria, most infections are community-acquired, linked to poor sanitation, contaminated water, and limited healthcare access[10]. *Pseudomonas aeruginosa*-associated diseases are treated with different classes of antibiotics, including beta-lactams, aminoglycosides, and fluoroquinolones. However, multidrug resistance often complicates treatments[11], thus categorizing it as a high-priority ESKAPE pathogen according to the World Health Organization, alongside *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*, and *Enterobacter* spp[12]. Developing countries face a major health challenge: rising multidrug-resistant (MDR) *P. aeruginosa*, resulting in prolonged hospital stays, increased costs, morbidity, and mortality, and have exacerbated global antimicrobial resistance (AMR) over decades[13]. *Pseudomonas aeruginosa* exhibits high antibiotic resistance, primarily due to its outer membrane acting as an intrinsic barrier, leading to alterations in porin proteins, reducing membrane permeability and enzyme activation in the periplasm[14]. MDR strains often carry high levels of virulence genes such as toxins exotoxin A (*toxA*), type III effectors (*ExoS/ExoU*), enzymes (phospholipases *PlcH/PlcN, LasB elastase*), and biofilm formation[15]. These facilitate tissue damage, immune evasion, and infection progression[16]. Multi-drug-resistant (MDR) Pseudomonas aeruginosa strains cause treatment failures due to genes encoding extended-spectrum β-lactamases (ESβL), AmpC β-lactamases, and carbapenemases[17]. Widespread use of third-generation cephalosporins, including cefotaxime, ceftriaxone, and ceftazidime, has driven the evolution of newer β-lactamases such as ESBL, a plasmid-mediated resistance mechanism enabling easy horizontal transfer between organisms[18]. This organism is among the high-priority pathogens designated by WHO, due to limited treatment options to carbapenem and third-generation cephalosporin antibiotics[12]. Reports from Guangzhou, China, indicate that carbapenem-resistant *Pseudomonas aeruginosa* infections are associated with 30-day mortality rates of 8.0%–18.4%[19]. While data from high-income countries highlight severity, low-resource settings like Nigeria face unique challenges; Nigeria's fragmented surveillance system limits accurate burden estimates. Only 15% of hospitals participate in AMR surveillance, with rural facilities largely excluded[20]. Diagnostic delays due to inadequate laboratory capacity further exacerbate underreporting. Therefore, this study investigated prevalence, antimicrobial resistance patterns, resistance, and virulence traits of *P. aeruginosa* from patients in Lagos.

Materials and Methods

Study Design: This cross-sectional study was conducted between August 2022 and July 2024 in two Local Government Areas (Ikeja and Badagry), Lagos State, Nigeria. A total of 550 patients aged ≥1 year were recruited from the surgical and general wards of Badagry General Hospital (Latitude: 6.4200° N, Longitude: 2.9100° E) and Lagos State University Teaching Hospital (LASUTH) (Latitude: 6.5770° N, Longitude: 3.3210° E). Participants had been hospitalised for ≥2 weeks and were suspected of bacterial infections.

Demographic and clinical data (age, gender, occupation, symptom duration, and prior antibiotic use) were obtained using a structured questionnaire. All participants were at increased risk of *P. aeruginosa* infection due to prolonged hospitalisation, surgical wounds, invasive devices, and frequent antibiotic exposure. The hospitals were selected as major reference centres with good accessibility.

Sample size

The minimum sample size of 118 was calculated based on an 8.4% prevalence from a previous study

(95% confidence level, 5% margin of error)[22].

Sample collection, processing and Bacterial isolation

Blood and other clinical samples were collected aseptically by phlebotomists and trained healthcare workers, respectively. Blood samples were pre-enriched in Brain Heart Infusion broth (Oxoid, UK) and incubated aerobically at 37°C for 18–24 hours, after which subcultures were plated on Cetrimide agar (Himedia, Maharashtra, India) and incubated under the same conditions; negative broths were subcultured daily for up to 7 days before being discarded. Urine samples were collected from patients aged one year and above who had been hospitalised for two weeks or more with suspected urinary tract infections (UTIs). Clean-catch midstream urine was obtained from non-catheterised inpatients, while 5 ml of urine was aseptically aspirated from the catheter port after disinfection in catheterised patients; wound swabs were collected after cleaning with physiological saline and transported in Stuart's transport medium. All samples (sputum, urine and wound) were directly streaked on the surface of highly selective medium Cetrimide agar (Himedia, Maharashtra, India) incubated at 37°C for 24 hours, and monitored for blue-greenish colonies, with isolates identified using conventional biochemical tests[23] and the API 20 NE system multi-test system (BioMérieux, France)

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing (AST) was performed on confirmed *P. aeruginosa* strains using commercial antibiotic discs (Mast Diagnostics, Merseyside, UK) for: Ciprofloxacin (5 µg), cefepime (30 µg), meropenem (10 µg), imipenem (10 µg), colistin sulphate (10 µg), tigecycline (15 µg), ceftazidime (30 µg), amoxicillin/clavulanic acid (20/10 µg), gentamicin (10 µg), piperacillin-tazobactam (100 µg), trimethoprim-sulfamethoxazole (4.75/0.25 µg), cefuroxime (30 µg), doripenem (10 µg), nalidixic acid (10 µg), cefoxitin (30 µg), and chloramphenicol (30 µg). Testing adhered to Clinical and Laboratory Standards Institute (CLSI) guidelines and breakpoint guidelines. The results of AST were then recorded as susceptible and resistant, strains showing intermediate resistance was considered susceptible dose dependent according to the CLSI guidelines[24].

