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Characterisation of Antibiotic Resistant *Pseudomonas* Species in Environmental and Clinical Samples in Lafia, Nasarawa State

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Abstract

Soil, water, and animals are reservoirs for resistance genes and multidrug resistant bacteria. The genus *Pseudomonas* is widely distributed in nature, with *Pseudomonas aeruginosa* being the most clinically significant. The aim of the study was to characterize antibiotic resistance in *Pseudomonas* species in environmental and clinical samples in Lafia, Nasarawa State. Water and soil (50 each) samples were collected five times per week, along with 50 urine samples. Samples with significant bacteriuria were analysed for positive isolates. The presence of virulence and antibiotic resistance genes on their DNA was confirmed with PCR analysis. Water samples had Pseudomonas counts ranging from $1.0x0^5$ to $17.0x10^5$ CFU/mL, and soil samples had highest count of $14.0x10^4$ CFU/g. *Pseudomonas* species were 86.7 % resistant to Augmentin and 75 % resistant to Ceftazidime, while Ofloxacin had the lowest resistant isolates at 25 %. The multidrug resistance was 72.7 % in the water isolates, 72.2 % in urine, and 60.0 % in soil isolates as the lowest. There was no significant association between sample source and MDR prevalence ($p > 0.05$). The resistance gene *bla_VIM* and virulence gene *pvdM* was each carried by 14 (82.4 %) of the *Pseudomonas* sp. and six of them co-carried *bla_VIM* and *bla_NDM* genes. *Pseudomonas mendocina* isolate haboured neither virulence nor resistance genes. The findings calls for concern as the genes haboured by the *Pseudomonas* species encode resistance to carbapenems, the last resort antibiotics. *Pseudomonas putida*, a non-pathogenic isolates haboured *bla_VIM* and *bla_NDM* genes making them a reservoir for horizontal transfer to other isolates and bacteria in the environment.

Keywords: *Pseudomonas*, antibiotic resistance, multidrug resistance, environment

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Introduction

Antibiotic resistance is a global health concern because of the threat to human and animal health, and the environment [1]. Antibiotics has widespread application in human health, agriculture, livestock breeding, and fish farming which increases indirect release to the environment [2]. Antibiotic resistance results from abuse, co-evolution among microorganisms and pathogens in soil and other environments. Resistance is no longer a hospital problem, it is now common within the community setting with a high incidence of poverty and overcrowding. Many of the resistance determinants are initiated when microbes mediate self-immunity, while others express the resistance attribute when plasmid, integron, or other extra-chromosomal agents are mobilized, or overexpressed in non-native hosts. Another challenge is the rise in the frequency and diversity of antibiotic resistant pathogens in healthcare institutions. Microbes from environmental and animal sources serve as channels for antibiotic resistance genes, which can be transmitted to humans. The increasing misuse of antibiotics in the treatment of infections, and the introduction of resistant strains lead to multidrug resistance [1]. This increases morbidity, mortality and, prolong hospital stay.

Pseudomonas species are widely distributed in nature. Their versatile nature enables them to adapt to different niches employing their virulence traits and pigment formation [3]. *Pseudomonas aeruginosa* is the most clinically recognized of the species due to its role in causing opportunistic infections in immune-compromised individuals. A major challenge in treating *Pseudomonas*-related infections is the high level of resistance to antibiotics [4]. The bacterium exhibits intrinsic resistance due to the low permeability of its outer membrane and its possession of antibiotics inactivating enzymes [5, 6]. It alsoeasily acquires resistance determinants via the horizontal gene transfer mechanisms [7]. These characteristics contribute to its multidrug-resistant (MDR) and extensively drug-resistant (XDR) traits [8, 9]. *Pseudomonas* from clinical samples are commonly associated with respiratory tract infections, urinary tract infections, wound infections and sepsis. They are problematic in the intensive care units (ICUs), where invasive procedures and immunosuppression are common [4]. Environmental studies have revealed that *P. aeruginosa* and other resistant bacteria can persist in aquatic and soil ecosystems for long period of time. While rivers receive discharges containing antibiotic residues from

hospitals, households, abattoirs, and agricultural lands, soil accumulate resistant bacteria through the application of animal manure, contaminated irrigation water, and runoff from nearby urban and industrial areas [10]. These contaminated environments promote the survival and exchange of antibiotic resistant genes (ARGs) among bacterial communities [11].

