

# MULTI-DRUG RESISTANT PROFILE OF CLINICAL AND ENVIRONMENTAL ISOLATES OF *Pseudomonas aeruginosa* FROM A TEACHING HOSPITAL AT AMAKU, ANAMBRA STATE, NIGERIA

Afunwa Ruth Asikiya<sup>1\*</sup>, Odiari Chineze Esther<sup>1</sup>, Nwofia Martin Chukwunonso<sup>1</sup>,  
Gbadamosi Francis Ayodele<sup>2</sup>

\*Corresponding author: Afunwa Ruth Asikiya, Tel: 2348027341073

<sup>1</sup>Department of Pharmaceutical Microbiology and Biotechnology, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria

<sup>2</sup>Department of Biological Sciences, Godfrey Okoye University, Enugu State, Nigeria

DOI: <https://doi.org/10.5281/zenodo.11517840>

Published Date: 07-June-2024

---

**Abstract:** The emergence of multiple antibiotic resistances in bacteria and the indiscriminate use of antibiotics contribute to the dissemination of resistant pathogens in the hospital and environment which may cause problems in therapy and is a serious public health issue. This study was conducted to determine the incidence of *Pseudomonas aeruginosa* isolates in clinical and environmental samples as well as to determine the antimicrobial susceptibility pattern of these isolates to some conventional antibiotics. A total of ninety six (96) samples were collected over a period of one month which includes fifty (50) clinical samples comprising of thirty (30) urine samples collected with sterile urine containers and twenty (20) ear swabs and forty-six (46) environmental samples comprising of twenty three (23) toilet seat swabs and twenty three (23) sink swabs were all collected using sterile cotton swab sticks. A selective medium, Cetrimide agar was used for the isolation of *Pseudomonas aeruginosa* from the specimens. After 24 hours incubation, there was presence of green, greenish-yellow colonies indicating the probable presence of *Pseudomonas aeruginosa*. Fifty-one (51) isolates were gotten which include twelve (12) urine isolates (12.50%), sixteen (16) ear swab isolates (16.67%), twelve (12) sink swab isolates (12.50%) and eleven (11) toilet swab isolates (11.45%). The presence of *Pseudomonas aeruginosa* was confirmed with the following biochemical tests, Gram staining, catalase test and oxidase test. Antibiotic sensitivity testing was carried out as well using Modified Kirby Bauer (Disc diffusion method). The antibiotics used were Amoxicillin clavulanate (30ug), Cefotaxime (25ug), Imipenem (10ug), Ofloxacin (5ug), Gentamycin (10ug), Nalidixic acid (30ug), Nitrofurantoin (300ug), Cefuroxime (30ug), Ceftriaxone sulbactam (45ug), Ampiclox (10ug), Cefexime (5ug) and Levofloxacin (5ug). This study showed the presence of multi-drug resistant (MDR) *Pseudomonas aeruginosa* from both clinical and environmental samples, with the clinical samples having a higher prevalence of multi-drug resistance compared to environmental samples. The results revealed that the isolates were more resistant to Amoxicillin clavulanate, Ampiclox and Cefexime; however, Imipenem could be the best drug of choice for the treatment of MDR *Pseudomonas aeruginosa* in South-eastern Nigeria.

**Keywords:** bacteria, resistance, hospital, environment, urine, *Pseudomonas aeruginosa*.

---

## 1. INTRODUCTION

### 1.1 Background of the study

Over the past decades, there has been a remarkable global increase in Multidrug-resistant (MDR) pathogens particularly among gram-negative bacteria. *Pseudomonas aeruginosa* is responsible for various healthcare-associated infections; while MDR *P. aeruginosa* causes significant morbidity and mortality (Mahmoud *et al* ...,2021). The discovery of antibacterial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antibacterial agents. Therefore, the emergence of bacterial resistance to most of the commonly used antibiotics is of considerable medical significance.

*Pseudomonas aeruginosa* is present throughout the world in soil and water. *Pseudomonas aeruginosa* is a non-fermentative Gram-negative bacterium that can thrive on damp atmosphere of most wards (Akinbuluma *et al*, 2017). These bacteria favor moist areas, such as sinks, toilets, inadequately chlorinated swimming pools, and outdated or inactivated antiseptic solutions. *Pseudomonas aeruginosa* infection ranges from minor, external infections to severe, life-threatening disorders. Infections occur more often and tend to be more severe in people who are weakened by certain severe disorders, have diabetes, cystic fibrosis, weakened immune systems and people that are hospitalized (Llanes *et al*, 2013).

*Pseudomonas aeruginosa* is the most common Gram-negative bacterium found in HAIs (Healthcare associated infections). It is responsible for about 10% - 20% of nosocomial infections (Ali *et al*, 2016). It has been reported in HAI outbreaks around the world, including pneumonia, bacteremia, urinary tract infection (UTI), and endocarditis, which are often complicated and potentially life-threatening. *Pseudomonas aeruginosa* wound infection is the leading cause of limb amputation in children (Ejike *et al*, 2016).

