

Multidrug-Resistant *Pseudomonas aeruginosa* from Wound Infections in Selected Tertiary Institution Hospitals in Ogbomosho, Oyo State, Nigeria

¹Mercy Temitope AKANDE, ²Ayanyinka Adeola Deborah,
³Adekunle Olugbenga OLOWE (PhD)

¹Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

²Centre for Emerging and Re-Emerging Infectious Disease, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

³Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

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Abstract: *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen commonly associated with hospital-acquired infections, predominantly affecting individuals with metabolic issues, burns, or immunocompromised conditions. This study investigates the prevalence of multidrug-resistant *P. aeruginosa* in wound infections across several tertiary institutions in Oyo State, Nigeria. To isolate the bacteria, 160 wound swabs were collected over six months from different patient groups and cultured. Confirmatory tests, including Gram staining and biochemical assays, identified 47 *P. aeruginosa* strains (25%). Antimicrobial susceptibility testing (AST) using the Kirby-Bauer disc diffusion method showed significant resistance levels, particularly to Ciprofloxacin (21.9%), Cefepime (20.6%), Ceftazidime (20%), and Vancomycin (18.8%). No resistance was observed to Meropenem. Molecular analysis detected resistance genes such as blaSHV (19.4%), blaTEM (19.4%), blaCTX (23.1%), and blaOXA (15.6%), along with virulence genes like exoS (15%) and lasB (21.9%). This study highlights the concerning increase of multidrug-resistant *P. aeruginosa* in wound infections, emphasising the pathogen's resilience and the urgent need for strategies to address its impact on healthcare outcomes.

Keywords: Antimicrobial; Multidrug; Resistance, *Pseudomonas aeruginosa*; Wound, Infections.

1. INTRODUCTION

In hospitals, wound infections remain a major cause of morbidity, particularly in low- and middle-income countries. *Pseudomonas aeruginosa*, a Gram-negative, non-fermenting opportunistic bacterium known for its inherent and acquired resistance to numerous antibiotics, is one of the most commonly identified pathogens in nosocomial and chronic wound infections (Breidenstein et al., 2011). The lack of effective treatments has been exacerbated by the increasing prevalence of multidrug-resistant *P. aeruginosa* (MDR-PA), which hampers clinical care. Multidrug resistance (MDR) is defined as resistance to at least three classes of antimicrobial agents (Magiorakos et al., 2012). Reports of resistance rates in various parts of Nigeria range from 50% to 80% (Adebayo et al., 2020; Iregbu et al., 2017).

Elastase (lasB), exoenzyme S (exoS), pyocyanin synthesis, and biofilm formation are virulence factors that enhance the pathogenicity of *P. aeruginosa* and contribute to delayed wound healing and persistent infections (Gonzalez et al., 2016). Furthermore, β -lactamases that hydrolyse β -lactam antibiotics are encoded by resistance genes such as blaTEM, blaSHV, blaCTX-M, and blaOXA, which reduce the effectiveness of treatment. The growing MDR-PA burden threatens effective wound management. There is limited information available about the molecular characteristics of MDR-PA in Ogbomoso, Oyo State, despite an increase in reports of the disease across Nigeria. The purpose of this study was to determine the frequency, patterns of antibiotic resistance, and genetic factors of multidrug-resistant *P. aeruginosa* from wound infections in some of Ogbomoso's tertiary institutions.

2. MATERIALS AND METHODS

Between January and June of 2024, a cross-sectional study was carried out in a few tertiary hospitals in Ogbomoso, Oyo State, Nigeria. Patients with burn wounds, diabetic ulcers, orthopaedic wounds, post-surgical wounds, and chronic wounds provided a total of 160 wound swab samples.

