Antibiotics Sensitivity Profile of \textit{Pseudomonas aeruginosa} Isolated from Wound Swabs and Urine Samples from University of Medical Sciences Teaching Hospital, Akure, Nigeria

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\textbf{Authors’ contributions}

This work was carried out in collaboration among all authors. Author MAA designed, conducted and analysed the data of the experiment. Author MTB corrected the manuscript and did the final proof read. Author JAA wrote the methodology and managed the literature searches. Authors JAA and IFA wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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\textbf{ABSTRACT}

\textbf{Aim:} To investigate the antimicrobial susceptibility profile of \textit{Pseudomonas aeruginosa} enumerated from wound swabs and urine samples from the University of Medical Sciences Teaching Hospital, Akure, Nigeria.

\textbf{Place of Study:} University of Medical Sciences Teaching Hospital, Akure, Ondo State, Nigeria, between January and May, 2019.

\textbf{Methodology:} Wound swabs and urine samples were collected from patients of University of Medical Sciences Teaching Hospital, Akure. Enumeration and identification of \textit{P. aeruginosa} isolates was employed. Antibiotic sensitivity test was conducted on the enumerated \textit{P. aeruginosa} strains from both clinical specimens via standard disc diffusion protocol. The susceptibility and resistance pattern of \textit{P. aeruginosa} isolates was established utilizing clinical laboratory standard institute (CLSI) standard.
Results: Ciprofloxacin was observed to display the highest zone of inhibition (ZOI) of 17.00±1.00 mm for P. aeruginosa isolate 1 and likewise the highest ZOI of 24.50±1.50 mm for P. aeruginosa strain 3. Fourteen strains of Pseudomonas aeruginosa exhibited highest resistance to septrin and augmentin for wound swabs as all 19 of the bacterial strains also exhibited the highest resistance to septrin, chloramphenicol, augmentin and streptomycin for urine specimens. Ten (10) of P. aeruginosa strains from wound swabs exhibited the highest intermediate susceptibility to perfloxacin. Eleven (11) strains of P. aeruginosa from urine specimens exhibited the highest intermediate susceptibility on sparfloxacin. All 19 strains of P. aeruginosa from urine specimens were susceptible to amoxicillin and gentamicin as completely minimal susceptibility was recorded for P. aeruginosa associated with wound swabs.

Conclusion: This study demonstrated the high resistance pattern of P. aeruginosa associated with wound swabs and urine samples and emphasizes the need for the regimentation of over-the-counter remedy and antibiotic susceptibility appraisal of anti-pseudomonal drugs.

Keywords: Pseudomonas aeruginosa; urine; wound swabs; antibiotics; susceptibility; resistance.

1. INTRODUCTION

An antibiotic is any substance that inhibits the growth of bacteria or completely kills it [1]. The first antibiotics, penicillin was discovered by Sir Alexander Fleming in the year 1928, which began the modern era of antibiotics and ever since antibiotics have greatly improved medicine, helped in saving numerous lives [2] and increased life expectancy by changing the results of bacterial infections [3]. The mechanism of activity of antibiotics includes inhibition of cell wall synthesis, inhibition of cell membrane function, metabolite antagonist, inhibition of protein synthesis and inhibition of nucleic acid synthesis [4].

The victory over bacterial infection won by the arrival of antibiotics several years ago now seems temporary due to the threat of resistant bacteria [5]. Antibiotic resistance of bacteria refers to its inherent ability to decline the efficiency of a particular antibiotic through its physiological or structural traits [6]. The consistent insurgence of resistant bacteria is now a global occurrence, compromising the efficiency of antibiotics, whose importance cannot be underestimated in medicine for its live saving essence [7,8]. The antibiotic resistance calamity has been associated with the frequent use and abuse of antibiotics, the failure of pharmaceutical industry to manufacture new drugs due to the financial implication in a capsizing economy is another probable cause [9].

