



Research Article

Evaluation of Wound Infection by Resistant Strains of  
*Pseudomonas aeruginosa* in Maiduguri, Northeastern  
Nigeria

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ABSTRACT

Following a loss of skin integrity (wound), the subcutaneous tissue is exposed, creating a moist, warm, and nutrient-rich environment that is favorable for microbial colonization and growth. The colonization of wound by pathogenic bacteria could be a source of localized or systemic infection and impair the wound healing process. Here, we report the incidence of infection by antibiotic resistant strains of *Pseudomonas aeruginosa* in wounds of patients attending hospitals in Maiduguri. Four hundred and twenty (420) wound swab samples were collected, using the Levine technique, from patients presenting with wounds in four (4) selected hospitals in Maiduguri (UMTH: University of Maiduguri Teaching Hospital; SSH: State Specialist Hospital; USH: Umaru Shehu Ultra-Modern; and MSH: Mohammed Shuwa Memorial Hospital). Wound swab samples were cultured on Blood agar, MacConkey agar and Cefrimide agar. Suspected colonies of *Pseudomonas aeruginosa* were further confirmed by biochemical tests and subjected to Kirby Bauer Disc Diffusion technique to ascertain their resistivity profile. Isolates were further classified as Multidrug resistant based on criteria set by European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC). Sixty eight (68) out of the four hundred and twenty (420) wounds analyzed yielded positive growth of *P. aeruginosa*. This gives a recovery rate of 16.2%, which was highest among male patients (10.7%) and patients between the ages of 21-40 years (6.0%) ( $p < 0.05$ ). Hospital based prevalence was found to be highest in UMTH (20.8%) and least in MSH (6.0%) ( $p > 0.05$ ). All isolates were resistant to Ceftazidime (100%) and prevalence of multidrug resistance was 44.1%, where patients attending SSH recorded the highest rate of 20.6%. Efficient care must be given to wounds from initial onset to the time of healing so as to prevent infection by pathogenic microorganisms

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## INTRODUCTION

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes wide array of infectious diseases. It is one of the leading cause of hospital-acquired infection and has been implicated in diseases such as Suppurative otitis media (Umar *et al.*, 2016), Cystic fibrosis (Winstanley *et al.*, 2016), and Chronic obstructive pulmonary disease (COPD) (Garcia-nunez *et al.*, 2017). It is a versatile gram negative rod that has the capacity to cause acute and chronic infections, albeit due to the large arsenal of virulence and antibiotic resistance factors at its disposal. The World Health Organization (WHO) has classified *P. aeruginosa* in its 'critical category' list of pathogenic bacteria that require urgent efforts in the area of new drug design and discovery due to its notoriety and reputation as a multiple drug resistant bacteria (WHO, 2017).

A wound is referred to as an injury to biological tissue, such as the skin, mucous membranes, or organ tissues. Wounds can be described as acute or chronic. Acute wounds arise as a result of external trauma to intact skin and comprises of burns wound, surgical wounds, abrasions, lacerations and gunshot wounds (Davis *et al.*, 1999). Chronic wounds on the other hand, are caused by intrinsic mechanisms that are associated with a predisposing factor which ultimately lead to dermal and epidermal tissue damage. Impaired venous drainage (venous hypertension) and metabolic diseases such as diabetes mellitus are examples of predisposing factors to chronic wounds (Bowler *et al.*, 2001). It is crucial to make sure that wounds are cleaned and correctly treated so as to prevent the development of infection (Wilkins and Unverdorben, 2013). Infection of wounds by opportunistic bacterial pathogens such as *P. aeruginosa* has been shown to disrupt wound healing/repair process (Ruffin and Brochiero, 2019). Individuals suffering from different categories of wound are at risk of *P. aeruginosa* infection (Mihai *et al.*, 2014). Abscess, cellulitis, or the presence of fluids, are some of the criteria

used to identify an infected wound (Herman and Bordoni, 2021).

Being an opportunistic pathogen, *Pseudomonas aeruginosa* affect mostly immunocompromised individuals and is difficult to treat due its intrinsic and acquired antimicrobial drug resistance which has been attributed to the presence of multiple resistance factors such as  $\beta$ -lactamases, porins channels and efflux pumps (Jurado-Martín *et al.*, 2021). It has a naturally low permeable outer-membrane and has an adaptive capacity towards increased or prolonged drug concentrations, the response to which can lead to mutation (insertions and deletions in its genome). Such adaptive indels lead to overproduction of  $\beta$ -lactamases, alteration of drug targets such as PBPs and increased expression of efflux pumps (Smith *et al.*, 2006; Llanes *et al.*, 2013; Castanheira *et al.*, 2014). In chronic infections, these factors play together in concert and lead to the formation of a typical multidrug resistant (MDR) phenotype.

