Antibiogram of P*seudomonas aeruginosa* Clinical Isolates Tested for Pan, Extensive and Multidrug Resistances

ABSTRACT

Background: *Pseudomonas aeruginosa* usually cause nosocomial infections with concurrent morbidity and mortality and is generally resistant to many antibiotics.

Aim: This study was aimed to determine the proportion of pan-drug-resistant (PDR), extensively drug-resistant (XDR), and multidrug-resistant (MDR) *P. aeruginosa* strains recovered from human samples.

Methodology: The retrospective study was conducted in the University of Nigeria Teaching Hospital Enugu in 2023. Clinical samples obtained from patients between October 2022 and April 2023 were analysed. A total of 100 *Pseudomonas aeruginosa* isolates recovered from 780 clinical samples were used. Standard microbiological techniques were used to identify and categorize the isolates. Antibiotic susceptibility pattern was determined using the Kirby Bauer disc diffusion method.

Results: Isolates recovered from wound was 38%, voided urine, catheter tip urine, ear swab, and high vaginal swab samples recorded 29%, 11%, 10% and 4%, respectively. Ceftazidime recorded the highest level of resistance (70.0%) and the least was Colistin (20%). Resistance patterns showed that 32(32.0%) bacterial strains were MDR, 68(68.0%) were XDR and no PDR was recorded.

Conclusions: For the best selection of empirical therapy, *P. aeruginosa* susceptibility monitoring is essential due to the high prevalence of antibiotic resistance. The resistance pattern raises the possibility of misuse of broad-spectrum antibiotics. Treatment for bacterial infections should be directed by the results of antimicrobial susceptibility tests.

Keywords:Pseudomonas aeruginosa, antibiotic, nosocomial, resistance, infections

1. INTRODUCTION

Pseudomonas species belong to the family Pseudomonadaceae, they are aerobic, Gram-negative, rod-shaped, and polar-flagellated organisms. The most widespread species of medically important bacteria is *Pseudomonas aeruginosa*, an organism that is present in a wide variety habitats and it is responsible for nosocomial infections in clinical settings [1, 2]. The organism is an opportunistic organism that is a principal contributor to morbidity and mortality, particularly among people with cystic fibrosis and other immune system disorders [3]. It causes infections in the blood, surgical sites, eye, external ear, urinary tract, respiratory tract, and wounds (particularly in burn victims) [1, 2].

The goal of initial antimicrobial regimen for patients suspected of severe *P. aeruginosa* infections is to decrease mortality. This therapy includes mono therapy and combination therapy [4, 5]. Nevertheless, because *P. aeruginosa* has the ability to resist the majority of the currently available antibiotics, treating *P. aeruginosa* infections has grown to be a significant concern [6]. *Pseudomonas aeruginosa* is one of the bacteria that was classified among 12 bacteria by the World Health Organization lists of antibiotic-resistant "priority pathogens", and was included in the first category of critical Priority 1[7].The occurrence of multidrug resistance in *P. aeruginosa* is accelerated by the misuse of antibiotics during treatment, rendering the empirical antimicrobial treatment ineffective against these bacteria [8]. *Pseudomonas aeruginosa* shows resistance to a wide range of antimicrobials [9, 3]. There are three main mechanisms *P. aeruginosa* uses to resist antimicrobials. This includes acquired, intrinsic, and adaptive resistances. Intrinsic resistance involves creation of efflux pumps that remove drugs from the cells, low outer membrane permeability, and the production of enzymes that render antibiotics inactive[10]. Adaptive resistance is concerned with the formation of biofilm [11] which can support the growth of multidrug-tolerant persister cells that survive the antimicrobial therapy [12].

Antibiotic resistance exists in all regions of the world.It is one of the most serious global public health threats in this century.The global rise in the antibiotic poses a significant threat but the patterns of resistances vary considerably across countries. Antimicrobial resistance in *P. aeruginosa* and other organisms has also been on the increase globally [13, 14]. Based on the degree of their resistance, the isolates have been labeled as MDR, XDR, and PDR. Since there are few effective antibiotic treatments available, infections with these resistant strains may lead to a rise in morbidity and mortality [8, 15].

Reviews on MDR *P. aeruginosa* have shown a wide range of definitions[16, 17]. However, in most of the research, multidrug resistance was defined asresistance to at least three drugs from different antibiotic group categories, including carbapenems, antipseudomonal penicillins, aminoglycosides,cephalosporins, and fluoroquinolones [8].

A team of experts got together to propound standardized international nomenclature for determining acquired resistance profiles in all bacteria that commonly cause nosocomial infections and are at risk for multidrug resistance [18]. For each bacterium, epidemiologically significant antimicrobial categories were

developed. The US Food and Drug Administration (FDA), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the Clinical Laboratory Standards Institute (CLSI) data and breakpoints were utilized. Acquired resistance to at least one antimicrobial agent across three or more different antimicrobial groups is referred to as MDR, whereas XDR refers to resistance to at least one antimicrobial agent across all categories except two or fewer. Resistance to all antimicrobial agents was defined as PDR.To be certain that these definitions are accurately applied, all or almost all of the antimicrobials mentioned under the antimicrobial categories must be employed. Additionally, results must be reported accurately [18].

In Nigeria, most studies on MDR in *P. aeruginosa* did not include these criteria used in the definitions and there is, therefore, a dearth of information on the prevalence of these phenotypes, hence this study. The purpose of this study, therefore, was to investigate the antimicrobial resistance profile of *Pseudomonas aeruginosa* and to evaluate the prevalence of MDR, XDR, and pan drug-resistance phenotypes in *P. aeruginosa* from human samples.