Gram-negative bacteria are part of the vital health problems globally, since they infect patients who have had a prolonged stay in the hospital particularly the intensive care unit (ICU) [10]. Pseudomonas aeruginosa is a Gram-negative bacterium that is ubiquitous [11] and identified as an opportunistic pathogen that is very often connected with nosocomial infections and ventilator-associated Pneumonia [12]. P. aeruginosa is ranked the second major Gram-negative pathogen causing hospital acquired infections and it is associated with increased hospitalization and death rate because of its persistent antibiotics resistance [13]. The pathogenicity of this bacterium gratifies its antibiotic resistance which in turn prolongs patients’ stay in the hospital and causes challenges in treatment [14]. Supposed urinary tract maladies caused by P. aeruginosa are escalating both in hospitals and in the public and it have been reported as one of the principal origin of multifarious antibiotic-resistant nosocomial bug, primarily amidst immune-weakened subjects [15-16]. P. aeruginosa has been reported to be one of the chief causal agent and bacterial consortia implicated in wound infection [17]. Alabi [18] also observed P. aeruginosa as the most copious bacteria associated with wound swabs obtained from in-patients attending a teaching hospital in Akure, Nigeria. Therefore this study is aimed at presenting the antibiotics susceptibility profile of P. aeruginosa isolated from wound and urine samples of patients in University of Medical Sciences Teaching Hospital, Akure, Nigeria.

2. METHODOLOGY

2.1 Study Area Catchment

This study was carried out at the University of Medical Sciences Teaching Hospital, Akure. Akure is the capital of Ondo State in Southwestern Nigeria. The University of Medical
2.2 Collection of Clinical Samples

Wound swabs were collected from patients in male medical, female medical, male surgical and female surgical wards of University of Medical Sciences Teaching Hospital, Akure, Ondo State, Nigeria. The samples were carefully collected by medical personnel and only one swab per patient was collected. The samples were immediately transported to the Department of Microbiology laboratory for analysis. Urine specimens were collected from medical laboratory unit of UNIMEDTH, Akure in sterile sample bottles and transported at a temperature of 4-8°C in a coolant pack to the Microbiology laboratory, FUTA for microbiological analysis within 1 hour of collection [19].

2.3 Isolation of P. aeruginosa from Wound Swab and Urine Samples

A total of 57 wound swab samples were collected using swab sticks. The swab samples were streaked on Nutrient agar and incubated at 37°C for 24 hours [20]. A total of 206 urine samples were collected. Bacteriological analysis of collected urine specimens was carried out according to the methods reported in literature [21-23]. The urine samples were immediately transferred aseptically using sterile and flame inoculating loop by streaking unto Nutrient Agar. All plates were incubated at 37°C for 24 hours.

2.4 Confirmation of Bacterial Isolate

Each isolate was sub-cultured on freshly prepared Nutrient agar to obtain pure cultures. Besides the morphological characteristic growth of P. aeruginosa on nutrient agar observed, the pure cultures were confirmed using standard biochemical tests as described by [24].

2.5 Determination of the Antibiotic Sensitivity

The antibiotic susceptibility test was carried out using standard disc diffusion techniques as described by Clinical Laboratory Standard Institute [25] using commercially available antibiotics. The isolates were sub-cultured on nutrient agar plates and incubated at 37°C for 18 hours. Mueller-Hinton broth was prepared following the manufacturer’s specification, inoculated with isolate and incubated for 6 hours. One millilitre (1ml) aliquot of the bacteria suspension equivalent to 0.5 McFarland standard was aseptically seeded unto freshly prepared Mueller-Hinton agar plates. The antibiotics disc was placed aseptically on the surface of the seeded plate and allowed to diffuse for 30 minutes and then incubated for 18 hours at 37°C, after which the zone of inhibition was measured. The antibiotics sensitivity test was done in duplicate.

2.6 Statistical Analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) software version 26. Data obtained were subjected to analysis of variance and results are presented as mean ± standard error. Statistical significance was obtained at values (p=0.05) and the means were separated using Duncan’s multiple range test.

