

## **Antibiogram of Pathogenic *Pseudomonasaeruginosa* Isolated from Hospital Environment**

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### **ABSTRACT**

*Pseudomonas aeruginosa* is an aerobic, non-fermentative, gram negative Bacillus belongs to the family Pseudomonadaceae. It is also a non-sporing, motile with polar flagellum, and straight or slightly curved rod-shaped bacterium that occurs as a single bacterium or in pairs and occasionally in short chains. Its antibiotic resistance to various drugs is of a major clinical importance. A total of fifteen (15) samples were collected individually from the various ward at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku, Awka, (COOUTH) using sterile swab sticks. The swab samples collected were properly labeled as sample 1 to sample 15 and were analyzed at Microbiology Laboratory of Nnamdi Azikiwe University, Awka, Nigeria. Isolation of *Pseudomonas aeruginosa* was carried out using a Cetrimide agar. Antimicrobial susceptibility patterns of the Isolated *Pseudomonas aeruginosa* was determined using specific antibiotics (Pefloxacin (10µg), Gentamycin (10µg), Amoxicillin (30µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septrin (30µg), Tarivid (30µg), Chloramphenicol (30µg), and Sparfloxacin (10µg) in which the various zones of inhibition of each antibiotic was measured and their results recorded. *Pseudomonas aeruginosa* isolated were more susceptible to Sparfloxacin (10µg) but resistant to Gentamycin (10µg) and Septrin (30µg).

**Keywords:** Antibiogram, *Pseudomonasaeruginosa*, Hospital, Antibiotic,

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Date of Submission: 09-03-2024

Date of acceptance: 23-03-2024

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### **I. INTRODUCTION**

In the second half of the last century, *Pseudomonas aeruginosa* has become an important hospital pathogen (Gretae *et al.*, 2007). It needs minimal nutritional requirements for growth. It is a commensal in healthy people and the rate of commensalism increases gradually with the increased duration of hospital stay (Altopark *et al.*, 2004). This bacteria is frequently isolated as an opportunistic pathogen in recurrent infections of hospitalized patients and has been isolated from a number of sites in the hospital environment (Shaheheragiet *et al.*, 2003; Vianelli *et al.*, 2006). Representative colonization rates for specific sites in humans are 0- 2% on skin, 0-3.3% for nasal mucosa, 0-6.6% for throat and 2.4-2.6% for faecal samples (Morrison and Wenzel, 1984). However, colonization rates may exceed 50% during hospitalization, especially among patients who have experienced trauma to or breached cutaneous or mucosal barriers by mechanical ventilation, tracheostomy, catheters, surgery or severe burns. Disruption in the normal microbial flora as a result of antimicrobial therapy has also shown to increase colonization with *P.aeruginosa* (Blanc *et al.*, 1998). *Pseudomonas aeruginosa* is the most important, resistant and dangerous organism infecting the burn patients (Estahbanatiet *et al.*, 2002). *Pseudomonas aeruginosa* is an aerobic, non-fermentative, gram negative Bacillus belonging to the family Pseudomonadaceae. It is non-sporing, motile with polar flagellum, and straight or slightly curved rod-shaped bacterium that occurs as a single bacterium or in pairs and occasionally in short chains. It is the most common bacteria routinely encountered in different clinical sample from human as well as in hospital and community based environment like water, soil, air, plant surfaces, medical equipments, moist environment. It is highly prevalent and persistent in hospital environment due to its ability to resist variety of antibiotics, physical conditions like temperature, high concentration of salts and antiseptics and also the nominal nutritional requirement (Ananthanarayan *et al.*, 2013; Koneman *et al.*, 1992; Okonet *et al.*, 2009; Remington *et al.*, 1989). It can grow in bottles containing disinfectants like quaternary ammonium compound, chloroxylenol and hexachlorophene due to its tendency to show resistant against all such disinfectants (Ananthanarayan and Jayaram, 2013). *P. aeruginosa* is a ubiquitous organism (Ananthanarayan and Jayaram, 2013) and human acquired infection from environment and very rarely transmitted from person-to-person (Anthony *et al.*, 2002). 10% -22.10% of nosocomial infections like bacteremia and septicemia in ICU patients, respiratory infection in cystic fibrosis, urinary tract infections, burns and other wound infection are caused by *P. aeruginosa* (Carmeliet *et al.*, 1999). It is numbered as the fourth common cause of nosocomial infections in the United States. It also

produces community acquired infection like otitis media, infantile diarrhoea and Shanghai fever (Ananthanarayan and Jayaram, 2013).

