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ORIGINAL ARTICLE

Multidrug-Resistant and Extremely Drug-Resistant *Pseudomonas aeruginosa* in Clinical Samples From a Tertiary Healthcare Facility in Nigeria

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ABSTRACT

Objectives: *Pseudomonas aeruginosa* has been globally implicated in healthcare-associated infection. The susceptibility pattern of clinical isolates of *P. aeruginosa* to anti-pseudomonal antibiotics is reported.

Materials and Methods: Clinical samples, namely blood, urine, tracheal aspirate, cerebrospinal fluid (CSF), wound swabs, high vaginal swabs, eye, and ear exudates were obtained from patients, processed and identified using standard microbiological protocols. Antibiotic susceptibility testing was undertaken using the Kirby Bauer Disc diffusion method. Results were reported following the Clinical and Laboratory Standards Institute guidelines.

Results: Of 104 *P. aeruginosa* isolates identified, males (52.88%) had a higher incidence of infection than female (47.11%) patients. The highest prevalence was recorded from wound swabs [46 (44.23%)] followed by ear exudates [23 (22.12%)], urine [22 (21.15%)], while eye exudates and samples from the CSF yielded the least [1 (0.96% each)]. From the antibiogram, imipenem had the highest antibiotic activity (91.3%) followed by polymyxin B (84.6%). The isolates exhibited the highest resistance to ceftazidime (73.1%) and piperacillin-tazobactam (61.5%). The antibiotic susceptibility pattern of *P. aeruginosa* isolates revealed 7.69% susceptible, 26% resistant, 61% multidrug resistance (MDR), 5% extremely drug resistance (XDR), and an absence (0%) of pandrug-resistant phenotypes.

Conclusion: The study recorded alarmingly high cases of MDR and some XDR phenotypes of *P. aeruginosa* in University of Port Harcourt Teaching Hospital. It will help identify existing gaps in antimicrobial resistance surveillance and assist in improving public health policies regarding antibiotic stewardship, initiatives, and interventions.

Key words: Antibiotics, antimicrobial resistance, multidrug resistance, extremely drug resistance, *Pseudomonas aeruginosa*, Nigeria

INTRODUCTION

Gram-negative bacteria account for many life-threatening hospital-associated infections.[1] *Pseudomonas aeruginosa* is an opportunistic Gram-negative bacterium implicated in many hospital infections especially in individuals with prolonged hospital stay, medical implants, weakened immune systems, or those with underlying conditions or disease states. Its ability to survive with minimal nutrition and tolerate various disinfectant conditions leads to its persistence on surfaces, where it can cause infection in the hospital and community settings.[2] Additionally, biofilms formed by *P. aeruginosa* enhance their capability of causing infection by protecting the bacteria from elimination by antibiotics and disinfectants.[3] Infections caused by *P. aeruginosa* include acute and chronic lung infections, urinary tract infections, ocular infections, and bacteremia, causing high mortality and morbidity.[4] They are usually difficult

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to treat and are life-threatening due to the intrinsic susceptibility of *Pseudomonas* to few antimicrobial agents and their ability to readily acquire antimicrobial resistance genes from other bacteria and the environment. These factors contribute to the reason, why infections caused by antibiotic resistant *P. aeruginosa* usually result in a longer duration of treatment, increased costs of treatment and higher mortality rates.[5]

Antibiotic resistance constitutes a major health challenge, especially in people in critical care conditions such as those in intensive care units (ICU); those with implanted medical devices; those who have recently undergone surgery; those on broad spectrum antibiotics or those with pre-existing severe underlying conditions such as cancer, diabetes, renal insufficiency, heart failure, liver cirrhosis, and autoimmune diseases.[6] Cases of multiple-drug and extremely-drug resistance have been described for *P. aeruginosa* and this limit the efficacy of empirical treatment of infections caused by *P. aeruginosa*.[7-9] Antibiotics recommended for the treatment of infections caused by *P. aeruginosa* are grouped into eight antibiotic classes namely: Fluoroquinolones (ciprofloxacin and levofloxacin); aminoglycosides (gentamicin, tobramycin, amikacin, and netilmicin); beta-lactam antibiotics and beta-lactamase inhibitors (ticarcillin-clavulanate and piperacillin-tazobactam); cephalosporins (ceftazidime and cefepime); carbapenems (imipenem, meropenem, and doripenem); monobactams (aztreonam); polymyxins (colistin and polymyxin B), and phosphonic acids (fosfomycin). Increasing rates of drug resistance in *P. aeruginosa* to many antibiotics have been recorded[10] and is attributed to many chromosomes or plasmid-mediated mechanisms. These include the production of antibodies inactivating enzymes *e.g.* β-lactamase production; modification of target sites; overexpression of target molecules; overexpression of drug efflux pumps, reduced drug permeability through the loss of porin proteins.[11,12]