2.1 Isolation and Identification

Isolation of *Pseudomonas aeruginosa* from wound infections starts with sterile collection of pus using a swab, avoiding contamination with skin flora. The specimen is transported to the microbiology lab to maintain viability and is inoculated onto selective Cetrimide agar that promotes *P. aeruginosa* growth. Identification involves biochemical tests that reveal it as oxidase-positive and catalase-positive, with Gram-negative rods observed via staining. Further confirmation utilizes Polymerase Chain Reaction for rapid identification. Morphological characteristics are recorded for distinct colonies, and identification follows Bergy's Manual of Bacteriology (2014), using nutrient agar medium and Gram staining for characterisation.

2.2 Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method was used to cultivate colonies from the pure culture. Mueller-Hinton Agar plates were used to cultivate the isolates. Using a sterile forcep, antibiotic discs containing a particular antibiotic were then placed on the agar surface. The discs were firmly pressed down to keep them from falling off the plate throughout the 24-hour incubation period at 37 °C. Diameters were recorded in two perpendicular planes, the zone of inhibition was measured, and averages were calculated. In order to ascertain whether the bacteria were sensitive (S), intermediate (I), or resistant (R) to the antimicrobials, the results were then evaluated using established tables. Amikacin, Meropenem, Ciprofloxacin, Cefepime, Ceftazidime, and Vancomycin are among the antibiotics utilised.

2.3 Molecular Detection of Resistance and Virulence Genes

2 μ L of 10X PCR buffer, 1.6 μ L of magnesium chloride, 0.2 μ L of dNTP mix, 0.2 μ L of Taq DNA polymerase, 0.5 μ L of forward and reverse primers, and 5 μ L of genomic DNA, adjusted to 20 μ L with nuclease-free water, were all used in each PCR process. The bla TEM, bla SHV, and bla CTX-M genes were amplified in an Eppendorf thermal cycler under particular conditions, resulting in anticipated amplicon sizes of 857 bp, 768 bp, and 464 bp, respectively. Both multiplex PCR techniques for β -lactamase gene detection and individual PCR for virulence genes like algD and lasB were employed in amplifications. Agarose gel electrophoresis was used to analyse the PCR results. The PCR products were resolved on the gel and seen under a UV lamp after being run on a 1% agarose solution and ethidium bromide was added to prepare the gel. A 100-base-pair DNA standard was used to verify the sizes of the PCR products.

3. RESULTS

3.1 Socio-Demographic and Clinical Characteristics

As shown in Table 1, the highest proportion of participants (48.8%) was within the 31–45-year age group, followed by 28.1% aged 46–60 years. Females constituted 56.9% of the study population, while males accounted for 43.1%. Post-surgical wounds were the predominant source of infection (60.6%), compared to accident-related (25.0%) and orthopaedic wounds (14.4%). This suggests that hospital-associated surgical wounds represent a major entry point for bacterial colonisation in the study area.

TABLE 1: Socio-Demographic and Clinical Characteristics of Participants (N = 160)

Variable	Category	Frequency (n)	Percentage (%)
Age Group (years)	16–30	28	17.5
	31–45	78	48.8
	46–60	45	28.1
	61–75	9	5.6
Gender	Male	69	43.1
	Female	91	56.9
Source of Wound	Accident	40	25.0
	Orthopedic	23	14.4
	Post-surgical	97	60.6

3.2 Culture and Biochemical Characteristics of Isolates

According to Table 2, out of 160 bacterial samples cultured on Nutrient Agar and selective Cetrimide media, 40 isolates exhibited bluish-green pigmentation, indicative of pyocyanin production. All 40 isolates were subjected to standard biochemical assays. The oxidase test yielded positive results, with a colour change to dark purple, confirming the presence of cytochrome c oxidase. Similarly, catalase activity was observed through vigorous bubbling upon exposure to hydrogen peroxide. The pigment production test confirmed pyocyanin synthesis in the majority of isolates, further supporting presumptive identification. 35.0% of samples showed growth on Nutrient Agar, while 25.6% demonstrated characteristic growth on Cetrimide Agar. All confirmed isolates were oxidase-positive and pyocyanin-producing, consistent with typical *Pseudomonas aeruginosa* characteristics. The reduction from general growth (35%) to selective growth (25.6%) confirms the specificity of Cetrimide Agar for isolating *P. aeruginosa*.

