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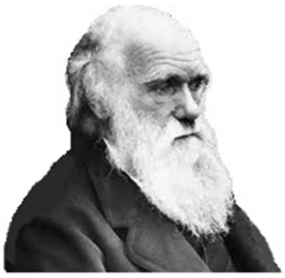

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Antibiotics resistance pattern of *Pseudomonas* Migula, 1894, species isolated from University of Calabar Teaching Hospital environment in Calabar, Nigeria

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ABSTRACT

This study aimed to assess the antibiotic resistance patterns of *Pseudomonas* species isolated from the environment of the University of Calabar Teaching Hospital (UCTH), Nigeria. A total of 53 *Pseudomonas aeruginosa* isolates were obtained from 201 clinical samples across various sites, including sinks, hand swabs, tabletops, gloves, and stethoscopes. Among the ten antibiotics tested, seven; Pefloxacin, Septrin, Ciprofloxacin, Gentamycin, Streptomycin, Rocephin, and Erythromycin - showed effectiveness against the isolates, while 30% exhibited resistance to Ampiclox, Zinnacef, and Amoxicillin. Rocephin demonstrated the highest sensitivity rate (33.3%) among isolates from sink samples. These findings underscore the prevalence of multidrug-resistant *P. aeruginosa* in the UCTH environment, highlighting a potential public health risk. Strengthened policies on antimicrobial stewardship and rigorous infection control measures are recommended to curb the spread of resistant strains.

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***Keywords*:** *Pseudomonas aeruginosa*, antibiotic resistance, healthcare associated infections, multi drug-resistant bacteria

1. INTRODUCTION

Pseudomonas aeruginosa (Schröter 1872) Migula 1900, is a non-fermentative aerobic, gram-negative rod that normally lives in moist environments (Goldberg, 2012), and has minimal nutrition requirements while being able to use several organic compounds for growth.

This metabolic versatility contributes to broad ecological adaptability and distribution and reflects a genome of larger size and complexity compared with that of many other bacteria species (Stover *et al*., 2013).

They are infrequently found as part of the human microflora in healthy individuals, widespread in natural environments, and serves as an opportunistic pathogen causing diseases in vulnerable individuals such as immuno-compromised, those whose host defenses have been breached, such as burn patients and infants in whom the immune system has not yet developed (Hu *et al*., 2012). *Pseudomonas aeruginosa* is an important nosocomial pathogen, they are gram-negative motile bacillus which is invasive, toxigenic, and produces pyocin (Gaynes, 2015).

Pseudomonas aeruginosa has been known to cause a broad spectrum of diseases such as urinary tract infections, burns, respiratory infections, septicemia, and it is the primary cause of ventilator-associated pneumonia.

However, the organisms have been reported to be an important cause of healthcare-associated infections particularly among patients and infants in neonatal intensive care units (Bouza *et al*., 2013).

In recent years, nosocomial infections caused by *Pseudomonas aeruginosa* have been recognized as an acute problem in hospitals due to its intrinsic resistance to many antibiotic classes and its capacity to acquire practical resistance to all effective antibiotics (Gaynes, 2015), together with the spread of these bacteria in hospital personnel, hospital equipment, wet places, sinks, mops, disinfectant solutions, respiratory equipment, food mixers and other moist environments within hospitals (Gaynes, 2015).

Unfortunately, the ability of the aforementioned to act as reservoirs for *Pseudomonas aeruginosa* within hospital settings remain worrisome, as it reduces the quality of healthcare systems, in addition to the fact that *Pseudomonas aeruginosa* is ubiquitous in the environment thereby making the sources of its outbreak difficult to identify.

Constant bacteriological monitoring of the pathogens isolated from clinical specimens from patients in special units is necessary to draw the attention of clinicians and infection control specialists to their current susceptibility pattern and how often specific pathogens are isolated (Breidenstein *et al*., 2011).

This will form the bedrock of appropriate surveillance studies in such settings that would lead to developing, implementing, and monitoring the impact of interventions such as the event-based, mutually agreed guidelines for the empirical antimicrobial therapy of common pathogens, effective infection control, and public health guidelines (Karlowsky *et al*., 2002).