The pathogenic success of *Pseudomonas aeruginosa* is as a result of its array of virulence factors and its tendency to colonize surfaces in an intractable biofilm form, making the cells impervious to therapeutic concentrations of antibiotics. It is innately tolerant to many antimicrobial products and disinfectants because of its outer membrane permeability barrier. In addition, it maintains resistance plasmids and can exchange the same with other bacteria with which it lives as normal flora, through the mechanism of Horizontal Gene Transfer (HGT), particularly conjugation and transduction (Mahmoud *et al*, 2013).

The aim of this study is to evaluate the multi-drug resistant profiles of clinical and environmental isolates of *Pseudomonas aeruginosa* in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku, Anambra state.

## 2. MATERIALS AND METHODS

### Sample site and collection:

The total number of sample size was obtained using formula for an infinite population (Israel, 1992).

$$no = Z^2 PQ / e^2$$

Where:

Z= confidence level (1.96)

P= expected prevalence (0.5)

Q= (1-p)

e= absolute precision (10%= 0.01)

Therefore;

$$no = (1.96)^2 \times (0.5) \times (0.5) / (0.1)^2$$

$$= 0.9604 / 0.01$$

$$no = 96.04 = 96 \text{ samples}$$

a total of 96 samples were obtained of which 50 were of clinical origin constituting 30 samples of urine and 20 ear swabs (both from random out-patients). Oral consent was obtained from the individuals. A total of 46 environmental samples constituting 23 swabs from toilet seats and 23 sink swabs were randomly collected from the hospital's student hostel. All samples were collected over a period of one month from Amaku teaching hospital, Awka.

### Isolation procedure:

**A. Clinical samples:** Samples were processed as follows:

1) **Urine:** The samples were mixed thoroughly by inverting the containers several times. Using a sterile wire loop, the samples were inoculated on Cetrimide agar plates. The plates were then incubated at 37°C for 24h.

2) **Ear swabs:** The ear swabs collected were inoculated into tubes of nutrient broth and incubated at 37°C for 24h. Isolates were inoculated on Cetrimide agar using the spread plate method. The plates were incubated at 37°C for 24h.

**B. Environmental samples:** Samples were processed as follows:

1) **Toilet seats:** The toilet seat swabs were collected using sterile swab sticks already soaked in peptone water. The swab sticks were inoculated into tubes of nutrient broth and incubated at 37°C for 24h. The isolates were incubated on Cetrimide agar plates using the spread plate method. The plates were then incubated at 37°C for 24h.

2) **Sinks:** The sink swabs were collected using sterile swab sticks already soaked in peptone water. The swab sticks were inoculated into tubes of nutrient broth and incubated at 37°C for 24h. The isolates were then incubated on Cetrimide agar plates using the spread plate method. The plates were incubated at 37°C for 24h.

All isolates recovered from both clinical and environmental samples were sub-cultured on selective media, **Cetrimide agar (Oxoid Ltd)**. The plates were incubated at 37°C for 24 to 48 hours. Isolates were confirmed by certain preliminary tests.

### PRELIMINARY IDENTIFICATION OF ISOLATES

All isolates were Gram stained and examined microscopically. Biochemical tests were carried out based on Gram reactions.

#### Oxidase test:

In this test, a few drops of 1% aqueous solution of oxidase reagent were added to a piece of filter paper. A smear of the culture was impregnated on the filter paper using a sterile wire loop. Purple coloration indicated an oxidase-positive test.

#### Catalase Test:

In this test, a smear of the culture was made on glass slides; 2-3 drops of hydrogen peroxide solution was poured using a sterile pipette. Bubbles of oxygen gas indicated a catalase positive test.

#### Antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolates

The antimicrobial susceptibility test was performed using the Disc diffusion method according to Bauer (1966) on Mueller-Hinton agar medium. The following antibiotics were used; Amoxicillin clavulanate (30ug), Cefotaxime (25ug), Imipenem (10ug), Nitrofurantoin (300ug), Cefuroxime (30ug), Ceftriaxone (45ug), Ofloxacin (5ug), Gentamycin (10ug), Nalidixic acid (30ug), Ampiclox(10ug), Cefexime(5ug), Levofloxacin(5ug).

Pure cultures of isolates were standardized by transferring colonies of the pure culture of the isolates using a sterile wire loop into 3mls of sterile nutrient broth. The suspension was incubated for 3 hours at 37°C to allow for the growth of test organism till the density was equivalent to the turbidity of 0.5 McFarland.

The standardized inoculums were swabbed onto Mueller-Hinton agar plate and the discs were placed on the inoculated plates and pressed firmly onto the agar plate for complete contact. The agar plates were left on the bench for 30 minutes to allow for diffusion of the antibiotics and were incubated at 37°C for 24h.

The susceptibility of each isolate to each antibiotic was shown by a clear zone of growth inhibition and this was measured using a meter rule in millimeters and the diameter of the zones of inhibition were interpreted using a standard chart, EUCAST 2023 (European Committee on Antimicrobial Susceptibility Testing).

