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Research Article

MULTIDRUG-RESISTANCE PATTERNS AND DETECTION OF *PstS* GENE IN CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* FROM NSUKKA, SOUTHEAST NIGERIA

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ABSTRACT

Objective: This study was aimed to determine the antibiotic resistance patterns of clinical *Pseudomonas aeruginosa* isolates and to detect the presence of *PstS* gene.

Methods: One hundred and ninety-two clinical isolates of *P. aeruginosa* were characterized using polymerase chain reaction (PCR) and 16S rDNA sequencing. Antibiotic resistance patterns were determined using the disk diffusion method, while the minimum inhibitory concentrations (MICs) of selected antibiotics against resistant isolates were determined by macro broth dilution and E-test strip methods. The resistant isolates were screened for the presence of *PstS* gene using PCR.

Results: Of 192 clinical isolates of *P. aeruginosa*, 136 (70.83%) were resistant to at least two antibiotics. Of these, 135 (99%) could be classified as multidrug-resistant *P. aeruginosa* (MDR-PA), 63 (46%) were extensively drug-resistant (XDR-PA), while 38 (28%) were pandrug-resistant (PDR-PA). The isolates exhibited high level of resistance to cefotaxime and ticarcillin, and low levels of resistance to meropenem and imipenem. The MIC values for meropenem against the resistant isolates were generally <32 mg/L, while the values for other antibiotics ranged from 32 to >128 mg/L. Multiple antibiotic resistance indexes of the MDR-PA ranged from 0.27 to 0.91 and the most prevalent pattern of resistance was Piperacillin^R – Ticarcillin^R – Piperacillin/Tazobactam^R – Cefotaxime^R – Ceftazidime^R – Gentamicn^R – Tobramycin^R– Ciprofloxacin^R. About 50% of the resistant isolates possessed the *PstS* gene.

Conclusions: The results confirmed the presence of XDR, PDRPA, and *PstS* gene in *P. aeruginosa* strains. There is an urgent need for healthcare practitioners to address the problem of multidrug resistance, by implementing a more rational and appropriate use of antibiotics.

Keywords: *Pseudomonas aeruginosa*, Antimicrobial resistance, Multidrug-resistant *Pseudomonas aeruginosa*, Extensively drug-resistant, *PstS* gene.

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INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative aerobic bacillus belonging to the Pseudomonadaceae family. It is highly ubiquitous in nature and may be found in most moist environments in the hospital, including sinks, cleaning buckets, drains, humidifiers, and toilet water [1]. *P. aeruginosa* is becoming an increasingly important cause of health care-associated infections [2-5]. It has been reported to be the sixth most common nosocomial pathogen overall and second most common pathogen in ventilator-associated pneumonia in US hospitals [4]. It has also been ranked second among Gram-negative pathogens reported to the United States National Nosocomial Infection Surveillance System [3]. *P. aeruginosa* constitutes a common pathogen in hospitals, particularly in intensive care units, due to its ubiquitous nature, ability to survive in moist environments, innate resistance to many antibiotics and antiseptics, and ability to acquire resistance to many drug classes [3,6,7]. It is a leading cause of septicemia, pneumonia, meningitis, wound, urinary tract, surgical wound, burn, and ear infections [8].

Resistance in *Pseudomonas* may be mediated through several distinct mechanisms, including the production of β-lactamases, efflux pumps, target site, and outer membrane modifications, but antimicrobial agents that have been found to be effective against *P. aeruginosa* include aminoglycosides (gentamicin, tobramycin, amikacin, and netilmicin); carbapenems (imipenem, meropenem, and doripenem); cephalosporins (ceftazidime and cefepime); fluoroquinolones (ciprofloxacin and levofloxacin); penicillins β-lactamase inhibitors (ticarcillin-clavulanic acid and piperacillin-tazobactam); monobactams (aztreonam); phosphonic acids (fosfomycin); and polymyxins (colistin and polymyxin B) [9,10]. Antibiotic resistant *P. aeruginosa* can be classified today Multidrug-resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant (PDR). MDR has been defined as a strain that develops resistance to at least one agent in three or more antimicrobial categories, XDR is a strain resistant to one agent in all categories except two or less, while PDR is a strain resistant to one agent in all categories [9,11].