In Nigeria, antimicrobial resistance problem is worsened by the widespread misuse of antibiotics, poor sanitation, poverty, and poor regulation of pharmaceutical wastes. In Lafia, increasing population and anthropogenic activities such as indiscriminate waste dumping, open defecation, and cattle rearing increased pollution within human environment [12]. River bodies within and around Lafia serve domestic use and irrigation purposes, which make them potential hotspots for the dissemination of resistant bacteria [13]. The study characterized antibiotic resistant *Pseudomonas* species in environmental and clinical samples in Lafia, Nigeria. The study provided the extent of resistance dissemination in these environments. The findings will lend to the understanding of *P. aeruginosa* resistance in Nigeria.

Materials and Methods

Sample collection

One hundred and fifty samples made of 50 each of urine, water, and soil samples were collected. The urine samples were collected from outpatients department, Federal University Teaching Hospital Lafia, Nasarawa State, while water and soil samples were collected from 10 streams and soils within the same vicinity of the streams. Collection of water and soil samples was done once in two weeks basis for 2½ months.

Isolation of *Pseudomonas* species

Soil sample (1 g) was diluted to 10^{-6}, water sample was diluted to 10^{-5} and 1 mL from each inoculated by the pour plate technique on Cetrimide agar. The cultures were incubated at 37 ℃ for 24 h. Urine samples (1 mL) were inoculated on CLED agar and Cetrimide agar and cultures incubated at 37 ℃ for 24 h. The CLED agar was to screen for urine samples with significant bacteriuria.

Identification of *Pseudomonas* species

Isolates were identified on the basis of their colonial morphology, Gram reaction, microscopy, and biochemical tests. The total bacteriuria for the urine samples was determined and samples with significant bacteriuria ($1x10^5$ CFU/mL) were used for *Pseudomonas* isolation.

Antibiotic susceptibility testing

The antibiotics tested against the *Pseudomonas* were ceftazidime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ceporex (30 µg), ceftriaxone (30 µg), streptomycin (10 µg), cefuroxime (30 µg), ofloxacin (5 µg), augmentin (20 µg), pefloxacin (5 µg). Kirby-Bauer diffusion test was carried out according to the CLSI protocol. The isolate culture ($1×10^8$ CFU/mL cells) were inoculated by streaking on the prepared Muller-Hilton agar plate evenly. The plates were inverted then incubated at 37 ℃ for 24 h after which the zone of inhibition were measured.

Plasmid extraction: Alkaline lysis and large-plasmid preparation

Pseudomonas species were inoculated in 5 mL LB broth and incubated at 37 °C and 180 rpm overnight. From the broth containing the culture, 1.5 mL was pelleted at 12,000×g for 1 min. The supernatant was discarded and the step repeated until 4.5 mL culture was centrifuged. The pellet was suspended in 200 µL Resuspension buffer (Solution I) and vortexed to obtain a homogenous suspension. Two hundred microliter (200 µL) Lysis buffer (Solution II) was added and it was inverted four to six times, then incubated 5 min on ice to get a clear solution. Neutralization buffer (Solution III) (300 µL) was added, and it was inverted 6 to 8 time to mix thoroughly and incubated again for 10 min on ice. The solution was centrifuged at 12,000×g, for 10 min at 4 °C and 300 µL isopropanol added. It was incubated for 10 min andinverted to precipitate the DNA before centrifuging at 12,000×g for 10 min again after which the supernatant was discarded. The pellet were washed in 70 % cold ethanol (500 µL), centrifuged at 12,000×g for 2 min, and air-dried between 5 to 10 min. The PCR run had the master mix made up of; 2× PCR Master Mix: 12.5 µL, Forward primer (10 µM): 1.0 µL (final 0.4 µM), Reverse primer (10 µM): 1.0 µL, Template DNA (plasmid prep): 1.0 µL (~10–50 ng plasmid DNA), and Nuclease-free water: 9.5 µL. The thermocycler program ran on initial denaturation of 95 °C for 3 min, 35 cycles of denaturation: 95 °C for 30 s, annealing for 52–60 °C for 30 s and extension for 1 min at 72 °C. The final extension was at 72 °C for 5 min and 4 °C hold. The amplicon were detected by running 5 µL of the PCR product on 1.5 % agarose gel with 1 kb ladder and the bands visualized. The genes screened included *blaCTX-M*, *bla_VIM*, *bla_NDM*, *exoS*, *toxA* and *pvdM*.