However, it is on this basis that this research work is focused on evaluating *Pseudomonas aeruginosa* and its possible threat to the quality of healthcare systems.

2. EXPERIMENTAL (MATERIALS AND METHODS)

2. 1. Study area

This study was carried out in the University of Calabar Teaching Hospital, Calabar.

2. 2. Collection of specimen/Sample collection

Multiple environmental swabs using swab sticks were collected under aseptic conditions from various sites of Intensive Care Units (ICU) wards including patients' tables, gloves, skin walls, hospital staff (hand swab), and hospital instruments (stethoscopes and ventilators) at the University of Calabar Teaching Hospital (UCTH).

The swab samples were then transported to the Microbiology Department laboratory at the University of Calabar for further processing, following standard microbiological procedures as described by Cheesbrough (2002) and reinforced in recent guidelines (Nicholas *et al*., 2021).

2. 3. Materials

The materials used in this research included glassware such as Petri dishes, measuring cylinders, conical flasks, test tubes, pipettes, glass slides, McCarthy bottles, Durham tubes, catty slides, sterile cotton wool, swab sticks, disinfectants, a microscope, oil immersion, and an autoclave

2. 4. Culture media and reagents

All the culture media used were prepared according to the manufacturer's instructions. These included nutrient agar, blood agar, and MacConkey agar. The reagents used included Korac's reagent, physiological saline, and peptone water, as well as tetramethyl-phenyl-diamine di-hydrochloride and Gram's reagents, including crystal violet, Lugol's iodine, acetone or ethanol, and safranin

2. 5. Sterilization of glassware

All the glassware used in this study were washed, rinsed with sterile water, and sterilized in a hot air oven for two hours at a temperature of 180 °C. This included Petri dishes, test tubes, conical flasks, pipettes, beakers, measuring cylinders, and Durham tubes.

Additional materials used included spatulas, foil paper, wire loops, wooden tongs, masking tape, Pasteur pipettes, and slides. The sterilization processes were carried out using the Sukmantara *et al*. (2024) method.

2. 6. Antibiotics sensitivity test

Antibiotics sensitivity test was carried out following the method of Kiranmai *et al*. (2022). In brief, commercially prepared antibiotic discs were used for this test.

They contained the following antibiotics: PEF (Pefloxacin) 10 µg, GN (Gentamicin) 10 µg, APX (Ampiclox) 30 µg, Z (Zinnacef) 20 µg, AM (Amoxicillin) 30 µg, R (Rocephin) 25 µg, CPX (Ciprofloxacin) 10 µg, S (Streptomycin) 30 µg, SXT (Septrin) 30 µg, and E (Erythromycin) 10 µg.

2. 7. Microscopic examination

A smear of each sub-cultured colony was prepared on a glass slide and stained using Gram's method. It was then observed under an oil immersion objective (40x) for identification. The method described by previous studies (Isenberg *et al*., 2011) was used to ensure accuracy.

2. 8. Preparation of stock cultures

Sterile nutrient agar was prepared and poured into sterile McCarthy bottles. These bottles were allowed to settle in a slanted position. Pure cultures of the isolates were streaked onto the surface of the slants and incubated for 24 hours. After incubation, the slants were stored in a refrigerator at 14 °C for future analysis (Nicholas *et al*., 2021).

2. 9. Characterization and identification of isolates

The cultural characteristics examined included shape, size, color, surface elevation, and edge morphology. These were observed using a microscope, and the various morphological types were recorded, following protocols described by Isenberg *et al*., (2011) and updated methodologies (Nicholas *et al*., 2021).

2. 10. Gram stain

The Gram stain test was used to differentiate between Gram-positive and Gram-negative bacteria, providing detailed cellular characteristics of the bacteria under the microscope. The process followed standard staining techniques outlined in recent research (Chen *et al*., 2023).

2. 11. Catalase test

Aerobic bacteria produce varying levels of catalase enzymes that break down hydrogen peroxide into water and oxygen. This test was carried out according to procedures updated by recent studies (Stefani *et al*., 2024).