## 3. RESULTS

### Percentage distribution of *Pseudomonas aeruginosa* isolates in both environmental and clinical samples:

In this study, a total of ninety-six (96) samples were collected, fifty-one (51) isolates of *Pseudomonas aeruginosa* were isolated and identified based on colony morphology and a series of biochemical tests were done as shown below;

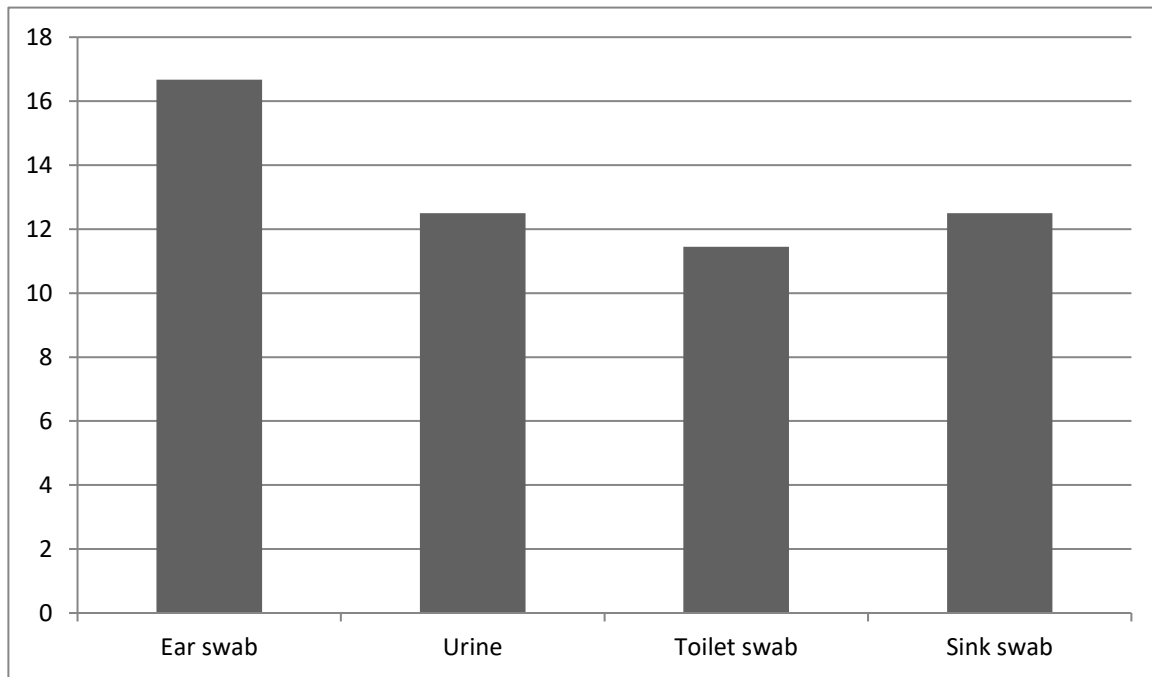


Figure 1: Percentage distribution of *Pseudomonas aeruginosa* isolates in both clinical and environmental samples:

TABLE 1: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ISOLATES

Samples	Total isolates	Cat	Oxi	G.S.	P.O.
Ear swab	16	+	+	-	<i>Pseudomonas aeruginosa</i>
Urine	12	+	+	-	<i>Pseudomonas aeruginosa</i>
Toilet swab	11	+	+	-	<i>Pseudomonas aeruginosa</i>
Sink swab	12	+	+	-	<i>Pseudomonas aeruginosa</i>

KEY: CET.A- Cetrimide agar; CAT-Catalase test; OXI-Oxidase test; PO-Probable organism; + Positive; - Negative; S-sink; T-Toilet; U-Urine; ES-Ear swab; GS-Gram staining