Results and Discussion

The Pseudomonad isolated from water (Table 1) had a total bacterial mean count of $5.4x10^5$ CFU/mL (water sample. In the first week, Kofankaura had $1.7x10^5$ CFU/mL, followed by Bukankwarto with $1.5x10^5$ CFU/mL then College with $1.0x10^5$ CFU/mL. There was a progressive decrease in colony counts from week one to week five, with week one having a total colony count of $9.3x10^5$ CFU/mL which decreased to $2.9x10^5$ CFU/mL in week five. The soil sample also had a total bacterial mean count of $7.1×10^4$ CFU/g. In week one, College had the highest colony count of $1.5×10^4$ CFU/g, followed by Shabu C and Anzaku with $1.4×10^4$ CFU/g each. A progressive decrease in the number of colonies was also observed in the soil samples collected. Week one had a total colony count of $12.2×10^4$ CFU/g, and a total of $3.4×10^4$ CFU/g.

Table 1: *Pseudomonas* species counts in water and soil samples

Location	W1 Water	W2 Water	W3 Water	W4 Water	W5 Water	W1 Soil	W2 Soil	W3 Soil	W4 Soil	W5 Soil
	x10^5 CFU/mL					×10^4 CFU/g				
Shabu A	0.9	0.5	0.4	0.3	0.1	1.0	0.9	0.5	0.5	0.3
Shabu B	0.7	0.4	0.2	0.2	0.1	1.3	0.8	0.6	0.3	0.2
Shabu C	0.6	0.5	0.4	0.4	0.3	1.4	1.2	0.5	0.4	0.4
Gandu	0.8	0.5	0.5	0.3	0.2	1.2	0.7	0.6	0.3	0.2
Bukankwarto	1.5	0.8	0.3	0.2	0.2	1.1	0.8	0.7	0.5	0.3
Anzaku	0.4	0.2	0.2	0.2	0.1	1.4	0.9	0.8	0.4	0.4
Elako	0.8	0.4	0.4	0.3	0.1	1.0	0.8	0.7	0.5	0.5
Kofankaura	1.7	1.2	1.1	0.9	1.0	1.3	1.2	0.9	0.6	0.6
Poly	0.9	0.8	0.7	0.7	0.6	1.0	0.8	0.6	0.3	0.2
College	1.0	0.8	0.5	0.3	0.3	1.5	0.9	0.8	0.6	0.3

Table 2: Antibiotic resistance profile of *Pseudomonas* species from urine, soil and water samples in Lafia

