



*P.aeruginosa* is an opportunistic human pathogen. It is “opportunistic” because it seldom infects healthy individuals. Instead, it often colonizes immunocompromised patients, like those with cystic fibrosis, cancer, or AIDS (Botzenhardt and Doring, 1993). It is such a potent pathogen that firstly, it attacks up two third of the critically-ill hospitalized patients, and this usually portends more invasive diseases. Secondly, *P.aeruginosa* is a leading Gram-negative opportunistic pathogen at most medical centers, carrying a 40-60% mortality rate. Thirdly, it complicates 90% of cystic fibrosis deaths; and lastly, it is always listed as one of the top three most frequent Gram-negative pathogens and is linked to the worst visual diseases (Fick, 1993). Furthermore, *P.aeruginosa* is a very important soil bacterium that is capable of breaking down polycyclic aromatic hydrocarbons and making rhamnolipids, quinolones, hydrogen cyanide, phenazines, and lectins. It also exhibits intrinsic resistance to a lot of different types of chemotherapeutic agents and antibiotics, making it a very hard pathogen to eliminate (Lederberg, 2000).

## 2. Methodology

### Collection of samples

Samples of environmental materials (soil, waste water and air) from which *P. aeruginosa* was isolated were collected from Mechanic village, Auchi Edo State at different locations, of which 20 samples each of soil and water were collected while 20 plates of prepared nutrient agar were taken to the site to collect air samples, by exposing the plates in the mechanic pit and allowing air to filtrate the agar.

### Bacteriological Analysis

The collected microbial samples were transported to the laboratory following Cheesbrough, (2000) method. Samples were plated primarily onto nutrient agar and Mac conkey agar and the plates were incubated at 37°C for 24–48 hrs. The Suspicious isolates were presumptively identified by using colony morphology, pigment formation, mucoidy, haemolysis on blood agar, positive oxidase test, grape-like odour, growth at 42°C on nutrient agar, positive motility test, and Gram reaction (Cheesbrough, 2000).

Further, the *P. aeruginosa* isolates were confirmed by biochemical tests which include; oxidase, catalase, coagulase, indole, urease, methyl red, voges prokuer, citrate, triple sugar iron and sugar fermentation test.

### Antibiotic Susceptibility Testing

The agar disc diffusion method of Bauer (Bauer *et al*, 1966) modified based on National Committee for Clinical Laboratory Standards (NCCL, 2000) CLSI was followed to perform the susceptibility test for the *P. aeruginosa* isolates.

A small inoculum of each bacterial isolate was emulsified in 3ml sterile normal saline in bijou bottles and the turbidity compared with barium chloride standard (0.5 McFarland). A sterile cotton swab was dipped into the standardized solution of *P.aeruginosa* cultures and used to evenly inoculate Mueller-Hinton plates and the plates were allowed to dry. Thereafter, the clinically pertinent 12 antibiotic discs (Abtek Biologicals Ltd Liverpool, L9 7AR UK) with the following drug contents Augmentin (Aug), Cloxacillin (Cxc), Gentamicin (Gen), Amikacin (Amk), Ceftazidime (Caz), Cefuroxime (Crx), Ceftriazone (Ctr), Ciprofloxacin (Cpr), Ofloxacin (OfI), Erythromycin (Ery), Imipenem (Imp) and Meropenem (Mer) were placed on the plate. After 24 hrs, clinical interpretation [resistant (R), and sensitive (S)] of the size of the zone was evaluated based on the MIC susceptibility value as determined by the diameter from the zone of inhibition and compared with ATCC 27853 strain of *P. aeruginosa*.

## 3. Result

A total of 72 *Pseudomonas aeruginosa* isolates from environmental sources (waste water, soil and air) were obtained from different spots in mechanic village in Auchi area of Edo State as shown in table 1.