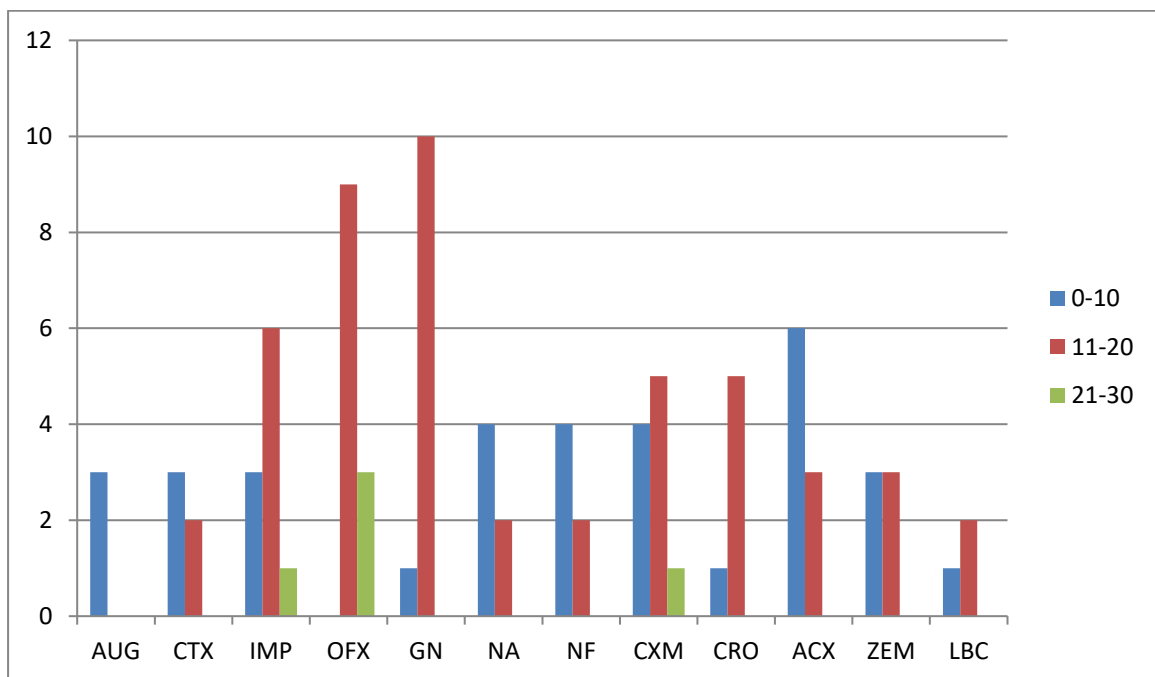


Figure 2: Range of Antibiotics susceptibility test results of isolates obtained from ear swabs

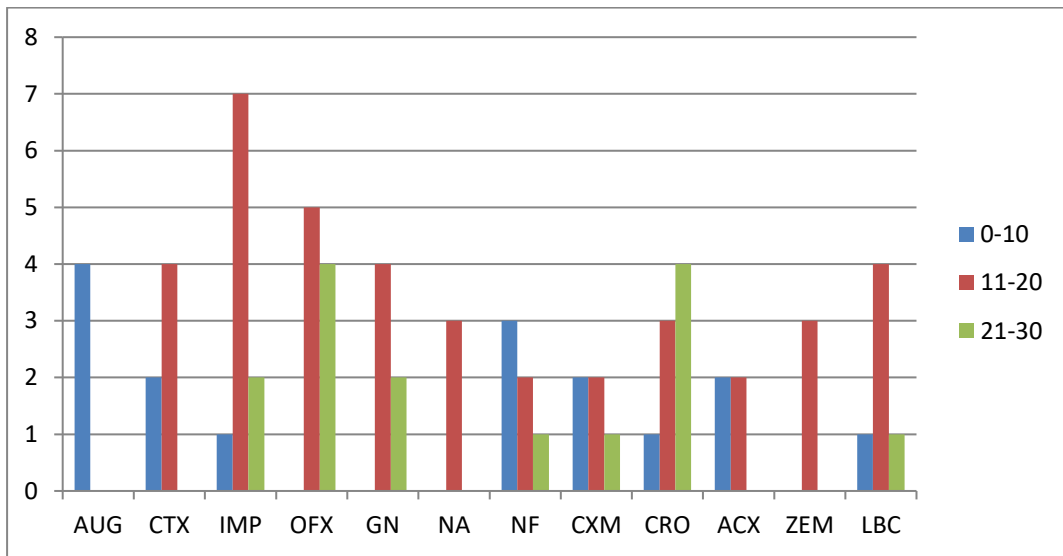


Figure 3: Range of Antibiotics susceptibility test results of isolates obtained from urine

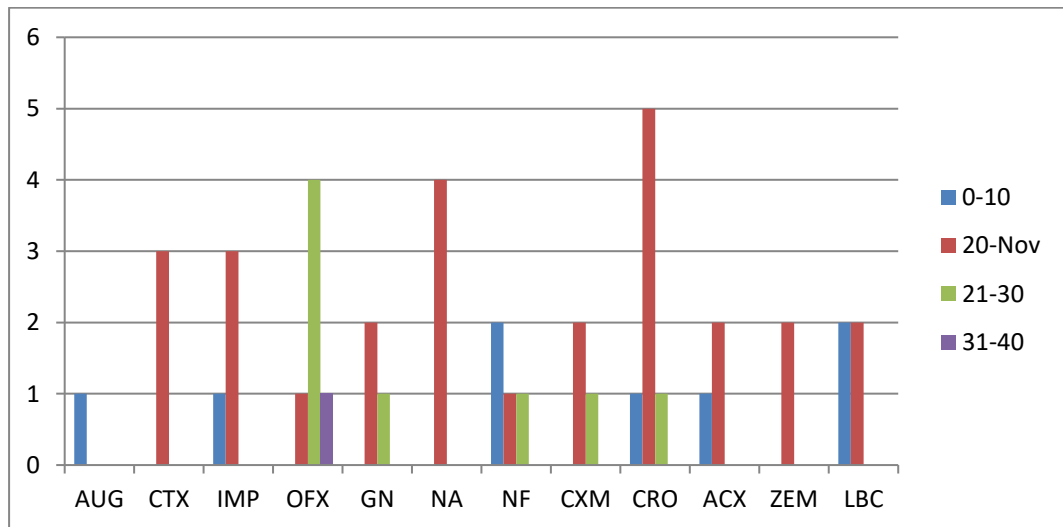


Figure 4: Range of Antibiotics susceptibility test results of isolates obtained from toilet seat swabs