Phenotypic detection of extended-spectrum β-lactamase (ESβL) production by double-disc synergy test (DDST)

All *P. aeruginosa* isolates were screened for ESβL production using the double disc diffusion method[25]. *Escherichia coli ATCC® 25922™* served as the negative control, while *Klebsiella pneumoniae* ATCC® 700603™ was the positive control. The test was considered positive if zones extended between any cephalosporin and amoxicillin-clavulanic acid.

Phenotypic identification of AmpC β-lactamase (AmpC)

AmpC β-lactamase production was screened using the AmpC disc test[26]. In-house filter paper discs were impregnated with 20µL saline and 100× Tris-EDTA, dried, and stored at 2–8°C. A lawn of cefoxitin-susceptible *E. coli* ATCC 25922 was spread on Mueller-Hinton agar. Discs were rehydrated with 20 µL saline, inoculated with the test organism, and placed adjacent to a 30 µg cefoxitin disc (with the inoculated side touching the agar). Plates were incubated overnight at 35°C. A positive result was indicated by indentation or flattening of the cefoxitin zone; a negative result showed no distortion[26]. *Escherichia coli* ATCC® 25922™ served as the negative control, while *K. pneumoniae* ATCC® 700603™ was used as the positive control for this assay[26].

Phenotypic detection of Carbapenemases by combined disc test (CDT)

Combined disc test (CDT) phenotypic assays detected carbapenemase production in *P. aeruginosa* isolates using meropenem and imipenem discs (one EDTA-embedded) placed opposite each other with cefoxitin (FOX) centred on Mueller-Hinton agar (Oxoid™ Massachusetts, USA)[27]. Carbapenemase production was considered positive if the zone of inhibition for the EDTA-impregnated carbapenem disc was ≥5 mm larger than that of the unmodified disc. *Escherichia coli* ATCC 25922 (negative control) and *Klebsiella pneumoniae* ATCC BAA-1705 (positive control) were used to validate the test.

Molecular detection of resistance and virulence genes

Genomic DNA was extracted using the Luna Universal qPCR protocol (New England Biolabs) according to the manufacturer's instructions,

involving bead beating (25 min) in a ZR BashingBead Lysis Tube with 0.5% β-mercaptoethanol, followed by centrifugation and Zymo-Spin column purification. Real-time TaqMan qPCR was carried out on a Qiagen Rotor-Gene Q 2plex to amplify resistance genes (*blaVIM* and *blaOXA-48*) and virulence genes (*oprL* and *toxA*) as described by Ajoseh *et al.*[25] using published primers.

Table 1: Target genes for PCR amplification, amplicon sizes, primer sequences and references used

Target gene	Primer sequence (5′→3′)	Reference
ToxA	F: CTGCGCGGGTCTATGTGCC R: GATGCTGGACGGGTCGAG	29
OprL	F: ATGGAAATGCTGAAATTCGGC F: CTTCTTCAGCTCGACGCGACG	30
Bla-VIM	F: TGGTCTACATGACCGCGTCT R: CGACTGAGCGATTTGTGTG	31
Bla oxa-48	F: TTCCCAATAGCTTGATCG R: CCATCCCACTTAAAGACTTGG	32

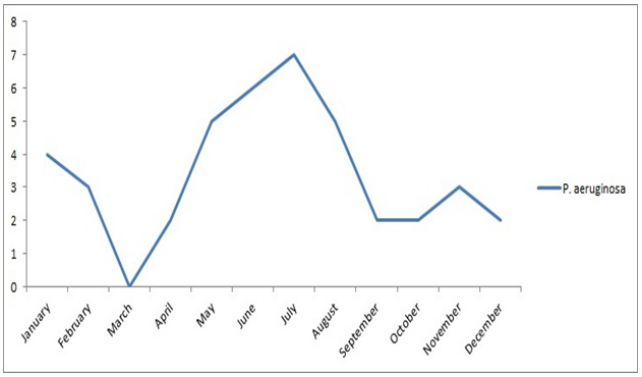

Figure 1: Seasonal distribution of positive samples of *P. aeruginosa* from human subjects

Statistical Analysis

Data were presented as numbers and percentages, compared using the chi-square test ($p < 0.05$ deemed significant), and analysed via SPSS version 20.0.2.10. The seasonal distribution was calculated by Percentage (%) of positive samples = (Number of *P. aeruginosa* -positive samples in the season ÷ Total number of *P. aeruginosa* -positive samples across the entire study period) × 100

Ethical Consideration

Ethical approval was granted by LASUTH's Health Research and Ethics Committee (registration no. LREC/06/10/2324).