**Table 1:** Sources of Isolates

Sources	Number of samples collected	Number of positive
Waste water	50	15(30%)
Soil	50	20(40%)
Air	50	37(74%)
<b>Total</b>	<b>150</b>	<b>72(48%)</b>

The antibiotic sensitivity and resistance patterns of various source isolates are shown in Table 2 - 4. Twelve (12) most commonly used drugs for *Pseudomonas* infection were used for antibiotic susceptibility assay which are; Augmentin (Aug), Cloxacillin (Cxc), Gentamicin (Gen), Amikacin (Amk), Ceftazidime (Caz), Cefuroxime (Crx), Ceftriazone (Ctr), Ciprofloxacin (Cpr), Ofloxacin (OfI), Erythromycin (Ery), Imipenem (Imp) and Meropenem (Mer).

Table 2, shows sensitivity and resistance patterns of 15 *Pseudomonas aeruginosa* isolates from waste water. Out of 15 isolates, 2(13.33%) were sensitive to Augumentin, 4(26.67%) to Gentamycin, 10(66.67%) to Amikacin, 8(53.33%) to Ciprofloxacin, 6(40%) to Ofloxacin, 2(13.13%) to Erythromycin, 9(60%) to Imipenem and 12(80%) to Meropenem. While all the 15 isolates were resistants to Cloxacillin, Ceftazidine, Ceftriazone and Cefuroxime.

**Table 2:** Antibiotic resistance patterns of the 15 *Pseudomonas aeruginosa* isolates from the waste Water

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug)	13(86.67%)	2(13.33%)
	Cloxacillin (5ug)	15(100.0%)	0 (0%)
Aminoglycoside	Gentamycin (10ug)	11(73.33%)	4(26.67%)
	Amikacin (30ug)	5(33.33%)	10(66.67%)
Cephalosporin	Ceftazidime (30ug)	15(100.0%)	0(0.00%)
	Cefuroxime (30ug)	15(100.0%)	0(0.00%)
	Ceftriaxone (30ug)	15(100.0%)	0(0.00%)

Quinolones	Ciprofloxacin (5ug)	7(46.67%)	8(53.33%)
	Ofloxacin (5ug)	9(60.00%)	6(40.00%)
Macrolides	Erythromycin (5ug)	13(86.67%)	2(13.33%)
Carbapenems (β-Lactamase inhibitors)	Imipenem (10ug)	6(40.00%)	9(60.00%)
	Meropenem(10ug)	3(20.00%)	12(80.00%)

The sensitivity and resistance patterns of 20 *Pseudomonas aeruginosa* isolates from soil sample are shown in Table 3. The result shows that 5(25%) of the isolate were susceptible to Augmentin, 7(35%) to Gentamycin, 10(50%) to Amikacin, 1(5%) to Ceftazidime, 1(5%) to Ceftriazone,

10(50%) to Ciprofloxacin, 8(40%) to Ofloxacin, 1(5%) to Erythromycin, 14(70%) to Imipenem and 16(80%) to Meropenem. Cloxacillin and Cefuroxime were not effective against the 20 isolates.

**Table 3:** Antibiotic resistance patterns of the 20 *Pseudomonas aeruginosa* isolates from the Soil

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug)	15(75.00%)	5(25.00%)
	Cloxacillin (5ug)	20(100%)	0 (0.00%)
Aminoglycoside	Gentamycin (10ug)	13(65.00%)	7(35.00%)
	Amikacin (30ug)	10(50.00%)	10(50.00%)
Cephalosporin	Ceftazidime (30ug)	19(95.00%)	1(5.00%)
	Cefuroxime (30ug)	20(100.00%)	0(0.00%)
	Ceftriaxone (30ug)	19(95.00%)	1(5.00%)
Quinolones	Ciprofloxacin (5ug)	10(50.00%)	10(50.00%)
	Ofloxacin (5ug)	12(60.00%)	8(40.00%)
Macrolides	Erythromycin (5ug)	19(95.00%)	1(5.00%)
Carbapenems (β-Lactamase inhibitors)	Imipenem (10ug)	6(30.00%)	14(70.00%)
	Meropenem(10ug)	4(20.00%)	16(80.00%)

The sensitivity and resistance patterns of 37 *Pseudomonas aeruginosa* isolates from air sample are shown in Table 4. The result shows that 2(5.41%) of the isolate were susceptible to Augmentin, 16(43.24%) to Gentamycin, 23(62.16%) to Amikacin, 1(2.70%) to Ceftazidime, 1(2.70%)

to Cefuroxime, 15(40.54%) to Ciprofloxacin, 13(35.14%) to Ofloxacin, 1(2.70%) to Erythromycin, 18(48.65%) to Imipenem and 25(67.58%) to Meropenem. Cloxacillin and Ceftriazone were not effective against the 37 isolates.