Of all the anti-*Pseudomonas* agents, the carbapenems have been widely used for empirical or directed therapy, whenever MDR *P. aeruginosa* is suspected. However, recent evidence indicates that resistance to the carbapenems is emerging in *P. aeruginosa* and improper use of these drugs could accelerate the occurrence [4,12]. This is a global concern, not just for *Pseudomonas*, but for other bacterial pathogens. However, for the developing world, especially, this concern appears to be more reality than speculation. The concern arises from certain practices observed in the developing world, including unregulated use/sale of antibiotics, over-prescription of antibiotics, release of antibiotics into wastewaters by drug manufacturers, poor sanitation, poor infection control, misuse of antibiotics, and among others [13]. These problems can, however, be solved by better surveillance and monitoring of drug resistance. To this effect, the World Health Organization (WHO) has proposed a new global surveillance network, although there are still questions about how the initiative will be funded [13].

Considering the constantly evolving pattern of antibiotic resistance in *P. aeruginosa* and high mortality rates associated with *Pseudomonas* infections, it is of great public health importance to continuously

track the occurrence and spread of antibiotic resistance in this rapidly emerging superbug. In a WHO global map of antibiotic resistance data from 194 member countries, Nigeria was one of the countries for which no information was obtained [13].

Studies have reported that expression of the *PstS* gene enhances the virulence of MDR *Pseudomonas* strains [14]. *PstS* proteins are the cell-bound phosphate binding elements of the ubiquitous bacterial ATP-binding cassette phosphate uptake mechanisms. This study was, therefore, undertaken to determine the prevalence of MDR among clinical isolates of *P. aeruginosa* in Nsukka, Southeast Nigeria, determine the patterns of resistance and screen the isolates for the presence of *PstS* gene, which has been reported to confer increased virulence on MDR *P. aeruginosa* (MDRPA).

METHODS

The bacterial strains

A total of 192 strains of *P. aeruginosa* were collected from Microbiology Laboratories from various hospitals in Nsukka, from March 2016 to February 2017. Ethical approval and informed consent were not required by our Institution Ethics Committee because all bacterial isolates were collected, processed, and stored as part of routine diagnosis by the hospitals. No patient information was associated with the data. The isolates obtained from the various laboratories were subcultured onto *Pseudomonas* cetrimide agar (Oxoid, U.K) which was supplemented with 10 ml/l of glycerol and characterized using standard microbiological techniques such as grade like odor, pigment production, positive oxidase test, growth at 42°C, and molecular method using 16S rRNA primer targeting *P. aeruginosa* consensus region (Inqaba Biotechnical Company, South Africa).

Antimicrobial susceptibility test

One hundred and ninety-two *P. aeruginosa* isolates were used for *in vitro* susceptibility testing. Susceptibility to ten antibiotics, namely – ticarcillin 75 µg; piperacillin 100 µg; piperacillin/tazobactam 100/10 µg; ceftazidime 30 µg; cefotaxime 30 µg; imipenem 10 µg; meropenem 10 µg; gentamicin 10 µg; tobramycin 10 µg; and ciprofloxacin 5 µg (Oxoid, UK) was evaluated on Muller–Hinton agar, using Kirby–Bauer disc diffusion method. The ten antibiotics represented six classes of antibiotic (Table 1). The isolates were considered susceptible or resistant according to the zone of inhibition recommended by the Clinical and Laboratory Standard Institute [15]. *P. aeruginosa* ATCC 27853 was used as the control strain. Isolates were considered MDR (MDRPA) if they showed resistance to three or more classes of the tested antibiotics.

