

# Antibiotic Resistance Pattern Of Pseudomonas Aeruginosa Isolated From Clinical Specimens In A Tertiary Hospital In Northeastern Nigeria

O KO, A PC, O W, B ST, U A

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## Abstract

A total of 106 greenish, pigmented consecutive non-duplicate Pseudomonas aeruginosa isolates from clinical specimens were examined. The mean age of the patients was 28.8±17.3 years, comprised of 56 (52.8%) males and 50 (47.2%) females. High prevalence of pseudomonal infections occurred more in age-group 20-29 years, 26 (24.6%) and least in 50-59 years and >60 years, 6 (5.7%), respectively. Majority of the isolates were recovered from wounds specimens, 42 (39%). Overall antibiotic susceptibility pattern showed that sparfloxacin demonstrated highest sensitivity of 84.9%, followed by ciprofloxacin, 69.8% and perfloracin, 52.8%. Other results were cefuroxime, 49.1%, and ceftazone, 13.2%. Majority of the isolates exhibited multidrug resistance pattern. In conclusion, the multidrug resistance pattern of P. aeruginosa isolates observed in this study posed a dire clinical consequence, especially in patients management with pseudomonal infections and infections control approach in hospital environment due to rapid dissemination of the strains

## INTRODUCTION

Pseudomonas aeruginosa, is a motile gram-negative rods that belongs to the family Pseudomonadaceae. It is a leading cause of nosocomial infections, especially among critically ill admitted in intensive care unit, immune-compromised patients.<sup>1-3</sup> It has been implicated in diverse nosocomial infection likes nosocomial pneumonis, urinary tract infection, surgical site infection, severe burns and infections of patient undergoing either chemotherapy for neoplastic disease or those on antibiotics therapy.<sup>4,5</sup> P.aeruginosa is widely distributed in nature, but has higher prevalence in hospital environment, as the wards encourages bacterial growth.<sup>3</sup> The characteristic features of P.aeruginosa isolates that allows the persistence in hospital is the ability to acquires resistance to variety of antibiotics, withstands physical conditions like temperature, high concentration of salts and antiseptics.

Epidemiologically, it is ranked as the fourth cause of nosocomial infections that accounts for 10% of all nosocomial infection in the United States. Overall prevalence in US hospital was approximately 4 per 1000 discharge and leading cause of high morbidity and mortality.<sup>6</sup> In studies conducted in Nigeria, it is one of the leading gram-negative bacteria isolated from clinical

specimens in hospital-based studies<sup>7-12</sup> P.aeruginosa isolates are naturally resistant to large number of antibiotics that can be acquired during treatment<sup>13</sup> of as a result of treatment failure.<sup>6</sup> Consequential effect of high resistance pattern is responsible for high mortality rate associated pseudomonal infections.<sup>14</sup>

Antibiotic resistance pattern of P. aeruginosa isolates varied with geographical location and hospitals environments. Therefore, chemotherapeutic approach of pseudomonal infection would depend on peculiarity of the isolates susceptibility pattern, in order to safeguard against treatment. In this context, this study examined the antibiotic resistance of P. aeruginosa isolates from clinical specimen in a tertiary hospital.

## MATERIALS AND METHODS

The study was conducted in the Medical Microbiology Laboratory of the University of Maiduguri Teaching Hospital between February and October 2009. It is a major referral centre for other tertiary hospital in the northeastern Nigeria, a 500-bed capacity with sub-specialties in Internal Medicine, Surgery, Paediatric, Obstetrics and Gynecology and Pathology. Approximately 23,000 clinical specimens are received in medical microbiology laboratory per year.

Over the study period, 106 greenish pigmented, non-duplicate consecutive P.aeruginosa isolated from the clinical specimens were identified by standard bacteriological methods( colonial morphology, citrate, and oxidase). The isolates were recovered from sputum, urine, pus/aspirate, wounds, ear swab and catheter tips. Demographic information on the isolates includes the age of the patient, sex, type of clinical specimens and wards/ clinics.

Antibiotic susceptibility testing was determined by disc diffusion method using Mueller-Hinton agar plates.<sup>15</sup> Bacterial suspension was prepared in Andrade peptones water to give concentration an equivalent of 0.5McFarlane standards. The bacterial suspension were inoculated on the Mueller-Hinton agar plate by swabbing to give a smooth lawn, and antibiotic discs were placed on it, incubated at 37°C overnight. The following antibiotic discs were tested , cefuroxime(30ug), ciprofloxacin(10ug), cotrimoxazole(30ug), erythromycin(15ug), ofloxacin(10ug), ceftriazone(30ug), amoxicillin (30ug), gentamycin (10ug), chloramphenicol (30ug), amoxicillin/clavunate (30ug) sparfloxacin (10ug), perfloxacin (30ug) and streptomycin (30ug).The zone of inhibition diameter was measured using calibrated ruler and interpreted as susceptible, intermediate or resistant in accordance to CSLI guidelines. Multidrug resistance is defined as isolates resistance to more than three classes of drugs.

