Original

Antibiotic Resistance Pattern of Pseudomonas *aeruginosa* Isolates from Patients Attending Estu Umaru Sanda General Hospital, Bida, Niger State, Nigeria

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ABSTRACT

Introduction: Pseudomonas aeruginosa is an opportunistic pathogen that represents a major nosocomial threat, particularly among patients on hospital admission. This study determined the prevalence of Pseudomonas aeruginosa infections in patients and assessed their antibiotic susceptibility patterns in order to provide up-to-date data that could support rational antibiotic use and contribute to improved management of infections caused by this pathogen.

Methods: One hundred clinical specimens were obtained from patients in Etsu Umaru Sanda General Hospital, Bida, Niger State, Nigeria. Samples were cultured on MacConkey agar and subcultured on cetrimide agar and colonies were Gram stained. Biochemical characterization was carried out and species were confirmed by 16S rRNA polymerase chain reaction assay. Antibiotic susceptibility testing of the isolates to ten antibiotics was done using the Kirby Bauer Disc diffusion method.

Results: Twenty-five Pseudomonas aeruginosa isolates were identified giving a 25% prevalence rate. The highest prevalence of infection was recorded from wound swabs (13 (52%), followed by ear swab (7 (28%) and urine (5 (20%%). From the antibiogram, the highest resistance rate was observed towards amoxicillin (68%) while isolates were remarkably susceptible to colistin (92%) and piperacillin/tazobactam (96%). Four isolates (16%) were multidrug-resistant (MDR) strains.

Conclusion: The presence of MDR *P. aeruginosa* is a cause for concern due to its potential to limit treatment options. Therefore, there is need for the establishment of a stringent and effective regime of antimicrobial stewardship and periodic monitoring system is of utmost importance to limit spread of multidrug resistant *P. aeruginosa* strains in the study area.

Keywords: *Pseudomonas aeruginosa*, opportunistic, wound swab, colistin, piperacillin/tazobactam, multidrug-resistant (MDR), surveillance

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INTRODUCTION

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium widely recognized as an important cause of hospital-acquired infections. *Pseudomonas aeruginosa* is commonly associated with infections of the respiratory tract, catheter-associated urinary tract infections, wounds, burn-related infections, and bloodstream, particularly among immunocompromised individuals and hospitalized patients. *Pseudomonas aeruginosa*, particularly multidrug-resistant variants cause serious infections that is usually associated with high rates of mortality that ranges between 18 – 61% and most often in cases of ventilator-associated pneumonia (VAP), bacteremia and those with chronic lung disease (cystic fibrosis)[1] that eventually lead to pulmonary damage and respiratory insufficiency[2]. The organism possesses numerous virulence factors and the ability to survive in diverse environmental conditions which allows its persistence in hospital environments, contributing significantly to nosocomial infections[3].

One of the major clinical challenges associated with *P. aeruginosa* infections is its intrinsic and acquired resistance to multiple classes of antimicrobial agents. The organism has the ability to develop resistance through various mechanisms including reduced membrane permeability, production of β-lactamases, efflux pump systems, and biofilm formation. These mechanisms significantly limit therapeutic options and contribute to treatment failure and prolonged hospital stay[4].

Globally, antimicrobial resistance among *P. aeruginosa* isolates has been increasing and has become a major public health concern. Multidrug-resistant (MDR) strains are resistant to several commonly used antibiotics, including fourth generation cephalosporins and advanced beta-lactams. In Nigeria, a number of studies have reported high resistance rates of *P. aeruginosa* to commonly used antibiotics such as cephalosporins, aminoglycosides, and β-lactam antibiotics [4,5,6,7]. This poses serious challenges for effective treatment and complicates infection management in healthcare settings.

The continuous emergence and spread of antibiotic-resistant *P. aeruginosa* strains emphasize the importance of monitoring antimicrobial susceptibility patterns in healthcare facilities. Such surveillance provides valuable information that guides clinicians in selecting appropriate antimicrobial therapy and assists in developing effective infection control policies. Despite several studies conducted in different parts of Nigeria, there is limited data on the resistance profile of *P. aeruginosa* from Bida, Niger State, Nigeria. Therefore, this study aimed to determine the antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from patients attending Etsu Umaru Sanda General Hospital, Bida, Niger State, Nigeria**,** in order to provide up-to-date data that could support rational antibiotic use and contribute to improved management of infections caused by this pathogen.