The term 'nosocomial infection' is usually restricted to the infections that patients acquire in the hospital, but has importance in occupational medicine as well. Nosocomial infection or hospital-acquired infections (HAIs) are one of the most important problems in the worldwide. These infections are more dangerous than other infections because they are caused by bacteria have a high resistance to antibiotics. These infections are an important cause of increased morbidity, mortality and health care costs worldwide (Maheswaran *et al.*, 2007). According to a study cited by the WHO (World Health Organization), over 1.4 million people worldwide suffer from HAI at any given time (Ducel *et al.*, 2002). The risk of nosocomial infection depends on a number of factors. These include the ability of pathogens to remain viable on a surface, the rate at which contaminated surfaces are touched by patients and healthcare workers, the context in which the patient is exposed, and the levels of contamination that result in transmission to patients (Boyce, 2007). For many healthcare-associated pathogens the degree of hospital surface contamination is closely correlated with the risk of transmission (Nseir *et al.*, 2011).

An antibiotic agent is a substance that kills (bactericidal) or inhibits (bacteriostatic) the growth of bacteria. They can also be classified based on their mode of action as follows; inhibitors of cell wall synthesis, inhibitors of protein synthesis, inhibitors of nucleic acid synthesis, inhibitors of metabolic pathways and inhibitors of cell membrane functions. The use of antibiotic agent medicines to treat infection is known as antibiotic agent chemotherapy, while the use of antibiotic agent medicines to prevent infection is known as antibiotic agent prophylaxis. Antibiotic agent that are effective only against a limited variety of bacteria are said to be narrow-spectrum drugs, example; gentamycin and penicillin are narrow-spectrum antibacterial drugs because they are effective only against Gram-negative and Gram-positive bacteria respectively. On the other hand, those that attack many different kinds of bacteria are known as broad-spectrum drugs, example; trimethoprim is a broad-spectrum antibacterial drug because it is effective against both Gram-positive and Gram-negative bacteria. Antibiotic agents can be synthesized by microorganisms (natural antibiotics) or manufactured by chemical procedures independent of microbial activity (synthetic antibiotics) and they can also be semi-synthetic that is natural antibiotics that have been chemically modified to make them less susceptible to inactivation by pathogens (Michigan State University Board of Trustees, 2011).

Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates (Koneman, 2006). Overall prevalence in US hospital was approximately 4 per 1000 discharge and leading cause of high morbidity and mortality (Okonet *et al.*, 2009). The overall prevalence of antibiotic resistant *P. aeruginosa* is increasing, with up to 10% of global isolates found to be multi-drug resistance. It is recognized as the second leading cause of gram negative nosocomial infection and a major treatment challenge for *Pseudomonas aeruginosa* (Nasreen *et al.*, 2015).

A general problem with nosocomial infections is the tendency of nosocomial pathogens to acquire new antibiotic resistance. Thus, infections caused by *P. aeruginosa* are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs. As an opportunistic infectious pathogen, *P. aeruginosa* can often lead to life-threatening diseases. Being gram-negative bacteria, *Pseudomonas* spp. are naturally resistant to penicillin and majority of related beta-lactam antibiotics, but a number are sensitive to Imepenem, Piperacillin+Tazobactam, Tobramycin, Polymyxin B. Overall effect of *P. aeruginosa* is due to Multidrug-resistant (MDR) nature. Its general resistance is due to a combination of factors. It is intrinsically resistant to antimicrobial agents, due to the low permeability of its cell wall and various mechanisms like multi-drug resistance, Efflux pumps, biofilm formation and production of  $\beta$ -lactamases (The high resistant to cephalosporins may be due to production of extended spectrum beta lactamases 'ESBL's)) and aminoglycoside modifying enzymes. It also has the genetic capacity to express a wide repertoire of resistance mechanisms through mutations in the chromosomal genes which regulate the resistance genes and can acquire additional resistance genes from other organisms through plasmids, transposons and bacteriophages (Betty *et al.*, 2007) as shown in fig.3. The carbapenems have been drug of choice for treatment of serious infections caused by Gram negative bacteria. However, carbapenem resistance has been observed frequently in *P. aeruginosa*. Resistance to carbapenems is predominantly mediated by MBL's i.e metallo-beta-lactamases that recognize bivalent metal ions, usually zinc for their activity (Gales *et al.*, 2001). In the absence of carbapenem hydrolyzing carbapenamases; mainly MBL's, resistance is usually multifactorial. Increased production of Amp C encoded cephalosporinase, reduced outer membrane porin Opr D expression, increased expression of efflux pump mechanisms are known to contribute to carbapenem resistance (Juan *et al.*, 2005; Livermore, 1992) as shown in fig.2. Success of antimicrobial therapy depends on the appropriateness of the

choice of antibiotics that should be used on the basis of prior knowledge of the susceptibility pattern of the agent.