2. 12. Oxidase test

This test was used to differentiate between oxidase-producing and non-oxidase-producing organisms. A 24-hour-old culture was used for the test. The method was based on the procedure described in recent literature (Miller *et al*., 2023).

2. 13. Motility test (Hanging drop method)

This test demonstrated the motility of microorganisms due to the possession of flagella. The method used was described by Jones *et al*. (2021).

2. 14. Citrate Test

This test was used to identify some members of the Enterobacteriaceae family based on their ability to utilize citrate as their sole carbon source and ammonia as their sole nitrogen source.

Simmons' citrate agar was prepared with the pH indicator bromothymol blue, which changes from green to blue when the medium becomes alkaline, indicating a positive result.

3. RESULTS

3. 1. Morphological characteristics of test isolates

Table 1 shows the morphological characteristics of the bacterial isolate obtained. These include large, opaque, irregular colonies and iridescent patches with slender rods, non-capsulated Gram-negative reactions as its microscopic features, which indicate *Pseudomonas aeruginosa.*

Table 1. Morphological characteristics of test isolates.

Culture Appearance	Microscopic feature	Probable Isolate
Large, opaque, irregular colonies, iridescent	Slender Rods Non-capsulated	*Pseudomonas aeruginosa*
Patches	Gram Negative	

3. 2. Total heterotrophic microbial counts of the isolated *Pseudomonas aeruginosa*

Table 2 presents the total heterotrophic microbial counts in CFU/g of the isolated *Pseudomonas aeruginosa* from the University of Calabar Teaching Hospital. From the results obtained, the male ward had the highest bacterial count of 4.8±0.24, followed by the general outpatient ward with 3.6±0.18, and the female ward had the lowest count of 2.2±0.11.

Table 2. Mean count of the isolated *Pseudomonas aeruginosa*

Hospital Unit	Total Heterotrophic Microbial Count (cfu/g)
Male ward	4.8 ±0.24
Female ward	2.2 ±0.11
General outpatients	3.6 ±0.18

Key: ± = Standard error

3. 3. Distribution of *Pseudomonas aeruginosa*

Out of 201 clinical samples analyzed, the total number of *Pseudomonas aeruginosa* isolates obtained was 53, as indicated in Table 3. The results show that from inpatients: sink swab 15 (28.30%), followed by hand swabs 12 (22.64%), then tabletop swab 10 (18.87%), glove swab 9 (16.98%), and stethoscopes had the lowest frequency and percentage of occurrence at 7 (13.21%). For outpatients: sink swab 25 (27.47%), hand swab 22 (24.18%), tabletop swab 18 (19.78%), glove swab 14 (15.38%), and stethoscopes had the lowest frequency and percentage of occurrence at 12 (13.19%).

Table 3. Distribution table showing *Pseudomonas aeruginosa* across sampling locations: inpatients and outpatients

Source of sample	Inpatients	Outpatients
Sink	15(28.30%)	25(27.47%)
Hand swab	12(22.64%)	22(24.18%)
Table top swab	10(18.78%)	18(19.78%)
Glove swab	9(16.98%)	14(15.38%)
Stethoscope	7(13.21%)	12(13.19%)
Total	53	91

3. 4. Biochemical test

Table 4 shows all the biochemical tests carried out for further identification and characterization of the bacterial isolate. These tests are consistent with those used in similar studies of *P. aeruginosa* characterization (Clinical and Laboratory Standards Institute, 2022).

Table 4. Biochemical tests of *Pseudomonas aeruginosa.*

Biochemical tests	Results
Cell shape	Rod
Gram's reaction	-
Motility	+
Oxidase test	+
Indole test	-
Citrate test	+
Catalase test	+

Key: Positive = +, Negative = -

3. 5. Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* according to source of specimens

The antibiotic susceptibility profile of *Pseudomonas aeruginosa* varied greatly depending on the antibiotics tested, as shown in Table 5. Rocephin was the most effective drug, with 5

(28.30%) isolates being sensitive to it, followed by Pefloxacin, which had 11 (20.75%) sensitive isolates. Gentamicin showed sensitivity in 5 (8.43%) isolates, Ciprofloxacin in 5 (9.43%), Septrin in 4 (7.55%), Erythromycin in 4 (8.77%), Ampiclox in 2 (3.77%), Zinnacef in 2 (3.77%), and Amoxicillin in 2 (3.77%) isolates. Out of a total of 15 isolates from sinks, 4 (33.33%) were sensitive to Rocephin, 3 (25.00%) isolates were sensitive to Pefloxacin, and 1 (8.33%) strain was sensitive to Gentamicin, Ciprofloxacin, Streptomycin, Septrin, and Erythromycin each.

