Antimicrobial Resistance and Associated Factors of Pseudomonas aeruginosa Isolated from wounds of Patients in Keffi Nigeria.

ABSTRACT

Aim: The current study was aimed at determining the antibiotic resistance pattern and associated risk factors of *P. aeruginosa* isolated from wound among patients in kefii metropolis.

Study Design: The investigation was a cross-sectional study which utilized random sampling of wounds from patients in three hospitals in Keffi.

Place and Duration of Study: Keffi local government is situated in Nasarawa State located in the North Central region of Nigeria, it is about 125 km from Lafia the State capital and about 50 kilometers from Abuja the Federal Capital Territory. It has an area of 138 km^2 and a population of about 92,664 at the 2006 census. The study was conducted from June to October 2024

Methodology: A total of 253 wound samples from three hospitals (Federal Medical Centre Keffi: 198, Silvercord Hospital Keffi: 43 and Amosun Hospital Keffi: 12) were analysed by standard bacteriological and biochemical methods. Antibiotic susceptibility test was carried out in line with the CSLI standard.

Results: The incidence of *P. aeruginosa* was highest among those aged 21-40 years 8(3.16%), while the least was seen among those aged greater than 60 0(0%). Similarly, females had a higher incidence 7(2.76%) compared to males (6(2.37%). Furthermore, the incidence of *P. aeruginosa* was highest among those that were self employed 5(1.98%) 6(2.37%) The *P. aeruginosa* isolates were significantly resistant to ceftazidime and streptomycin 13(100%), but sensitive to imipenem 8(61.53%). Only 3(23.08)%) were found to be ESBL producers.

Conclusion: This study revealed that *P. aeruginosa* isolated from wound had a high level of resistance to the antibiotics used even though only three were ESBL producing, there is a need for survelance and increased publichealth awareness on wound management including prescription and indiscreminate use of antibiotics.

Key Words: Wound infection; *P. aeruginosa*; Antibiotic susceptibility; Risk factor; Keffi.

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•Consider adding a sentence to highlight the clinical implications of high antibiotic resistance rates for local healthcare.

Introduction

A breach in intact skin surface whether it is caused by trauma, accident, surgical operation, or burn provides an open door for bacterial infections [1]. The invasion of a wound by proliferating microorganisms to an extent where local spreading and/or systemic response in the host is invoked within the wound leads to the development of a range of virulence factors to overcome the host defences resulting to local tissue damage and impeding wound healing [2,3]. The characteristics of the individual (host), the type of wound, and the environment influences the risk of wound infection. Factors specific to each host that influence the development of wound infection are systemic, multifactorial and encompass many variables. The type of wound (i.e. aetiology) also contributes to the risk of infection, with acute wounds having a range of different risk factors for infection as compared to chronic wounds [4]. *Pseudomonas aeruginosa* is a Gram negative bacterial pathogen that has generated increased clinical importance as it is associated with morbidity, mortality, and poor quality of life in a variety of human infections including cystic fibrosis, burn wounds, surgical wounds, immunodeficiency, chronic obstructive pulmonary disorder (COPD), cancer, leg ulcers, and severe infection requiring ventilation, such as COVID-19 and other lung infections [5,6,7]. *Pseudomonas aeruginosa* is a predominant pathogen that causes various chronic infections. Relapse infections promote the adaptation and evolution of antimicrobial resistance and virulence of *P. aeruginosa*, which obscure evolutionary trends and complicate infection management [8]. The organism was first described by the French Pharmacist Carle Gessard in 1882. It produces a unique number of pigments in culture, known as pyocyanin, (a blue/green phenazine compound) that has both antimicrobial and toxin properties. The name Pseudomonas is derived from two Greek words: Pseudo meaning ‘false’ and Monas meaning ‘single unit’; while aeruginosa ‘greenish-blue’ is from the latin aerūgō meaning ‘rusted copper’ [9]. *Pseudomonas aeruginosa* is a multi-drug resistance (MDR) opportunistic pathogen and is