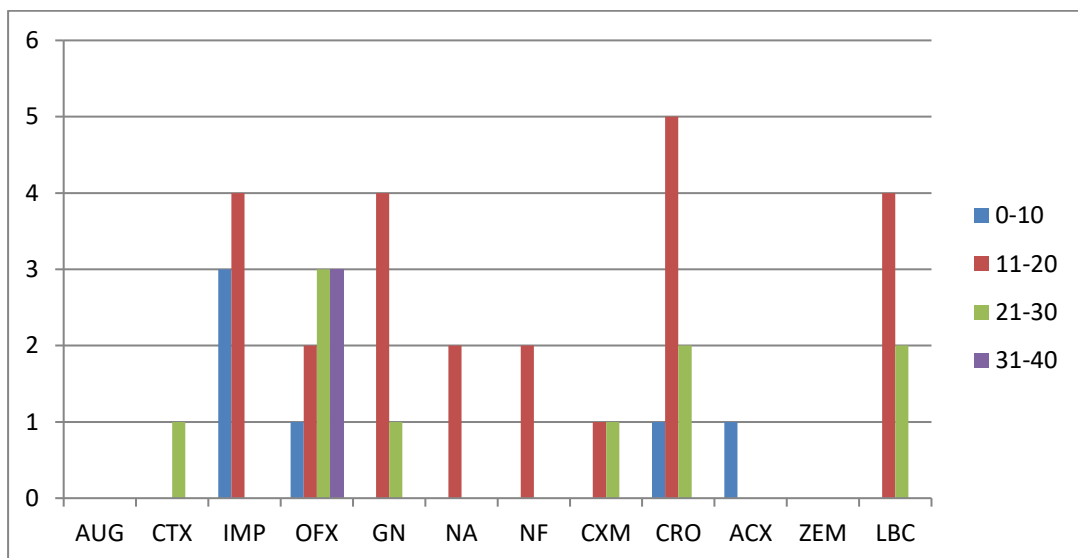


Figure 5: Range of Antibiotics susceptibility test results of isolates obtained from Sink swab

#### 4. DISCUSSION

Multiple drug resistances in bacterial population are currently one of the greatest challenges in the effective management of infections. Many factors contribute to the antibiotic resistance observed in *P. aeruginosa* (Pang Z *et al.*, 2019). Antimicrobial drugs have been proved remarkably effective for the control of bacterial infections. However, it was soon evidenced that bacterial pathogens were unlikely to surrender unconditionally and some pathogens rapidly became resistant to many antibiotics. Antimicrobial resistance in bacterial pathogens is a challenge that is associated with high morbidity and mortality. Multidrug resistance patterns in gram-negative and gram-positive bacteria are difficult to treat and may even be untreatable with conventional antibiotics. (Marianne *et al.*, 2017)

This study reports the incidence, prevalence and antibiotic resistance pattern of 51 isolates of *P. aeruginosa* from some clinical and environmental samples. Both clinical and environmental samples studied harbor this microorganism. Clinical samples which include urine, ear swab and environmental samples including toilet seat swab and sink swabs were examined for the presence of multidrug resistant *Pseudomonas aeruginosa*. Out of the ninety-six (96) samples analyzed, fifty-one (51) isolates (53.12%) were obtained on a selective medium, Cetrimide agar.

The prevalence rate recorded showed the isolation of *Pseudomonas aeruginosa* in the different samples collected as follows; toilet seat swabs 11 (11.45%), sink swabs 12 (12.50%), urine 12 (12.50%) and ear swabs 16 (16.67%). This results show ear swab having the higher number of isolates, which disagrees with the findings of (basak *et al.*, 2012) where pus and wound swab yielded the highest number of *Pseudomonas aeruginosa* strains in that study. The variations in *Pseudomonas aeruginosa* prevalence may be due to differences in geographical location, differences in study population, number of specimens, exposure to broad spectrum antibiotics and contact with hospital settings.

The EUCAST 2023 standard breakpoints was used to interpret results. Analysis revealed high level resistance to a significant number of antibiotics; this is in agreement with similar studies that have shown high incidence of antimicrobial resistance in *P. aeruginosa*. (Alnimir and Alamri *et al.*, 2020). The isolates showed some degree of resistance to all the antibiotics, this agrees with the findings of (Manikandan *et al.*, 2011) which reported multidrug resistance by bacteria isolated from UTI. Overall, more than 70% of isolates tested were resistant to Amoxicillin clavulanate (84.32%), cefexime (78.43%) and nalidixic acid (70.59%) while over 50% were resistant to cefotaxime (66.67%), Gentamicin (56.86%), Nitrofurantoin (62.75%), Cefuroxime (58.82%), Ampiclox (64.71%) and Levofloxacin (60.78%). Of all antibiotics tested, imipenem recorded the highest number of susceptibility (87.52%) followed by ofloxacin and ceftriaxone, these findings are in line with reports by (Amaka *et al.*, 2022) this report showed that imipenem was the most effective drug against *Pseudomonas aeruginosa* with a susceptibility value of 91.3%. The high susceptibility pattern of this drug could be associated to less drug abuse by the population being that the cost of this antibiotic prevents patient self-medication. The high activity of ofloxacin against *Pseudomonas aeruginosa* could be associated to less drug abuse by the population. This study revealed a resistance of 33.34% to ofloxacin. Increasing resistance to broad-spectrum antibiotics might be as a result of monotherapy on the part of the clinicians or as a result of selective pressure due to its frequent use in Nigeria.