TABLE 2: Culture and Biochemical Characteristics of Isolates

Test Parameter	Category	Frequency (n)	Percentage (%)
Nutrient Agar	No Growth	104	65.0
	Growth	56	35.0
Cetrimide Agar	No Growth	119	74.4
	Growth	41	25.6
Oxidase Test	Positive	40	25.0
Catalase Test	Negative	9	5.6
	Positive	30	18.8
Pyocyanin Production	Positive	40	25.0

3.3 Antibiotic Susceptibility Pattern

As presented in Table 3, high resistance rates were observed among the isolates. Antibiotic susceptibility testing was carried out for all the bacteria 40 bacteria isolates. It was observed that for Amikacin 16 bacteria isolates were sensitive (10%), only 1 was intermediate (0.6%), and 23 isolates were resistant (14.4%). For Cefepime 7 isolates (4.4%) were sensitive and 33 isolates (20.6%) were resistant. 7 isolates were sensitive to ceftazidime, 1 (0.6%) was intermediate and 32 isolates (20%) were resistant. 16 isolates (10%) were sensitive to Meropenem while 24 isolates (15%) were resistant. For Ciprofloxacin, 4 (2.5%) of the isolates were sensitive, only 1(0.6%) was intermediate and 35 isolates (21.9%) were resistant. For Vancomycin 7 isolates (4.4%) were sensitive, 3 isolates (1.9%) were intermediate and 30 isolates (18.8%) were resistant. These findings indicate a worrying pattern of resistance to both fluoroquinolones and third-/fourth-generation cephalosporins, suggesting possible β -lactamase-mediated resistance mechanisms.

TABLE 3: Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* Isolates (n = 40)

Test Parameter	Category	Frequency (n)	Percentage (%)
Nutrient Agar	No Growth	104	65.0
	Growth	56	35.0
Cetrimide Agar	No Growth	119	74.4
	Growth	41	25.6
Oxidase Test	Positive	40	25.0
Catalase Test	Negative	9	5.6
	Positive	30	18.8
Pyocyanin Production	Positive	40	25.0
Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Amikacin (AK)	16 (10.0)	1 (0.6)	23 (14.4)
Cefepime (FEP)	7 (4.4)	—	33 (20.6)
Ceftazidime (CAZ)	7 (4.4)	1 (0.6)	32 (20.0)
Meropenem (MRP)	16 (10.0)	—	24 (15.0)
Ciprofloxacin (CIP)	4 (2.5)	1 (0.6)	35 (21.9)
Vancomycin (VA)	7 (4.4)	3 (1.9)	30 (18.8)

