# Antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* isolates from patients attending health care facilities, Ebonyi State, Nigeria

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## ABSTRACT

**Aim**: To investigate the antibiotic susceptibility profile of *Pseudomonas aeruginosa* isolates from wounds of patients and to determine the age group commonly infected with the bacteria.

**Study Design**: This is a cross-sectional study conducted among patients suspected of having wound infection.

**Place and Duration of Study:** The study was conducted between May, 2015 and June, 2016 at the Microbiology Laboratory of Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.

**Methodology**: A total of 165 wound swabs were analysed for the presence of *P. aeruginosa.* Standard microbiology laboratory tests were used to isolate and identify the isolates. Antibiotic susceptibility testing of the isolates was carried out using the disc diffusion method.

**Results**: A total of 56 (33.94 %) *P. aeruginosa* isolates were identified. The age group, 31 – 40 years recorded the highest number 28 (50.00 %) of *P. aeruginosa* infection. Within this age group, females 15 (51.72 %) were slightly more infected than males 13 (48.15 %). In the tertiary hospital (MMH), the highest sensitivity was observed for ofloxacin (32 strains, 78.05 % of *P. aeruginosa* isolates) followed by ciprofloxacin (29 strains, 70.73 %of *P. aeruginosa* isolates) and ceftazidime (26 strains, 63.41 % of *P. aeruginosa* isolates). The number of isolates resistant to amoxicillin/clavulanate, cefixime and cefuroxime

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were 34 strains (82.93 % of *P. aeruginosa* isolates), 26 strains (63.41% of *P. aeruginosa* isolates) and 23 strains (56.10 % of *P. aeruginosa* isolates) respectively. The number of *P. aeruginosa* isolates from the teaching hospital (FETHA) sensitive to ofloxacin and ciprofloxacin were 9 strains (60.00 % of *P. aeruginosa* isolates) and 8 strains (53.33 % of *P. aeruginosa* isolates) respectively. In FETHA, the isolates showed their highest resistance to amoxicillin/clavulanate (14 strains, 93.33 % of *P. aeruginosa* isolates) followed by cefixime (12 strains, 80. 00 % of *P. aeruginosa* isolates). A total of 32 strains (57.14 % of *P. aeruginosa* isolates) were found to be multidrug-resistant (MDR). Regular monitoring of antimicrobial susceptibility profile is essential to guide the physicians in drug prescription against *P. aeruginosa* strains.

*Keywords: Susceptibility, Infection, P. aeruginosa, Multidrug resistance.*

## 1. INTRODUCTION

Wound infection is universal and the bacterial type varies with geographical location, resident flora of the skin, clothing at the site of wound, time between wound and examination [1]. The development of wound infection depends on the integrity and protective function of the skin [1]. Many bacterial pathogens are involved in wound infection [2].

*P. aeruginosa* can be found in most environments including soil, water and various types of vegetation. The organisms are a clinically important pathogen responsible for a variety of systemic infections such as urinary tract infections, respiratory system infections, gastrointestinal infections, dermatitis, bacteremia, soft tissue infections, bone and joint infections [3]. *P. aeruginosa* causes infection in immunocompromised patients such as those suffering from burn wounds or receiving cancer chemotherapy [4]. It is a major nosocomial pathogen, particularly dangerous to cystic fibrosis patients [5].

This organism is resistant to many antibacterial drugs [6]. Multidrug-resistant *P. aeruginosa* phenotype is defined as resistant to one antimicrobial agent in three or more anti-pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins/cephalosporins and aminoglycosides) [7, 8]. Multidrug-resistant *P. aeruginosa* is an important public health issue because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs [9]. Multidrug-resistant *P. aeruginosa* develops resistance by various mechanisms like multidrug resistance efflux pumps, biofilm formation, production of β-lactamases and aminoglycoside modifying enzymes [8]. Broad-spectrum anti-pseudomonal drugs such as imipenem, ceftazidime, amikacin have been

recommended for treatments of infections caused by multiple drug resistant *P. aeruginosa* [10]. However, resistance to one or more of these anti-pseudomonal drugs during therapy has been widely observed.