A multidrug resistant *Pseudomonas aeruginosa* (MDR-PA) is a strain that is resistant to at least one (1) antibacterial drug in three (3) or more different classes (Magiorakos *et al.*, 2012). MDR *Pseudomonas aeruginosa* is of significant public health importance due to the severity of infections it causes, especially in a nosocomial setting and its ability to spread rapidly *in vivo*. More worrisome is the global spread of MDR *Pseudomonas aeruginosa* strains designated as high risk clones that have exceptional capacity for the spread of the resistance trait across wild type strains. The slow pace of discovery of new antibiotics and lack of therapeutic options has made MDR *Pseudomonas aeruginosa* infections highly fatal. This study seeks to examine the infection of wounds by *Pseudomonas aeruginosa*. It also has as its aim, the analysis of the resistivity pattern of identified *P. aeruginosa* isolates to selected commonly prescribed antimicrobial drugs with the goal of identifying MDR strains and their distribution in relation to patient demographic characteristics.

Materials and Method

## METHODOLOGY

### Study Area

The study was conducted in Maiduguri, the capital city of Borno State, Nigeria. Maiduguri shares borders with neighboring countries, such as Niger Republic, Chad, and Cameroon. Within Nigeria, Maiduguri shares borders with Adamawa, Yobe, and Gombe and has Sahel savannah vegetation. The annual average temperature of Maiduguri ranges from 19.1°C to 34.7°C. It is a city with rich cultural heritage and home to the Kanem Borno Empire (Udoh, 1978). Four selected hospitals namely; University of Maiduguri Teaching Hospital (UMTH), State Specialist Hospital (SSH), Umaru Shehu Ultra-Modern (USH), and Mohammed Shuwa Memorial Hospital (MSH) were included in this study.

### Ethical Approval

Ethical approval was obtained from the research and Ethics Committees of the UMTH, SSH, MSH, USH and Borno State Hospitals

$$n = Z^2 pq / d^2$$

Z= the standard normal deviate (1.96 which corresponds to 95% confidence level)

P= 51.5% (0.515) (Zubairu and Iregbu, 2017)

q= 1.0-p (1.0-0.515)

d= 0.05 (5%)

n = 383.

Taking care of possible attrition, additional thirty-seven (37) samples were added.

Therefore, n = 420.

### Study Design, Sampling Technique and Sample Collection

This is a cross-sectional study where wound swab samples were collected (via simple-formal random sampling) from four hundred and twenty (420) patients attending the selected hospitals. Sample collection was done by adhering to standard aseptic technique to prevent contamination. The Levine technique was applied in the collection of wound swab samples and it involves rotating a swab over a 1 cm<sup>2</sup> region of the wound while applying pressure that

Management Board (HMB) before commencement of the study. Informed consent of the patients was obtained before sample collection.

### Inclusion criteria

This study include and was restricted to patients (in-patients and out-patients) that attended the selected hospitals (UMTH, SSH, MSH, and USH) and presented with wounds that belong to all four classes (Class I, II, III, and IV) of wound classification.

### Exclusion criteria

Excluded from this study are patients that are attending other hospitals or clinics in Maiduguri, or patients that are attending the selected hospitals but presented with non-wound related cases.

### Sample Size Determination

The sample size (n) was calculated from the general formula as described by Zubairu and Iregbu (2017),

will cause fluid to be released from the wound tissue (Levine *et al.*, 1976). Samples were collected from patients of both sexes (males and females) and different age groups. The swab containers were capped, labelled and taken to the Bacteriology Laboratory of the Department of Microbiology, University of Maiduguri, Nigeria for further analysis.

### Bacteriological analysis (Phenotypic

#### Identification of *P. aeruginosa*)

The swab samples were directly inoculated on MacConkey and Blood agar and incubated for

24 hours Bacterial growth was phenotypically examined by the presence of visible bacterial growth. Preliminary identification of *P. aeruginosa* was based on colony characteristics of the organism which include beta-hemolysis, non-lactose fermentation and pigment production on MacConkey and Blood Agar. Isolates were further sub-cultured onto freshly prepared plates of ceftrimide selective agar (Oxoid, UK) for the selective isolation of *P. aeruginosa*. The plates were incubated overnight at 37°C. And the presence of *P. aeruginosa* on the culture plates was determined qualitatively and quantitatively based on colony morphology or characteristics, Gram stain reaction and further confirmed using biochemical tests.