increasingly encountered from a variety of infections including wound and burns. Antibiotics are substances that are used for inhibiting the growth and treating infections caused by specific bacteria. They play an important role in treating bacterial infections, including life-threatening ones. Antibiotics are one of the wonder discoveries of the twentieth century. Antibiotic resistance occurs when a normally active antibiotic no longer shows activity against susceptible bacteria [10]. However, antibiotics resistance does not necessarily mean that the human body is resistant to antibiotics, instead, this phenomenon occurs when the growth of microbes is no longer inhibited or destroyed by antibiotics [11]. *P. aeruginosa* aquisation of resistance depends primarily on multiple intrinsic and acquired antibiotic resistance mechanisms, including the biofilm-mediated formation of resistant and multi-drug-resistant persistent cells [5]. The World Health Organization (WHO) reported that *P. aeruginosa* poses one of the greatest threats to humans in terms of antibiotic resistance [12]. Furthermore, treatments of *P. aeruginosa* infection are extremely difficult due to its rapid mutations and adaptation to gain resistance to antibiotics [13,14]. Studies have shown that the spread of plasmid–encoded Extended-Spectrum Beta-Lactamase (ESBL) genes confers resistance to third generation cephalosporin [15,16].

2.0 Materials and Methods

2.1 Study Design and Population

The investigation was a cross-sectional study which utilized random sampling of wounds from patients in three hospitals in Keffi from June to October 2024.

2.2 Study Location and Population

Keffi local government is situated in Nasarawa State located in the North Central region of Nigeria, it is about 125 km from Lafia the State capital and about 50 kilometers from Abuja

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•The statistical methods need more detail, such as specifying how confidence intervals were calculated and the justification for the sample size determination. Additionally, the ethics statement is repeated in two sections, which could be streamlined.

the Federal Capital Territory. It has an area of 138 km^2 and a population of about 92,664 at the 2006 census [17].

2.3 Inclusion Criteria.

Patients of all age group and gender with wound assessing healthcare in the selected health facilities in Keffi metropolis were included in this study.

2.3.1 Exclusion Criteria.

Patients without wounds or those with wound but on antibiotics attending the selected health facilities were excluded from this study

2.4 Sample Size Determination.

A prevalence rate of 18.3% reported by [18] was used to estimate the minimum sample for the study using the formula described by [19] as follows;

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

Where, N = Sample size Z = Statistics for a level of 95% confidence interval = 1.96

P = prevalence rate of *P. aeruginosa* infection from previous studies = 18.3% [18].

d = level of significance (allowable error) = 5% or 0.05

q = 1-p

Thus, $N = (1.96)^2 \times 0.183 \times (1-0.183)/ (0.05)^2 = 3.8416 \times 0.183 \times 0.817 /0.0025 = 229.7445 \approx 230$

N = 230

However, actual sample size = Calculated sample size + 10% Attrition rate. But 10% Attrition rate = 23

Therefore, actual sample size = 230 + 23 = 253

2. 5 Ethical Consideration

Ethical clearance for this research was obtained from the Nasarawa State Ministry of Health in line with the Declaration of Helsinki on the conduct of biomedical research involving human subjects. All participants gave their consent to participate in the study.

2.6 Sample Collection

The sterile labeled swab sticks were rolled over the wound site gently to obtain adequate samples. The swab sticks were aseptically returned into their containers and transported immediately in an ice box to the Microbiology laboratory for processing within one hour of collection [20]. In addition, the socio-demographic and clinical data for each patient was obtained by the use of a structured questionnaire.

2.7 Preparation of Culture Media

The media used were weighed and prepared according to manufacturer's specification. The prepared media were carefully packed into the autoclave and sterilized at 121˚C for 15 minutes. Thereafter, they were allowed to cool to 45˚C before pouring into Petri dishes or MacCartney bottles as the case may be.

2.8 Sample Analysis

Swabs were gently pressed directly onto the prepared MacConkey and Blood agar to make a primary inoculum before streaking using sterile wire loop and incubated at 37˚C for 24 hours [21].

2.8.1 Isolation and Identification

Isolates were identified on the basis of standard bacteriological methods like morphology, colonial characteristics, smell in culture, haemolysis, as well as pigment production on media.