Table 5. Antibiotics susceptibility profile of *P. aeruginosa* based on source of specimen.

Source of specimen	**No. of *Pseudomonas aeruginosa***	**Susceptibility profile of p aeruginosa**						
		R	PEF	CN	CPX	S	SXT	E
Sink	5	4(33.3)	3(3.25.00)	1(8.33)	1(8.33)	1(8.33)	1(8.33)	1(8.33)
Hand	2	4(26.67)	2(13.33)	1(13.33)	2(13.33)	1(13.33)	1(13.33)	1(13.33)
Tabletop	0	3(30.00)	2(20.00)	1(10.00)	1(10.00)	1(10.00)	1(10.00)	1(10.00)
Gloves		2(22.22)	2(22.22)	1(11.11)	1(11.11)	1(11.11)	1(10.00)	1(10.00)
Stethoscope		2(28.30)	2(28.57)	1(14.29)	1(14.29)	1(14.29)	1(10.00)	1(10.00)

Key: R - Rocephin; PEF - Pefloxaxin; CN - Gentamycin; CPX - Ciprofloxacin, SXT - Septrin, S - Streptomycin, E - Erythromycin.

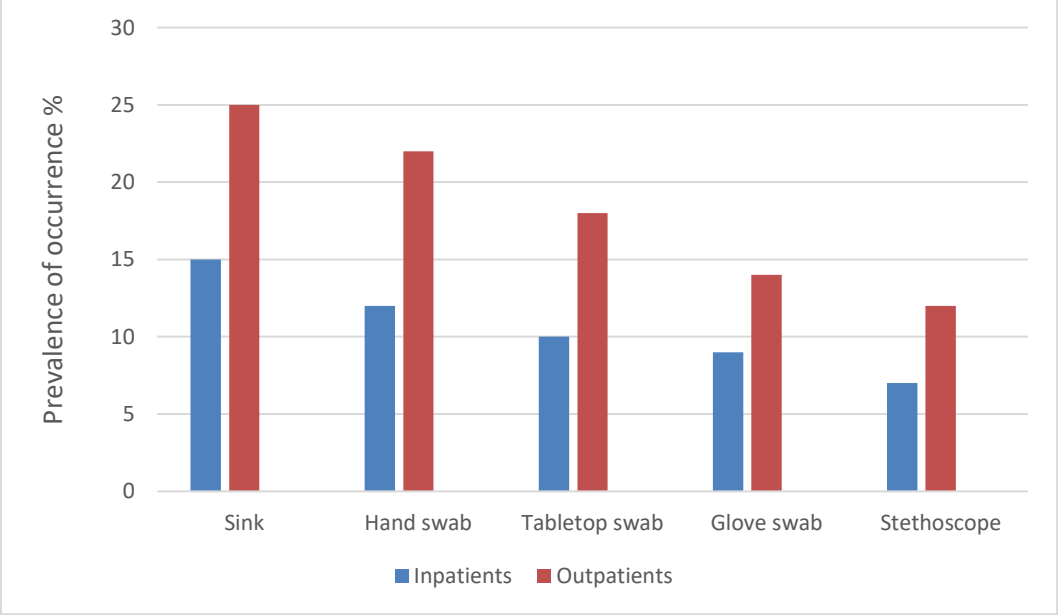

Figure 1. Shows the susceptibility pattern amongst inpatients and outpatients

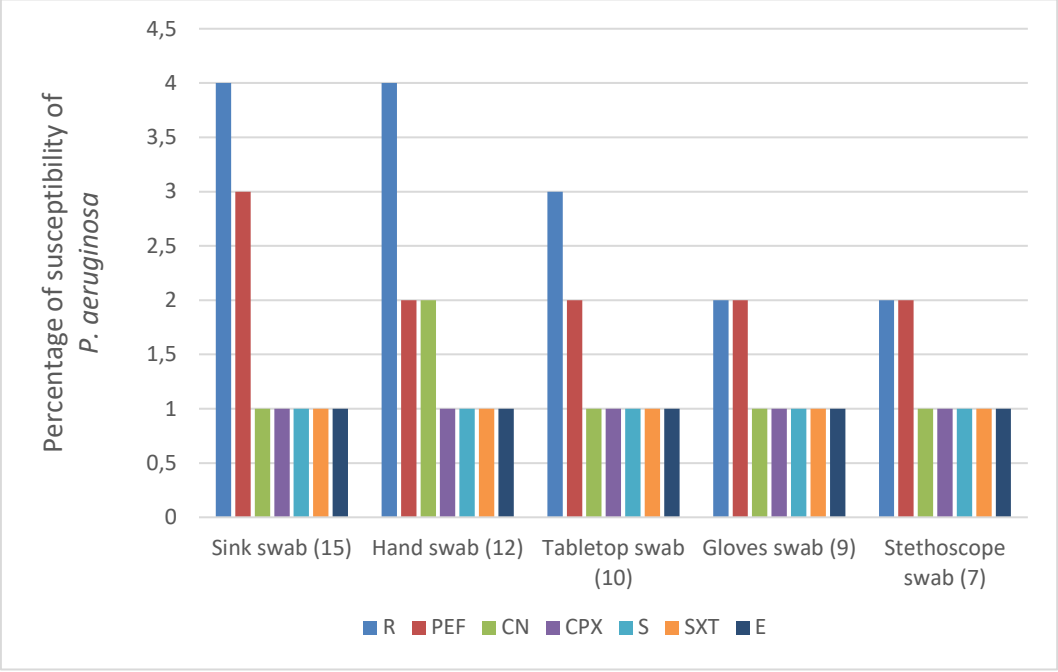

Figure 2. Shows the susceptibility pattern of *P. aeruginosa*
Key: R = Rocephin, PEF = Pefloxacin, CN = Gentamycin, CPX = Ciprofloxacin, SXT = Septrin, S = Streptomycin, E = Erythromycin.