#### **Antimicrobial Susceptibility Test**

Antimicrobial susceptibility test was performed on Mueller Hinton agar according to the Kirby Bauer Disc Diffusion Technique recommended by the Clinical and Laboratory Standards Institute (CLSI, 2022). This was done by dipping a sterile swab in *P. aeruginosa*-inoculated 0.5 MacFarlands solution and then carefully swabbing the entire surface of Mueller Hinton agar plates. With the aid of sterile forceps, antibiotic discs were placed on the surface of the inoculated plates and gently pressed. The antibiotics used in this study were as follows: Meropenem (10 µg), Ceftazidime (30 µg), Gentamicin (10 µg), Ciprofloxacin (10 µg), Aztreonam (30 µg) and Piperacillin-tazobactam (100 µg). All antibiotics were procured from Oxoid limited (Oxoid, UK). The plates were incubated at 37°C for 24 hours. Diameter of the zone of inhibition was measure in millimetre, recorded and interpreted based on the CLSI criteria for the interpretation of zone sizes of inhibition. Accordingly, isolates were classified as Sensitive, Intermediate or Resistant (CLSI, 2022).

#### **Identification of Multidrug Resistant *P. aeruginosa* (MDR-*P. aeruginosa*)**

Multidrug resistant *P. aeruginosa* isolates were classified according to the definition given by the joint initiative of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC). MDR- *P. aeruginosa* are defined as isolates that are resistant to at least one drug in three (3) or more antimicrobial drug classes (Magiorakos *et al.*, 2012).

#### **Data analysis**

Data was entered into a Microsoft Excel spread sheet and analyzed using SPSS package version 21.0 (IBM, Chicago). Data were described as frequencies and percentages and presented as tables and graphs.

### **RESULTS**

#### **Distribution of *Pseudomonas aeruginosa* in Wounds of patients according to their demographic properties**

Four hundred and twenty (420) samples were analyzed for the identification of *Pseudomonas aeruginosa*, out of which sixty eight (68) samples yielded positive growth. This gives a bacterial recovery rate of 16.2%, which was observed to be highest among male patients (10.7%) compared to female patients (5.5%), an approximate ratio of 2:1. Differences in the recovery of *P. aeruginosa* in relation to the age of patients has shown that patients that are between the age interval of 21-40years (6.0%) recorded the highest, followed by those less than 20years (4.0%). The least was observed among patients in the higher age category of ≥61years (2.6%). The distribution of *Pseudomonas aeruginosa* in relation to difference in age of patients was statistically significant ( $X^2=11.7463$ ,  $p\text{-value}=0.008305$ ;  $p<0.05$ ) (Table 1).

**Table 1:** Distribution of *Pseudomonas aeruginosa* according to the demographic properties of patients attending selected hospitals in Maiduguri metropolis

Demographic properties	No of samples collected (%)	<i>Pseudomonas aeruginosa</i> (%)		X <sup>2</sup>	p-value ( $\alpha=0.05$ )
		Positive	Negative		
<u>Sex</u>					
Male	252 (60.0)	45 (10.7)	207 (49.3)	1.2897	0.256105
Female	168 (40.0)	23 (5.5)	145 (34.5)		
Total	420 (100)	68 (16.2)	352 (83.8)	-	-
<u>Age (years)</u>					
≤ 20	139 (33.1)	17 (4.0)	122 (29.0)	11.7463*	0.008305
21-40	155 (36.9)	25 (6.0)	130 (31.0)		
41-60	97 (23.1)	15 (3.6)	82 (19.5)		
≥ 61	29 (6.9)	11 (2.6)	18 (4.3)		
Total	420 (100)	68 (16.2)	352 (83.8)	-	-

\*statistically significant  $p < 0.05$

**Frequency of occurrence of *Pseudomonas aeruginosa* according to sampling area (hospitals attended by patients) in Maiduguri metropolis**

Rate of isolation of *Pseudomonas aeruginosa* across the sampling area (Hospitals) has shown that patients sampled from SSH had the highest

infection rate of 8.3% followed by those attending UMTH (6.0%). The hospital based prevalence has shown that UMTH had the highest prevalence rate of 20.8% followed by SSH (16.7%), USH (12.5%) and MSH (6.0%) (Table 2).