The alarming rate of resistance in bacteria pathogens raises concern for the effectiveness of antibiotic therapy [11]. The spread of multidrug-resistant pathogens is a real threat to public health and a major concern for infection control practitioners globally [12]. This spread has paved way for the re-emergence of previously controlled diseases and a high frequency of opportunistic and chronic infection cases in developing countries like Nigeria [12].

This study aims at investigating the antibiotic susceptibility profile of *P. aeruginosa* isolates from wounds of patients and to determine the age group commonly infected with the organism.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

One tertiary hospital (MMH) and one teaching hospital (FETHA) in Ebonyi State, Nigeria was used for this study. These hospitals were selected because they receive a large number of patients seeking medical attention and also serve as a referral centre for the state and neighbouring states.

### 2.2 Sample Collection

A total of 165 wound swabs were collected from patients aged 11 years and above. No specimen was collected from the age group 0 -10 years. All wound swabs collected at the hospitals were inoculated into 10 ml cooked meat broth and incubated at 37 $^{o}$C for 48 hours [13].

### 2.3 Isolation of Bacteria

A loopful of organisms growing in the cooked meat broth above was inoculated onto Cetrimide agar plates using the streaking technique. The inoculated Petri dishes were incubated at 37 $^{o}$C for 24 hours [14]. Suspected discrete colonies of *P. aeruginosa* that appeared green on cetrimide agar was inoculated into the nutrient broth and nutrient agar slants and incubated at 37 $^{o}$C for 24 hours. The organisms were confirmed by adopting a standard microbiological procedure which includes: colony morphology, Gram stain reaction and biochemical reaction such as catalase test, indole test, motility test, citrate utilization test, oxidase test, triple sugar iron agar test and urease test.

### 2.4 Antibiotic Susceptibility Testing

In vitro, **a**ntibiotic susceptibility testing of the isolates was carried out using the disc diffusion method as recommended by the Clinical and Laboratory Standard Institute [15]. The antibiotic disc used include: Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamycin (10 µg), Cefixime (5 µg), Ofloxacin (5 µg), Amoxycillin/clavulanate (30 µg), and Ciprofloxacin (5 µg) (Abtek Biologicals Ltd, Liverpool, UK). A sterile Pasteur pipette was used to drop 0.2 ml of the standardized inoculum equivalent to 0.5 McFarland turbidity standards (1.0 $x10^8$ cfu/ml) on the surface of dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. The agar was left for about 10 minutes for the inoculum to dry. Thereafter, antibiotic discs were aseptically placed on the surface of the inoculated Mueller-Hinton agar plate using heat-sterilized forceps. They were incubated at 37 $^oC$ for 18-24 hours. The diameter of zones of inhibition for each antibiotic was measured in millimetre and compared with values provided by the Clinical and Laboratory Standard Institute [15].

### 2.5 Statistical Analysis

Statistical analysis was carried using the statistical package for social sciences (SPSS) version 15. $P < 0.05$ was considered significant.

## 2. RESULTS AND DISCUSSION

Out of 165 wound swabs analysed, 56 (33.94 %) *P. aeruginosa* isolates were obtained (Table 1).The most frequent isolates of *P. aeruginosa* was from trauma 39 (44.32 %) followed by burn wound 3 (25.00 %).

**Table 1**: Distribution *of P. aeruginosa* from wound swabs

| **Type of wound** | **Number** | **Number of bacterial isolates obtained** | **Percentage (%) bacterial isolates** |
|---|---|---|---|
| Burn wound | 12 | 3 | 25.00 |
| Abscess | 65 | 14 | 21.54 |
| Trauma | 88 | 39 | 44.32 |
| **Total** | **165** | **56** | **33.94** |

The age group 31 – 40 years had the highest number 28 (50.00 %) of *P. aeruginosa* infection. (Fig. 1). Within this age group, females 15 (51.72 %) were slightly more infected than males 13 (48.15 %).