METHODOLOGY

Study area and sample size: The study adopted a cross-sectional descriptive approach. A total of 100 different clinical specimens including 20 each of urine, high vaginal swab, ear swabs, wound/burn swabs and urethra swabs were retrieved from the laboratory units of Estu Umaru Sanda General Hospital, Bida, Niger State, Nigeria from December, 2021 to November, 2022. Socio-demographic data of the patients were obtained from the laboratory request forms using a predesigned questionnaire.

Isolates culture and characterization: Samples were cultured on MacConkey agar plates prepared according to manufacturer's instructions. Colourless colonies due to non-lactose fermentation were subculture on cetrimide agar, incubated at 37^{o}C for 24 h. Morphological and biochemical characterization were carried out by Gram staining, catalase test, oxidase test and citrate utilization test.

Molecular identification of isolates: *Pseudomonas aeruginosa* specie was confirmed using the molecular method of 16s rRNA polymerase chain reaction assay.

DNA extraction: The boiling method of DNA extraction was used. In an Eppendorf tube, 3 colonies of an overnight culture of each isolate were mixed in 100 µL of sterile distilled water. This was boiled for 15 minutes, then centrifuged at 10,000 rpm for 5 minutes. The supernatant was centrifuged and then transferred to a new tube. This was used as DNA template for PCR assay

DNA amplification A 25 µL PCR mixture was prepared containing 12.5 µL of OneTaq Quick-Load 2X Master Mix with standard buffer, 0.5 µL of 10 µM forward and reverse primers, 3 µL of template DNA, and 8.5 µL of nuclease-free water. The primers used for amplifying the *Pseudomonas aeruginosa* gene were 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGATCCAGCC-3'). Polymerase chain reaction (PCR) was performed in a GeneAmp 9700 PCR

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System (Applied Biosystems Inc., USA) using the following cycling conditions: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 60 seconds, and extension at 72°C for 1 minute 30 seconds. A final termination step was performed at 72°C for 10 minutes. The amplicon (10 µL) was electrophoresed at 120V for 45 minutes on a 1.5% agarose gel pre-stained with 3 µL of 0.5 µg/mL ethidium bromide in 1X Tris-Acetate-EDTA (TAE) buffer. The gel was visualized under ultraviolet light and photographed (Avebury, UK). The size of the PCR products was estimated by comparison with a 100 base pair molecular weight marker run alongside the experimental samples.

Antibiotic susceptibility testing: All isolates were subjected to antibiotic susceptibility testing (AST) using the Kirby-Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021). Commercial antibiotic discs (Mast Discs, UK) were placed 20 mm apart, center to center, and the plates were incubated at 37°C for 24 hours. The antibiotics used were imipenem (IPM: 10 µg), meropenem (MEM: 10 µg), ceftazidime (CAZ: 30 µg), cefotaxime (CTX: 30 µg), gentamicin (GEN: 10 µg), amoxicillin (AMX: 30 µg), piperacillin/tazobactam (TZP: 100/10 µg), colistin (CST: 10 µg), aztreonam (ATN: 30 µg), and ciprofloxacin (CIP: 5 µg) (Oxoid, UK). The diameters of the inhibition zones were measured using a metered ruler and classified as susceptible, intermediate, or resistant according to the CLSI (2022).

Determination of multidrug resistance: An isolate was termed multidrug resistant if it was resistant to at least one antibiotic in three or more antibiotic classes.

Data analysis: Descriptive statistics (frequencies and percentages) was used to present the data in this study.

Ethical approval Ethical approval for the conduct of this study was granted by the Hospitals Ethics Committee of the Hospitals Management Board, Niger State.

RESULTS

During the sample collection which lasted from December 2021 to November, 2022, a total of 100 clinical samples were collected from Etsu Umaru Sanda General Hospital, Bida. Twenty-five isolates of *Pseudomonas aeruginosa* (25%) were recovered. Ten (40.0%) and 15(60.0%) were recovered from the male and female patients respectively. Majority (20, 80.0%) out of the 25 *P. aeruginosa* isolates were recovered from in-patients (patients on hospital admission) and the remaining 5(20%) from out-patients. As shown in Table 1, wound swabs accounted for the highest number of isolates (13, 52%), followed by ear swabs (7, 28%) and urine accounted for the least (5, 20%). Eleven (44%) out of the 25 isolates of *P. aeruginosa* were recovered from patients in the age range of 21-30 years, 8 (32.0%) from patients in the age range of 31 – 40 years, 3 (12.0%) from patients between 10 – 20 year, 2 (8.0%) from 51 – 60 years old patients and 1 from 41 – 5 0 years old patient. while the least was from the age range of 51-60 years.