3. RESULTS AND DISCUSSION

Table 1 shows the morphological and biochemical characteristics of P. aeruginosa. This confirms the biochemistry of P. aeruginosa coupled with the macro-morphological repertoire of the bacteria. This finding is in consonance with the observation of the study conducted by [26] who investigated P. aeruginosa resistance pattern of antibiotics in urine specimens and wound swabs from Iranian educational hospital. Table 2 gives the details of the zones of inhibition (mm) of P. aeruginosa strains isolated from wound swab samples against commercial antibiotics. Ciprofloxacin was observed to display the highest zone of inhibition (ZOI) of 17.00±1.00 mm for P. aeruginosa isolate 1 while P. aeruginosa strains 3, 4, 5, 6, 8, 9, 11, 12 and 14 all had least ZOI of 7.00±1.00 mm on chloramphenicol, sparflxacin, amoxicillin, augmentin, gentamicin, and streptomycin. This outcome bears semblance with the findings of Rostamzadeh et al. [26] who observed ciprofloxacin as one of the most effective antimicrobial agent for the management of P. aeruginosa associated with wound infection. Alabi [18] also detailed considerable P. aeruginosa susceptibility profile in a study on the antibiotics sensitivity profile of wounds’ bacterial isolates. Table 3 gives the details of the zones of inhibition (mm) of P. aeruginosa strains isolated from urine samples against commercial antibiotics. Ciprofloxacin also displayed the
highest zone of inhibition (ZOI) 24.50±1.50 mm for *P. aeruginosa* strain 3 while streptomycin, septrin, chloramphenicol and augmentin all had the least ZOI 7.00±0.00 mm. This result could be connected to the *P. aeruginosa* resistance prevalence mostly among beta-lactams which are chiefly prescribed by clinicians in hospital setting for the management of *P. aeruginosa* causing suspected urinary tract infection as supported by Bayode et al. [16] who worked on multiple antibiotic resistant index and detection of qnrS and qnrB genes in bacterial consortium of urine samples from clinical settings in their study. Fig. 1 shows the number of *P. aeruginosa* isolated from wound swabs and urine samples resistant to commercial antibiotics tested. Fourteen (14) strains of *P. aeruginosa* exhibited highest resistance to septrin and augmentin for wound swabs while just two strains exhibited resistance to perfloxacin. This finding is analogous to the outcome of the study conducted by Rostamzadeh et al. [26]. All 19 of the bacterial strains also exhibited the highest resistance to septrin, chloramphenicol, augmentin and streptomycin for urine specimens while 7 strains of *P. aeruginosa* exhibited the least resistance to sparfloxacin. Similarly, *P. aeruginosa* isolated from urine, and wound swabs was found resistant to chloramphenicol which corroborated the findings of this study [27-28]. This might be connected to the self medication of this drug implicated in this study. The findings of this study is parallel to the observation of Ahmed-Hassan et al. [29] who observed the clinical isolates of *P. aeruginosa* were highly resistance to beta-lactams antimicrobials including; augmentin, amoxicillin, ampicillin and Cefixime which could be linked to the elevated assembly of beta lactamase enzyme via the resistance genes and trans-mutational machinery of the implicated bacteria as stated by CDC [30], Lister et al. [31] and Okon et al. [32]. Fig. 2 shows the number of *P. aeruginosa* isolated from wound swab and urine samples intermediate to commercial antibiotics tested. Ten (10) of *P. aeruginosa* strains from wound swabs exhibited the highest intermediate susceptibility to perfloxacin while only one strain of the bacterium exhibited intermediate susceptibility to septrin and amoxicillin. Eleven (11) strains of *P. aeruginosa* from urine specimens exhibited the highest intermediate susceptibility on sparfloxacin while only one strain of the bacterium exhibited intermediate susceptibility to ciprofloxacine and perfloxacin. The rationale for this could be related to the voracious spread of plasmid/chromosomally-mediated resistance genes dispersal in *P. aeruginosa* isolates in clinical milieu as augmented by Bayode et al. [16]. Fig. 3 shows the number of *P. aeruginosa* isolated from wound swab and urine samples susceptible to commercial antibiotics tested. All 19 strains of *P. aeruginosa* from urine specimens were susceptible to amoxicillin and gentamicin as completely minimal susceptibility was recorded for *P. aeruginosa* associated with wound swabs. Though this finding is at odds with the observation of Bayode et al. [16] who observed ultra-resistance of *P. aeruginosa* to gentamicin in their report.