All suspected isolates were further characterized and identified by observing biochemical reactions such as oxidase test, urease test, indole test, citrate test, sugar fermentation test, Tsia (triple sugar iron agar), methyl red test and Voges Proskauer test. The tested isolates were stored on nutrient agar slants for further use [21,22].

2.9 Antibiotic Susceptibility Testing

The susceptibility profile of antibiotics commonly prescribed for *P. aeruginosa* infections was determined using Kirby-Bauer disc diffusion method in accordance with CLSI guidelines [23]. The antimicrobial agents tested included: ofloxacin (5µg), augmentin (10µg), ceftazidime (30µg), ceftriaxone (30µg), gentamicin (10µg), ciprofloxacin (5µg), streptomycin (30µg), chloramphenicol (30µg), imipenem (10µg), and amoxicillin (10µg). Briefly, 3 pure colonies of the isolate was inoculated into 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland standard. The McFarland's standard was prepared as follows; 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ was added into 99.5 ml of 1% (w/v) H_2SO_4 [23]. A sterile swab stick was dipped gently into the standardized bacteria suspension while pressing the swab lightly on the wall of the tube to remove excess broth. This was followed by streaking on Mueller Hinton agar plates before placing the antibiotic discs aseptically and allowed to stand for 1 hour for pre-diffusion. The plates were then incubated at 37 ^{0}C for 24hours. The diameter zone of inhibition in millimeter was measured and the result of the susceptibility interpreted in accordance with the susceptibility break point described by Clinical and Laboratory Standards Institute [23].

2.10 Ethical Consideration

Ethical clearance for this research was obtained from the Nasarawa State Ministry of Health NHREC protocol number: 18/06/2017. Additionally, informed consent was required from all participants prior to the collection of their samples.

2.11 Data Analysis

The Smith's Statistical Package (version 2.8, California, USA) was used to analyse data obtained. The chi-square test at a 95% confidence interval and P values ≤ 0.05 was considered statistically significant.

3.0 Results

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•The study's findings on gender and age differences in prevalence could benefit from further exploration and discussion, especially if related studies have found differing trends.

3.1 Distribution of Factors Associated with Isolation of *P. aeruginosa* in Keffi

Out of the 253 wound swab collected in the selected hospitals, the prevalence was higher among those aged between 21-40 years 8(3.16%), followed by 41-60 years 4(1.58%) and 1(0.39%) for those aged less than 20. The least prevalence was recorded among those greater than 60 years 0(0%) (P=0.000). Regarding the gender of participants, females had a higher prevalence 7(2.76%) compared to males 6(2.37%) (P=0.457). In this study, the distribution of the isolate regarding occupation of the participants was found to be higher among those that were self employed 5(1.98%) followed by Farmers 3(1.18%), Drivers 2(0.79%) and Students 2(0.79%) while the least was recorded among Civil servants 1(0.39%) (P=0.612). Finally the distribution regarding the type of wound revealed that those who had traumatic wound 6(2.37%) had the highest prevance followed by those who had surgical wound 3(1.18%) while for those that had burns and non-traumatic wounds it was 2(0.79%) being the least respectively with P= 0.797 as shown in Table 1

Table 1 Distribution of Factors Associated with Isolation of *P. aeruginosa* Isolates in Keffi

Age	**Number examined**	**Number of Isolates (%)**	***P.* value**
< 20	47	1(0.39)	
21-40	105	8(3.16)	0.000

41-60	76	4(1.58)	
>60	25	0(0)	
Total	253	13 (5.13)	
Gender			
Male	142	6(2.37)	
Female	111	7(2.76)	0.457
Total	253	13 (5.13)	
Ocupation			
Farmer	64	3(1.18)	
Self employed	65	5(1.98)	0.612
Civil servant	44	1(0.39)	
Student	22	2(0.79)	
Driver	58	2(0.79)	
Total	253	13 (5.13)	
Wound type			
Surgical	54	3(1.18)	
Traumatic	107	6(2.37)	0.797
Non-traumatic	66	2(0.79)	
Burns	26	2(0.79)	
Total	253	13 (5.13)	

3.2 Antibiotic Resistance Profile of the *P. aeruginosa* Isolated in Selected Hospitals of Keffi, Nasarawa State, Nigeria.