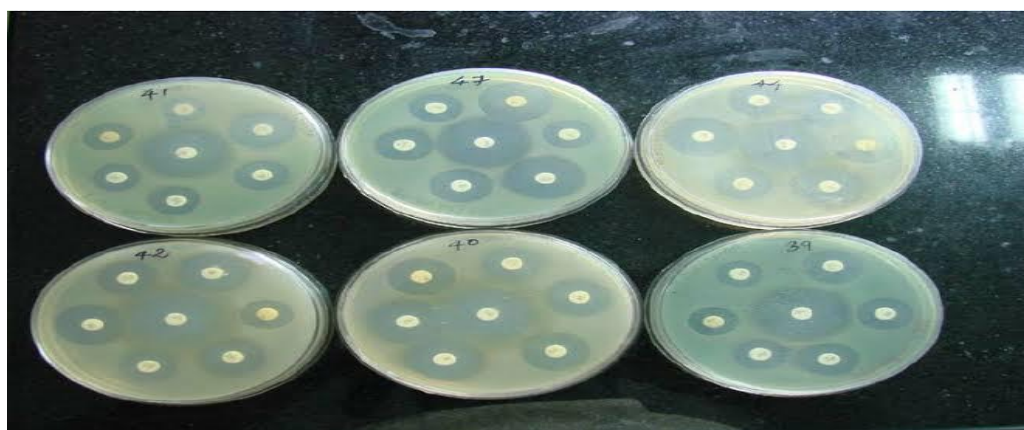


Fig. 1: Antibiotic Susceptibility Testing

3.4 Molecular Distribution of Virulence and Resistance Genes

As shown in Table 4, PCR analysis revealed the following gene prevalence. The blaCTX gene was the most prevalent resistance gene (23.1%), followed by lasB (21.9%). Other genes detected included blaSHV (19.4%), blaTEM (19.4%), blaOXA (15.6%), and exoS (15.0%). The high prevalence of β -lactamase genes correlates with the phenotypic resistance observed in Table 3, reinforcing the molecular basis of multidrug resistance among isolates.

TABLE 4: Summary Distribution of Virulence and Resistance Genes Among Isolates (n = 40)

Gene	Negative n (%)	Positive n (%)
exoS	16 (10.0)	24 (15.0)
lasB	5 (3.1)	35 (21.9)
blaSHV	9 (5.6)	31 (19.4)
blaTEM	9 (5.6)	31 (19.4)
blaCTX	3 (1.9)	37 (23.1)
blaOXA	15 (9.4)	25 (15.6)

TABLE 5: EXOs Summary

EXO S							
Variables	Categories	Negative	Positive	Total	Pearson Chi-Square X^2	Df	P-value
Age	16-30 years	1 (2.5%)	5 (12.5%)	6 (15.0%)	3.005	3	0.391
	31-45 years	9 (22.5%)	13 (32.5%)	22 (55.0%)			
	46-60 years	6 (15.0%)	5 (12.5%)	11 (27.5%)			
	61-75 years	0 (0.0%)	1 (2.5%)	1 (2.5%)			
Group	Male	6 (15.0%)	10 (25.0%)	16 (40.0%)	0.069	1	0.792
	Female	10 (25.0%)	14 (35.0%)	24 (60.0%)			
Source of Wound	Accident	1 (2.5%)	5 (12.5%)	6 (15.0%)	4.194	2	0.123
	Orthopaedic	6 (15.0%)	3 (7.5%)	9 (22.5%)			
	Post Surgical	9 (22.5%)	16 (40.0%)	25 (62.5%)			

TABLE 6: LASB Summary

LASB							
Variables	Categories	Negative	Positive	Total	Pearson Chi-Square X^2	Df	P-value
Age	16-30 years	1 (2.5%)	6 (15.0%)	7 (17.5%)	1.371	3	0.71
	31-45 years	3 (7.5%)	24 (60.0%)	27 (67.5%)			
	46-60 years	1 (2.5%)	3 (7.5%)	4 (10.0%)			
	61-75 years	0 (0.0%)	2 (5.0%)	2 (5.0%)			
Group	Male	1 (2.5%)	15 (37.5%)	16 (40.0%)	0.024	1	0.876
	Female	2 (5.0%)	22 (55.0%)	24 (60.0%)			
Source of Wound	Accident	1 (2.5%)	4 (10.0%)	5 (12.5%)	0.641	2	0.725
	Orthopaedic	1 (2.5%)	6 (15.0%)	7 (17.5%)			
	Post Surgical	3 (7.5%)	25 (62.5%)	28 (70.0%)			