**Table 4:** Antibiotic resistance patterns of the 37 *Pseudomonas aeruginosa* isolates from the Air

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug)	35(94.59%)	2(5.41%)
	Cloxacillin (5ug)	37(100.0%)	0 (0.00%)
Aminoglycoside	Gentamycin (10ug)	21(56.76%)	16(43.24%)
	Amikacin (30ug)	14(37.84%)	23(62.16%)
Cephalosporin	Ceftazidime (30ug)	36(97.30%)	1(2.70%)
	Cefuroxime (30ug)	37(100.0%)	0(0.00%)
	Ceftriaxone (30ug)	36(97.30%)	1(2.70%)
Quinolones	Ciprofloxacin (5ug)	22(59.46%)	15(40.54%)
	Ofloxacin (5ug)	24(64.86%)	13(35.14%)
Macrolides	Erythromycin (5ug)	36(97.30%)	1(2.70%)
Carbapenems (β-Lactamase inhibitors)	Imipenem (10ug)	19(51.35%)	18(48.65%)
	Meropenem(10ug)	12(32.43%)	25(67.57%)

Table 5, shows sensitivity and resistance patterns of the 72 *Pseudomonas aeruginosa* isolates from the mechanic village (environment). Out of 72 isolates, 9(12.5%) were sensitive to Augmentin, 27(37.5%) to Gentamycin, 43(59.72%) to Amikacin, 2(2.78%) to Ceftazidime, 2(2.78%) to

Ceftriazone, 33(45.83%) to Ciprofloxacin, 27(37.5%) to Ofloxacin, 4(5.56%) to Erythromycin, 41(56.94%) to Imipenem and 53(73.61%) to Meropenem. While all the 15 isolates were resistant to Cloxacillin and Cefuroxime.

**Table 5:** Antibiotic resistance patterns of the 72 *Pseudomonas aeruginosa* isolates from environmental sources

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug)	63(87.5%)	9(12.5%)
	Cloxacillin (5ug)	72(100%)	0 (0%)
Aminoglycoside	Gentamycin (10ug)	45(62.5%)	27(37.5%)
	Amikacin (30ug)	29(40.28%)	43(59.72%)
Cephalosporin	Ceftazidime (30ug)	70(97.22%)	2(2.78%)

	Cefuroxime (30ug)	72(100.00%)	0(0%)
	Ceftriaxone (30ug)	70(97.22%)	2(2.78%)
Quinolones	Ciprofloxacin (5ug)	39(54.17%)	33(45.83%)
	Ofloxacin (5ug)	45(62.5%)	27(37.5%)
Macrolides	Erythromycin (5ug)	68(94.44%)	4(5.56%)
Carbapenems ( $\beta$ -Lactamase inhibitors)	Imipenem (10ug)	31(43.06%)	41(56.94%)
	Meropenem(10ug)	19(26.39%)	53(73.61%)

#### 4. Discussion

In this study, twelve antibiotics were tested against *P. aeruginosa* from different environmental samples. These were (penicillin) augmentin, cloxacillin, (aminoglycosides) gentamicin, amikacin, (cephalosporins) ceftazidime, cefurozime, ceftriazone, (quinolones) ciprofloxacin, ofloxacin, (macrolide) erythromycin, (carbapenems) imipenems and meropenems. The reason choosing this antimicrobial was their wide use in the hospital as antipseudomonal agents. Therefore, this kind of study could provide appropriate guidelines to the hospital regarding the prescription of these antimicrobials according to their sensitivity to *P. aeruginosa*.