![Fig. 1. Number of *P. aeruginosa* isolated from wound swabs and urine samples resistant to commercial antibiotics tested](image)
### Table 1. Morphological and biochemical characteristics of *P. aeruginosa* in wound swabs and urine specimens from UNIMEDTH

| Morphological characteristics | Colour | Elevation | Surface | Edge | Shape |
|------------------------------|--------|-----------|---------|------|-------|
| Gram's reaction             | Green  | Flat      | Smooth  | Entire| Round |
| -                            | +      | +         | +/-     |       |       |
| +                            | -      | -         |         |       |       |

#### Biochemical characteristics

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### Table 2. Zones of inhibition (mm) of *P. aeruginosa* isolated from wound swabs against antibiotics

| Strains | SXT | CH | SP | CPX | AM | AU | CN | PEF | OFX | S |
|---------|-----|----|----|-----|----|----|----|-----|-----|----|
| R ≤ 10  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 12  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 15  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 16  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 17  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 18  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 19  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 20  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 21  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 22  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 23  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 24  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 25  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 26  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 27  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 28  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 29  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 30  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 31  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 32  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 33  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 34  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 35  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 36  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 37  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 38  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 39  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 40  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 41  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 42  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 43  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 44  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 45  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 46  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 47  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 48  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 49  |     |    |    |     |    |    |    |     |     |    |
| R ≤ 50  |     |    |    |     |    |    |    |     |     |    |

Results are presented as mean ± SE. Values carrying the same alphabet in similar row are not significantly dissimilar (P=0.05). Key: SXT= Septrin (30µg); CH= Chloramphenicol (30µg); SP= Sparfloxacin (10µg); CPX= Ciprofloxacin (30µg); AM= Amoxicillin (30µg); AU= Augmentin (10µg); CN= Gentamicin (30µg); PEF= Pefloxacin (30µg); OFX= Ofloxacine (10µg); S= Streptomycin (30µg)
Table 3. Zones of inhibition (mm) of *P. aeruginosa* isolated from urine against antibiotics

| Strains   | OFX | S   | SXT | CH  | SP  | CPX | AM  | AU  | CN  | PEF |
|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|           | S = ≥16 | S ≥15 | S ≥16 | S ≤12 | R ≤11 | R ≤10 | R ≤12 | R ≤15 | R ≤13 | R ≤12 | R ≤12 |
| R = ≤12  | 1   | 17.50±2.50 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 14.00±1.00 | 23.00±1.00 | 23.00±1.00 | 6.00±0.00 | 15.00±1.00 | 19.50±1.50 |
|           | 2   | 15.00±1.00 | 15.00±1.00 | 15.00±1.00 | 15.00±1.00 | 22.00±1.00 | 22.00±1.00 | 22.00±1.00 | 7.00±1.00 | 15.00±1.00 | 17.00±2.00 |
|           | 3   | 18.50±0.50 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 15.50±2.50 | 21.50±1.00 | 21.00±1.00 | 6.00±0.00 | 17.50±0.50 | 21.50±2.50 |
|           | 4   | 7.00±0.00 | 7.00±0.00 | 7.00±0.00 | 17.50±1.50 | 22.00±1.00 | 22.00±1.00 | 22.00±1.00 | 7.00±1.00 | 15.00±1.00 | 17.00±2.00 |
|           | 5   | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 14.50±5.00 | 20.00±1.00 | 20.00±1.00 | 20.00±1.00 | 6.00±0.00 | 14.50±0.50 | 17.50±1.50 |
|           | 6   | 7.00±0.00 | 6.00±0.00 | 6.00±0.00 | 14.50±5.00 | 22.00±1.00 | 22.00±1.00 | 22.00±1.00 | 7.00±1.00 | 17.50±1.00 | 17.50±2.00 |
|           | 7   | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 14.50±5.00 | 22.00±1.00 | 22.00±1.00 | 22.00±1.00 | 7.00±1.00 | 17.50±1.00 | 17.50±2.00 |
|           | 8   | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 14.50±5.00 | 22.00±1.00 | 22.00±1.00 | 22.00±1.00 | 7.00±1.00 | 17.50±1.00 | 17.50±2.00 |
|           | 9   | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 14.00±1.00 | 22.00±1.00 | 22.00±1.00 | 22.00±1.00 | 7.00±1.00 | 17.50±1.00 | 17.50±2.00 |
|           | 10  | 7.00±1.00 | 6.00±0.00 | 6.00±0.00 | 7.00±1.00 | 16.50±2.00 | 16.50±2.00 | 16.50±2.00 | 7.00±1.00 | 17.50±1.00 | 17.50±2.00 |
|           | 11  | 7.00±1.00 | 7.00±1.00 | 7.00±1.00 | 17.00±1.50 | 20.00±1.00 | 20.00±1.00 | 20.00±1.00 | 7.00±1.00 | 17.50±1.00 | 17.50±2.00 |
|           | 12  | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 15.00±1.00 | 21.00±1.00 | 21.00±1.00 | 21.00±1.00 | 6.00±0.00 | 15.00±1.00 | 15.00±1.00 |
|           | 13  | 7.00±1.00 | 7.00±1.00 | 7.00±1.00 | 17.00±1.00 | 20.00±1.00 | 20.00±1.00 | 20.00±1.00 | 6.00±0.00 | 14.50±0.50 | 14.50±0.50 |
|           | 14  | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 14.00±0.00 | 22.00±1.00 | 22.00±1.00 | 22.00±1.00 | 7.00±1.00 | 15.00±1.00 | 15.00±1.00 |
|           | 15  | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 16.00±2.00 | 19.00±1.00 | 19.00±1.00 | 19.00±1.00 | 7.00±1.00 | 15.00±1.00 | 15.00±1.00 |
|           | 16  | 7.00±1.00 | 6.00±0.00 | 6.00±0.00 | 15.00±1.00 | 21.50±1.00 | 21.50±1.00 | 21.50±1.00 | 6.00±0.00 | 14.50±0.50 | 14.50±0.50 |
|           | 17  | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 16.00±1.00 | 20.00±1.00 | 20.00±1.00 | 20.00±1.00 | 6.00±0.00 | 14.50±0.50 | 14.50±0.50 |
|           | 18  | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 16.50±1.00 | 23.00±1.00 | 23.00±1.00 | 23.00±1.00 | 7.00±1.00 | 17.50±1.00 | 17.50±2.00 |
|           | 19  | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 15.00±1.00 | 23.50±2.00 | 23.50±2.00 | 23.50±2.00 | 7.00±1.00 | 16.50±2.00 | 20.50±1.50 |