This study is aimed at determining the Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Hospital Environment. Thus, the significance of this research is to understand the sensitivity (effectiveness of an antimicrobial drug) and/or resistance of these isolated microbes to the various antimicrobial drugs used in times of the occurrence of nosocomial illness caused by the exposure to Hospital environmental-related *Pseudomonad* infection/disease.

## II. MATERIALS AND METHODS

### 3.1. Study Area

Awka the capital of Anambra State of Nigeria and a pre-colonial city situated on latitude 6° 12' 15.00" Nand longitude 7° 04' 2.40" E. It lies within the rainforest area but is now classified within the Guinea Savannah because of its derived vegetation, as the original vegetation has been removed by man. The mean annual rainfall is about 1524 mm with a relative humidity of 80% at dawn. Its location and its climatic characteristic high ambient temperature and rainfall add to the problem of waste management because of rapid rate of putrefaction with its attendant odour. Awka is about 40 km on the direct route from Onitsha with East–West extension of 8 km on Enugu- Onitsha express way corridor (Okonkwo, 2014). Amongst the various Hospitals in Awka metropolis, the one worked on with regards to this research work is Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku, Awka (COOUTH).

### 3.2. Sample Collection

Samples were collected at random from various hospital wards, including, the intensive care unit (floor, the respirator, and the bed), male surgical ward (window, the floor, and the side table), female surgical ward (door handle, the bed rail, and the curtain), theatre (the bed, the floor and air condition), and the delivery room (the bedsheet, the mop). Samples were collected using swabsticks by random sampling from four different wards in the different sections of the hospital. A total of fifteen (15) samples were collected individually from the hospital environment. Samples were properly labelled, sealed and transported to Microbiology Lab of Nnamdi Azikiwe University, Awka for bacterial analyses. All samples were transported to the laboratory for analyses immediately after collection.

### 3.3. Isolation of Microorganisms

One (1) g of distilled water is added into each swab stick and properly agitated to allow the microbes obtained on the swab sticks to be dislodged into the water, and aseptically, a 10-fold serial dilution was performed to obtain 10<sup>-4</sup> dilution. 0.1ml of each dilution (10<sup>-4</sup>) was inoculated onto Cetrimide agar medium using pour plate method and incubated at 37°C for 18-24 hours. Discrete colonies were obtained by streaking. Where the isolate was picked and stored on agar slants at 4°C.

### Characterization and Identification of bacteria

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Chessbrough, 1984). The characterization of the isolates were performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, Methyl Red and Voges proskauer test as described by Bergey's Manual of Determinative Bacteriology, 9th edition (1994).

### Gram reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolorized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (× 100). Purple colour indicated Gram-positive organisms while red or pink colour indicated Gram-negative organisms.

### Catalase test

Exactly 3ml of 3% solution of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was transferred into a sterile test tube. Then, 3 loopful of a 24 hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

### **Motility test (Hanging Drop Method)**

A loopful of 18-24 hour broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound microscope. Care was taken to not interpret “drift” or “Brownian motion” as motility. Results were recorded as motile or non-motile.

### **Oxidase Test**

All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

Whatmann No.1 filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. The filter paper was moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared in the filter paper. The inoculum was observe the area around the inoculated paper for a color change to deep blue or purple within 10-30 seconds. Positive and negative quality controls were also set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*). **Positive** was indicated by development of dark purple color (indophenols) within 10 seconds. **Negative:** Absence of color.

### **Urease Test using Christensen’s Urea Agar**

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive *Proteeae* from other *Enterobacteriaceae*.

Heavy inoculum from an 18- to 24-hour pure culture was used to streak the entire **Christensen’s Urea Agar** slant surface. Adequate care was taken not to stab the butt as it will serve as a color control. The tubes were incubated loosened caps at 35 °C. The slants were observed for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production would be indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was also set up. Rapidly urease-positive *Proteeae* (*Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish color if the organism is urease negative

### **Indole Test**

A loopful of an 18-24 hour culture was used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation was done at 35–37 °C first for 24 hours and further for up to 48 hours. Test for indole was done by adding 0.5 ml of Kovac’s reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

### **Methyl Red test**

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

### **Voges-Prausker test**

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR-VP broth was aseptically transferred into a sterile test tube. Then 0.6ml of 5%  $\alpha$ -naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that this reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