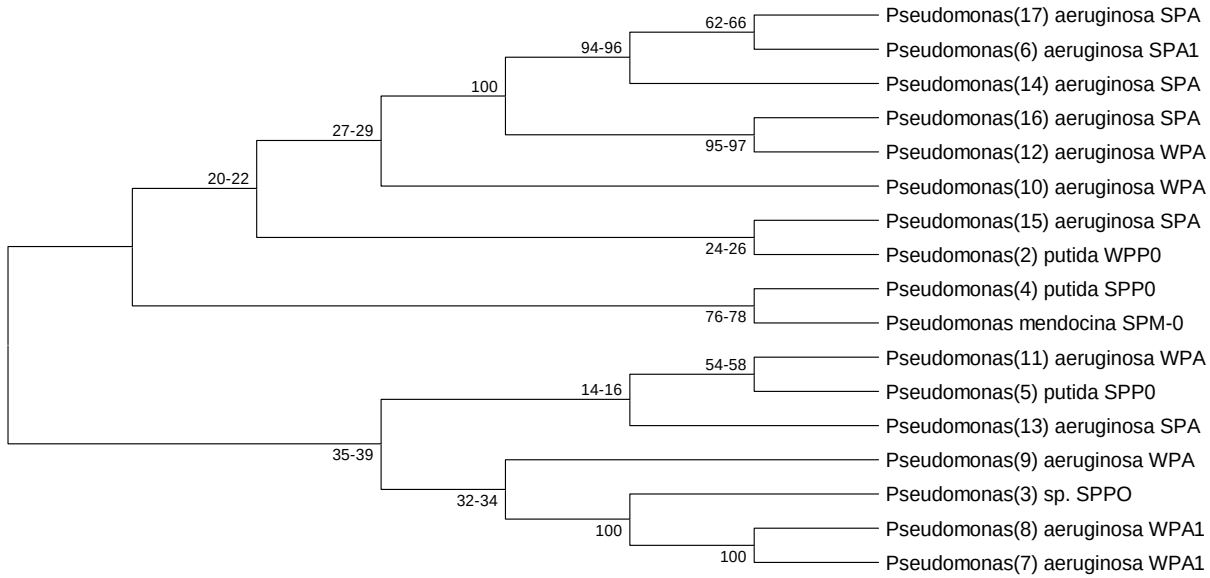
Isolate ID	Ceftazidime	Gentamicin	Ciprofloxacin	Ceporex	Ceftriaxone	Streptomycin	Cefuroxime	Ofloxacin	Augmentin	Pefloxacin
	Zone of Inhibition (mm)									
PO1	13(R)	13(S)	12(R)	11(R)	10(R)	15(S)	9(R)	25(S)	19(I)	17(S)
PO3	12(R)	13(S)	15(I)	13(R)	12(R)	14(S)	12(R)	28(S)	15(R)	15(I)
PO6	10(R)	11(R)	13(R)	10(R)	11(R)	9(R)	12(R)	22(R)	16(R)	14(R)
PO11	16(S)	15(S)	15(I)	14(I)	15(S)	14(S)	14(I)	20(R)	18(R)	11(R)
PO12	9(R)	16(S)	20(S)	9(R)	10(R)	11(I)	12(R)	25(S)	14(R)	18(S)
PO16	10(R)	11(R)	14(R)	13(R)	12(R)	11(I)	10(R)	19(R)	16(R)	12(R)
PO17	18(S)	10(R)	19(S)	18(S)	13(I)	12(S)	19(S)	27(S)	18(R)	20(S)
PO18	11(R)	12(I)	21(S)	12(R)	11(R)	15(S)	14(I)	28(S)	15(R)	15(I)
PO22	13(R)	11(R)	14(R)	12(R)	10(R)	9(R)	13(R)	18(R)	13(R)	12(R)
PO25	17(S)	10(R)	16(S)	12(R)	15(S)	10(R)	14(I)	24(I)	14(R)	19(S)
PO27	9(R)	15(S)	17(S)	13(R)	12(R)	17(S)	12(R)	29(S)	15(R)	16(S)
PO29	13(R)	11(R)	19(S)	12(R)	10(R)	16(S)	10(R)	24(I)	17(R)	15(I)
PO33	10(R)	11(R)	13(R)	9(R)	11(R)	9(R)	13(R)	17(R)	14(R)	12(R)
PO38	14(I)	12(I)	19(S)	20(S)	13(I)	17(S)	14(I)	23(R)	16(R)	13(R)
PO42	12(R)	11(R)	13(R)	10(R)	10(R)	11(I)	9(R)	28(S)	18(R)	12(R)
PO45	10(R)	14(S)	14(R)	12(R)	11(R)	15(S)	9(R)	24(I)	19(I)	18(S)
PO46	12(R)	12	17(S)	12(R)	10(R)	11(I)	18(S)	26(S)	15(R)	15(I)
PO50	13(R)	11(R)	14(R)	10(R)	9(R)	10(R)	13(R)	15(R)	17(R)	12(R)
SSHA01	11(R)	18(S)	15(I)	12(R)	10(R)	15(S)	11(R)	26(S)	19(I)	15(I)
SSHA02	12(R)	9(R)	16(S)	11(R)	13(I)	13(S)	10(R)	25(S)	17(R)	15(I)
SSHB01	10(R)	10(R)	18(S)	14(I)	11(R)	9(R)	12(R)	24(I)	16(R)	17(S)
SSHB02	13(R)	12(I)	19(S)	12(R)	11(R)	15(S)	16(S)	20(R)	15(R)	20(S)
SSHC01	12(R)	9(R)	13(R)	9(R)	15(S)	16(S)	12(R)	19(R)	15(R)	12(R)
SSHC02	13(R)	12(I)	14(R)	16(S)	10(R)	15(S)	14(I)	28(S)	17(R)	16(S)
SGD01	10(R)	18(S)	11(R)	16(S)	12(R)	11(I)	13(R)	25(S)	14(R)	19(S)
SGD02	11(R)	10(R)	18(S)	19(S)	12(R)	9(R)	12(R)	22(R)	15(R)	15(I)
SBK01	14(I)	16(S)	15(S)	12(R)	11(R)	11(I)	12(R)	29(S)	13(R)	16(S)
SBK02	12(R)	11(R)	17(S)	14(I)	11(R)	10(R)	11(R)	25(S)	16(R)	17(S)
SAZK01	18(S)	11(R)	16(S)	17(S)	15(S)	11(R)	13(R)	27(S)	12(R)	15(I)