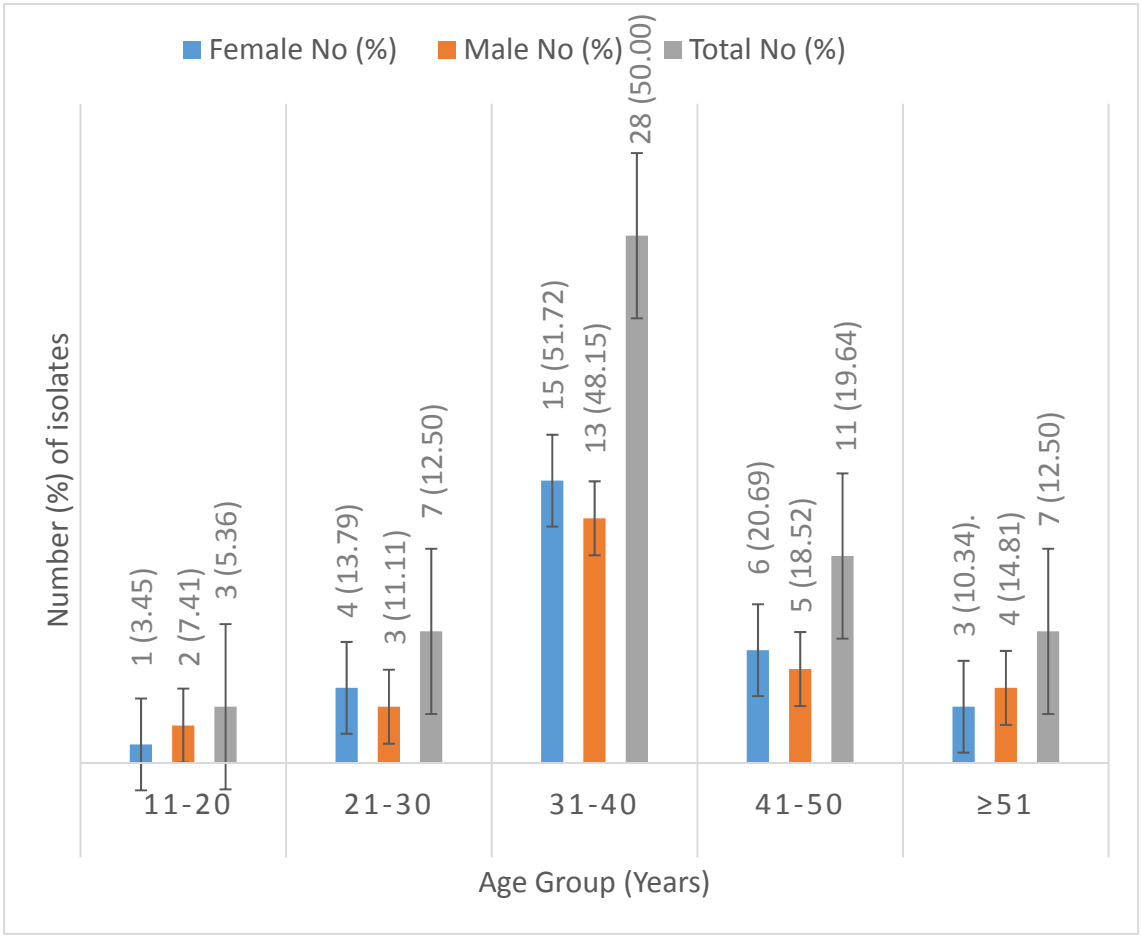


**Figure 1**: Mean age group and gender distribution of *P. aeruginosa* isolates from wound swabs.

**Key**: % = Percentage in bracket

Antibiotic susceptibility profile of *P. aeruginosa* from MMH is as shown in Table 2. The highest sensitivity was observed for ofloxacin (32 strains, 78.05 % of *P. aeruginosa* isolates) followed by ciprofloxacin (29 strains, 70.73 % of *P. aeruginosa* isolates) and ceftazidime (26 strains, 63.41 % of *P. aeruginosa* isolates).The number of isolates resistant to amoxicillin/clavulanate, cefixime and cefuroxime were 34 strains (82.93 % of *P. aeruginosa* isolates), 26 strains (63.41% of *P. aeruginosa* isolates) and 23 strains (56.10 % of *P. aeruginosa* isolates) respectively.