The antibiotic resistance profile of *P. aeruginosa* is shown in Table 2 where the isolates were highly resistant to ceftazidime and streptomycin 13(100%), followed by amoxicillin,

ceftriaxone, cefurexime and gentamycin 12(92.30%), augmentin and ciprofloxacin 10(76.92%), while it was least resitant to ofloxacin 9(69.23%) and imipenem 8(61.53%) respectively (P = 0.044).

Table 2. Antibiotic Resistance Profile of the *P. aeruginosa* Isolated in Selected Hospitals of Keffi, Nasarawa State, Nigeria.

Antimicrobial Agent	**Disc Content (µg)**	**Number of tested isolates**	**Sensitive** **Number (%)**	**Intermediate** **Number (%)**	**Resistance** **Number (%)**
Ofloxacin(OFX)	05	13	4(30.76)	0(0)	9(69.23)
Augmentin (AU)	30	13	1(7.69)	2(15.38)	10(76.92)
Ceftazidime (CTZ)	30	13	0(0)	0(0)	13(100)
Gentamycin (CN)	10	13	1(7.69)	0(0)	12(92.30)
Ciprofloxacin (CPX)	10	13	1(7.69)	2(15.38)	10(76.92)
Imipenem (IMP)	30	13	2(15.38)	3(0)	8(61.53)
Ceftriaxone (TRX)	30	13	0(0)	1(0)	12(92.30)
Streptomycin (S)	30	13	0(0)	0(0)	13(100)
Cefuroxime (CEF)	30	13	1(7.69)	0(0)	12(92.30)
Amoxicillin (AM)	30	13	0(0)	1(7.69)	12(92.30)

P = 0.044

3.3. Antimicrobial Resistance Phenotypes of the *P. aeruginosa* Isolated from Selected Hospitals of Keffi.

The antibiotic resistance profile was significantly associated to *P. aeruginosa* isolates as shown in Table 3. The highest resistance profile was seen in the following antibiotics ; AM, AU, CEF,

CN, CPX, CTZ, IMP, OFX, S, TRX 9(69.2%), while the least resistance was seen in AM, AU, CEF, CN, CTZ, IMP, OFX, S, TRX; AM, AU, CEF, CN, CTZ, IMP, S, TRX; AM, AU, CEF, CN, CTZ, S, TRX and AM, CPX, CTZ, IMP, S, TRX 1(7.7%) respectively (P=0.000).

Table 3. Antimicrobial Resistance Phenotypes of the *P. aeruginosa* Isolated from Selected Hospitals of Keffi.

Antibiotic resistance phenotypes	**Number *of P. aeruginosa* isolates (%)**	**P value**
AM, CPX, CTZ, IMP, S, TRX	1(7.7)	
AM, AU, CEF, CN, CTZ, S, TRX	1(7.7)	0.000
AM, AU, CEF, CN, CTZ, IMP, S, TRX	1(7.7)	
AM, AU, CEF, CN, CTZ, IMP, OFX, S, TRX	1(7.7)	
AM, AU, CEF, CN, CPX, CTZ, IMP, OFX, S, TRX	9(69.2)	
Total	13(100)	

AM= Amoxycillin, AU= Augmentin, CEF= Cefurexime, CN= Gentamycin, CPX= Ciprofloxacin, CTZ= Ceftazidime, OFX= Ofloxacin, S= Streptomycin, TRX= Cefriaxone, IMP= Imipenem

3.4 Phenotypic Detection of ESBL-Producing *P.aeruginosa*

The production of ESBL from cephalosporin resistant *P. aeruginosa* isolated from the respective hospitals was not statistically significant as shown in Table 4. Out of 13 resistant isolates, 3(23.08)%) were found to be ESBL producers (P= 0.103).