Ventilator-associated-pneumonia (VAP) is a common infectious disease in the ICU, caused by multidrug resistant (MDR) *P. aeruginosa*.[13] In 2016, Infectious Disease Society of America and the American Thoracic Society published a clinical practice guideline. In cases of VAP caused by *P. aeruginosa*, an empirical treatment consisting of imipenem, meropenem or aztreonam plus aminoglycoside, or colistin may be used.[14] As reported cases of carbapenem resistance (CR) increase, the guidelines have been updated to accommodate newer anti-pseudomonal antibiotics in the order of preference: Ceftolozane-tazobactam, ceftazidime-avibactam, meropenem, ceftazidime, or piperacillin-tazobactam plus amikacin or colistin.[15] Over the years, many countries have reported CR[2,16,17] and this has become a global health challenge. In 2016, World Health Organization (WHO) classified CR *P. aeruginosa* as a critical pathogen for new drug research, discovery and development.[18]

This study determined the incidence, prevalence, and resistance pattern of clinical isolates of *P. aeruginosa* obtained from a tertiary healthcare facility in Southern Nigeria. We report the prevalence of MDR and extremely drug resistant (XDR) phenotypes of clinical isolates of *P. aeruginosa* in Nigeria.

MATERIALS AND METHODS

Sample collection

The isolates used in this study were recovered from blood, urine, sputum, cerebrospinal fluid (CSF), wound swabs, high vaginal swabs, eye, and ear exudates. These were obtained from both in-patients and out-patients that presented to the University of Port Harcourt Teaching Hospital (UPTH), Nigeria. All samples that were collected were immediately transferred under aseptic conditions to the Pharmaceutical Microbiology Laboratory of the University of Port Harcourt for the isolation and identification of *P. aeruginosa*.

Isolation and identification of P. aeruginosa

P. aeruginosa was isolated and cultured on cetrimide agar (Himedia, India) using spread plate technique, and then, incubated at 37°C for 24 h. Identification followed the Clinical and Laboratory Standards Institute (CLSI) guidelines.[19,20] Species identification was determined based on differential growth characteristics; a Gram-negative reaction; pyocyanin production, and positive reactions to oxidase test, mannitol, and maltose fermentation.

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were carried out using the Kirby-Bauer disc diffusion method.[21] A colony of each isolate was grown in Muller Hinton broth. Broth cultures were diluted with normal saline to obtain similar turbidity as 0.05 McFarland standard. Standardized microbial cultures were spread on sterile Muller Hinton agar to generate a lawn culture. Single antibiotic disks (Oxoid, UK) namely: piperacillin-tazobactam (110 µg); cefepime (30 µg); aztreonam (30 µg); imipenem (10 µg); polymyxin B (300 units); ceftazidime (30 µg); gentamicin (10 µg); ciprofloxacin (5 µg), and levofloxacin (5 µg) were aseptically placed on agar plates 24 mm apart, the centre to centre between disks on the same plates. All plates were left to stand for 1 h on the bench at room temperature for pre-diffusion of the antibiotics before incubation at 37°C for 24 h. After incubation, the zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant following the CLSI guidelines for antimicrobial susceptibility testing.[19,20]

Statistical analysis

Data analysis was carried out using the IBM Statistical Package for Social Science (SPSS) version 27.0 with values expressed as mean and percentage. Descriptive analysis was carried out on different isolates against the different antibiotics. The different inhibition-zone-diameter measurements in triplicate were compared by performing a one-way ANOVA. A significant difference at 95% of confidence level was set at $p<0.05$.

RESULTS

Prevalence of P. aeruginosa in clinical specimens

In total, 104 isolates of *P. aeruginosa* were recovered over the 3-month study period (Table 1). The age and gender distribution of patients from whom clinical specimens *P. aeruginosa* were recovered are shown in Tables 1 and 2, respectively.

The frequency of *Pseudomonas* infection was highest among children younger than 10 years of age (26.92%) with ear infections and least among the 11-20 age group (3.85%). The mean age of the patients was 31.94 years (range 0-70 years). More isolates were obtained from males (52.88%) than females (47.11%). *P. aeruginosa* was isolated mostly from wound swabs (44.23%), ear exudates (22.12%), and urine samples (21.15%). The least was obtained from eye exudates and CSF samples (0.96% each).