**Table 2**: Antibiotic Susceptibility Profile of *P. aeruginosa* isolates from Wound swabs, MMH.

| **Antibiotic** | **Disc Content** | **Number of sensitive strains (%)** | **Number of intermediate strains (%)** | **Number of resistant strains (%)** |
|---|---|---|---|---|
| Ceftazidime | 30 µg | 26 (63.41) | 4 (9.76) | 11 (26.83) |
| Cefuroxime | 30 µg | 13 (31.71) | 5 (12.20) | 23(56.10) |
| Gentamycin | 10 µg | 15 (36.59) | 5 (12.20) | 21 (51.22) |
| Cefixime | 5 µg | 6 (14.63) | 9 (21.95) | 26 (63.41) |
| Ofloxacin | 5 µg | 32 (78.05) | 0 (0.00) | 9 (21.95) |
| Amoxicillin/ Clavulanate | 30 µg | 3 (7.32) | 4 (9.76) | 34 (82.93) |
| Ciprofloxacin | 5 µg | 29 (70.73) | 3 (7.32) | 9 (21.95) |

**Key**: % = Percentage in bracket; MMH = Code of tertiary hospital used

The number of *P. aeruginosa* isolates from FETHA sensitive to ofloxacin and ciprofloxacin were 9 strains (60.00 % of *P. aeruginosa* isolates) and 8 strains (53.33 % of *P. aeruginosa* isolates) respectively (Table 3). The isolates showed their highest resistance to amoxicillin/clavulanate (14 strains, 93.33 % of *P. aeruginosa* isolates) followed by cefixime (12 strains, 80. 00 % of *P. aeruginosa* isolates).

**Table 3**: Antibiotic Susceptibility Profile of *P. aeruginosa* isolates from Wound swabs, FETHA.

| **Antibiotic** | **Disc Content** | **Number of sensitive strains (%)** | **Number of intermediate strains (%)** | **Number of resistant strains (%)** |
|---|---|---|---|---|
| Ceftazidime | 30 µg | 6 (40.00) | 1 (6.67) | 8 (53.33) |
| Cefuroxime | 30 µg | 5 (33.33) | 1 (6.67) | 9(60.00) |
| Gentamycin | 10 µg | 4 (26.67) | 2 (13.33) | 9(60.00) |
| Cefixime | 5 µg | 2 (13.33) | 1 (6.67) | 12 (80.00) |
| Ofloxacin | 5 µg | 9 (60.00) | 1 (6.67) | 5 (33.33) |
| Amoxicillin/ Clavulanate | 30 µg | 1 (6.67) | 0 (0.00) | 14 (93.33) |
| Ciprofloxacin | 5 µg | 8 (53.33) | 0 (0.00) | 7 (46.67) |

**Key**: % = Percentage in bracket; FETHA = Code of tertiary hospital used

A total of 57.14 % of *P. aeruginosa* isolates from the two hospitals were found to be multidrug resistant. (Table 4). The prevalence was slightly higher in MMH (58. 54 %) than FETHA (53.33 %).

**Table 4**: Prevalence of MDR Isolates of *P. aeruginosa* from Wound swabs.

| Hospital | Total No. of *P. aeruginosa* isolates | Number of MDR *P. aeruginosa* strains | Percentage of MDR *P. aeruginosa* strains |
|---|---|---|---|
| MMH | 41 | 24 | 58.54 |
| FETHA | 15 | 8 | 53.33 |
| Total | 56 | 32 | 57.14 |

**Key**: MDR = Multidrug-Resistant

*P. aeruginosa* is known to be associated with wound infections. The result of this study was in contrast with similar studies carried out by Garba et al. [2] who isolated 11 % of *P. aeruginosa* from wounds of patients attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Also, Hyder et al. [16] and

Mama et al. [17] reported a lower prevalence of 6 (3.95 %) and 11 (8 %) respectively. Similarly, Ezebialu et al. [18] isolated 27.3 % of *P. aeruginosa* from wounds in Enugu, which is slightly lower than the 33.94 % observed in this study. The findings of this study is similar to the reports of Nwachukwu et al. [19] and Anupurba et al. [1] who isolated 32.90 % and 32 % *P. aeruginosa* respectively from wound swabs.