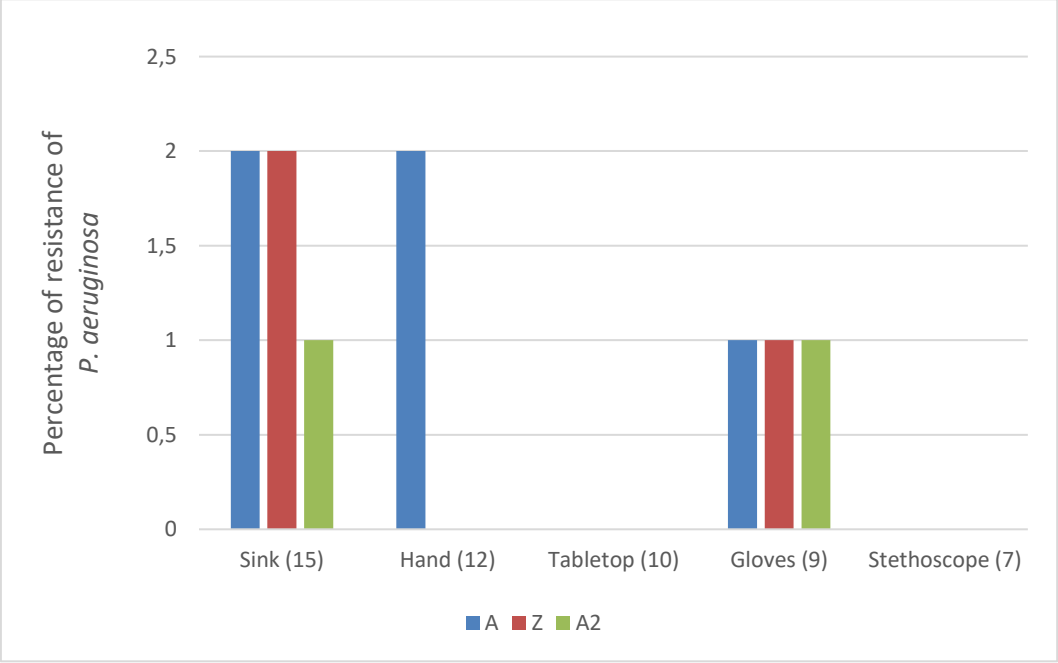

Figure 3. Bar chart showing the prevalence resistance pattern of *P. aeruginosa*
Key: A = Ampiclox**,** Z = Zinnacef, A = Amoxacillin

Table 6. Shows antibiotics resistance profile of *P. aeruginosa* from UCTH antibiotics resistance profile of *P. aeruginosa*

Source of specimen/no. of isolates	Resistance profile of isolates		
	A	**Z**	**A**
Sink (15)	2(3.77)	2(3.77)	1(2.78)
Hand (12)	2(33.3)	0(0.0)	0(0.0)
Tabletop(10)	0.(0.0)	0(0.0)	0(0.0)
Gloves (9)	1(20.0)	1(20.0)	1(25.0)
Stethoscope (7)	0(0.0)	0(0.0)	0(0.0)

Key: A - Ampiclox, Z - Zinnacef, A - Amoxacillin

4. DISCUSSION

Microorganisms are commonly attached to hospital environments and indwelling medical devices (such as urinary catheters, trolleys, tubing, and suction apparatus, among others) to form biofilms made up of extracellular polymers (Dulworth & Pyenson, 2012). The high frequency and percentage occurrence of *Pseudomonas aeruginosa* observed in the different intensive care units, hospital sites and equipment investigated was not surprising, as this observation corroborates with reports from similar research. Hossein *et al*., (2012) reported to have isolated *Pseudomonas aeruginosa* from hospital means and hospital personnel in a selected hospital in Iran, Jefferies *et al*., (2012) also reported having identified *Pseudomonas aeruginosa* outbreaks in the neonatal intensive care unit at University Hospital Southampton. A similar study by Olayinka *et al*., (2014) reported a high prevalence rate of *Pseudomonas aeruginosa* in the Federal Medical Centre Makurdi, General Hospital and Gboko, General Hospital Otukpo and General Hospital North Bank, Makurdi.

Pseudomonas aeruginosa is a ubiquitous microorganism that could affect individuals with immunocompromised situations and are responsible for nosocomial infection (Yang *et al*., 2011). It has not only metabolic versatility and remarkable ability to adapt and colonization in a wide variety of ecologic environments but also its intrinsic