SAZK02	12(R)	16(S)	17(S)	15(S)	13(I)	12(S)	15(S)	18(R)	15(R)	15(I)
SELK01	9(R)	12(I)	13(R)	14(I)	12(R)	15(S)	13(R)	26(S)	18(R)	16(S)
SELK02	16(S)	11(R)	15(I)	17(S)	13(I)	13(S)	15(S)	15(R)	14(R)	17(S)
SKK01	13(R)	10(R)	16(S)	14(I)	12(R)	9(R)	11(R)	25(S)	15(R)	16(S)
SKK02	11(R)	14(S)	17(S)	13(R)	12(R)	15(S)	13(R)	26(S)	13(R)	19(S)
SPOL01	10(R)	13(S)	15(I)	12(R)	11(R)	13(S)	12(R)	27(S)	14(R)	17(S)
SPOL02	12(R)	11(R)	16(S)	10(R)	11(R)	9(R)	10(R)	28(S)	17(R)	15(I)
SCOL01	11(R)	10(R)	12(R)	13(R)	15(S)	12(S)	10(R)	22(R)	15(R)	13(R)
SCOL02	12(R)	12(I)	17(S)	13(R)	12(R)	11(I)	12(R)	26(S)	18(R)	17(S)
WSHA01	13(R)	14(S)	18(S)	12(R)	11(R)	13(S)	12(R)	26(S)	17(R)	16(S)
WSHA02	14(I)	11(R)	17(S)	16(S)	17(S)	10(R)	18(S)	25(S)	14(R)	17(S)
WSHB01	14(I)	12(I)	14(R)	15(S)	13(I)	12(S)	14(I)	17(R)	15(R)	16(S)
WSHB02	15(S)	13(S)	13(R)	17(S)	15(S)	13(S)	16(S)	19(R)	18(R)	14(R)
WSHC01	16(S)	14(S)	11(R)	18(S)	17(S)	15(S)	16(S)	20(R)	18(R)	13(R)
WSHC02	12(R)	14(S)	15(I)	12(R)	11(R)	13(S)	13(R)	26(S)	16(R)	15(I)
WGD01	13(R)	16(S)	18(S)	13(R)	14(R)	13(S)	11(R)	28(S)	19(I)	14(R)
WGD02	15(S)	13(S)	13(R)	15(S)	14(R)	11(I)	15(S)	19(R)	15(R)	11(R)
WBK01	14(I)	11(R)	16(S)	15(S)	17(S)	10(R)	18(S)	28(S)	18(R)	17(S)
WBK02	14(I)	10(R)	17(S)	18(S)	16(S)	9(R)	15(S)	25(S)	19(I)	16(S)
WAZK01	13(R)	12(I)	13(R)	12(R)	11(R)	10(R)	12(R)	18(R)	17(R)	14(R)
WAZK02	18(S)	16(S)	12(R)	18(S)	15(S)	13(S)	16(S)	20(R)	14(R)	13(R)
WELK01	13(R)	12(I)	14(R)	20(S)	12(R)	17(S)	14(I)	25(S)	15(R)	17(S)
WELK02	12(R)	12(I)	19(S)	18(S)	11(R)	10(R)	12(R)	28(S)	13(R)	20(S)
WKK01	11(R)	14(S)	15(I)	13(R)	12(R)	12(S)	13(R)	26(S)	16(R)	22(S)
WKK02	10(R)	12(I)	16(S)	12(R)	11(R)	11(I)	12(R)	25(S)	14(R)	16(S)
WPOL01	14(I)	11(R)	17(S)	15(S)	14(S)	10(R)	16(S)	29(S)	16(R)	17(S)
WPOL02	14(I)	11(R)	16(S)	18(S)	17(S)	9(R)	18(S)	27(S)	19(I)	17(S)
WCOL01	18(S)	10(R)	18(S)	14(I)	13(I)	9(R)	16(S)	24(I)	15(R)	19(S)
WCOL02	12(R)	14(S)	19(S)	14(I)	12(R)	9(R)	10(R)	26(S)	13(R)	16(S)
CLS I Standard	**≤ 14**	**≤ 12**	**≤ 15**	**≤ 14**	**≤ 13**	**≤ 11**	**≤ 14**	**≤ 24**	**≤ 19**	**≤ 15**