Results

Of the 230 blood, 170 urine, 50 sputum, 50 wound swabs, and 50 oral swabs, an overall prevalence of 6.7% (37/550) *Pseudomonas* species, comprising 4.2% (23/550) *P. aeruginosa*, 2% (11/550) *P. alcaligenes*, and 1.3% (7/550) *P. maltophilia* (Table 2). *P. aeruginosa* prevalence was higher in 5.2% (13/250) females than in 3.3% (10/300) males. Furthermore, 17.4% (4/23) of *P. aeruginosa* isolates were in age groups 0-17 and 18-30 years, 60.9% (14/23) in 31-49 years, and 4.3% (1/23) in ≥50 years. However, no significant association was found between *P. aeruginosa* and sex, age, or socio-economic status ($p > 0.05$), while significant associations existed with prognosis ($p = 0.002$) and education level ($p = 0.0236$) (Table 3). *P. aeruginosa* infections occurred year-round, peaking in July, with none in March (Figure 1). All 23 *Pseudomonas* isolates were 100% resistant to trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, colistin, tigecycline, and chloramphenicol; 91.3% to cefepime, 82.6% to nalidixic acid, 78.3% to meropenem, 73.9% to ceftazidime, and 65.2% to cefoxitin, but 95.65% were sensitive to gentamicin and piperacillin-tazobactam (Table 4). Interestingly,

Table 2: Prevalence and distribution of *Pseudomonas spp.* isolated from different sources

Category	Sub-category				Bacterial Isolates identified		
Clinical	Hospital	Parameters	No. of Samples	No. of samples	*P. aeruginosa*	*P. alcaligenes*	*P. maltophilia*
		BGH	220	20	10	7	3
		LASUTH	330	17	13	2	2
		Total	**550**	**37**	**23**	**9**	**5**
	Samples collected	Urine	170	19	1	2	3
		Blood	230	25	20	5	0
		Sputum	50	0	0	0	0
		Wound	50	3	1	1	1
		Oral swab	50	3	1	1	1
		Total	**550**	**37**	**23**	**9**	**5**
	Sex	Male	300	19	10	6	3
		Female	250	18	13	3	2
		Total	**550**	**37**	**23**	**9**	**5**
	Age	0-17	100	07	4	1	2
		18–30	150	06	4	1	1
		31 – 49	250	20	14	5	1
		>50	50	04	1	2	1
		Total	**550**	**37**	**23**	**9**	**5**
	Economic status	G/P	200	18	10	5	3
		Self	300	14	9	3	2
		Not employed	50	05	4	1	0
		Total	**550**	**37**	**23**	**9**	**5**
	Education status	Primary	80	03	2	1	0
		Secondary	100	08	5	2	1
		Tertiary	220	16	9	4	3
		None	150	10	7	2	1
		Total	**550**	**37**	**23**	**9**	**5**

Table 3: Prevalence and risk factors associated with *Pseudomonas aeruginosa* isolated from hospitalized patients.

		Type and number of positive samples						95% CI, df=1	
Category	Parameter	Urine	Blood	Sputum	Wound	Oral swab	Total	Chi-square	P-value
Hospital	LASUTH	1	7	0	1	2	220 (11)		
	BGH	0	12	0	0	0	330 (12)	5.15	0.2725
	TOTAL	**1**	**19**	**0**	**1**	**2**	**550 (23)**		
Diagnosis	Sepsis	0	15	0	0	0	150 (15)		
	Kidney disease	0	2	0	0	0	100 (2)		
	Liver disease	0	2	0	0	0	100 (2)		
	RTI	0	0	0	0	1	50 (1)	35.03	0.0002
	UTI	1	0	0	0	0	100 (1)		
	WI	0	0	0	1	1	50 (2)		
	TOTAL	**1**	**19**	**0**	**1**	**2**	**550 (23)**		
AGE	0-17	0	4	0	0	0	100 (4)		
	18-30	0	3	0	0	1	150 (4)		
	31-49	1	11	0	1	1	200 (14)	5.59	0.6708
	> 50	0	1	0	0	0	50 (1)		
	TOTAL	**1**	**19**	**0**	**1**	**2**	**550 (23)**		
SEX	Male	0	9	0	0	1	300 (10)		
	Female	1	9	0	1	1	250 (13)	2.17	0.7048
	TOTAL	**1**	**19**	**0**	**1**	**2**	**550 (23)**		
Economic status	G/P employed	1	9	0	0	0	200 (10)		
	Self-Employed	0	8	0	1	1	300 (9)	6.16	0.6267
	Not Employed		3	0	0	1	50 (4)		
	Total	**1**	**19**	**0**	**1**	**2**	**550 (23)**		
Education	Primary	1	0	0	0	1	80 (2)		
	Secondary	0	5	0	0	0	100 (5)		
	Tertiary	0	7	0	1	1	220 (9)	17.72	0.0236
	None	0	7	0	0	0	150 (7)		
	Total	**1**	**19**	**0**	**1**	**2**	**550 (23)**		