TABLE 7: Bla SHV Summary

Bla SHV							
Variables	Categories	Negative	Positive	Total	Pearson Chi-Square X^2	Df	P-value
Age	16-30 years	1 (2.5%)	5 (12.5%)	6 (15.0%)	6.026	3	0.11
	31-45 years	8 (20.0%)	14 (35.0%)	22 (55.0%)			
	46-60 years	0 (0.0%)	11 (27.5%)	11 (27.5%)			
	61-75 years	0 (0.0%)	1 (2.5%)	1 (2.5%)			
Group	Male	3 (7.5%)	13 (32.5%)	16 (40.0%)	0.215	1	0.643
	Female	6 (15.0%)	18 (45.0%)	24 (60.0%)			
Source of Wound	Accident	1 (2.5%)	5 (12.5%)	6 (15.0%)	0.812	2	0.666
	Orthopaedic	3 (7.5%)	6 (15.0%)	9 (22.5%)			
	Post Surgical	5 (12.5%)	20 (50.0%)	25 (62.5%)			

TABLE 8: Bla Tem Summary

Bla Tem							
Variables	Categories	Negative	Positive	Total	Pearson Chi-Square X^2	Df	P-value
Age	16-30 years	1 (2.5%)	5 (12.5%)	6 (15.0%)	2.637	3	0.451
	31-45 years	7 (17.5%)	15 (37.5%)	22 (55.0%)			
	46-60 years	1 (2.5%)	10 (25.0%)	11 (27.5%)			
	61-75 years	0 (0.0%)	1 (2.5%)	1 (2.5%)			
Group	Male	4 (10.0%)	12 (30.0%)	16 (40.0%)	0.096	1	0.757
	Female	5 (12.5%)	19 (47.5%)	24 (60.0%)			
Source of Wound	Accident	2 (5.0%)	4 (10.0%)	6 (15.0%)	0.494	2	0.781
	Orthopaedic	2 (5.0%)	7 (17.5%)	9 (22.5%)			
	Post Surgical	5 (12.5%)	20 (50.0%)	25 (62.5%)			

TABLE 9: Bla CTX Summary

Bla CTX							
Variables	Categories	Negative	Positive	Total	Pearson Chi-Square X^2	Df	P-value
Age	16-30 years	0 (0.0%)	6 (15.0%)	6 (15.0%)	2.654	3	0.448
	31-45 years	3 (7.5%)	19 (47.5%)	22 (55.0%)			
	46-60 years	0 (0.0%)	11 (27.5%)	11 (27.5%)			
	61-75 years	0 (0.0%)	1 (2.5%)	1 (2.5%)			
Group	Male	1 (2.5%)	15 (37.5%)	16 (40.0%)	0.060	1	0.806
	Female	2 (5.0%)	22 (55.0%)	24 (60.0%)			
Source of Wound	Accident	1 (2.5%)	5 (12.5%)	6 (15.0%)	1.465	2	0.481
	Orthopaedic	0 (0.0%)	9 (22.5%)	9 (22.5%)			
	Post Surgical	2 (5.0%)	23 (57.5%)	25 (62.5%)			

TABLE 10: Bla Oxa Summary

Bla Oxa							
Variables	Categories	Negative	Positive	Total	Pearson Chi-Square X^2	Df	P-value
Age	16-30 years	3 (7.5%)	3 (7.5%)	6 (15.0%)	3.345	3	0.341
	31-45 years	6 (15.0%)	16 (40.0%)	22 (55.0%)			
	46-60 years	5 (12.5%)	6 (15.0%)	11 (27.5%)			
	61-75 years	1 (2.5%)	0 (0.0%)	1 (2.5%)			
Group	Male	8 (20.0%)	8 (20.0%)	16 (40.0%)	1.778	1	0.182
	Female	7 (17.5%)	17 (42.5%)	24 (60.0%)			
Source of Wound	Accident	2 (5.0%)	4 (10.0%)	6 (15.0%)	0.178	2	0.915
	Orthopaedic	3 (7.5%)	6 (15.0%)	9 (22.5%)			
	Post Surgical	10 (25.0%)	15 (37.5%)	25 (62.5%)			