Multiple antibiotic resistance (MAR) index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested [16].

$$\text{MAR index} = \frac{\text{Number of antibiotics isolate is resistant to}}{\text{Total number of antibiotics tested}}$$

Determination of minimum inhibitory concentrations (MIC)

The MIC for the MDR *P. aeruginosa* was determined using macro broth dilution method and E-test method according to the CLSI standard [15]. *P. aeruginosa* isolates that were resistant to meropenem; ceftazidime; ciprofloxacin; and gentamicin were used for MIC study. Macro broth dilution was used to determine the MIC for ciprofloxacin and gentamicin while E-strip test was used for meropenem and ceftazidime.

Genomic DNA extraction

The genomic DNA was extracted from 20 MDR *P. aeruginosa* strain using Zymo research fungal/bacterial DNA Miniprep Kit (Zymo Research, USA) according to the manufacturer's protocol.

DNA amplification

The extracted DNA was amplified with 16S rRNA primer targeting *P. aeruginosa* consensus region and another primer targeting *PstS* gene that enhances the virulence of MDR *Pseudomonas* strains (Inqaba Biotechnical Company, South Africa), Table 2. The polymerase chain reaction (PCR) was carried out using the New England Biolabs one Taq 2X master mix with standard buffer. PCR reaction mixture was prepared in a 25 µl reaction volume containing 12.5 µl of 1X Master mix with standard buffer, 20 mM Tris-HCl, 1.8 mM $MgCl_2$, 22 mM NH_4Cl, 22 mM KCl, 0.2 mM DNTPS, 5% glycerol, 0.06% GEPAL CA-630, 0.05% Tween 20, 25 units/ml Taq DNA polymerase (Biolab, England), 0.5 µl (10 µM), each of the forward and reverse primers (Inqaba Biotechnical, South Africa), 5 µl of the extracted DNA, and 6.5 µl of sterile nuclease-free water (Norgen Biotek, Canada) to make up to 25 µl of reaction volume. This was vortexed at low speed and placed in a thermal cycler machine (BIBBY) – Scientific Ltd., UK. The parameters for amplification were as follows initial denaturation of 94°C for 5 min, followed by 35 amplification cycles of denaturation at

Table 1: Zone diameter interpretive standards and equivalent MIC breakpoints for *Pseudomonas aeruginosa* (Clinical and Laboratory Standard Institute, 2014)

Antimicrobial class	Antimicrobial agent and disk concentration	Zone diameter (mm)			Equivalent MIC break pointing (ug/ml)		
		R	I	S	R	I	S
Penicillins	Ticarcillin 75 µg	≤15	16–23	≥24	≥128		≤64
	Piperacillin 100 µg	≤14	15–20	≥21	≥128		≤64
β-lactam/β-lactamase inhibitor	Piperacillin-tazobactam 100/10 µg	≤14	15–20	≥21	≥128/4		≤64/4
Cephems	Ceftazidime 30 µg	≤14	15–17	≥18	≥32	16	≤8
	Cefotaxime 30 µg	≤14	15–17	≥21	≥64		≤8
Carbapenems	Imipenem 10 µg	≤15	16–18	≥19	≥16		≤4
	Meropenem 10 µg	≤15	16–18	≥19	≥16		≤4
Aminoglycosides	Gentamicin 10 µg	≤12	13–14	≥15	≥8		≤4
	Tobramycin 10 µg	≤12	13–14	≥15	≥8		≤4
Fluoroquinolones	Ciprofloxacin 5 µg	≤15	16–20	≥21	≥4		≤1

R: Resistant, 1: Intermediate, S: Sensitive, MIC: Minimal inhibiting concentration

Table 2: Primers used for Identification of *Pseudomonas aeruginosa* and detection of *PstS* gene