In general, the clinical isolates harbor this organism and are more resistant to the antibiotics than environmental isolates; this is in line with the report by (Indu *et al.*, 2014) where a total of fifty eight strains of *P. aeruginosa* were obtained of which two were isolated from environmental samples (one from sink and the other from door wall). The high level resistance to these antibiotics might be attributed to antibiotic bacterial emergence because of improper and extensive use of these antibiotics, antibiotic discharge in various amounts in the environment, indiscriminate use of antibiotics in medical, veterinary and agricultural practices leads to multiple antibiotic resistances in bacterial pathogens. Prompt and accurate detection of MDR bacteria from clinical samples is critical to proper patient care, since these measures will provide sound epidemiological data that will guide antimicrobial therapy during treatment. This measure will also help to suppress the emergence and the spread of drug-resistant bacteria in the both the community and hospital environment.

#### 5. CONCLUSION

This study showed the presence of multi-drug resistant *Pseudomonas aeruginosa* from clinical and environmental samples. It also showed that there is a high antibiotic usage among patients (as antibiotics can be obtained in Nigeria without a prescription), which is a well identified risk factor for the emergence of drug resistant strains in this environment. High antibiotic resistance to third generation cephalosporins as well as  $\beta$ -lactam inhibitors as obtained in this study is very disturbing as these drugs are mainly used against Gram-negative resistant microorganisms. However, imipenem could be the best drug of choice for the treatment of MDR *Pseudomonas aeruginosa* in South-eastern Nigeria.

## 6. RECOMMENDATION

There should be public enlightenment to create awareness on the effects and risks of indiscriminate use and self-medication of antibiotics.

A good antibiotic surveillance system should be set up to ensure proper monitoring and control of drugs, also the indiscriminate use and prescription of drugs without prior susceptibility tests should be discouraged.

### REFERENCES

- [1] Akinbuluma, M. D., Ewete, F. K., & Yeye, E. O. (2017). Phytochemical investigations of Piper guineense seed extract and their effects on Sitophilus zeamais ( Coleoptera : Curculionidae ) on stored maize. 6(1), 45–52. <https://doi.org/10.18869/modares.jcp.6.1.45>
- [2] Ali, U., Ominyi, M. C., Ebenyi, L. N., Orinya, O. F., Ogbanshi, M. E., & Ezenwali, M. (2016). *Comparative Study on the Effects of Ethanol Extracts of Piper guineense and Gongronema latifolium Plants on Hematological Parameters in Albino Rats Exposed to Ethanol*. 13(3), 196–203. <https://doi.org/10.5829/idosi.wjms.2016.196.203>
- [3] Alnimir AM, Alamri AM. Antimicrobial activity of cephalosporin beta-lactamase inhibitor combinations against drug-susceptible and drug resistant *Pseudomonas aeruginosa* strains. J Taibah Univ Mes Sci. 2020;15:203-210.
- [4] Amaka Marian AWANYE, Chidozie Ngozi IBEZIM, Catherine Nonyelum STANLEY, Hannah ONAH, Iheanyi Omezurike OKONKO and Nkechi Eucharia EGBE. (2022). Multidrug –Resistant and Extremely Drug-Resistant *Pseudomonas aeruginosa* in Clinical Samples from a Tertiary Healthcare Facility in Nigeria. 19(4): 447-454. <https://doi.org/10.4274/tjps.galenos.2021.66066>
- [5] Basak, S., Attal, R. O. & Rajurkar, M. (2012). *Pseudomonas aeruginosa* and newer  $\beta$ -lactamases: an emerging resistance threat. In C. Sudhakar (Ed). Infection control- updates. Shanghai: InTech, pp. 181-198.
- [6] Ejike ugwu C, Esimone C, Iroha I, Ugwu C, Ezeador C, Duru C, Adikwu M. (2016). *Phenotypic detection of AmpC beta-lactamase among anal Pseudomonas aeruginosa isolates in a Nigerian abattoir*. *Archives of Clinical Microbiology*, 7(2):1-5.
- [7] Indu Biswal, Balvinder Singh Arora, Dimple Kasana and Neetushree. (2014). Incidence of Multidrug Resistant *Pseudomonas aeruginosa* Isolated From Burn Patients And Environment of Teaching Institution. 8(5): DC26-DC29. <https://doi.org/10.7860/JCDR/2014/7483.4383>
- [8] Israel, Glenn D 1992. *Determining sample size*. Program Evaluation and Organizational Development, IFAS, University of Florida. PEOD-6. November
- [9] Llanes, C., Pourcel, C., Richardot, C., Plesiat, P., Fichant, G., Cavallo, J. & Merens, A. (2013). Diversity of  $\beta$ -lactam resistance mechanisms in cystic fibrosis isolates of *Pseudomonas aeruginosa*: a French multicentre study. *Journal of Antimicrobial Chemotherapy*, 68, 1763 – 1771
- [10] Mahmood AI-Orphaly, Hamad Abdel Hadi, Faiha Kamaledin Eltayeb, Hissa AI-Hail, Bincy Gladason Samuel, Ali A. Sultan and Sini Skariah. (2021). Epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in the Middle East and North Africa region. *Journal of Antimicrobial Chemotherapy*. DOI: <https://doi.org/10.1128/msphere.00202-21>
- [11] Mahmoud, A.B., W.A. Zahran, A.Z. Hindawi Labib, and R. Galal (2013). *Prevalence of Multidrug-Resistant Pseudomonas aeruginosa in Patients with Nosocomial Infections at a University Hospital in Egypt, with Special Reference to Typing Methods*. *Journal of Virology and Microbiology*, 4:1-13.
- [12] Manikandan, S., Ganesapandian, S., Singh, M. and Kumaraguru, A. (2011). Antimicrobial susceptibility pattern of urinary tract infection causing- pathogenic bacteria. *Asian Journal of Medical Sciences*, 3(2):56-60.
- [13] Marianne Frieri, Krishan Kumar & Anthony Boutin (2017). *Journal of infection and public health*. Antibiotic resistance. 10, 369-378.
- [14] Pang Z., Raudonis R, Glick BR, Lin TJ, Cheng Z . Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnol Adv*. 2019;37:177-192.