Table 4. Phenotypic Detection of ESBL-Producing *P.aeruginosa*

Facility	**No. Resistant**	**PCDDT**	**DDST**	***P* value**

		ESBL Pos (%)	ESBL Neg		ESBL Pos (%)	ESBL Neg	
FMCK	6	6(46.15)	0	$X^2 = 0.000$	3(23.08)	3 (23.08)	
SCHK	4	4(30.77)	0	P = 0.000	0(0)	4(30.77)	0.103
AHK	3	3(23.08)	0		0(0)	3 (23.08)	
Total	13	13 (100)	0		3 (23.08)	10 (76.92)	

FMCK= Federal Medical Centre Keffi, SCHK= Silvercord Hospital Keffi, AHK= Amosun Hospital Keffi. ESBL= Extended spectrum betaLactamase, PCDDT= Phenotypic Confirmatory disc difusion test, DDST= Double disc synergy test

4.0 Discussion

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•Include more interpretation on how local factors (e.g., healthcare practices, environmental factors in Keffi) may explain the observed resistance patterns.

P. aeruginosa has been placed as a priority pathogen by the WHO regarding antibiotic resistance [24]. It is one of the most frequent pathogen associated with severe complication of wound infection [25]. In this study, out of the 253 wound swab collected in the selected hospitals, an overall prevalence of 13(5.13%) was recorded. The prevalence obtained in this study was similar to that reported by [26] in Portharcourt. On the contrary the prevalence obtained in this study is lower than 24.1 % reported by [27] in Keffi, 54.5% by [28] in Sokoto and 26.67% reported by [29] in Jos, Also, a higer prevalence of 96.0 % was reported by [30] in South-West Nigeria. Similarly, higher prevalence of 12.86% was reported by [31] in Ethiopia, 30.2% by [32] in Ghana, [33] reported a prevalence of 40% in Egypt. Several authors have also reported high prevalence accross other continents [25,34,35,36,37,38,39]. The differnce in regional prevalence could be as a result of inherent resistance of Pseudomonas spp. to frequently administered antibiotics as reported by [40], also, biofilm-mediated resistance and formation of multidrug-tolerant persister cells, contribute to recalcitrance and relapse of infections requiring more prescription of antibiotics [5]. Furthermore, [31] reported that the type of study design, the different types of selective/differential media used for culturing the target organism and the protocols for sampling could also result in the differences.[41] reported

a potential zoonotic transmission of *P. aeruginosa* between animals, the environment, and human populations may contribute to varying occurence and distribution of accross the globe.

The prevalence of *P. aeruginosa* in the study are was also determined with respect to some sociodemographic factors. Out of the 253 wound swab collected in the selected hospitals, the prevalence was higher among those aged between 21-40 years 8(3.16%), followed by 41-60 years 4(1.58%) and 1(0.39%) for those aged less than 20. The least prevalence was recorded among those greater than 60 years 0(0%) (P=0.000).

The result revealed a significant association between rate of infection and age. Similar association have been reported by [26,28,30] in different parts of Nigeria and other parts of the world [34]. Interestingly, isolation of *P. aeruginosa* from wounds of patients much younger than that reported in this study have been documented [34,38,42,43].

Regarding the gender of participants, females had a higher prevalence 7(2.76%) compared to males 6(2.37%) although there was no significant association (P= 0.457). This is similar to several reports within and outside Nigeria [26,29,30,31,44,42]

Converesely, [27,28,34,36,45,46,47] all reported hiher prevalence in males.

In this study, the distribution of the isolate regarding occupation of the participants was found to be higher among those that were self employed 5(1.98%) followed by Farmers 3(1.18%), Drivers 2(0.79%) and Students 2(0.79%) while the least was recorded among Civil servants 1(0.39%) (P=0.612). Finally the distribution regarding the type of wound revealed that those who had traumatic wound 6(2.37%) had the highest prevance followed by those who had surgical wound 3(1.18%) while for those that had burns and non-traumatic wounds it was 2(0.79%) being the least respectively with P= 0.797 as shown in Table 1. On the contrary, [25] reported a higher prevalence among those that had traumatic wound followed by surgical

wounds while the least was reported from burns wound. [42] reported higher prevalence (66.6%) among patients that had burns wound followed by those that had post-surgigal wound (62%).