In a similar study carried out by Kirecci and Kareem [20], the age group mostly infected with *P. aeruginosa* was 41 – 60 years representing 45.33 %. Their finding differ from the present study where the age group of 31 – 40 years recorded the highest number (50.00 %) of *P. aeruginosa* infection. Rajat et al. [21] reported an isolation rate of 29 % in the age group of 31–45 years. This result is similar to our study and that carried out by Chander and Raza [22] that found 20% *P. aeruginosa* infection in age group of 21–40 years.

*P. aeruginosa* has been reported to have an innate resistance to several antibiotics due to the presence of lipopolysaccharide in the outer membrane [16]. In Nigeria, the trend in resistance phenotype of *P. aeruginosa* to commonly prescribed antibiotics in various hospitals is increasing since the last decade [10]. In line with the present study, Jombo *et al.* [23] and Garba *et al*. [2] reported a high resistance of 100 % and 81.8 % respectively, to amoxicillin/clavulanate among *P. aeruginosa* isolates from urine and wound swabs. Rajeevan *et al*. [24] found 40 % and 20 % resistance to ceftazidime and gentamycin respectively. This is low compared to the finding of this study in FETHA with ceftazidime and gentamycin resistance of 53.33 % and 60.00 % respectively. In MMH, the resistance to gentamycin was high (51.22 %) compared to 20 % resistance reported by Rajeevan *et al*. [24]. Ahmed *et al*. [8] reported a high (91 %) resistance to ceftazidime. In comparison to the present study in the two hospitals, Hyder *et al*. [16] observed a higher (70.83 %) resistance to gentamycin and a lower (31.25 %) resistance to ciprofloxacin in Hilla Teaching hospital, Babylon. The resistance observed against ceftazidime is worrisome, being among the broad-spectrum anti-pseudomonal drugs recommended for treatments of infections caused by MDR *P. aeruginosa* [10]. The high resistance of the isolates to antibiotics may be due to the practice of self-medication, lack of diagnostic laboratory services or unavailability of guidance regarding the selection of drugs, thereby leading to inappropriate use of antibiotics [17].

Various researchers have reported lower rates of MDR *P. aeruginosa* in their study. Compared to this study, Ahmed et al. [8] detected a slightly low prevalence rate of 52 % in patients with nosocomial

infections at a University hospital, Egypt. In Iran, Zahra and Moniri [25] reported 30 % prevalence of MDR *P. aeruginosa.* Siveraj et al. [26] reported 12 % MDR among *P. aeruginosa* isolates from India. In Abeokuta, Nigeria, Okonko *et al*. [27] reported multidrug-resistant to 5 antibiotics (ampicillin, chloramphenicol, cotrimoxazole, nitrofuratoin and tetracycline) by *P. aeruginosa.* The level of MDR recorded in this study shows that *P. aeruginosa* can develop resistance to many antibacterial. *P. aeruginosa* can develop resistance to many antibacterial both through the resistance genes on extrachromosomal genetic elements and through mutational processes [28].

## 3. CONCLUSION

In this study, 56 (33.94 %) of *P. aeruginosa* isolates were obtained from the wound swabs analyzed. The age group mostly infected with *P. aeruginosa* was 31 – 40 years. The *P. aeruginosa* strains were more sensitive to ofloxacin while they showed their highest resistance to amoxicillin/clavulanate. Exactly 57.14 % of *P. aeruginosa* isolates were found to be multidrug resistant. A regular monitoring of antimicrobial susceptibility profile is essential to guide the physicians in prescribing the right drugs and prevent the emergence of multidrug-resistant strains of *P. aeruginosa*.

## COMPETING INTEREST

Authors have declared that competing for interest does not exist.

## AUTHORS' CONTRIBUTION

This work was carried out in collaboration between all authors. Authors OVU and NVO designed and wrote the protocol and first draft of the manuscript. Author OVU managed the literature search and analysis of the study. Authors OMO, KMC, ONU and OI revised the manuscript. All authors read and approved the final manuscript.

## CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this case report. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

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