Target gene	Function	Sequence (5^1---3^1)	Amplicon size (pb)	Accession No:
16S rRNA	Consensus region	AGAGTTTGATCCTGGCTCAG ACGGCTACCTTGTTACGACTT	1499	HM045838
PstS	Enhances the virulence of MDR	GGCTTTCGAGCAGAAGTACG ATGTAGCCGTCCTTGACCAC	606	EF601159

Table 3: Resistance patterns of multidrug-resistant strains of *Pseudomonas aeruginosa*

Resistance pattern	Classification of resistance	No. of isolates
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, IMP^R, CN^R, TOB^R, CIP^R	PDR	4
PRL^R, TZP^R, TIC^R, CTX^R, CAZ^R, MEM^R, CN^R, TOB^R, CIP^R	PDR	1
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, IMP^R, CN^R, TOB^R	PDR	2
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, CN^R, TOB^R, CIP^R	PDR	16
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, IMP^R, CN^R, TOB^R, CIP^R	PDR	3
PRL^R, $gTIC^R$, TZP^R, CTX^R, CAZ^R, CN^R, TOB^R, CIP^R	XDR	2
PRL^R, TIC^R, CTX^R, CAZ^R, MEM^R, CN^R, TOB^R, CIP^R	XDR	2
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, CN^R, CIP^R	PDR	1
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, CN^R, TOB^R, CIP^R	XDR	17
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, IMP^R, CIP^R	XDR	1
PRL^R, TIC^R, TZP^R, CTX^R, MEM^R, CN^R, TOB^R, CIP^R	PDR	1
PRL^R, TIC^R, TZP^R, CTX^R, IMP^R, CN^R, TOB^R, CIP^R	PDR	1
PPL^R, TIC^R, CTX^R, CAZ^R, CN^R, TOB^R, CIP^R, MEM^R	XDR	1
TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, CN^R, TOB^R, CIP^R	PDR	1
PRL^R, TIC^R, CTX^R, CAZ^R, MEM^R, IMP^R, CN^R, TOB^R	XDR	1
TIC^R, CTX^R, CAZ^R, MEM^R, CN^R, TOB^R, CIP^R	XDR	1
TIC^R, TZP^R, CTX^R, CAZ^R, CN^R, TOB^R, CIP^R	XDR	1
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, CN^R, CIP^R	XDR	1
PRL^R, TIC^R, CTX^R, CAZ^R, IMP^R, CN^R, TOB^R	XDR	1
PRL^R, TIC^R, TZP^R, CTX^R, IMP^R, TOB^R	XDR	1
PRL^R, TIC^R, TZP^R, CTX^R, CN^R, TOB^R, CIP^R	XDR	1
PRL^R, TIC^R, CTX^R, CAZ^R, CN^R, TOB^R, CIP^R	XDR	7
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, TOB^R	XDR	2
TIC^R, TZP^R, CTX^R, CAZ^R, TOB^R, CIP^R, CN^R	XDR	2
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, CN^R, CIP^R	XDR	4
PRL^R, TIC^R, TZP^R, CTX^R, IMP^R, CN^R, CIP^R	PDR	2
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, MEM^R, CIP^R	XDR	2
TIC^R, CTX^R, CAZ^R, CN^R, TOB^R, CIP^R, MEM^R	XDR	3
PRL^R, TIC^R, TZP^R, CTX^R, IMP^R, CIP^R	XDR	1
PRL^R, TIC^R, CTX^R, MEM^R, CN^R, TOB^R	XDR	2
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, CN^R	XDR	7
TIC^R, CTX^R, CAZ^R, CN^R, TOB^R, CIP^R	XDR	3
PRL^R, TIC^R, CTX^R, CN^R, TOB^R, CIP^R	XDR	2
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, CIP^R	XDR	2
PRL^R, TIC^R, CTX^R, CAZ^R, CN^R, TOB^R	MDR	1
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R, TOB^R	XDR	1
PRL^R, TIC^R, TZP^R, CTX^R, CAZ^R	MDR	4
TIC^R, CTX^R, CAZ^R, CN^R, CIP^R	MDR	1
TIC^R, CTX^R, CAZ^R, CN^R, TOB^R	MDR	4
TIC^R, CTX^R, CAZ^R, TOB^R, CIP^R	MDR	1
PRL^R, TIC^R, TZP^R, CTX^R, CN^R	MDR	1
TIC^R, CTX^R, CN^R, TOB^R	MDR	1
PRL^R, TIC^R, TZP^R, CIP^R	MDR	1
PRL^R, TZP^R, CTX^R, TOB^R	MDR	1
TIC^R, TZP^R, CTX^R, CAZ^R	MDR	1
TIC^R, CTX^R, CN^R, TOB^R	MDR	3
TIC^R, CTX^R, CAZ^R, CN^R	MDR	3
TIC^R, CTX^R, CAZ^R, MEM^R	MDR	8
PRL^R, TZP^R, CTX^R	MDR	1
TIC^R, CTX^R, CAZ^R		1
PRL^R, TIC^R, CTX^R		2
TIC^R, CTX^R, CN^R	MDR	1
TIC^R, CTX^R, CAZ^R		1