Regarding the antibiotic resistance profile of *P. aeruginosa*, the isolates were found to be highly resistant to ceftazidime and streptomycin 13(100%), amoxicillin, ceftriaxone, cefurexime and gentamycin 12(92.30%), augmentin and ciprofloxacin 10(76.92%), while it was least resitant to ofloxacin 9(69.23%) and imipenem 8(61.53%) respectively (P = 0.044). this is in agreement with [28] who reported high resistance to cefuroxime, 50 (90.9%) and ceftrizone, 54 (98.1%) while the least resistance was imipenem (54.5%), also, [48] reported similar partern of resistance.

[49] reported high sensitivity to imipenem (90.6%) and (56.3% to ciprofloxacin). [26] also reported a high resistance to cefurexime (96.30%) while the highest sensitivity was seen with ciprofloxacin (37.04%), [30] reported highest resistance for gentamycin (35.4%). In a study by [50] in Ethiopia, *P.aeruginosa* showed resistance against gentamicin at 62.2%, ceftazidime 51.4%, cefepime 50%, amikacin 29.7%, imipenem 28.4% and ciprofloxacin 14.9%. while Ahmed *et al*. (2023) reported a higher sesitivity to ciprofloxacin (79.2%). Higher resistance to ciprofloxacin was also reported by [51]. On the contrary, a higher sensitivity to the antibiotics was reported by [34].

The antibiotic resistance profile of *P. aeruginosa* isolates was investigated revealing a significantly high 9(69.2%) multidrug resistance (MDR) profile (P=0.000). The value optained in this study is similar to 58.4% reported by[51], but was higher than 12.8% and 29.63% reported by [26] and [30] in Nigeria. Similarly, lower prevalence were reported in other countries [31] and [50]. In contrast however, the value obtained in this study was lower than 95.8% reported by [52] and [53]. The reason for the continued spread of MDR *P. aeruginosa*

could be as a result of extensive use or misuse of broad spectrum antibiotics, extended periods of hospital admission, community spread of MDR organisms, lack of proper healthcare facilities, ability to form biofilm and increased expression of MDR genes [54,55,56,57].

4.0 CONCLUSION

The result of this study shows a notably low prevalene of *P. aeruginosa* 13 (5.13%) isolated from wound of patients in Keffi metropolis. Though the prevalence was low, there is a strong indication that *P. aeruginosa* is an important pathogen in wound infections. The significant occurrence of multidrug resistance to commonly prescribed antibiotics recorded in this study and the detection of ESBL resistance genes is worrisome and should be considered as a public health concern. The relatively low resistance to imipenem is noteworthy as it has been reported to be effective in the treatment of chronic infections. Data obtained in this study will support the ongoing public health initiatives aimed at controlling infection and guiding effective treatment protocols in the study area and the country at large.

5.1 RECOMMENDATIONS

The following recommendations were proffered to curb the menace of the rising antibiotic resistance:

I. The continous monitoring af antibiotic resistance in the studty area through the establishment of antimicrobial resistance surveillance network in health facilities and in the community.

II. Ensuring strict compliance towards infection control measures

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III. The fight to combat antimicrobial resistance requires a holistic approach and concerted efforts by policy makers, researchers, clinicians, Medical Laboratory Scientists, Pharmacists, Community health workers and the patient.

IV. Further research in the study area to cover more health facilities and incorporate molecular methods over time is recommended.

CONSENT

Written informed consent was taken from each participant.

ETHICAL APPROVAL

Ethical clearance for this research was obtained from the Nasarawa State Ministry of Health (NHREC Protocol number: 18/06/2017) in line with the Declaration of Helsinki on the conduct of biomedical research involving human subjects.

REFERENCE

Commented [w6]: •Some references appear incomplete or formatted inconsistently, which affects credibility. Correctly formatting citations and ensuring accurate reference information is essential.
•Consistent table formatting, especially in terms of data alignment and abbreviations, would enhance the readability of results.

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