Table 4: Susceptibility and resistance profiles of *P. aeruginosa* isolates against major antibiotic classes

Antibiotics class	Antibiotic used	Resisted n (%)	Susceptible n (%)
Aminoglycoside	Gentamicin	1 (4.4)	22 (95.6)
Fluoroquinolones	Ciprofloxacin	15 (65.2)	8 (34.8)
	Nalidixic acid	19 (82.6)	4(17.4)
Cephalosporin	Ceftazidime (3rd Gen)	17 (73.9)	6 (26.1)
	Cefepime (4th Gen)	21 (91.3)	2(8.7)
	Cefotaxime (3rd Gen.)	23 (100)	0(0)
	Cefoxitin	15 (65.2)	8 (34.8)
Carbapenems	Imipenem	3 (13.0)	20(87)
	Meropenem	18 (78.3)	5 (21.7)
	Doripenem	14 (60.9)	9 (39.1)
Penicillin with beta-lactam inhibitor	Piperacillin tazobactam	1 (4.4)	22 (95.6)
	Amoxicillin clavulanic	23 (100)	0(0)
Glycylcyclines	Tigecycline	23 (100)	0(0)
Polymyxin	Colistin	23 (100)	0(0)
Chloramphenicol	Chloramphenicol	23 (100)	0(0)
Sulphonamides	Trimethoprim-sulphamethoxazole	23 (100)	0(0)

Pseudomonas isolates were multidrug-resistant (MAR index 0.56–0.81), showing 18 resistance patterns, with pattern P (CO-NA-TGC-CIP-TS-C-AUG-CAZ-CTX-CPM-MEM-FOX-DOR) in 4 strains and pattern F (CO-TGC-CIP-TS-C-AUG-CPM-MEM-FOX-DOR) in 3 strains. However, for the other 16 strains of *P. aeruginosa*, a dissimilar resistance pattern was observed (Tables 5 and 6). Furthermore, of the 23 *P. aeruginosa* isolates, 13% (3/23) were AmpC-positive, 18.9% (6/23) expressed ESBL, and 74% (17/23) produced carbapenemase. Strains C15 and C18 showed AmpC, ESBL, and carbapenemase co-expression. Additionally, 21.7% (5/23) co-expressed all three resistance mechanisms, 13% (3/23) carried *blaOXA-48* and *blaVIM* genes, 95.7% (22/23) expressed *ToxA*, and 100% (23/23) carried *OprL* (Table 7).

Discussion

Pseudomonas aeruginosa is a public health concern, causing serious infections in healthy individuals and invasive infections in hospitalized, intubated, or immunocompromised patients[8] often after catheterization, urinary procedures, or surgery with consequences including discomfort, pyelonephritis, morbidity, and occasional death[33]. In this study, the overall prevalence of *P. aeruginosa* was 4.2%, which is comparable to the report by Ezeador et al.[34], who recorded an 18.3% prevalence rate of *P. aeruginosa* in Zaria, Kano State, Nigeria. This also differs from the report by Odoi et al.[35] from Ghana, which recorded a 12.9% prevalence in clinical samples. This discrepancy in prevalence rate could be associated with differing sample sizes, locations, sample distributions, and types of samples collected. Furthermore, a prevalence rate of 5.2% was found in females, and 3.3% in males, which is in contrast to a report in India, with a prevalence rate higher in males (58%) compared to female patients (42%). This was further corroborated by a study in Afghanistan, which reported a higher prevalence in males (61.8%)[36]. These observed differences in the prevalence rates may be due to biological, social, and behavioural differences between genders, resulting in higher infection acquisition in females. Notably, this study revealed a high rate of *P. aeruginosa* in the 31-49 age range. This finding contrasts with Ranjan and co-workers' report, which identified a higher prevalence in the 21-year age bracket in India[37]. These differences may be attributed to several factors, including increased exposure to healthcare