4. DISCUSSION

In this study, the prevalence rate of isolation was 25%. This aligns with previous Nigerian reports identifying *P. aeruginosa* as a predominant wound pathogen (Ogunsanya et al., 2018). Hence, establishing the predominance of post-surgical wounds highlights the risk of nosocomial acquisition of *P. aeruginosa* in tertiary healthcare facilities. Similar findings have been reported in Nigerian tertiary hospitals, where surgical sites serve as reservoirs for opportunistic pathogens. The isolation rate observed aligns with reports indicating that *P. aeruginosa* remains a major cause of chronic wound infections in developing countries. Its oxidase positivity and pyocyanin production confirm its virulent phenotype. These findings are consistent with previous reports identifying *P. aeruginosa* as a leading cause of chronic and nosocomial wound infections (Turner et al., 2019; Ogunsanya et al., 2018; Rahim et al., 2017; Gjodsbol et al., 2006).

The high prevalence of blaCTX, blaTEM, and blaSHV genes explains resistance to β -lactam antibiotics. Indicating molecular findings provide genetic confirmation of resistance mechanisms. The detection of virulence genes such as lasB and exoS suggests enhanced pathogenic potential, which may contribute to chronicity and delayed wound healing. Similar resistance trends have been reported in Nigerian tertiary hospitals, with MDR prevalence ranging from 50–80% (Adebayo et al., 2020; Iregbu et al., 2017). Similarly, Page et al. (2009) reported increasing ineffectiveness of β -lactam + aminoglycoside combinations.

The concordance between phenotypic resistance and genotypic findings strengthens the validity of the study and underscores the importance of molecular surveillance in antimicrobial resistance monitoring. High resistance to fluoroquinolones and cephalosporins observed in this study reflects increasing selective pressure from inappropriate antibiotic use. Implying that the presence of blaCTX, blaTEM, and blaSHV genes confirms the molecular basis for β -lactam resistance. Similarly, the detection of blaTEM, blaSHV, and blaCTX confirms ESBL-mediated β -lactam resistance. Although meropenem showed comparatively lower resistance, emerging carbapenem resistance remains concerning. The detection of virulence genes such as lasB and exoS suggests enhanced pathogenic potential, contributing to chronicity and delayed wound healing. These findings underscore the need for continuous antimicrobial surveillance, strict infection control practices, and antibiotic stewardship programs to mitigate further spread of MDR strains.

5. CONCLUSION

This study demonstrated a considerable burden of multidrug-resistant *Pseudomonas aeruginosa* among wound infection patients in selected tertiary hospitals in Ogbomoso, Oyo State, Nigeria. The isolation rate of 25% confirms that *P. aeruginosa* remains a significant opportunistic pathogen associated with wound infections, particularly post-surgical wounds which showed the highest prevalence. The high occurrence among adults aged 31–45 years may reflect increased exposure to hospital procedures and occupational risks. Antimicrobial susceptibility testing revealed high resistance rates to commonly used antibiotics including ciprofloxacin, cefepime, and ceftazidime, indicating the growing challenge of treating *P. aeruginosa* infections with conventional therapies. The relatively lower resistance to meropenem suggests that carbapenems remain important therapeutic options, although the risk of emerging resistance cannot be ignored.

Molecular analysis confirmed the presence of key resistance genes (blaCTX, blaTEM, blaSHV, and blaOXA), which explains the phenotypic resistance patterns observed. The detection of virulence genes such as lasB and exoS further indicates the high pathogenic potential of the isolates and their role in chronic wound infections and delayed healing. The strong correlation between phenotypic and genotypic resistance highlights the importance of integrating molecular surveillance into routine diagnostic practices.

Overall, the findings emphasize the urgent need for antimicrobial stewardship programs, improved infection control practices, rational antibiotic use, and regular monitoring of resistance patterns in healthcare facilities to curb the spread of multidrug-resistant *P. aeruginosa* in the study area and beyond.

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