Antimicrobial susceptibility pattern of P. aeruginosa

A minimum of one antimicrobial agent for each anti-pseudomonal antibiotic category was tested on every isolate of *P. aeruginosa*. As shown in Table 3, the susceptibility pattern of *P. aeruginosa* to antibiotics showed the highest sensitivity to imipenem (91.3%) followed by polymyxin B (84.6%), gentamicin (78.8%), levofloxacin (75.0%), ciprofloxacin (68.3%), cefepime (47.1%), aztreonam (32.7%), piperacillin-tazobactam (27.9%), and least to ceftazidime (23.1%).

Patterns of antibiotic resistant phenotypes

The term "non-susceptible" describes those isolates showing resistant and intermediate levels of resistance following the CLSI guidelines.[19] The non-susceptible phenotype was further described as follows: Resistant when an isolate is unsusceptible to at least one drug in one or two antimicrobial classes; multi-drug-resistant when an isolate is unsusceptible to at least one drug in three or more antimicrobial categories; extremely-drug-resistant when an isolate is unsusceptible to at least one drug from six antibiotic categories and pandrug resistant when an isolate is unsusceptible to at least one drug in all antibiotic categories.

The susceptibility pattern of *P. aeruginosa* phenotypes is shown in Table 4. Our data show that 8% were susceptible, 26% were resistant, 61% were MDR and 5% were extremely-drug-resistant (Figure 1). No isolate showed resistance to all antibiotics tested. Figure 2 shows the response of individual isolates to anti-pseudomonal antibiotics. The antibiotic resistance profiles of individual isolates are shown in Table 5.

Table 1. Age distribution of sources of *Pseudomonas aeruginosa* isolates

Age group (years)	Frequency of cases	Relative frequency	Specimen site							
			Blood	CSF	Ear	Eye	HVS	Sputum	Urine	Wound
0 - 10	28	26.92	2	0	17	0	1	2	0	6
11-20	4	3.85	0	0	2	0	0	0	0	2
21-30	11	10.58	0	0	0	0	3	0	3	5
31-40	18	17.31	0	0	0	0	1	0	7	10
41-50	20	19.23	0	1	2	1	1	1	4	10
>51	23	22.12	0	0	2	0	0	0	8	13
Total	**104**	**100**	**2**	**1**	**23**	**1**	**6**	**3**	**22**	**46**

HVS: High vaginal swab, CSF: Cerebrospinal fluid

Table 2. Gender distribution of sources of *Pseudomonas aeruginosa* isolates

Specimen	Male	Female	Frequency (n)	Relative frequency (%)
Blood	0	2	2	1.92
CSF	0	1	1	0.96
Ear	11	12	23	22.12
Eye	1	0	1	0.96
HVS	0	6	6	5.77
Sputum	3	0	3	2.88
Urine	10	12	22	21.15
Wound	30	16	46	44.23
Total	**55**	**49**	**104**	**100.00**

HVS: High vaginal swab, CSF: Cerebrospinal fluid

Table 3. Antimicrobial categories and antibiotics used to classify *Pseudomonas aeruginosa* phenotypes

Antibiotic category	Anti-pseudomonal antibiotic	Antibiotic code	Antibiotic concentration	Number of isolates, n: 104		
				Susceptible	Intermediate	Resistant
β-Lactam + β-lactamase inhibitor	Piperacillin - tazobactam	TZP	110 µg	29 (27.9%)	11 (10.6%)	64 (61.5%)
Cephem	Ceftazidime	CAZ	30 µg	24 (23.1%)	4 (3.85%)	76 (73.1%)
Cephem	Cefepime	FEP	30 µg	49 (47.1%)	5 (4.81%)	50 (48.1%)
Monobactam	Aztreonam	ATM	30 µg	34 (32.7%)	28 (26.9%)	42 (40.4%)
Carbapenem	Imipenem	IMP	10 µg	95 (91.3%)	1 (0.96%)	8 (7.69%)
Polymyxin	Polymyxin B	PB	300 units	88 (84.6%)	-	16 (15.4%)
Aminoglycoside	Gentamicin	CN	10 µg	82 (78.8%)	3 (2.88%)	19 (18.3%)
Fluoroquinolone	Ciprofloxacin	CIP	5 µg	71 (68.3%)	7 (6.73%)	26 (25.0%)
Fluoroquinolone	Levofloxacin	LEV	5 µg	78 (75.0%)	1 (0.96%)	25 (24.0%)