**Table 2:** Distribution of *Pseudomonas aeruginosa* according to the hospitals attended by patients in Maiduguri metropolis

Hospital	No of samples collected (%)	<i>Pseudomonas aeruginosa</i> (%)		Hospital based prevalence (%)	X <sup>2</sup>	p-value ( $\alpha=0.05$ )
		Positive	Negative			
UMTH	120 (28.6)	25 (6.0)	95 (22.6)	20.8	6.1695	0.103651
SSH	210 (50.0)	35 (8.3)	175 (41.7)	16.7		
USH	40 (9.5)	5 (1.2)	35 (8.3)	12.5		
MSH	50 (11.9)	3 (0.7)	47 (11.2)	6.0		
Total	420 (100)	68 (16.2)	352 (83.8)	-	-	-

**Resistivity profile of *Pseudomonas aeruginosa* identified from wounds of patients attending selected hospitals in Maiduguri metropolis**

Antimicrobial susceptibility pattern of isolates revealed a 100% resistance against Ceftazidime, which is followed by Aztreonam (82.4%). Isolates were highly sensitive to Piperacillin-

tazobactam (73.5%) and Ciprofloxacin (70.5%). Differences in resistance or sensitivity demonstrated by *Pseudomonas aeruginosa* towards antimicrobial drugs tested was found to be statistically significant ( $X^2=201.1817$ ;  $p < 0.05$ ) (Table 3).

**Table 3:** Antimicrobial Susceptibility Profile of *Pseudomonas aeruginosa* isolated from wounds of patients attending hospitals in Maiduguri metropolis

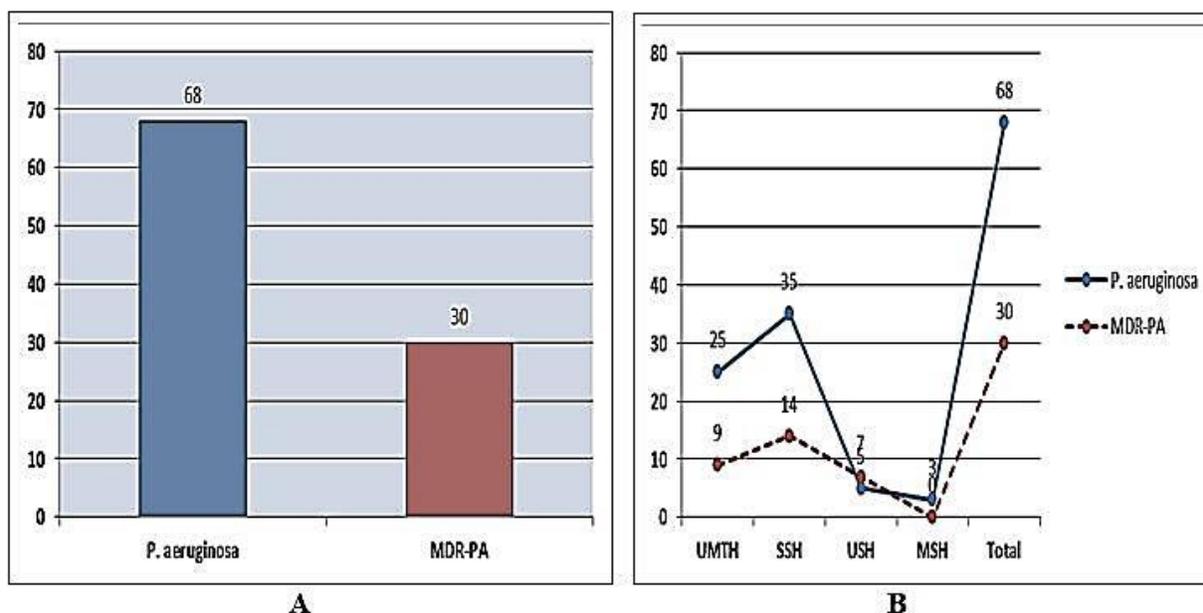
Antimicrobial drugs	Concentration (µg)	Breaking points			Antimicrobial Susceptibility Test (n=68) (%)		
		S≥	I	R≤	S	I	R
Ciprofloxacin	10	23	13-22	12	70.5	1.5	28.0
Gentamicin	10	18	-	17	35.3	35.3	29.4
Piperacillin-tazobactam	100	25	-	24	73.5	22.1	4.4
Ceftazidime	30	24	-	23	0.0	0.0	100
Aztreonam	30	36	20-35	19	1.5	16.1	82.4
Meropenem	10	20	16-19	15	38.2	26.5	35.3
X <sup>2</sup>	-	-	-	-	201.1817*		
p-value (α=0.05)	-	-	-	-	0.00001		

\*statistically significant p<0.05; S:sensitive; I:intermediate; R:resistant

**Multidrug resistance (MDR) profile of *Pseudomonas aeruginosa* identified from wounds of patients attending selected hospitals in Maiduguri metropolis**

Thirty (30) out of the sixty eight (68) *P. aeruginosa* isolated were multidrug resistant. This yields an MDR rate of 44.1%.