## DATA ANALYSIS

Data analysis was carried using SPSS version 15.0<sup>16</sup>, with values expressed in means and percentage. Statistical significance difference was determined by chi-square test (p<0.05).

## RESULTS

A total of 5000 bacterial pathogens were isolated over the nine month study period, P.aeruginosa isolates accounted for 106 (2.1%). The mean age of the patient was 28.81±17.27 years (range 2-79 years), gender distribution showed that male patients were 56 (52.8%) and 50(47.2%) females. High frequency of pseudomonal infection was more within the age-group of 20-29 years (20.7%) and least in 50-59years (5.7%) (Table I). Significant proportion of isolates were recovered from wounds specimen 42(39.6%), followed by ear swabs 32 (30.2%), catheter tips 16 (15.1%) urine 8 (7.5%), aspirate 4 (3.8%) and least 2 (1.9%) from urethral swab and high vaginal swab respectively (Table II).

The antibiotic susceptibility pattern of P. aeruginosa isolates

as presented in Tables III, showed that the isolates were highly susceptible to sparfloxacin (84.9%0, ciprofloxacin (69.8%), and moderately to perfloxacin (52.8%) and least to ofloxacin (30.2%). High level of resistance was observed with erythromycin (100%), gentamicin (100%), streptomycin (71.1%), all the penicillins, amoxicillin (92.5%), ampiclox (100%), ampicillin-clauvante (100%), cotrimoxazole (100%) and chloramphenicol (98.3%). The two cephalosporin tested, cefuroxime (50.9%) and ceftriaxone (86.8%) respectively. Majority of the isolates that exhibited multidrug resistant pattern to a least 8 antibiotics were 22.6% and to all the drugs 9.4%.

**Figure 1**

Table I. Age and gender distribution of isolates

Age-group(years)	Males (%)	Females (%)	Total (%)
<10	10(9.4)	14(13.2)	24(22.6)
10-19	4(3.8)	4(3.8)	8(7.6)
20-29	6(5.7)	20(18.9)	26(24.6)
30-39	14(13.2)	8(7.5)	22(20.7)
40-49	12(11.3)	2(1.9)	14(13.2)
50-59	4(3.8)	2(1.9)	6(5.7)
>60	6(5.7)	0(0)	6(5.7)
<b>Total</b>	<b>56 (52.8)</b>	<b>50 (47.2)</b>	<b>106(100)</b>

**Figure 2**

Table II. Distribution of isolates among clinical specimens

Clinical specimens	Frequency (%)
Wound swab	42(39.2)
Ear swab	32(30.2)
Catheter tip	16 (15.1)
Aspirate	4 (3.8)
Urine	8 (7.5)
Urethral swab	2 (1.9)
High vaginal swab	2 (1.9)
<b>Total</b>	<b>106 (100)</b>

**Figure 3**

Table III. Antibiotic susceptibility pattern of isolates

Antibiotics	Sensitivity (%)	Resistance (%)
<b>Quinolones</b>		
Ciprofloxacin	74(69.8)	32(30.2)
Sparfloxacin	90(84.9)	16(15.1)
Ofloxacin	32(30.2)	74(69.8)
Perfloxacin	56(52.8)	50(47.2)
<b>Macrolides</b>		
Erythromycin	2(1.9)	104(98.1)
<b>Aminoglycosides</b>		
Gentamycin	0(0)	106(100)
Streptomycin	30(28.3)	76(71.7)
<b>Penicillins</b>		
Amoxicillin	8(7.5)	98(92.5)
Ampiclox	0(0)	106(100)
Ampicillin/clavulanate	0(0)	106(100)
<b>Sulfonamides</b>		
Co-trimoxazole	0(0)	106(100)
<b>Cephalosporins</b>		
Cefuroxime	52(49.1)	54(50.9)
Ceftriaxone	14(13.2)	92(86.8)
Chloramphenicol	2(1.9)	104(98.1)

## DISCUSSION

*Pseudomonas aeruginosa* is ranked second among gram-negative bacteria isolated in hospital environmental, and leading cause of nosocomial infections responsible for high morbidity and mortality rate. High prevalence of pseudomonal infections is common among critically ill patients on admission on intensive care unit and those with underlying clinical conditions.<sup>17</sup>