Results are presented as mean ± SE. Values carrying similar alphabet in the same row are not extensively dissimilar (P>0.05). Key: SXT = Septrim (30µg); CH= Chloramphenicol (30µg); SP= Sparfloxacin (10µg); CPX= Ciprofloxacin (30µg); AM= Amoxicillin (30µg); AU= Augmentin (10µg); CN= Gentamicin (30µg); PEF= Pefloxacin (30µg); OFX= Ofloxacin (10µg); S= Streptomycin (30µg)
4. CONCLUSION

*P. aeruginosa* is considered one of the most persistent nosocomial and difficult to treat pathogen, its antibiotics resistance tendency makes it more difficult to completely eradicate. Testing the bacteria on different commercially-available antibiotics in this research demonstrated the efficacy of ciprofloxacin in the management of *P. aeruginosa* infections. *P. aeruginosa* from wound swab samples showed high resistance to septrin and augmentin while *P. aeruginosa* from urine samples showed high resistance to chloramphenicol, septrin, augmentin and streptomycin. This implicates the resistance of *P. aeruginosa* to several over the counter remedies and susceptibility to only few antibiotics. It is therefore essential to understand the pattern of antibiotics resistance on this bacterium and modify antibiotics used in treatment, to curb its prevalence in hospital patients and broaden the study scope on antibiotic resistant bacteria. The misuse and abuse of antibiotics are one of the vital factors...
causing the persistence of resistant bacteria. Therefore, measures are advised to be put in place to curb the sale of antibiotics without doctor's prescription and the public be sensitized on the danger of misuse of antibiotics as well as not completing antibiotics treatment dosage.

CONSENT
As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL
Ethical approval for the collection of wound swabs and urine samples from in-patients and outpatients of Medical Sciences Teaching Hospital, Akure were collected from Ondo State Health Research Ethics Committee, Ministry of Health, Ondo State, Nigeria.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

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