Multidrug resistance was more common among isolates identified from SSH (20.6%). This is followed by UMTH (13.2%) and the least was observed among isolates isolated from USH (10.3%) (Figure 1).



**Figure 1:** 44.10% of *Pseudomonas aeruginosa* identified were multidrug resistant (MDR-PA) (A). It can be seen from (B) that 71.0% of *P. aeruginosa* identified from the wounds of patients attending USH were Multidrug resistant (5/7).

## DISCUSSION

The infection of wounds by *P. aeruginosa* is a recipe for chronic infection that could jeopardize wound healing and lead to secondary infection, especially if it gains access to body tissues. In this study, we identified *P. aeruginosa* in the wounds of patients attending selected hospitals in Maiduguri metropolis. The infectivity rate was found to be highest among male patients than female patients, and patients within the age category of 21-40years. Similar findings were reported by Tom *et al.* (2019) in a research conducted in the same study area. They observed that *P. aeruginosa* is among the top two (2) gram negative pathogens found to be infecting wounds of hospitalized patients in Maiduguri metropolis. Tom *et al.* (2018) and Bahashwan and El Shafey, (2013) explicated that individuals whose age fall within the third decade of life are exposed to high risk of wound/and or trauma because they constitute the majority of workforce involved in hard and risky labour. Sports and farming are two engagements where young adults are actively participating and these activities expose them to the risk of acquiring injuries which in-turn, could be infected by *P. aeruginosa*. Although the infection rate observed in this study was low, the presence of *P. aeruginosa* in wounds is of utmost clinical significance due to its status as an opportunistic pathogen and its high virulence. Wound infection usually arises due to lack of proper care. A neglected wound can be open to infection by various pathogens. In the hospital environment, *P. aeruginosa* can be contracted through contact with surfaces such as sinks, bedrails, vital signs monitor, and supply carts. A more direct means of contracting the pathogen is through the hands of healthcare workers (Bennett, 2020). In this study, *P. aeruginosa* wound infection was found to be highest among patients attending UMTM followed by SSH. UMTM is the largest hospital of the four in terms of the number of patients admitted and services provided. The high prevalence observed in this hospital can be attributed to its large size and the number of patients it handles at a given time.

To prevent outbreak or infection of wounds by *P. aeruginosa*, the hospital management must establish effective infection prevention protocol that can be followed by healthcare professionals during patient care/handling. These professionals span from physicians, to nursing staff clinical microbiologists, hospital pharmacists, central sterile supply service, food service, housekeeping service, laundry and maintenance service. Efficient decontamination of hospital equipment and disinfection of hands and other body surfaces of healthcare workers will go a long way in minimizing the rate of transmission of *P. aeruginosa*. Routine surveillance must be embarked upon to monitor trends, to identify the needs for robust strategies and to appraise the effectiveness of the established prevention protocols (WHO, 2002). All isolates identified in this study were resistant to Ceftazidime. Resistance to Ceftazidime by *P. aeruginosa* is a preliminary indicator for extended-spectrum beta-lactamases (ESBLs) production. This is an important finding in the understanding that ESBLs production makes treatment difficult because it confers resistance to *P. aeruginosa*, against a wide variety of beta-lactam antibiotics. The genes coding for ESBLs such as *bla<sub>TEM</sub>*, *bla<sub>CTX</sub>*, and *bla<sub>SHV</sub>* are found on plasmids that can be transferred across bacterial populations. The proliferation of resistance-gene carrying plasmids is a recipe for Multidrug resistance by pathogenic bacteria. A significant number off *P. aeruginosa* isolates identified in this study were observed to be multidrug resistant with varying occurrence rates across the four hospitals, where patients attending USH harbour the highest.

## CONCLUSION

We report the isolation of multidrug resistant *P. aeruginosa* in wounds of patients attending selected hospitals in Maiduguri. The rate of infection was higher among male patients and patients within the third decade of life. We also revealed that all *P. aeruginosa* identified in this study were resistant to Ceftazidime, which is a preliminary indication of beta-lactamase production.

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**CONFLICT OF INTEREST**

The authors hereby declare that no conflict of interest exists.

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