## APPENDICES - A

## APPENDIX 1

TABLE 1: Percentage distribution of *Pseudomonas aeruginosa* isolates in both clinical and environmental samples:

Samples	Sample type	Total sample	No. of isolates	Percentage (%)
Clinical	Ear swab	20	16	16.67
	Urine	30	12	12.50
Environmental	Toilet seat swab	23	11	11.45
	Sink swab	23	12	12.50
Total		96	51	53.12

## APPENDIX 2

TABLE 2A: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ISOLATES

S/N	ISOLATES	CET A	CAT	OXI	G.S	P.O
1	T1	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
2	T2	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
3	T3	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
4	T4	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
5	T5	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
6	T6	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
7	T7	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
8	T8	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
9	T9	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
10	T10	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
11	T11	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
12	S1	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
13	S2	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
14	S3	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
15	S4	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
16	S5	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
17	S6	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
18	S7	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa

KEY: CET.A- Cetrinide agar; CAT-Catalase test; OXI-Oxidase test; PO-Probable organism; + Positive; - Negative; S-sink; T-Toilet; U-Urine; ES-Ear swab; GS-Gram staining

TABLE 2B: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ISOLATES

S/N	ISOLATES	CET A	CAT	OXI	G.S	P.O
19	S8	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
20	S9	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
21	S10	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
22	S11	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
23	S12	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
24	U1	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
25	U2	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
26	U3	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
27	U4	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
28	U5	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
29	U6	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
30	U7	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
31	U8	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
32	U9	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
33	U10	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
34	U11	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
35	U12	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
36	ES1	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
37	ES2	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
38	ES3	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa

KEY: CET.A- Cetrinide agar; CAT-Catalase test; OXI-Oxidase test; PO-Probable organism; + Positive; - Negative; S-sink; T-Toilet; U-Urine; ES-Ear swab; GS-Gram staining



TABLE 2C: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ISOLATES

S/N	ISOLATES	CET A	CAT	OXI	G.S	P.O
39	ES4	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
40	ES5	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
41	ES6	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
42	ES7	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
43	ES8	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
44	ES9	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
45	ES10	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
46	ES11	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
47	ES12	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
48	ES13	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
49	ES14	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
50	ES15	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa
51	ES16	Green pigmentation, convex, opaque, mucoid	+	+	-	P. aeruginosa

KEY: CET.A- Cetrinide agar; CAT-Catalase test; OXI-Oxidase test; PO-Probable organism; + Positive; - Negative; S-sink; T-Toilet; U-Urine; ES-Ear swab; GS-Gram staining

## APPENDIX 3

TABLE 3A: ANTIBIOTICS SENSITIVITY TEST RESULTS (inhibition zone diameter measured in mm)

S/N	P.O.	AUG	CTX	IMP	OFX	GN	NA	NF	CXM	CRO	ACX	ZEM	LBC
1	P. aeruginosa	0	17	12	17	11	0	0	17	0	12	0	0
2	P. aeruginosa	0	0	14	0	13	0	8	10	0	7	12	0
3	P. aeruginosa	0	0	0	22	12	0	11	0	0	9	0	0
4	P. aeruginosa	0	9	7	28	15	0	10	0	11	0	12	25
5	P. aeruginosa	0	0	6	11	0	0	0	0	0	0	0	12
6	P. aeruginosa	9	7	9	0	18	0	0	11	0	0	0	0
7	P. aeruginosa	0	5	0	15	9	7	13	0	0	0	0	0
8	P. aeruginosa	0	0	11	13	0	5	0	7	0	0	6	0
9	P. aeruginosa	0	0	18	12	13	0	0	11	0	8	10	0
10	P. aeruginosa	8	0	0	0	15	8	0	10	10	16	0	0
11	P. aeruginosa	0	0	10	18	11	0	0	15	12	8	10	0
12	P. aeruginosa	8	20	21	30	0	6	0	25	17	0	0	0
13	P. aeruginosa	0	0	16	0	0	0	0	0	0	0	0	0
14	P. aeruginosa	0	0	20	12	13	13	8	8	13	11	15	0
15	P. aeruginosa	0	0	0	17	11	12	0	0	12	10	0	25
16	P. aeruginosa	0	0	12	14	0	0	9	16	0	8	0	0