In this study, the prevalence of *P. aeruginosa* isolates in clinical specimens examined over the study period was 2.1%, this level is relatively low when compared with similar studies with higher prevalence level. In Zaria, Olayinka et al.<sup>11</sup> reported a level of 10.5%, while 30% was reported in a study conducted in Pakistan<sup>18</sup>, and 20.3% in India.<sup>19</sup> Comparison of epidemiological data of bacterial pathogens as in this study might be difficult as there are other variables that influences the outcome of results such as, clinical specimens received for examination, studied population, type of hospitals and geographical locations.<sup>19</sup> Prevalence of *P. aeruginosa* isolates varied with clinical

conditions and specimens. In the European Prevalence of Infection in Intensive Care (EPIC), *P. aeruginosa* was predominant gram-negative bacteria isolated from bronchopulmonary infections and accounts for 17% of health care-associated pneumonia and late-onset ventilator associated pneumonia<sup>20</sup> and accounts for significant cases of cystic fibrosis.<sup>21</sup> The distribution of isolates differs with studies and clinical specimens, In Zaria, Olayinka et al.<sup>11</sup> reported 51.1% in urine, 41.3% in wound and 1.1% in sputum, while 4.6% in urine in Jos. In Ile-Ife, southwestern Nigeria, prevalence of 11.1% in open musculoskeletal injuries<sup>8</sup>, and in Ibadan, isolate rate of 16.8% with 41.9% and 39.35 from ear and wound swab respectively.<sup>22</sup> However, the possibility of *P. aeruginosa* contamination of wounds and catheter tips cannot be ruled out. This is possible in hospital environment where strict hand washing procedure is not strictly adhered to and unhygienic procedure especially in wound dressing and insertion of indwelling catheter may be a contributory factor. Majority of isolates were recovered from patient on admission, this observation affirmed the significant role of this organism in nosocomial infection, similarly was the pattern in wounds and catheter tip specimens.

The unique feature of *P. aeruginosa* isolates is the resistance to variety of antibiotics, primarily attributed to low permeability of the cell wall, production of inducible cephalosporinase, active efflux and poor affinity for the target (DNA gyrase).<sup>23</sup> In this study, members of quinolones family exhibited high susceptibility pattern, sparfloxacin (84.9%) as the most effective, followed by ciprofloxacin (68.9%), perfloxacin (52.8%), while ofloxacin (30.2%) demonstrated the least susceptibility pattern. This pattern observed with ofloxacin is consistent with report from Bangkok, Thailand with with ofloxacin resistance value of 37.5%.<sup>14</sup> The pattern presented by sparfloxacin may not be surprising, as it is new drug in clinical armamentarium, that are seldom prescribed or abuse in the community. Erythromycin and chloramphenicol resistance value of 98.1% recorded in this study is similar with the report from Gujarat, India.<sup>24</sup> Penicillin are narrow spectrum antibiotics, therefore the high resistance pattern observed in this study, amoxicillin (92.5%), ampiclox and amoxicillin/clavulanate (100%). Similar pattern had been reported in studies in Enugu and Abakaliki, Nigeria<sup>25</sup> and in Malaysia.<sup>26</sup>

Aminoglycosides, especially gentamicin and streptomycin are known frontline antibiotics in the treatment of bacterial

infection due to gram-negative bacteria. However, emerging reports showed increased prevalence of resistance to these drugs. In the study, resistance value of 100% and 71.7% was observed which higher than values reported in study conducted in Ilorin, with resistance value of 40.2% and 36.0%<sup>12</sup> and in Lagos, 75% and 30%.<sup>27</sup> Difference in the resistance pattern may be attributable to factors like exposure to antibiotics, studied population, type of clinical specimen examined. Cephalosporins, are known antipseudomonal drugs, especially the third-generation ceftazidime, that has demonstrated high susceptibility pattern with *P. aeruginosa* isolates, however cefuroxime and ceftriaxone were the two of the cephalosporin drug tested in this study, with resistance value of 50.9% and 86.8% respectively. These high resistance value observed were comparable with the report from Gujarat, India with resistance value of 73.2% and 75%<sup>25</sup>, but higher than reports from Malaysia of 40% and 31%.<sup>26</sup> In contrast, cephalosporins tested in a study conducted in Ibadan, southwestern Nigeria, showed that 90% of the isolates were sensitive.<sup>28</sup> This pattern observed with cephalosporins, underscores the emerging resistance trends to antipseudomonals drugs in different parts of the world. The clinical implication, is that there is need for evaluation of the efficacy of cephalosporin in the treatment of pseudomonal infections in order to prevent treatment failure, a scenario that often common in management of pseudomonal infections.

Most disturbing pattern observed in this study was the multidrug resistance exhibited by most of the isolates. Although, similar pattern had been reported in studies conducted in Zaria<sup>11</sup>, in Jamaica<sup>29</sup>, in Italy<sup>30</sup>, Saudi Arabia<sup>31</sup> and Brazil<sup>32</sup>. In conclusion, the multidrug resistance by *P. aeruginosa* isolated in this study posed dire clinical consequence in term of patient management and infection control approach in hospital environment.

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**Author Information**

**Okon KO, M.Sc**

Department of Medical Laboratory Sciences, College of Medical Sciences, University of Maiduguri

**Agukwe PC, BMLS**

Department of Medical Laboratory Sciences, College of Medical Sciences, University of Maiduguri

**Oladosu W, Ph.D**

National Institute of Pharmaceutical Research

**Balogun ST, M.Sc**

Department of Clinical Pharmacology & Therapeutic, College of Medical Sciences, University of Maiduguri

**Uba A, Ph.D**

Department of Biological Sciences, Abubakar Tafawa Balewa University