Table 4. Phenotypic resistance pattern of *Pseudomonas aeruginosa*

Number of antibiotic categories	Number of isolates (frequency)	Relative frequency (%)	Antibiotic phenotype
0	8	7.69	Susceptible
1	12	11.54	Resistant
2	15	14.42	Resistant
3	35	33.65	Multi-drug resistant
4	16	15.38	Multi-drug resistant
5	13	12.50	Multi-drug resistant
6	5	4.81	Extremely drug resistant
7	0	0.00	Extremely drug resistant

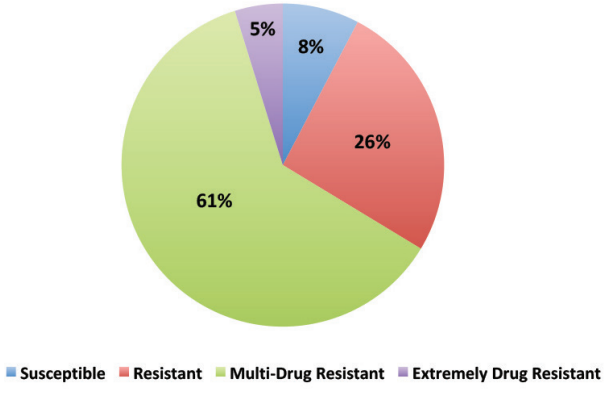

Figure 1. Prevalence of *Pseudomonas aeruginosa* drug-resistant phenotypes

DISCUSSION

MDR in microorganisms has raised much global concerns. Many factors contribute to the antibiotic resistance observed in *P. aeruginosa*.[11] This study reports the incidence, prevalence, and antibiotic resistance pattern of 104 isolates of *P. aeruginosa* recovered from various body fluids, swabs, and exudates. All clinical samples studied harbour this microorganism irrespective of age groups. Most isolates were obtained from wound swabs, ear exudates, and urine samples. The wound samples were mostly from adults above 30 years of age; urine samples from more than 20 years, while isolates from ear exudates were mostly children younger than 10 years of age.

The capability of some anti-pseudomonal antibiotics to clear infections caused by identified isolates was investigated *in vitro*. Our findings show that only 7.69% isolates were sensitive to all antibiotics tested, while others were unsusceptible to a minimum of one antibiotic. This is in agreement with similar studies that have shown a high incidence of antimicrobial resistance in *P. aeruginosa*.[8,9,22] Of all antibiotics tested, imipenem recorded the highest number of susceptibility (91.3%) followed by polymyxin B (84.6%). Our findings show that imipenem remains a potent drug for clearing infections caused by *P. aeruginosa*. However, the development of resistance to carbapenems has emerged, thus the need for research on new drugs. *P. aeruginosa* showed a high degree of resistance to penicillins and cephalosporins with ceftazidime expressing the highest level of resistance (73.1%). This is because *P. aeruginosa* is capable of acquiring genes encoding antimicrobial resistant determinants. The most common resistance mechanism is the