Among the twelve antibiotics, maximum sensitivity was found with meropenem (73.61%) among carbapenems followed by amikacin (59.72%) among the aminoglycoside, while other drugs showed decrease in susceptibility pattern. Maximum sensitivity was demonstrated by these two classes of drugs in comparison to other antibiotics used in this study. One of the reasons for these drugs still remaining sensitive might be due to their restricted use in ICU (intensive care unit) and also limited use in critical care unit.

In earlier studies by Quinn (1998), it was reported that increased resistance rates of *Pseudomonas aeruginosa* have been detected against carbapenems, quinolones and third-generation cephalosporins across the globe. In this study, resistance rates against carbapenems such as meropenems and imipenem, were 26.39% and 43.06% and against aminoglycosides such as amikacin was 40.28%. In yet another study by Bouza *et al* (1999), it was reported that resistance to imipenem was 14% in Spain, 19.3% in Italy and 68% in Saudi Arabia. The National Nosocomial Infections Surveillance (NNIS) system reported the incidence of imipenem resistance as 18.5% among isolates of *Pseudomonas aeruginosa* from ICU patients (CDC, 1999).

The resistance of *P. aeruginosa* to the antibiotic in the quinolone group is variable in different centers. In a prospective study, resistance to ciprofloxacin was reported as 8-31% in ICU patients (Tassios *et al.*, 1998). This study reveals that the resistance rate against ciprofloxacin was found as 54.17% while it was 32% in Indian (Sivaraj *et al.*, 2012), 23% in Spain (Bouza *et al.*, 1999), 31.9% in Italy (Bonfiglio *et al.*, 1998), and 28.8% in Latin America. *P. aeruginosa* isolates had high resistant against ofloxacin 62.5% another quinolone used in the study. The difference in the resistance patterns to the various quinolones is similar to a study in Turkey where a wide range of resistance status against various quinolones was also recorded (Algun *et al.*, 2004). The main mechanism of resistance to

fluoroquinolones has been reported to be the decrease in binding of the target quinolones to enzymes as a result of changes in DNA gyrase and or topoisomerase enzymes. Mutations occur in gyr A and par C genes. This is usually against all quinolones. However, resistance due to mutations of gyr B, though less common may not be against all quinolones (Algun *et al.*, 2004).

The resistant rate of ceftazidime (97.22%) was very high compared to Ciprofloxacin. According to earlier reports, resistance to ceftazidime was 15%-22% in the world (Jones, 2001), 36% in Indian (Sivaraj *et al.*, 2012). Resistance to Ceftriaxone and cefuroxime was higher 97.22% and 100.00%, similar to ceftazidime. Resistance rates of antipseudomonal antibiotics were quite low in the United Kingdom: 5% for ceftazidime, 10% for ciprofloxacin, and 11% for imipenem (Spencer, 1996). In a study by Akingbade, *et al* (2012), *Pseudomonas aeruginosa* isolates showed 72.7% resistance to erythromycin which is in line 94.44% resistance to erythromycin in this study.

The air isolates were more resistant to the antibiotics which were more effective against the isolates when compared to the soil and waste water isolates; from the air isolates, resistant to meropenem, amikacin and imipenem were 32.43%, 37.84% and 51.35% respectively, in soil isolates, resistant to meropenem, amikacin and imipenem were 20.00%, 50.00% and 30.00% respectively, while in waste water isolates, resistant to meropenem, amikacin and imipenem were 20.00%, 33.33% and 40.00% respectively. All the isolates were resistant to five or more antibiotics; of this 6(8.33%) of isolates were resistance to all twelve antibiotics used.

#### 5. Conclusion

Our results indicate that the resistance of *P. aeruginosa* is on the rise which may be due to the activities of industrial effluent in the environment. As a result, immunization may fail to recover by constant exposure of resistance microbes. Even though of medical improvement, the antimicrobial resistance still becomes an age - old problem. So, proper implementation of antibiotic policies and guideline must be there in every hospital to local susceptibility pattern. Currently, the treatment of *P. aeruginosa* infections is based on combination antibiotic therapy that traditionally includes  $\beta$ -lactam agents and aminoglycosides, in addition to this; treatment with fluoroquinolones has offered new perspectives. The development of effective vaccine against *P. aeruginosa* is necessary in the modern world.

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