ability to resistance to wide variety of antimicrobial agents as well as its mucoid form of adaptation mechanism in surviving in environments that are concerned to polysaccharide net as called alginate (Nseir *et al*., 2012. The high prevalence *Pseudomonas aeruginosa* in the intensive care units of this selected hospital studied was not surprising, as this observation was in line with that of Jarlier *et al*., (2014) who reported a higher incidence of *Pseudomonas aeruginosa* (52.35%) in ICU studied.

Also Naze *et al*., (2010) in their studies, reported nosocomial outbreaks of *Pseudomonas aeruginosa* colonization or infection of infant in neonatal intensive care units from 17 different hospitals. Intensive care patients are more prone to infection because of the debilitating effect of a prolonged hospitalization and instrumentation. Intensive care units are generally considered epicenter of multi drug resistant (MDR) organisms, with the most important risk

factors been excessive use of antibiotics exerting selective pressure on bacteria, the frequent use of invasive devices and relative density of immuno-suppressed patient population with severe underlying diseases (Ramprasad *et al*., 2010). In support of the aforementioned observations in this study, various studies reviewed have provided evidence that *Pseudomonas aeruginosa* can be introduced into hospital intensive care units via several routes, including environmental contamination, transmission by healthcare workers, transfer of colonized patterns and through the use of contaminated water to prepare milk or other nutrition (Hu *et al*., 2010). In this study a high prevalence of *Pseudomonas aeruginosa* was observed in various sites and instruments (floor, nurse hands gloves, and patient trolley, patient sinks) used in this selected hospital. In support of this observation, Grasle-Guen *et al*., (2013) reported hospital water baths and pasteurizers used to sterilize milk to be possible reservoirs of *Pseudomonas aeruginosa*.