Table 2 shows the antibiotic resistance profile of Pseudomonad isolated from urine, soil and water samples. The urine isolates demonstrated resistance to augmentin (88.9 %), ceporex (83.3 %), ceftazidime (77.8 %), ceftriaxone (77.8 %) and cefuroxime (66.7 %). A partial resistance was recorded against gentamycin, ciprofloxacin with 44.4 % respectively for each, while pefloxacin, streptomycin and ofloxacin with 38.9 % each. The most resistant isolates were those obtained from the clinical samples. The isolates PO6, PO22, PO33, and PO50 were resistant to all the ten (10) antibiotics tested. PO16, PO42 and WANZ1 were resistant to Nine (9) antibiotics followed by SSHC1 and SCOL1 were resistant to 8 (eight) antibiotics. The most resistant antibiotic was Augmentin and the most effective drug was perfloxacin which was closely followed by Ciprofloxacin.

As presented in Table 3, fifteen (88 %) of the isolates had *bla_VIM*, while 9 (53 %) carried *bla_NDM*, which is another metallo-β-lactamase. *bla_CTX-M* were detected in 7 (41 %) of the isolates. The virulence gene *exoS* was detected in 8 (47 %) of the isolates, *toxA* was detected in 8 isolates, and *pvdM* was detected in 82 % (14) of the isolates. The *bla_VIM*genes demonstrated a 100 % significant effect on patients as seen that it was present in all patient isolates examined as compared to the environmental isolates. Although the result shows that *bla_VIM*gene is predominant in both environmental and clinical settings. On the other hand, *bla_CTX-M* appears to be the least detected gene in both the clinical and environmental isolates with positive cases found in 2 out of 6 examined clinical isolates.

Table 3: Distribution of resistance and virulence genes carried by the *Pseudomonas* Isolates

S/N	Isolate	Resistance genes			Virulence genes		
		bla_VIM	***bla_NDM***	***bla_CTX-M***	***exoS***	***toxA***	***pvdM***
1	*Pseudomonas aeruginosa* SSHA2	+	+	+	-	+	+
2	*Pseudomonas aeruginosa* SSHB2	+	-	-	+	-	+
3	*Pseudomonas aeruginosa* SSHC1	+	-	+	+	+	+
4	*Pseudomonas aeruginosa* SGD2	+	+	-	+	+	+
5	*Pseudomonas aeruginosa* WELK2	+	+	+	-	-	+
6	*Pseudomonas aeruginosa* WCOL2	-	+	+	+	+	+
7	*Pseudomonas putida* SBK1	+	-	-	-	-	-
8	*Pseudomonas putida* SKK1	+	+	-	-	-	+
9	*Pseudomonas putida* WANZ1	+	-	-	-	-	+
10	*Pseudomonas putida* SPOL2	+	+	-	-	-	-
11	*Pseudomonas mendocina* SCOL1	-	-	-	-	-	-
12	*Pseudomonas aeruginosa* PO50	+	+	+	-	+	+
13	*Pseudomonas aeruginosa* P06	+	-	+	+	+	+
14	*Pseudomonas aeruginosa* PO16	+	+	-	-	+	+
15	*Pseudomonas aeruginosa* PO22	+	-	-	+	+	+
16	*Pseudomonas aeruginosa* PO33	+	-	-	+	-	+
17	*Pseudomonas aeruginosa* PO42	+	+	-	+	-	+



Isolates 17=PO42, 6=WCOL2, 14=PO16, 16=PO33, 12=PO50, 10=SPOL2, 15=PO22, 2=SSHB1, 4=SGD2, SPM-O=SSHA2, 11=SCOL1 5=WELK2, 13=PO6, 9=WANZ1, 3=SSHC1, 8=SKK1, 7=SBK1

Figure 1: The phylogenetic tree of the relationship between the *Pseudomonas* isolates