PRL^R: Piperacillin resistant, TZP^R: Piperacillin/Tazobactam resistant, TIC^R: Ticarcillin resistant, CTX^R: Cefotaxime resistant, CAZ^R: Ceftazidime resistant, MEM^R: Meropenem resistant, IMP^R: Imipenem resistant, TOB^R: Tobramycin resistant, CN^R: Gentamicin resistant, CIP^R: Ciprofloxacin resistant, MDR: Multidrug-resistant, XDR: Extensively drug-resistant, PDR: Pandrug-resistant

Table 4: MAR indexes of *Pseudomonas aeruginosa* isolates

MAR index	No. of resistant *Pseudomonas aeruginosa* isolates	% Prevalence of MAR
0.27	18	13.23
0.36	20	14.71
0.45	16	11.76
0.55	17	12.50
0.64	20	14.71
0.73	25	18.38
0.82	19	13.97
0.91	1	0.74

MAR: Multiple antibiotic resistance

94°C for 1 min, annealing at 50°C and 55°C for 1 min for 16S rRNA and *PstS* primers, and extension at 72°C for 1 min. This was followed by a final extension step of 5 min at 72°C. The amplification products (amplicons) were separated on 1.5% agarose gel stained with ethidium bromide and electrophoresis was carried out at 70 volts for 90 min and visualized/illuminated under ultraviolet transilluminator. A 100 bp DNA ladder (Norgen Biotek Corp., Canada) was used as DNA molecular weight marker.

Data analysis

Frequency of MDRPA and percentage of resistance to antibiotics were calculated.

RESULTS

Antimicrobial sensitivity

Of 192 isolates of *P. aeruginosa* that were tested, 136 (70.83%) were resistant to at least two antibiotics. The resistance of the isolates against a panel of 10 antibiotics was cefotaxime, 88.02%; ticarcillin, 87.50%; ceftazidime, 64.06%; ciprofloxacin, 62.50%; piperacillin, 58.33%; tobramycin, 57.29%; gentamicin, 56.25%; piperacillin/tazobactam, 55.73%; meropenem, 24.48%; and imipenem, 12.50%. Evaluation of resistance of the isolates to different classes of antibiotics revealed that 135 (99%) were resistant to at least one antibiotic in three different classes (MDR-PA); 63 (46%) were resistant to all except two or three classes (XDR-PA), while 38 (28%) were resistant to all six classes of antibiotics (PDR-PA), as shown in Table 3. The most prevalent pattern of resistance was (PRL^R TIC^R TZP^R CTX^R CAZ^R CN^R TOB^R CIP^R).

Analysis of MAR index

Analysis of the MAR index showed that the isolates had MAR indexes ranging from 0.27 to 0.91 (Table 4) while evaluation of the MIC of some of the antibiotics against the isolates ranged from 0.12 to 128 µg/ml (Table 5).