Table 5: Antibiotic Susceptibility Test of *P. aeruginosa* isolates

Strains code	GM	CO	PTZ	NA	TGC	CIP	TS	C	AUG	CAZ	CTX	CPM	MEM	FOX	IMI	DOR	No. of Resistance	No. of Sensitive	MAR Index
C1	S	R	R	R	R	S	R	R	R	S	R	R	R	R	S	R	12	4	0.75
C2	S	R	S	S	R	R	R	R	R	S	R	R	R	R	S	R	11	5	0.69
C3	S	R	S	S	R	R	R	R	R	S	R	R	R	R	S	R	11	5	0.69
C4	S	R	S	R	R	R	R	R	R	R	R	R	R	R	S	R	13	3	0.81
C5	S	R	S	R	R	R	R	R	R	S	R	R	R	R	S	R	12	4	0.75
C6	S	R	S	S	R	S	R	R	R	R	R	R	R	R	S	R	11	5	0.69
C7	S	R	S	R	R	S	R	R	R	R	R	R	R	R	S	R	12	4	0.75
C8	S	R	S	R	R	R	R	R	R	R	R	R	R	R	S	R	13	3	0.81
C9	S	R	S	R	R	S	R	R	R	R	R	R	R	S	S	S	10	6	0.63
C10	S	R	S	R	R	R	R	R	R	R	R	R	R	R	S	R	13	3	0.81
C11	S	R	S	R	R	S	R	R	R	S	R	R	S	R	R	R	11	5	0.69
C12	S	R	S	R	R	S	R	R	R	S	R	R	R	R	R	R	12	4	0.75
C13	S	R	S	R	R	R	R	R	R	R	R	R	R	R	S	R	13	3	0.81
C14	S	R	S	R	R	R	R	R	R	R	R	R	S	R	R	S	12	4	0.75
C15	S	R	S	S	R	S	R	R	R	R	R	S	R	R	S	R	10	6	0.63
C16	R	R	S	R	R	R	R	R	R	R	R	R	R	S	S	S	12	4	0.75
C17	S	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S	9	7	0.56
C18	S	R	S	R	R	R	R	R	R	R	R	R	R	S	S	S	11	5	0.69
C19	S	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S	10	6	0.63
C20	S	R	S	R	R	S	R	R	R	R	R	S	S	S	S	S	9	7	0.56
C21	S	R	S	R	R	R	R	R	R	R	R	R	R	R	S	S	12	4	0.75
C22	S	R	S	R	R	R	R	R	R	R	R	R	R	S	S	S	11	5	0.69
C23	S	R	S	R	R	R	R	R	R	R	R	R	R	S	S	R	12	4	0.75

R: Resistant, S: Sensitive, GM: Gentamicin, CO-Colistin, PTZ: Piperacillin-tazobactam, NA: Nalidixic acid, TGC: Tigecycline, CIP: Ciprofloxacin, TS: Trimethoprim-sulphamethoxazole, C: Chloramphenicol, AUG: Amoxicillin-Clavulanic acid, CAZ: Ceftazidime, CTX: Cefotaxime, CPM: Cefepime, MEM: Meropenem, FOX: Cefoxitin, IMP: Imipenem, DOR: Doripenem, MARI: Multiple antimicrobial resistance index, TNS: Total number susceptible. TNR: Total number resistant.NR: Not required.

Table 6: Multidrug resistance pattern of *P. aeruginosa* isolated from different sources

S/ No	Resistant pattern	Sample code/	Resistant pattern code
1.	CO-NA-TGC-CIP-TS-C-AUG-CAZ-CTX	C17	A
2.	CO-NA-TGC-TS-C-AUG-CAZ-CTX-CPM	C20	B
3.	CO-NA-TGC-CIP-TS-C-AUG-CAZ-CTX-CPM	C19	C
4.	CO-NA-TGC-TS-AUG-CAZ-CTX-CPM-MEM-DOR	C9	D
5.	CO-NA-TGC-TS-C-AUG-CTX-CPM-FOX-IMI	C11	E
6.	CO-TGC-CIP-TS-C-AUG-CPM-MEM-FOX-DOR	C2, C3, C15	F
7.	CO-NA-TGC-CIP-TS-C-AUG-CAZ-CTX-CPM-MEM	C18	G
8.	CO-NA-TGC-TS-C-AUG-CAZ-CTX-CPM-FOX-IMI	C14	H
9.	CO-TGC-TS-C-AUG-CAZ-CTX-CPM-MEM-FOX-DOR	C6	I
10.	CO-NA-TGC-CIP-TS-C-AUG-CTZ-CPM-MEM-FOX-DOR	C5	J
11.	CO-NA-TGC-TS-C-AUG-CAZ-CTX-CPM-MEM-FOX-DOR	C7	K
12.	CO-NA-TGC-TS-C-AUG-CTX-CPM-MEM-FOX-IMI-DOR	C12	L
13.	CO-NA-TGC-TS-CIP-TS-C-AUG-CAZ-CTX-CPM-MEM	C22	M
14	CO-PTZ-NA-TGC-TS-C-AUG-CTX-CPM-MEM-FOX-DOR	C1	N
15.	GM-CO-NA-TGC-CIP-TS-C-AUG-CAZ-CTX-CPM-MEM	C16	O
16.	CO-NA-TGC-CIP-TS-C-AUG-CAZ-CTX-CPM-MEM-FOX-DOR	C4, C8, C10, C13	P
17.	CO-NA-TGC-TS-CIP-TS-C-AUG-CAZ-CTX-CPM-MEM-DOR	C23	Q
18.	CO-NA-TGC-TS-CIP-TS-C-AUG-CAZ-CTX-CPM-MEM-FOX	C21	R

settings, higher rates of chronic illnesses, or weakened immune systems within this demographic. The relationship between *P. aeruginosa* and sex, age distribution, and socio-economic status revealed a non-significant association with a P-value > 0.05 at 95% CI. This suggests that demographic factors may not influence infection rates, prompting a need to explore other potential risk factors[38,39]. In this study, *P. aeruginosa* -associated infections occurred throughout the year except in March, with peak infection in July. This finding aligns with a report from India, which recorded the highest isolation of *P. aeruginosa* in June[40]. These fluctuations may be attributed to weather conditions that coincided with the rainy season of the year in Lagos, which could promote the organism's proliferation in July.