Figure 1 provides the phylogenetic relationship between isolates. This phylogenetic tree shows the evolutionary relationships among *Pseudomonas* isolates, mainly *P. aeruginosa*, *P. putida* and *P. mendocina* from different sources. The cluster I includes *Pseudomonas aeruginosa* isolates which are PO42, PO16, PO33, WCOL2, PO50, SPOL2 and PO22 with bootstrap of 94-100 which implies high confidence. This isolates form a strongly supported monophyletic cluster, which are highly similar genetically. The cluster II contains *Pseudomonas putida* SSHB1, SGD2, and *P. mendocina* SSHA2, with bootstrap of 24-26, 76-78 with a moderate confidence (Fig. 1). This group separates from *P. aeruginosa* cluster, which is expected since *P. putida* and *P. mendocina* species within the *Pseudomonas* genus which shows moderate evolutionary divergence from *P. aeruginosa* but some conserved genetic similarity within the genus. The third cluster (cluster III) also known as the lowest clade include *P. aeruginosa* SCOL1,WANZ1, PO6, SBK1, SKK1, and *Pseudomonas* SSHC1, WELK2 with a bootstrap of 100 which has very strong for SBK1 and SKK1. This group shows internal similarity especially between *P. aeruginosa* SBK1 and SKK1 which represent closely related or identical strains. The *Pseudomonas* species SSHC1 and WELK2 show some divergence but remain connected to the *P. aeruginosa* branch, indicating possible intermediate genetic relatedness or unresolved species-level distinction. The tree distinctly separates the three *Pseudomonas* species consistent with taxonomic expectations. High bootstrap values close to 100 indicate high reliable clade formation while low bootstrap values (20-40) implies less genetic certainty of high divergence or diversity within those groups.

The population of isolated *Pseudomonads* was high in the water samples; this was regardless of their location within Lafia. The dry season which the samples collected affected the counts obtained [14]. During this period, there was no more rainfall, and the flow rate also had reduced considerably, this enhanced and made attachments and biofilm formation more pronounced [15]. Other reasons adduced for the high count included runoff from surrounding soils, cattle drinking from streams in large numbers around the metropolis, human activities (washing and other domestic uses), and defecation [16, 17]. The soil samples had lower counts compared to the water samples. The counts could be attributed to high solar radiation, which makes the soil environment unfriendly for microbial growth. Despite feacal deposits around these locations, the *Pseudomonas* counts were not significantly affected. It could be that the bacteria are less versatile than their counterparts in the water environment.

The mid-stream urine samples showed that about 17 of the collected samples had significant bacteriuria. This indicates that the patients had contamination or were infected. Bacteriuria is caused mostly by Enterobacteriaceae, made up of *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Enterobacter* sp., *Pseudomonas auruginosa*, and *Enterococcus faecalis*. Other bacterial strains reported include *Staphylococcus aureus*, *Corynebacterium* sp., and *Streptococcus agalactiae* [18]. These pathogens can create polymicrobial activities that increase the severity of infection and cause recurring infections [19]. The *Pseudomonas* count in the urine of the patient with significant bacteriuria was concerning because these pathogens are opportunistic, taking advantage when the immune system is suppressed.

Ofloxacin was the most effective antibiotic with antibacterial activity against the isolates, followed by ciprofloxacin and perfloxacin. Most of the isolates showed multidrug resistance to ceftazidime, gentamicin, streptomycin, Augmentin, and cefuroxime. The isolates had differential resistance to fluoroquinolones. Fluoroquinolones have broad-spectrum activity and are less commonly used compared to β-lactams and aminoglycosides, which may explain their promising activity. The beta-lactam antibiotics showed poor activity against the isolates, possibly due to β-lactamase production. The observed resistance in the isolates could be due to frequent or inappropriate antibiotic use in the environment from which the isolates were obtained. The reduced activity observed in gentamycin and streptomycin could be attributed to the production of modifying enzymes, reduced membrane permeability, and efflux mechanisms in the isolates [20]. Poor activity of the antibiotics reduces their reliability for treatment, thus limiting treatment options, increasing treatment failures, and spreading resistance genes within the environment. Resistance to β-lactams and aminoglycosides suggests the presence of resistance plasmid.