Also, Zabel *et al*. (2004), reported that hospital humidifying of equipment for ventilators are possible reservoirs of *Pseudomonas aeruginosa*. In support of this, various researchers have reported *Pseudomonas aeruginosa* to be primarily an environmental organism that is adapted to survive in numerous conditions and is particularly well adapted to wet conditions. Nevertheless, environmental reservoirs such as sinks have the potential to lead to outbreaks. An outbreak due to splash back from contaminated sink drains was reported from the ICU and transplant unit of a Canadian hospital in 2009 (Hota *et al*., 2009). The high prevalence rate of *Pseudomonas aeruginosa* observed in the ICU, different sites, and instruments in the investigated hospitals were worrisome, as the pathogen has been implicated with numerous disease conditions ranging from pneumoniae, bacteremia, urinary tract infections, meningitidis, among others (Hota *et al*., 2009). These findings align with microbial distribution patterns in clinical settings as reported in recent studies (Perinbam *et al*., 2020). Nowadays, the prevalence of multidrug-resistant strains of *Pseudomonas aeruginosa* is observed mainly in hospital-acquired infections due to selective pressure exerted on bacteria by over-usage of broad-spectrum antibiotics (Jones, 2011).

The results obtained from this study showed a percentage rate of resistance to antibiotics by *P. aeruginosa*. These antimicrobial susceptibility results reflect patterns reported in recent antimicrobial resistance studies in clinical settings (Kumar *et al*., 2021). This resistance to some antibiotics may be due to the permeability barrier provided by its outer membrane, which makes the organism impervious to therapeutic concentrations of antibiotics. Of all the (10) antibiotics used for this study only Pefloxacin, Septrin, Ciprofloxacin, Gentamycin, Streptomycin, Rocephin, and Erythromycin, were found to be effective against the isolates. This somehow supports the assertion by (Anyanwu *et al*., 2023), that *P. aeruginosa* is naturally susceptible to aminoglycosides (Gentamycin) and quinolones (Ciprofloxacin). However, acquired antibiotic resistance of *P. aeruginosa* during treatment is a very common phenomenon. All the isolates obtained in this study were found to be resistant to about (3) antibiotics.

This therefore reveals the resistance *P. aeruginosa* strains within the hospital environment. The trend of resistance is increasing with time and this may be due to the indiscriminate use of antibiotics by patients either within the hospital or their homes. This is because the emergence of a resistant population of organisms from previously sensitized ones depends on several factors such; as the initial organism present, the frequency of exposure to the drug, concentration of the drug. Also, resistance to this opportunistic human pathogen may be due to the presence of drug resistance plasmid, the outer membrane, and mutation caused by the use of excessive drugs by the population.

(Bouza *et al*., (2013) advise that in order to prevent the emergence of multidrug-resistant *P. aeruginosa* strains, it will be necessary to improve the administration of antibiotics in the treatment of infections, and employ the methods of the determination of phenotype and genotype makers, to evaluate and control the source and prevalence of multiple resistant strains in hospitals.

5. CONCLUSION

P. aeruginosa is a highly adaptable and versatile organism. This reveals its ability to contaminate and grow in a wide variety of substrates and various anatomical sites causing diverse infections. It is intrinsically resistant to a wide range of antimicrobial agents and this study reveals the prevalence resistant *P. aeruginosa* strains within UCTH Calabar. One should always remember that the spread of resistant organisms from patients to patients can be reduced by appropriate infection control measures. The results of this study are not generalizable to organisms other than *Pseudomonas aeruginosa*.

From the study, it could be recommended that policies governing the antimicrobial use in the country be formulated, ensuring proper sterilization of hospital equipment, and maintaining improved environmental hygiene to reduce contamination and the spread of resistant strains. The findings emphasize the need for careful antimicrobial prescription especially in *P. aeruginosa* in hospital settings.

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