The result of antimicrobial susceptibility testing in this study indicated that over 50% of *P. aeruginosa* isolates were MDR. This study recorded 100% resistance of P. aeruginosa to trimethoprim-sulfamethoxazole, amoxicillin-clavulanic, colistin, tigecycline, and cefotaxime; 91.3% resistance to cefepime; 73.9% to ceftazidime; 82.6% to nalidixic acid; 78.3% to meropenem; and 65.2% to both ciprofloxacin and cefoxitin. This finding is similar to the study conducted in Egypt, in which high resistance of *P. aeruginosa* to fluoroquinolones, aminoglycosides, and carbapenems was reported[41], but in contrast to a report from Dhaka city in Bangladesh, where *P. aeruginosa* strains were susceptible to aminoglycosides and carbapenems[42], and Wuppertal in Germany, in which low resistance to ceftazidime was recorded[43]. Resistance to trimethoprim-sulfamethoxazole in *P. aeruginosa* isolates was corroborated by a study in Dhaka, Bangladesh, which similarly documented the antibiotic's ineffectiveness against this pathogen[44], and Harmadan in Iran[45], where elevated resistance of *Pseudomonas* isolates among COVID-19 patients to trimethoprim-sulfamethoxazole was observed, but contrary to a report in South Africa, which reported its susceptibility[46]. Furthermore, there was a high resistance of *P. aeruginosa* to cefotaxime and cefepime. This result is consistent with a study conducted by Hoque *et al*., who reported that clinical isolates of *P. aeruginosa* demonstrated high resistance to ceftriaxone (82.82%), ceftazidime (81.82%), and cefuroxime (100%)[47]. Similarly, Javiya *et al*.[48] reported that over 60% of *P. aeruginosa* strains from clinical samples were resistant to ceftazidime in a study conducted in Gujarat, India. Furthermore, this study revealed that over 60% of *P. aeruginosa* strains were resistant to ciprofloxacin.

Table 7: Occurrence and Co-existence of β-lactamase Production in *P. aeruginosa* clinical isolates with prognosis, age, and sex of the subjects

S/N	Sample Code/Source	Sex	Age	Diagnosis	Phenotypic Expression			Co-Existence	Genotypic detection			
					AmpC	CPPA	ESBL		Virulence gene		Resistance gene	
									Oprl	*ToxA*	bla_{OXA-48}	bla_{VIM}
1.	C17	M	10	LV	-	-	+	NIL	+	+	-	-
2.	C20	F	48	SS	-	-	-	NIL	+	+	+	+
3.	C19	M	27	SS	-	+	-	-	+	-	-	-
4.	C9	F	AD	SS	-	-	-	NIL	+	+	-	-
5.	C11	F	38	SS	-	-	-	NIL	+	+	+	+
6.	C2	M	34	SS	-	+	+	CRPA-ESBL	+	+	+	+
7.	C3	F	38	RS	-	+	-	NIL	+	+	-	-
8.	C15	F	15	SS	+	+	+	AmpC+-CPPA+ESBL	+	+	+	+
9.	C18	F	9	KD	+	+	+	AmpC-CPPA-ESBL	+	+	-	-
10.	C14	M	34	SS	+	+	-	AMPC-CPPA	+	+	-	-
11.	C6	F	AD	SS	-	+	-	NIL	+	+	-	-
12.	C5	M	29	KD	-	+	-	NIL	+	+	-	-
13.	C7	F	58	SS	-	-	-	NIL	+	+	-	-
14.	C12	F	17	LV	-	+	-	NIL	+	+	-	-
15.	C22	F	26	SS	-	+	+	CPPA-ESBL	+	+	-	-
16.	C1	M	34	SS	-	+	-	NIL	+	+	-	-
17.	C16	F	35	SS	-	+	-	NIL	+	+	-	-
18.	C4	M	46	KD	-	+	-	NIL	+	+	-	-
19.	C8	M	40	SS	-	+	-	NIL	+	+	-	-
20.	C10	F	32	WD	-	-	-	NIL	+	+	-	-
21.	C13	M	21	SS	-	+	-	NIL	+	+	-	-
22.	C23	F	AD	PN	-	+	+	CPPA-ESBL	+	+	-	-
23.	C21	M	31	LV	-	+	-	NIL	+	+	-	-

CPPA: Carbapenemase Resistance P. aeruginosa, ESBL: Extended-spectrum beta-lactamases, AmpC: Ambler class C β-lactamases, F: Female, M: Male, C: Clinical sample, MARI: Multiple Antimicrobial Resistance, SS: Septicaemia, KD: Kidney disease. LV: Liver disease, PN: Pneumonia infection, AD: Adult whose age not disclosed but above 30 and below 50