Table 1. Demographic characteristics of patients with *Pseudomonas aeruginosa* infections

Parameters	***Pseudomonas aeruginosa* isolate**	**(%)**
Gender		
Male	10	40.0
Female	15	60.0
Patient category		
Out-patient	5	20.0
In-patient	20	80.0
Age groups (years)		
10-20	3	12.0
21-30	11	44.0
31-40	8	32.0
41-50	1	4.0
51-60	2	8.0
Sample Type		
Urine	5	20.0
HVS	0	0
Ear swab	7	28.0
Wound swab	13	52.0
Urethra swab	0	0

HVS: high vaginal swab

The PCR result was used to confirm the isolates as *Pseudomonas aeruginosa.* The 16s rRNA gene of *P. aeruginosa* yielded 1400 bp as shown in Figure 1.

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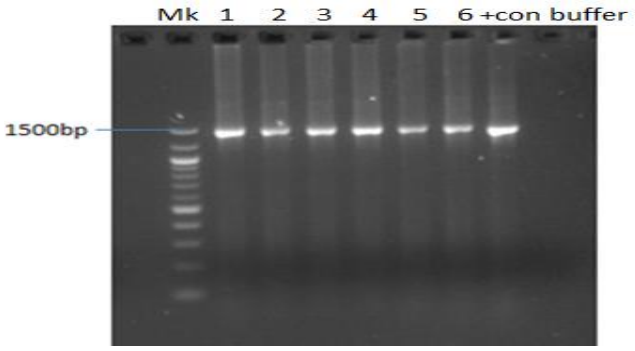

Figure 1: Confirmation of *Pseudomonas aeruginosa* by 16S rRNA: lane MK-100bp molecular marker; Lane 1-6 are positive isolates; Lane+con-positive control *P. aeruginosa* ATCC27853; Lane buffer: negative control

Out of the 25 *P. aeruginosa* isolates recovered, 24 (96%) and 23 (92%) were susceptible to piperacillin/tazobactam and colistin respectively while 21 (84%) and 20 (20%) were susceptible to imipenem and meropenem respectively. As shown in Table 2, amoxicillin was the least active antibiotic where 17 (68%) were resistant. Followed by gentamicin (15 ,60%) and ciprofloxacin (12, 48%). Among the cephalosporins, ceftazidime was least active with 13 (52%) of the isolates were found resistant.

Four isolates were multidrug-resistant. All were from in-patients and had high multiple antibiotic resistance index as shown in Table 3.

Table 2. Antibiotic susceptibility profile of the isolates

Antibiotics	Susceptible	Intermediate	Resistant
Imipenen	21 (84.0)	2 (8.0)	2 (8.0)
Meropenem	20 (80.0)	3 (12.0)	2 (8.0)
Amoxicillin	8 (32.0)	0 (0)	17 (68.0)
Piperacillin/tazobactam	24 (96.0)	0 (0)	1 (4.0%)
Gentamicin	9 (39.2)	1 (4.0)	15 (60.0)
Cefotaxime	16 (64.0)	0 (0)	9 (36.0)
Ceftazidime	13 (52.0)	0 (0)	12 (48.0)
Ciprofloxacin	11 (44.0)	2 (8.0)	12 (48.0)
Aztreonam	17 (68.0)	2 (8.0)	6 (24.0)
Colistin	23 (92.0)	0 (0)	2 (8.0)

Table 3. Resistance phenotype of multidrug resistant strains

IN	Resistance phenotype	MI
06	IMP, MEM, AMX, GT, CEF	0.5
11	IMP, MEM, AMX, GT, CEF, CTX, COL,	0.7
13	PIP/TAZ, AMX, GT, CEF	0.4
19	GT, CTX, CEF, AMX	0.4

IN – Isolate Number; MI – MAR INDEX
IMI:imipenem, MEM: meropenem, AMX:amoxicillin, GT: gentamicin, CEF: ceftazidime, CTX: cefotaxime, COL: colistin, PIP/TAZ: piperacillin/tazobactam

DISCUSSION

Pseudomonas aeruginosa is an organism that is widespread in nature, particularly in the hospital environment where it is known as one of the leading nosocomial pathogens that cause severe infections[2]. In this study, the prevalence of *P. aeruginosa* infections was 25%. Daji *et al.*[8] in Taraba State, Nigeria reported 17% prevalence rate of *P. aeruginosa* infections. The prevalence rate in our study is much lower than 96.0% reported in a study by Adejobi *et al.*[5] in Ile Ife, a more humid zone in Nigeria while a recent report by Bawa *et al.*[9] reported 12.7% prevalence of *P. aeruginosa* infection in a study from Sokoto State. The observed prevalence rate of *P. aeruginosa* in our study may be attributed to study population, types of clinical samples involved or some environmental and climatic conditions related to geographical location.