KEY: AUG-Amoxicilin Clavulanate; CTX-Cefotaxime; IMP-Imipenem/Cilastatin; NF-Nitrofurantoin; CXM-Cefuroxime; CRO-Ceftriaxone Sulbactam; OFX-Ofloxacin; GN-Gentamicin; NA-Nalidixic Acid; ACX-Ampiclox; ZEM-Cefexime; LBC;Levofloxacin

TABLE 3B: ANTIBIOTICS SENSITIVITY TEST RESULT (inhibition zone diameter measured in mm)

S/N	P.O.	AUG	CTX	IMP	OFX	GN	NA	NF	CXM	CRO	ACX	ZEM	LBC
17	P. aeruginosa	5	0	16	28	23	0	0	0	0	0	0	10
18	P. aeruginosa	0	0	18	16	11	12	20	11	18	12	16	0
19	P. aeruginosa	0	15	0	0	0	12	0	12	27	9	0	0
20	P. aeruginosa	0	8	25	19	0	0	0	22	0	0	0	18
21	P. aeruginosa	0	6	10	23	22	0	7	0	10	0	0	25
22	P. aeruginosa	7	0	17	15	11	0	10	0	0	0	0	15
23	P. aeruginosa	0	16	20	29	0	0	0	0	25	0	0	0
24	P. aeruginosa	0	13	18	27	0	12	0	0	24	15	14	0
25	P. aeruginosa	0	0	17	16	19	0	15	7	12	7	0	18
26	P. aeruginosa	0	15	16	0	0	0	13	10	28	0	17	0

27	P. aeruginosa	7	0	0	0	0	0	0	0	0	0	0	0
28	P. aeruginosa	8	10	23	15	19	0	6	0	18	0	0	20
29	P. aeruginosa	0	18	13	32	0	0	0	20	22	0	18	0
30	P. aeruginosa	0	0	0	20	23	0	15	0	0	0	0	17
31	P. aeruginosa	0	0	0	0	0	15	0	12	18	0	0	0
32	P. aeruginosa	0	0	0	0	0	15	21	0	11	13	12	0
33	P. aeruginosa	0	11	14	23	0	0	6	0	0	8	0	0
34	P. aeruginosa	0	15	0	0	15	0	8	0	10	0	0	0
35	P. aeruginosa	0	0	16	23	0	0	0	0	0	0	0	18
36	P. aeruginosa	0	0	0	29	15	16	0	0	0	0	0	0

**KEY:** AUG-Amoxicilin Clavulanate; CTX-Cefotaxime; IMP-Imipenem/Cilastatin; NF-Nitrofurantoin; CXM-Cefuroxime; CRO-Ceftriaxone Sulbactam; OFX-Ofloxacin; GN-Gentamicin; NA-Nalidixic Acid; ACX-Ampiclox; ZEM-Cefexime; LBC;Levofloxacin

**TABLE 3C: ANTIBIOTICS SENSITIVITY TEST RESULT (inhibition zone diameter measured in mm)**

S/N	P.O.	AUG	CTX	IMP	OFX	GN	NA	NF	CXM	CRO	ACX	ZEM	LBC
38	P. aeruginosa	0	0	0	24	0	0	0	11	15	0	0	6
39	P. aeruginosa	0	0	8	0	0	11	0	0	16	0	0	0
40	P. aeruginosa	0	0	0	0	0	0	0	0	18	17	0	10
41	P. aeruginosa	0	22	12	13	12	0	12	0	12	0	0	13
42	P. aeruginosa	0	0	0	31	18	15	0	0	0	0	0	12
43	P. aeruginosa	0	0	5	10	0	0	0	0	0	0	0	0
44	P. aeruginosa	0	0	0	25	0	0	0	0	13	0	0	0
45	P. aeruginosa	0	0	17	0	0	0	0	13	0	0	0	0
46	P. aeruginosa	0	0	8	0	0	0	0	0	6	0	0	13
47	P. aeruginosa	0	0	18	17	12	0	12	0	22	0	0	13
48	P. aeruginosa	0	0	7	26	0	14	0	0	17	0	0	0
49	P. aeruginosa	0	0	18	35	12	0	0	21	22	0	0	0
50	P. aeruginosa	0	0	0	30	22	0	0	0	18	8	0	22
51	P. aeruginosa	0	0	7	28	0	0	0	0	12	0	0	27

**KEY:** AUG-Amoxicilin Clavulanate; CTX-Cefotaxime; IMP-Imipenem/Cilastatin; NF-Nitrofurantoin; CXM-Cefuroxime; CRO-Ceftriaxone Sulbactam; OFX-Ofloxacin; GN-Gentamicin; NA-Nalidixic Acid; ACX-Ampiclox; ZEM-Cefexime; LBC;Levofloxacin