This is similar to the work in Tamil Nadu, India, where 61.53% was recorded[49], compared to over 50% recorded in Dhaka, Bangladesh[47]. However, in a recent study conducted in Egypt, 100% of *P. aeruginosa* strains recovered from patients were resistant to ciprofloxacin[50]. The discrepancies recorded in this study compared to other studies may be attributed to several factors, such as differences in the source of isolates, the rise of empiric antibiotic use, the existence or lack of antibiotic surveillance, horizontal gene transfer among the isolates, and methods adopted, an assertion that has been well documented in the literature[14,51]. In this study, all *P. aeruginosa* isolates were 100% colistin-resistant. This result is worrisome because colistin has been known to be one of the last resorts for the treatment of respiratory infections, such as pneumonia[52]. In other developing countries, high resistance rates (>80%) of *P. aeruginosa* isolates have been documented, such as in Bangladesh[47] and Nepal[53]. Thisalarming pattern may be driven by irrational use of colistin antibiotic as a result of over-the-counter sale, a practice that is common in most developing nations. It is therefore suggested that the use of colistin for the treatment of *P. aeruginosa*-associated diseases should be suspended for a while in this part of the world, to prevent treatment failure and prolong hospital stay. Nonetheless, *P. aeruginosa* exhibited 78.3% resistance to meropenem. This result is similar to the findings, which recorded resistance to meropenem at 67.85%[47], and also in contrast to the report by Varaiya *et al*.,[54] where lower resistance of *P. aeruginosa* isolates to meropenem 20.8% was recorded. The discrepancy in the percentage variation may be due to different geographical locations, types of samples collected, and indiscriminate use of antibiotics.

This study found 100% resistance to amoxicillin-clavulanic acid in *P. aeruginosa* strains, mirroring the 100% resistance rates reported in Egyptian studies[55] and 95.1% in Pakistan[44]. Additionally, this study recorded a high level of resistance to tigecycline, which aligns with the findings of Farhan et al[55], who also reported significant resistance to tigecycline in Egypt. The elevated levels of antimicrobial resistance observed in *P. aeruginosa* and other bacterial pathogens are likely driven by poor adherence to antibiotic stewardship policies, as well as the excessive, inappropriate, and indiscriminate use of broad-spectrum antibiotics. The overuse and misuse of antibiotics is a common global phenomenon that has substantially increased. This resistance pattern complicates and prolongs infection treatment, a trend consistent with our study's findings, and poses severe threats to human and animal healthcare due to high environmental antibiotic levels and rapid strain dissemination[56].

This study revealed that all isolates were 100% MDR *P. aeruginosa*, being resistant to more than 3 classes of antibiotics. These findings are in contrast with those reported: a low MDR to be 10.7% and 19.6% in Mekkah and Jeddah, respectively,[57,58], and 7% Malaysia[35]. Variations in drug resistance among *Pseudomonas aeruginosa* isolates may stem from differences in sample size, collection methods, and antibiotic misuse/overuse. These factors prolong the treatment of *P. aeruginosa* -associated infections. Therefore, continuous surveillance of resistance patterns and the adoption of combined therapy are recommended to manage multidrug-resistant (MDR) strains. Furthermore, eighteen distinct antibiotic

resistance patterns were identified in this study. The most prevalent pattern (Pattern P) occurred in 4 strains (C4, C8, C10, C13), conferring resistance to multiple antibiotics (e.g., cefoperazone, gentamicin, ciprofloxacin). Three strains (C2, C3, and C15) shared Pattern F, while the remaining 16 isolates exhibited unique resistance profiles. This finding is consistent with a similar study conducted in Lagos, Nigeria, where the same resistance profile was recorded in some strains of *P. aeruginosa* from clinical and environmental sources[59]. Extensive antibiotic use in clinical and agricultural settings elevates environmental concentrations, driving the evolution of bacterial resistance. This promotes persistence in the environment and facilitates transmission to humans and animals, a phenomenon well-documented in the literature[60].

This study revealed that 13% *P. aeruginosa strains* from septicemia/nephrosis patients were AmpC producers, which is consistent with separate studies conducted in India that reported 17.3-59.4% and 17.3% AmpC production from diverse sources, respectively[61,62]. However, this is contrary to the report in Tehran, Iran, where 68.6% of AmpC production was recorded[63]. The disparity in prevalence may be due to differences in the number of isolates, different geographical locations, and the selection of antibiotics prescribed[64]. This study detected 18.9% phenotypic ESBL production in *P. aeruginosa strains*, a rate comparable to the 20.27% reported in India[65], 45.19% in Tamilnadu, India[49], and 39.2% in Tehran, Iran, on ESBL-producing *P. aeruginosa strains*[63], and a higher rate 88% in Ashanti, Ghana[35]. The varying prevalence of ESBL production across countries and healthcare institutions reflects differences in antibiotic prescribing practices and the circulation of pathogens carrying ESBL traits.