In our study, the majority of the *Pseudomonas aeruginosa* isolates were recovered from wound swabs (13, 52%), ear swabs (28%) and urine (5, 20%). This pattern of isolation is in agreement with the findings of Gad *et al.*[10], Awanye *et al.*[11] and Lawal *et al.*[12] where urine, wound swabs and ear swabs gave the highest yields of *P. aeruginosa.* Similar to our findings, some studies within and outside Nigeria have reported wound specimen as the clinical specimen which account for the highest number of isolates [2,5,11,13,14,15]. These highlights wound as one of the major regular specimen sources for the recovery of *P. aeruginosa. Pseudomonas aeruginosa* thrives well in moist environments, forming biofilms on medical devices and causing tissue damage. Urinary catheterization provides an avenue for the colonization of the urinary tract while wounds and burns provide an enabling nutrient-rich surface for invasion. The higher isolation rates observed in these samples may also be

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attributed to large sample numbers. Urine, wound and ear swab samples were the most common sample types in this study.

Results of antibiotics susceptibility testing revealed that isolates were most resistant to amoxicillin (68%), which belongs to the β-lactam class of antibiotic. This resistance rate is lower than the result of Umar *et al.*[2] where *P. aeruginosa* isolates were reportedly 98.1% resistant to amoxicillin. In another study by Egwin *et al.*[16], *P. aeruginosa* was reportedly 97.4% resistant to amoxicillin. The high resistance rate exhibited by *Pseudomonas aeruginosa* towards amoxicillin may be attributable to their intrinsic resistance mechanisms such as chromosomal AmpC β-lactamase production, lower outer membrane permeability of *P. aeruginosa*, efflux pumps, poor affinity to penicillin-binding proteins and biofilm formation/selective pressure from misuse[17]. In Nigeria, most antibiotics are freely sold which aids misuse of antibiotics, thereby promoting resistance. Sometimes, for some economic reasons, many patients are prematurely discharged from hospitals to complete their treatment at home.

The β-lactam inhibitor piperacillin/tazobactam was the most active antibiotic with the highest susceptibility rate of 96% in our study. High susceptibility of *P. aeruginosa* to piperacillin/tazobactam has been reported in several studies highlighting its continuous effectiveness as an anti-pseudomonal β-lactam antibiotic. Our finding agrees with 84.5% susceptibility rate reported by Zubair & Iregbu[14], 94% susceptibility rate reported by Adejobi *et al.*[5] and 87.6% reported by Murtala *et al.*[19] where piperacillin/tazobactam was the most active antibiotic against *P. aeruginosa*, recording low resistance rates. In contrast, 27.9% susceptibility rate of piperacillin/tazobactam was reported by Awanya *et al.*[11] from Rivers State, Nigeria and 35.6% susceptibility rate reported by Wang & Wang,[15] from China. The high susceptibility rate in our study may be due to the fact that piperacillin/tazobactam is not common prescribed in the study areas, thus, still a potent drug for clearing infections caused by *P. aeruginosa.*

In this study, 4 (16%) of the isolates were resistant to the carbapenems: 8% resistance each to imipenem and meropenem respectively. The prevalence in our study is lower than reports from previous studies in Nigeria: 33.2% resistance reported by Zubair & Iregbu,[14] in a study from Abuja, the Federal Capital Territory and 54.5% resistance reported by Umar *et al.*[2] in a study from Sokoto while Wang & Wang,[15] from China reported 77.5% incidence of resistance to imipenem and 64.7% resistance to meropenem. About 58.8% resistance was reported by Murtala et al.[18]. In Niger State where this study was conducted, carbapenems are reserved majorly as among the last resort drugs for the treatment of *P. aeruginosa* infections. Nontheless, Olowo-okere *et al.* [19] listed importation of resistance strains by patients returning from other parts of the country or other countries where carbapenem resistance is endemic, cross-resistance between carbapenem and other β-lactam antibiotics which may result in independent emergence of carbapenems resistance and localization of most carbapenemase genes on highly ambulatory genetic elements which aid the ease of acquisition and transmission of acquired carbapenem resistance among bacterial isolates as possible situations that may result in carbapenem-resistance in Enterobacteriaeceae among patients with no prior history of exposure to carbapenems. The carbapenem-resistant *P. aeruginosa* in this study may be results arising from any of the aforementioned situations.