The genes *blaVIM* (Metallo-β-lactamase (MBL)), *blaNDM* (New Delhi Metallo-β-lactamase), and *blaCTX-M* (Extended-spectrum β-lactamases) encode enzymes that hydrolyze β-lactam antibiotics, especially the cephalosporins and carbapenems [21]. Most isolates haboured *blaVIM* gene which explains the high resistance to β-lactam antibiotics (ceftazidime, ceftriaxone, cefuroxime, ceporex, and augmentin). The gene confers resistance to β-lactams and is associated with multidrug resistance. The *blaNDM* gene was carried by some *P. aeruginosa* isolates from water, soil and urine, while two *P. putida* (SKK1 and SPOL2) isolates harboured the same gene. This gene encode enzyme carbapenemase. The co-carriage of *blaVIM and blaNDM* genes in isolates SSHA2, SGD2, WELK2, PO50, and PO42 could lead to stronger resistance to β-lactam and carbapenem antibiotics and the induction of plasmid-mediated horizontal gene transfer. The presence of *the (blaCTX-M)* gene could be attributed to the resistance some of the isolates presented against ceftazidime and ceftriaxone, both third-generation cephalosporins.

The virulence gene *exoS* (exoenzyme S) encodes a Type III secretion system effector associated with host cell damage, invasion, and immune evasion [22]. It was mostly carried by *Pseudomonas aeruginosa* isolates. The*toxA*gene on the other hand, encodes exotoxin A in *P. aeruginosa* isolates. The toxin inhibits protein

synthesis in host cells, leading to tissue necrosis and worsening infection severity [23]. The *pvdM*, detected in most *P. aeruginosa* and some *P. putida* isolates, regulates pyoverdine synthesis, which is necessary for iron acquisition and survival in host tissues. *Pseudomonas aeruginosa* isolates that co-harboured resistance genes and *toxA* could be described as high-risk strains and are of clinical concern [24]. These strains of *Pseudomonas* could be associated with severe hospital-acquired infections, treatment failure, increased mortality, and rapid dissemination of resistance genes. No resistance or virulence gene was detected in *Pseudomonas mendocina.* The effectiveness of ofloxacin observed in the study showed that fluoroquinolone resistance mechanisms are either absent or not yet widespread in these isolates.

The isolates clustered according to species, with *Pseudomonas aeruginosa* forming the largest and most closely related clade. The isolates were genetically related, with limited genetic divergence, despite being sampled from different locations (water and soil). The *P. aeruginosa* isolates had common ancestry, or related strains were disseminated across different locations. This clustering pattern correlates with the shared resistance and virulence gene profiles observed in their genetic make-up, suggesting the possibility of clonal spread of multidrug-resistant lineages among *P. aeruginosa*. *Pseudomonas putida* and *Pseudomonas mendocina* formed distinct branches separate from the *P. aeruginosa* cluster, demonstrating clear interspecies divergence. The phylogenetic tree showed that *P. putida* and *P. mendocina* had distinct genetic make-up despite sharing certain resistance genes. This finding suggests possible horizontal gene transfer, rather than evolutionary relatedness, as the mechanism for resistance dissemination between the two strains.

Conclusion

The isolation of *Pseudomonas* species from both environmental and clinical samples indicates the presence of potentially harmful bacteria in the environment. *Pseudomonas*, an opportunistic bacterium is known for its versatility and ability to thrive and adapt to various environmental conditions. While some strains are harmless and even beneficial, others can pose a significant risk to human health, especially in immunocompromised patients and the ecosystem. The organism *P. aeruginosa* was prevalent in the environmental and the clinical samples collected, soil, water and urine respectively. The antibiotic resistance shown by *Pseudomonas* in this study is alarming. The isolated *P. aereuginosa* from samples showed multidrug resistance to beta-lactam, flouroguinolones and amynoglycosides antibiotics which pose significant challenge to clinicians in the treatment of infection caused by this species. Although it is generally believed that the resistant genes are found mostly in the environment (water), however, the AST result showed that the isolates obtained from soil were more resistant as compared to those gotten from the water. Fifteen (88 %) of the isolates had *bla_VIM*, while 9(53 %) carried *bla_NDM* and *bla_CTX-M/ESBLS* was detected in 7(41 %). The virulence genes detected were *exoS* which was 47 % of the isolates, *toxA* was detected in 8 isolates and *pvdM* was detected in 82 % (14) isolates. This study confirmed the circulation of multidrug-resistant and potentially virulent *Pseudomonas* strains in water, soil, and human samples in Lafia, Nigeria.

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