Occurrence of *PstS* gene

The result of the PCR revealed that of 20 MDRPA isolates that were screened for *PstS* gene, lane 1, 3–7, 9, 10, 15, and 16 showed positive amplification of the 606 bp *PstS* gene (Fig. 1).

DISCUSSION

In this study, 192 clinical isolates of *P. aeruginosa* were screened against a panel of ten antibiotics, representing six classes of antibiotics.

Table 5: The MIC and MBC (µg/ml) of multidrug resistant *Pseudomonas aeruginosa*

Organisms	MIC (µg/ml)			
	Meropenem (S ≤4µg, I=8 µg/ml and R ≤16 (µg/ml)*	**Ceftazidime (S ≤8, I=16 µg/ml and R ≥32 µg/ml)***	**Ciprofloxacin (S ≤1 µg/ml, I=2 and R ≥4 µg/ml)***	**Gentamicin (S ≤4 µg/ml, I=8 µg/ml and R ≥16 µg/ml)***
ATCC 27853	0.25	1.00	2.00	4.00
1	8.00	4.00	32.00	16.00
2	0.12	2.00	64.00	64.00
3	0.50	64.00	32.00	16.00
4	1.00	16.00	16.00	8.00
5	8.00	32.00	128.00	32.00
6	0.50	4.00	32.00	16.00
7	2.00	16.00	16.00	8.00
8	8.00	64.00	64.00	32.00
9	32.00	8.00	128.00	64.00
10	2.00	32.00	8.00	16.00

Grey shade indicates resistant strains. *CLSI breakpoints. S: Sensitive, I: Intermediate, R: Resistant, MIC: Minimal inhibiting concentration, MBC: Minimal bactericidal concentration, CLSI: Clinical and Laboratory Standard Institute

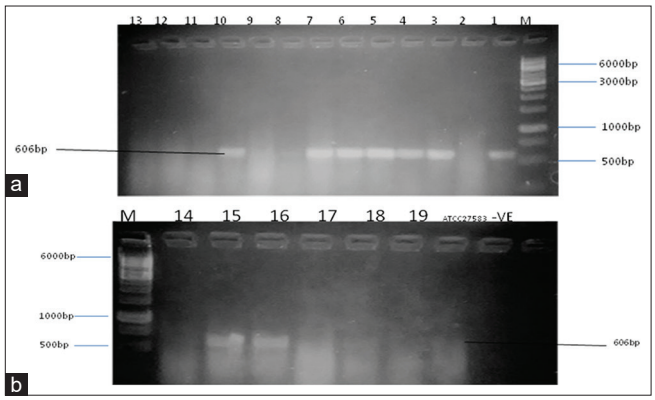

Fig. 1: (a and b) Polymerase chain reaction detection of 606 bp amplicons of *PstS* primer for identification of multidrug-resistant *Pseudomonas aeruginosa*, Lane M shows bands for 1kb (1000bp) molecular weight standard. Lanes 1, 3–7, 9, 10, 15, and 16 show a positive amplification band. Indicating the presence of *PstS* gene in *P. aeruginosa* isolates analyzed. Lanes 14, 17, 18, 19, and ATCC 27583 show negative amplification and produced no visible band