In this study, *P. aeruginosa* exhibited high rate of resistance to gentamicin (64.4%). This finding aligns with a previous report by Gad *et al.* (2007) who reported a 59% resistance of *P. aeruginosa* to gentacmicin. Some previous studies have revealed that gentamicin-resistant *P. aeruginosa* has being on the increase since the last decade [2,13]. The high resistance rate observed in our study may in part be a reflection of the antibiotic prescription pattern in the study area and the fact that gentamicin has been in use since the '60s. This suggests that the antibiotic has long been in use and this is a driver for resistance.

In the face of multi-drug-resistant *P. aeruginosa*, colistin, sometimes remains the only effective antibiotic. In this study, 92% of the isolates were susceptible to colistin while 8% were-resistant. High susceptibility rate in our study is comparable to 84% observed in previous reports by Zubair & Iregbu[14] The low resistance rate observed in this study may be attributed to the fact that colistin is an antibiotic that is not commonly prescribed in the hospital because they are reserved as a last resort therapeutic option for multidrug resistant infections, implying less exposure of this pathogen to the antibiotic which results in lesser chances of resistance development. Colistin resistance in *P. aeruginosa* may result from either modification of lipid A or loss of lipopolysaccharide. Colistin resistance in *P. aeruginosa* isolates may also arise from plamid-related resistance

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mechanisms and from chromosomal mutations invoving regulatory systems such as *pmrA*/*pmrB* and *phoP*/*phoQ* [20]

High rates of multiple drug-resistant isolates which are resistant to aminoglycosides, cephalosporins and fluoroquinolones have been reported in previous studies from Nigeria and other parts of the world. In this study, 4 (16%) isolates were multidrug resistant. Umar *et al.*[2] in a study on the prevalence of MDR *P. aeruginosa* from ear and wound swabs at two specialist hospitals in Sokoto, Nigeria, reported high degree of in-vitro antimicrobial resistance (90-96%) to the third generation cephalosporins, moderate resistance to quinolone (56%) and 36.7% prevalence of MDR *P. aeruginosa*. In another study by Adejobi *et al.*[5]in Ile Ife, Ogun State, MDR *P. aeruginosa* strains accounted for 82.4% of the isolates. Awanye *et al.*[11] reported 61% prevalence of MDR *P. aeruginosa* in a study on the prevalence of MDR and XDR *P. aeruginosa* from tertiary healthcare facilities in Rivers State. Onyi *et al.*[7] in a study on the antibiogram of multidrug-resistant *Pseudomonas aeruginosa* with oprL gene associated with surgical site infections in Bauchi, Nigeria reported 80% multidrug resistant *P. aeruginosa* isolates with a significant potion resistant to β-lactams and cephalosporins. From the aforementioned, it is evident that multidrug resistant P. aeruginosa has been since emerged and being in circulation. In the case of our study, low prevalence may be attributed to small sample size , sample types or study population, limited prior exposure to the carbapenems, β-lactam inhibitor (piperacillin/tazobactam) and also colistin. Additionally, Estu Umaru Sanda general hospital is not tertiary health institution where invasive procedures such as urinary catheterization and mechanical ventilation are commonly done and these increase chances of acquiring *P. aeruginosa* infections and resistant strains. It is therefore safe to say that there is still a relatively low misuse/overuse of antibiotics in the community.

The findings of this study show that though of low prevalence, multidrug resistant *Pseudomonas aeruginosa* has emerged in the study area. Therefore, there is need for the establishment of a stringent and effective regime of antimicrobial stewardship and periodic monitoring system is of utmost importance to limit spread of multidrug resistant *P. aeruginosa* strains in the study area.

Declarations

Authors' contributions: NUA designed the study, ZJK collected, analyzed the data and carried out the laboratory work, NUA, HNB and AA performed the literature review for the manuscript, supervised the study. The final version of the manuscript was read and approved by all authors.

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Conflicts of Interest: none to declare.

Disclosure in AI use: There was very minimal use of AI tools in the manuscript.

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