Additionally, 74% of *P. aeruginosa* strains were positive for carbapenemase production. The result of this study is at variance with the 36% recorded in Taiwan[66]. This disparity in reports may be partly attributed to geographical variation as well as the attitude towards the usage of the carbapenem antibiotics. These results confirm that carbapenemase resistant *P. aeruginosa* (CRPA) poses a significant health threat, as it exhibits resistance to a broad spectrum of antibiotics and produces carbapenemase, rendering carbapenems (often last-resort treatments for severe Pseudomonas aeruginosa infections) ineffective. Interestingly, 21.7% of strains co-expressed all three resistance genes, including the subgroups: 8.7% both for CRPA + ESBL and AmpC + ESBL producers, and 4.3% showed AmpC + carbapenemase co-expression. The result in this study is comparable with 7.3%[67], 8.0%[68], and 6.59% respectively[69], who reported different resistance genes in India. This was further corroborated by Rafiee *et al*.,[63] revealed 11.8% documented slightly higher co-existence of AmpC along with ESBL and MBL. Nonetheless, phenotypic coexistence of AmpC, ESBL, and CRPA occurred in *P. aeruginosa strains* C15 and C18 that exhibited extensive drug resistance with MAR indexes of 0.63 and 0.81, isolated from the blood of patients diagnosed with septicaemia and nephrosis in female subjects. The presence of the co-existence of carbapenemases and ESBL in these strains suggests a potential role in the severity of the diseases and clinical outcomes, an observation that has been recorded elsewhere[70]. Furthermore, this study revealed a 13% prevalence of co-carriage of *blaOXA-48* and *blaVIM* in *P. aeruginosa* from septicaemia/nephrosis cases, suggesting emerging co-occurrence but remains low relative to regional and global patterns. However, this rate remains low compared to a 2024 Egyptian study of 57 CRPA clinical isolates, blaVIM was detected in 58% and 51% *blaOXA-48*, with co-carriage inferred in 30-40% based on overlapping positives, thus, high *blaOXA-48* may reflect horizontal transfer from Enterobacterales in shared hospital environments[71]. Furthermore, a 2021 retrospective analysis of 328 carbapenemase-producing *P. aeruginosa* found no *OXA-48* positives, with *VIM* at 50% and *IMP* at 39%[72]. Another Colombian study reported 150 *P. aeruginosa* clinical isolates found 41% CRPA, with *blaOXA-48* in 22%, *blaVIM* in 9%, and co-occurrence in 2.8%[73]. However, prevalence of 13% of co-resistance determinants observed in this study, warrants intensified monitoring via networks like GLASS (WHO) to track multi-carbapenemase trends, with co-production detected in 2.6% globally in 2025 data[74]. Hospital infection control, e.g., isolation, hand hygiene, is critical to curb horizontal spread. In this study, 95.7% genetically expressed the *ToxA* gene, and 100% carried the *OprL* virulence gene. However, similar to the study conducted in Brazil, which reported 81.2% for *ToxA* and 100% for *oprL* genes[75], 95.4% for *ToxA* and 100% for *oprL*

were reported in Nepal[76]. The differences in the distribution of virulence factor genes across various populations may arise from geographical differences, variations in sample size and methodology, strain diversity, and environmental factors influencing gene expression[77,78]. The coexistence of these virulence genes with resistance mechanisms such as AmpC β-lactamase, extended-spectrum β-lactamase (ESBL), and carbapenemase production has been documented, indicating that strains with high virulence potential can also acquire significant antibiotic resistance[79,80]. For instance, CRPA isolates have been shown to frequently possess ToxA and other virulence genes while also expressing resistance through mechanisms like AmpC overproduction, ESBL production, and carbapenemase genes[79,81]. This co-occurrence poses a significant clinical challenge due to increased pathogenicity, treatment failure, and limited treatment options due to reduced activity against the last resort antibiotic[82]. Studies have shown that *P. aeruginosa* isolates frequently harbour virulence genes like *oprL* and *ToxA,* which contribute to pathogenicity and are prevalent in MDR and extensively drug-resistant (XDR) strains[76]. Furthermore, studies have shown *oprL* facilitates bacterial attachment to host cells, biofilm development and boosting infectivity[83] while *ToxA* impairs immune responses and causes direct tissue injury, driving severe disease outcomes[84]. This study is limited by a small sample size of *P. aeruginosa* isolates from Lagos, Nigeria, restricting generalizability, and lacks comprehensive molecular characterization and clinical metadata to fully elucidate resistance mechanisms.

Conclusion

The study revealed the escalating public health threat of *Pseudomonas aeruginosa* in Lagos, Nigeria, with 4.2% prevalence in septicaemia and nephrosis cases. *Pseudomonas aeruginosa* was 100% resistant to trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, colistin and tigecycline, 78.3% to meropenem, and 73.9%. Carbapenemase production was detected in 73.9% of *P. aeruginosa*, EsβL in 26.1%, and AmpC β-lactamase in 13%. Interestingly, 100% of P. aeruginosa expressed oprL, and 95.7% expressed *toxA,* while 13% carried *blaOXA-48* and *blaVIM*, from septiceamia cases. A need for antimicrobial stewardship and molecular surveillance to mitigate the effects.

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