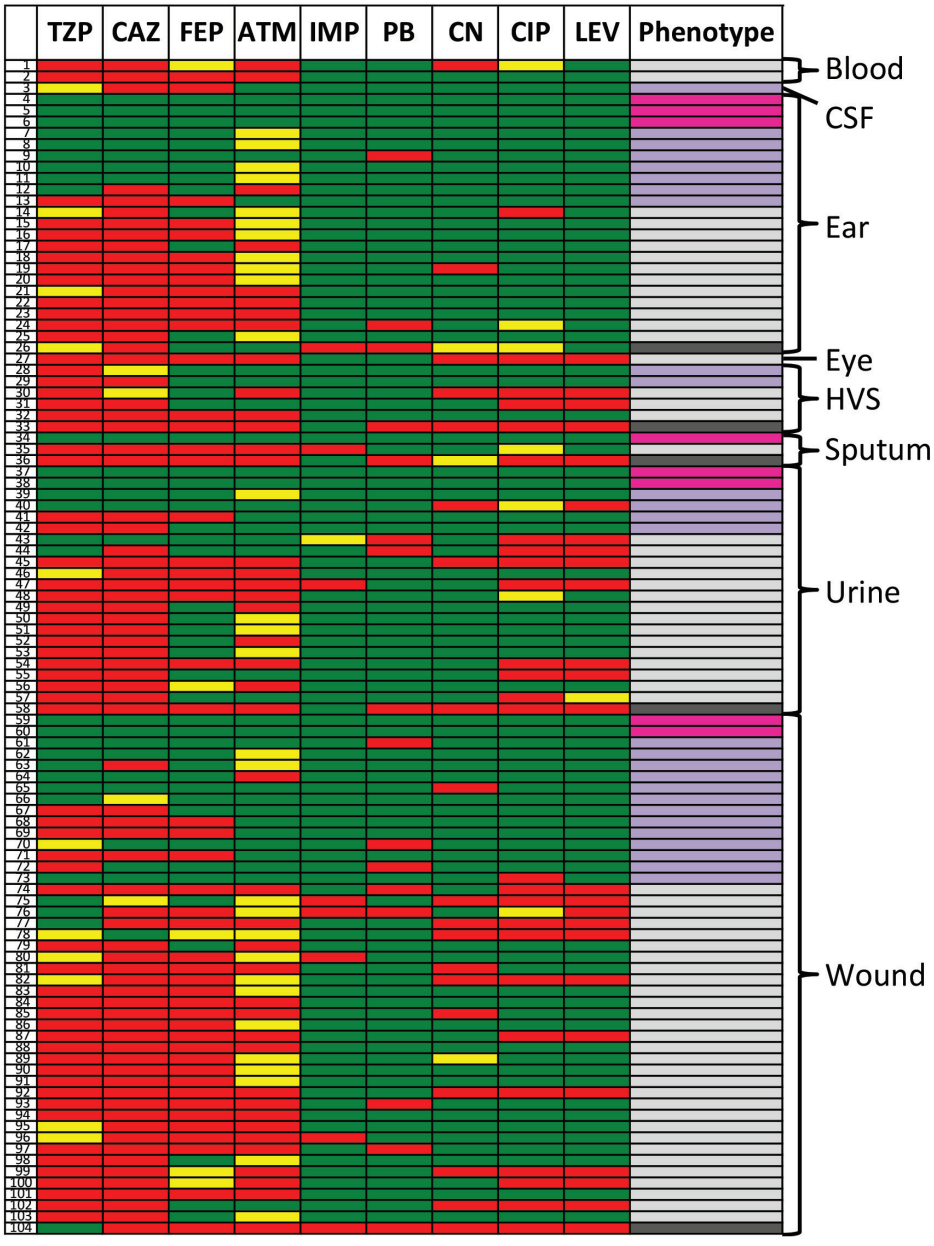

Figure 2. Heat map showing the response of individual isolates to anti-pseudomonal antibiotics

TZP: Piperacillin-tazobactam (110 µg), CAZ: Ceftazidime (30 µg), FEP: Cefepime (30 µg), ATM: Aztreonam (30 µg), IMP: Imipenem (10 µg), PB: Polymyxin B (300 units), CN: Gentamicin (10 µg), CIP: Ciprofloxacin (5 µg), LEV: Levofloxacin (5 µg), Green: Sensitive, Yellow: Intermediate, Red: Resistant, Pink: Susceptible phenotype (sensitive to all antibiotic categories), Lilac: Resistant phenotype (not susceptible to at least one drug in one or two antibiotic categories), Light grey: Multidrug-resistant phenotype (not susceptible to at least one drug in three or more antibiotic categories), Dark grey: Extremely-drug-resistant phenotype (not susceptible to at least one drug in all but one antibiotic category)

production of β-lactamase enzymes that inactivate penicillins and cephalosporins.[23] The next highest level of resistance was recorded for piperacillin-tazobactam (61.5%) showing that other mechanisms of resistance besides β-lactamase production contribute to the resistant phenotypes observed.

An international group of experts from the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention came together to establish standardized terminologies that will define the resistance profiles in some selected bacteria associated with nosocomial infections, which are prone to MDR.[24] The selected bacteria include *P. aeruginosa, Acinetobacter* spp., *Enterobacteriaceae* (excluding *Salmonella* and *Shigella*), *Staphylococcus aureus*, and *Enterococcus* spp. An isolate is classified as MDR, when it is non-susceptible to at least one drug in three or more antimicrobial classes. Extremely-drug-resistant isolates are non-susceptible to at least one