### Citrate test

A 24h old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

### Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

### 3.6 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing on all the isolates was done using disc diffusion method. For bacterial isolates, sensitivity disks containing conventional antimicrobials which are Pefloxacin (10µg), Gentamycin (10µg), Amoxicillin (30µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septrin (30µg), Tarivid (30µg), Chloramphenicol (30µg), and Sparfloxacin (10µg). Antimicrobial activity screening was conducted using the modified agar well diffusion method, following the procedure outlined by (Agu *et al.*, 2013; Agu *et al.*, 2014; Awah *et al.*, 2016; Adindu *et al.*, 2017; Awah *et al.*, 2017; Saeed and Tariq, 2017; Ubaoji *et al.*, 2020). Duplicate plates of Mueller-Hinton Agar (MHA) were prepared, with 20 ml poured into each sterile Petri dish and allowed to solidify. Subsequently, each plate was inoculated with 0.1 ml of the suspension of isolates obtained from an overnight broth culture. The turbidity of the suspension was adjusted to 0.5 of the McFarland scale, equivalent to approximately  $1 \times 10^8$  cfu/ml. The inoculum was evenly spread across the agar surface and allowed to air-dry for 30 minutes. Three holes were aseptically made approximately 1 cm away from the edges of each plate using a sterile cork borer with a diameter of 6 mm. The extracts were reconstituted and the aqueous extracts were dissolved in distilled water, while the ethanol extracts were dissolved in 6% dimethyl sulfoxide (DMSO). A stock solution with a concentration of 100 mg/ml was prepared by dissolving 1 g of each plant extract in 10 ml of the respective diluent. Next, the agar wells were filled with 0.01 µl of the extract solution at different concentrations, including 50 mg/ml, 25 mg/ml, and the last well with only the diluent to serve as the control. This entire process was performed in duplicates. The Petri dishes were then incubated at 37°C for a duration of 16 to 18 hours. After incubation, the zones of inhibition around each well were measured using a transparent meter rule and recorded, to the nearest millimeter (mm).

## IV. RESULTS

### 4.1 Colony Count of the *Pseudomonas aeruginosa* Isolates.

Colony count of the *Pseudomonas aeruginosa* isolated from samples collected from Chukwuemeka Odumwenu Ojukwu University Teaching Hospital, Amaku, Awka, (COOUTH) were recorded. The results are represented below using bar charts in figures 1 and 2:

**TABLE 2:** Colony Count of *Pseudomonas aeruginosa* after 24hr Incubation of Samples Obtained from COOUTH

S/N	Hospital ward	Sample collection site	Sample number (labelling)	Total Pseudomonas count (cfu/ml)
1	Intensive Care Unit	Floor	1	TFTC
		Respirator	2	TFTC
		Bed	3	$3.07 \times 10^5 \pm 1.15$
2	Male surgical ward	Window	4	TFTC
		Floor	5	$4.60 \times 10^5 \pm 0.58$
		Side table	6	$3.27 \times 10^5 \pm 1.00$
3	Female surgical ward	Door	7	TFTC
		Bed rail	8	$3.13 \times 10^5 \pm 0.58$
		Curtain	9	TFTC
4	Theatre	Bed	10	TFTC
		Floor	11	$3.43 \times 10^5 \pm 0.58$
		Air condition	12	$3.23 \times 10^5 \pm 0.58$
5	Delivery room	Bed sheet	13	$3.13 \times 10^5 \pm 0.58$
		Cleaning mop	14	$6.10 \times 10^5 \pm 1.00$
		Nurse's tray	15	TFTC



## VI. CONCLUSION

Despite its significance to the society or community, hospital as seen in this project work and many other related work, are niche for countless microorganism of medical importance such as *Pseudomonas aeruginosa* because different sick people go to the hospital to be treated of their illnesses thereby depositing microbes in the environment. As earlier stated, these pathogenic microbes could be present on surrounding surfaces as a result of poor sanitary and hygiene practices during cleaning and disinfection process. Thus, this is a wake-up call for the hospital to use an effective disinfectant during cleaning routines so as to reduce the microbial load on their surrounding surfaces and also reduce the risk of infection of its personnel patients and visitors. With respect to the antibiotic susceptibility patterns of the isolate (*Pseudomonas aeruginosa*), results showed that most of the test antibiotics were effective against the isolate, with sparfloxacin being the most effective against *Pseudomonas*. Thus, sparfloxacin should be the first line of prescription in cases of hospital related infection and disease. It should be noted that *Pseudomonas* resistance was seen among drugs such as gentamycin and septrin respectively. Reducing cases of *Pseudomonas* resistance against antibiotic agents is very paramount and could be achieved if the most effective drug is administered during disease cases (that is after comparing the antibiotic susceptibility pattern), misuse and abuse of drugs is avoided and also by ensuring the completion of the administered dosage during treatment.

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