The highest levels of resistance were recorded to the cephalosporin (cefotaxime and ceftazidime) and β-lactam (ticarcillin) antibiotics, while the lowest resistance levels were recorded to the carbapenems (meropenem and imipenem). *P. aeruginosa* is known to be intrinsically resistant to many antimicrobial agents including β-lactams, the older quinolones, chloramphenicol, tetracycline, macrolides, trimethoprim-sulfamethoxazole, and rifampin [10,16]. Until recently, the most important antipseudomonal agents were some β-lactams (ticarcillin and piperacillin), cephalosporins (cefoperazone, ceftazidime, and cefotaxime), aminoglycosides (gentamicin, tobramycin, and amikacin), fluoroquinolones (notably ciprofloxacin), and β-lactam-β-lactamase inhibitor combinations [3,10]. Recently, however, there are increasing reports of emerging resistance to these antipseudomonal agents in clinical settings, worldwide [3,10,17-20]. In this study, *P. aeruginosa* isolates exhibited high levels of resistance to different antibiotics used. Although fluoroquinolones are regarded as a good option in many parts of the world, in this study, the *P. aeruginosa* isolates exhibited high levels of resistance to this class of antibiotics.

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by β-lactam resistant bacteria and they have been reported by many surveillance studies to be the drug of choice for MDR-*P. aeruginosa* [4,10,12,21]. In this study, the carbapenems (meropenem and imipenem) were found to be among the most active agents.

Although many studies have reported low resistance or outright 100% sensitivity for imipenem and meropenem [22-29], 12.5% and 24.5% resistance were recorded for both drugs, respectively, in this study, which is a cause for concern. It is also worrisome that about 46% of the clinical isolates in this study have gone beyond exhibiting mere MDR to extensive drug-resistance (XDR), while up to 28% may now be classified as PDR, as shown in Table 3. The most prevalent pattern of resistance among the isolates was PRL^R-TIC^R-TZP^R-CTX^R-CAZ^R-CN^R-TOB^R-CIP^R.

It is evident from these findings that a serious public health problem already exists in the environment under study, as a significant number of isolates is exhibiting resistance not only to the classical antipseudomonal drugs but also to the β-lactam-β-lactamase inhibitor combination and the last line carbapenem group. This can only indicate that this environment would be experiencing a high degree of treatment failure. The high degree of resistance recorded in this study is in agreement with a 2012 study in Southwest Nigeria [30]. It has been suggested that this trend may be due to selective pressure from the overuse and misuse of antibiotics, prevalent in the country.

Some authors have suggested that the MAR index may give an indirect indication of the probable source of an organism, an index of greater than 0.2, indicating that an organism must have originated from an environment where antibiotics are often used [16,31]. As can be seen in Table 4, all the MDR (XDR and PDR inclusive) isolates had MAR indexes ranging from 0.27 to 0.91. These observations tend to validate the assertion by some authors that antibiotic resistance is sweeping the developing world [13] and point to the need for tighter restrictions on the sale/use of antibiotics if the rapid spread of resistance is to be checked.

Studies have reported that expression of the *PstS* gene enhances the virulence of MDR *Pseudomonas* strains. The gene encodes a periplasmic phosphate-binding protein, which, when present, confers a highly virulent phenotype on MDR strains of *P. aeruginosa.* In addition, the development of MDR may be related to the overproduction of the *PstS* gene [14]. About 50% of the isolates in this study possessed the *PstS* gene, which further raises alarm about the impending public health crises due to *P. aeruginosa* in Nsukka, Southeast Nigeria.

CONCLUSIONS

There is the presence of XDR, PDRPA, and *PstS* gene in *P. aeruginosa* strains in Southeast Nigeria. The findings from this study show an alarming degree of resistance among *P. aeruginosa* isolates in Nsukka, Nigeria and an urgent need for healthcare practitioners and policymakers to address the problem of MDR by implementing a more rational and appropriate use of antibiotics. Establishment of an effective surveillance program and strict disinfection policy in hospital

environments would also help to control the spread of MDR, XDR, and PDRPA in hospital settings.

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AUTHORS' CONTRIBUTIONS

Martina C Agbo, as the corresponding author, carried out the experiments and drafted the manuscript. Ifeoma M Ezeonu conceived, organized, and supervised this research work and reviews the manuscript. Anthony C Ike helped in review of the work, offered advice, participated in PCR, and read through the manuscript. Celestina C Ugwu assisted during the sample collection and helped in some bench work.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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