agent in all but one or two anti-microbial categories. The pan-drug-resistant isolates are non-susceptible to all antimicrobial categories. An isolate is considered as non-susceptible to an antibiotic, when it tests resistant or intermediate using clinical breakpoints as interpretive criteria provided by Clinical & Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing or US Food and Drug Administration. From this classification, *P. aeruginosa* were grouped based on the number of antibiotics they were resistant to. Our findings revealed a high prevalence (62%) of MDR strains. These isolates were resistant to at least one drug in 3-5 antibiotic categories. The highest recorded MDR occurred between piperacillin-tazobactam + ceftazidime and cefepime + aztreonam. The antibiotic categories, whose MDR patterns commonly occur, includes cephem » fluoroquinolone › monobactam= β-lactam/β-lactamase-inhibitor » aminoglycoside › polymyxin › carbapenem. Although the least amount of resistance was against imipenem, the incidence of resistance to this antibiotic class poses a global health concern. CR *P. aeruginosa* was ranked second after *Acinetobacter baumannii* as the most critical antimicrobial resistant bacteria, of which WHO placed it in the priority list for new drug R&D.[18] However, the lack of any new drugs and limited therapeutic options indicate that surveillance efforts

Table 5. Antibiotic resistance pattern of Pseudomonas aeruginosa clinical isolates

Antibiotic resistance patterns	Number of non-susceptible isolates	Drug resistance phenotype
CAZ/FEP	1	Resistant
ATM	7	Resistant
PB	2	Resistant
CN	1	Resistant
CIP/LEV	1	Resistant
TZP + CAZ/FEP	10	Resistant
TZP + PB	2	Resistant
CAZ/FEP + ATM	2	Resistant
CN + CIP/LEV	1	Resistant
TZP + CAZ/FEP + ATM	30	MDR
TZP + CAZ/FEP + CIP/LEV	3	MDR
PB + CAZ/FEP + CIP/LEV	1	MDR
PB + IMP + CIP/LEV	1	MDR
TZP + CAZ/FEP + ATM + IMP	2	MDR
TZP + CAZ/FEP + ATM + PB	2	MDR
TZP + CAZ/FEP + ATM + CN	4	MDR
TZP + CAZ/FEP + ATM + CIP/LEV	5	MDR
TZP + CAZ/FEP + CN + CIP/LEV	1	MDR
CAZ/FEP + ATM + CN + CIP/LEV	1	MDR
CAZ/FEP + IMP + CN + CIP/LEV	1	MDR
TZP + CAZ/FEP + ATM + PB + CIP/LEV	2	MDR
TZP + CAZ/FEP + ATM + IMP + CIP/LEV	2	MDR
TZP + CAZ/FEP + ATM + CN + CIP/LEV	8	MDR
CAZ/FEP + ATM + IMP + PB + CIP/LEV	1	MDR
TZP + CAZ/FEP + ATM + PB + CN + CIP/LEV	3	XDR
TZP + CAZ/FEP + IMP + PB + CN + CIP/LEV	1	XDR
CAZ/FEP + ATM + IMP + PB + CN + CIP/LEV	1	XDR

TZP: Piperacillin-tazobactam (110 µg), CAZ: Ceftazidime (30 µg), FEP: Cefepime (30 µg), ATM: Aztreonam (30 µg), IMP: Imipenem (10 µg), PB: Polymyxin B (300 units), CN: Gentamicin (10 µg), CIP: Ciprofloxacin (5 µg), LEV: Levofloxacin (5 µg), MDR: Multidrug resistant, XDR: Extremely drug resistant

and rigorous monitoring for MDR among *Pseudomonas* isolates is critical especially as given that *P. aeruginosa* has become a cause of many nosocomial infections.

CONCLUSION

In summary, we analyzed the incidence, prevalence, and antibiotic susceptibility pattern of *P. aeruginosa* in a healthcare facility in Nigeria. We report disturbingly high prevalence of MDR and some XDR phenotypes of *P. aeruginosa* in Nigeria. Our findings call for antimicrobial stewardship and improved public health policies regarding proper antibiotic use.

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Ethics

Ethics Committee Approval: The Research and Ethics Committee of the University of Port Harcourt approved this study (reference number: UPH/CEREMAD/REC/MM76/053). Samples were taken from patients after they or their legal guardians had given their informed voluntary consent to partake in the study. Standard protocols were followed to ensure the confidentiality of patient information.

Informed Consent: Approval received.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.M.A., Design: A.M.A., Data Collection or Processing: C.N.I., C.N.S., H.O., I.O.O., N.E.E., Analysis, or Interpretation: A.M.A., C.N.I., C.N.S., H.O., I.O.O., N.E.E., Literature Search: A.M.A., C.N.I., C.N.S., H.O., I.O.O., N.E.E., Writing: A.M.A., C.N.I., C.N.S., H.O., I.O.O., N.E.E.

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