2. METHODOLOGY

2.1 Study Area

This study was conducted in Enugu Metropolis. It is located in southeast Nigeria with a population of 820,000 based on the last census. There are three tertiary hospitals including the University of Nigeria Teaching Hospital (UNTH) Ituku/Ozalla, National Orthopedic Hospital, and Enugu State University Teaching Hospital.

2.2 Bacteriological Analysis

The isolates were recovered from clinical samples that were submitted to the Microbiology Laboratory of the UNTH,Ituku-Ozalla. They were sub-cultured from the stock cultures unto nutrient agar (Neogen, LAB008, KR) and then cultured unto Centrimide agar (HiMedia, MH024, IND).*Pseudomonas aeruginosa* were isolated from the various clinical samples using the cetrimide Agar selective medium. The formation of pigment by the production of fluorescein and pyocyanin appearing as greenish colonies was a prominent confirmatory identification characteristic of the colonies after 24hours incubation at 37^{o}C. The oxidase enzyme test for the presence of cytochrome oxidase enzymes was also carried out. The change of tetra-methyl- p-phenylenediamine dihydrochloride reagent to deep purple colour indicated positive. Other Biochemical confirmatory tests were carried out according to standard methods [19].A total of 100 non-duplicates *Pseudomonas. aeruginosa* were positive among 780 clinical isolates from samples of urine, wound swab, high vaginal swab, catheter tip, ear swabs, and sputum.

2.3 Antimicrobial Susceptibility Testing

The Kirby Bauer's disk diffusion technique as set out in the recommendations of the Clinical Laboratory Standard Institute (CLSI) was used [20]. The disc diffusion technique involved swabbing a standardized inoculums onto the surface of Muller Hinton agar (Oxoid, CMO337B, UK). The agar surface was inoculated by using a swab dipped in the pseudomonas cells suspension adjusted to the turbidity of a 0.5 McFarland standard and was spread evenly over the surface, avoiding the edges of the plates during the swabbing. There after the antibiotic disks were picked by sterile forceps and dropped on the surface of the agar seeded with the *Pseudomonas* inoculums. The antibiotics were allowed to diffuse into the agar before incubation. The plates were then incubated at 37°C for 18 to 24 hours. After the incubation, the Inhibition Zone Diameter (IZD) was measured and recorded. The zone sizes were read using standardized chart to record the result as sensitive, resistant, or intermediate. The following antibiotics were included; ceftazidime (30µg), Cefepime (30µg), Piperacillin-tazobactam (110 µg) Amikacin (30µg), gentamicin (10µg), levofloxacin (5µg), ciprofloxacin (5µg), imipenem (10 µg), Meropenem (10 µg) Aztreonam (30µg), and colistin (10 µg). *P. aeruginosa* ATCC 27853 was used for the quality control. It was done in parallel with test isolates for each susceptibility test.

2.4 Detection of MDR, XDR, and PDR

MDR, XDR, and PDR in *P. aeruginosa* isolates were defined following a structured global report [17], and by the outcome of antimicrobial susceptibility profile against all antimicrobial categories. Non-susceptibility to at least one agent in ≥3 antimicrobial categories was recorded MDR, non-susceptibility to at least one agent in ≥ 6 antimicrobial categories were reported XDR, and the isolates that showed non-susceptibility to all the antibiotic categories were called PDR. Phosphonic acids (Fosfomycin) were not used because of the absence of susceptibility breakpoints for the drug against *P. aeruginosa*.

2.5 Statistical Analysis

For all statistical calculations, SPSS for Windows version 20 (SPSS, Chicago, IL, USA) was used. Descriptive statistics (frequencies and percentages) were used to describe categorical variables. Pearson's Chi-square test (X^2) was used to test for significant association between variables at a 95% confidence interval. Statistical significance was defined as a P-value of 0.05 or lower.

3. RESULTS

Table 1 shows the distribution of clinical isolates according to source, of the 100 isolates of *Pseudomonas aeruginosa,* the highest number of isolates was recovered from wound 38 (38.0%), followed by voided urine 29 (29.0%). Others were catheter tips 11 (11.0%), ear swab 10 (10.0%), sputum 8 (8.0%) and the least was from HVS 4 (4.0%).

Table 1: Distribution of Isolates According to the Source

Isolate Source	No/ %
Wound	38 (38.0)
Urine	29 (29.0)
Sputum	8 (8.0)
Catheter tip	11 (11.0)
Ear swab	10 (10.0)
High Vaginal Swab (HVS)	4 (4.0)
Total	**100 (100.0)**

Table 2 shows the resistance rates of the antimicrobials, Gentamicin, Amikacin, Imipenem, Meropenem, Ceftazidime, Cefepime, Ciprofloxacin, Levofloxacin, Piperacillin-tazobactam, Aztreonam, and Colistin as 40.0%, 44.0%, 68.0%, 62.0%,70.0%, 55.0%, 50.0%, 51.0%. 55.0%, 58.0%, and 20.0%, respectively.

Table 2: Antibiogram of *P. aeruginosa* from different clinical samples

Antimicrobial categories	Antimicrobial agents	Susceptible No/%	Intermediate No/%	Resistant No/%
Aminoglycosides	Gentamicin	50 (50.0)	10 (10.0)	40 (40.0)
	Amikacin	52 (52.0)	4 (4.0)	44 (44.0)
Carbapenems	Imipenem	31 (31.0)	1 (1.0)	68 (68.0)
	Meropenem	34 (29.0)	4 (3.0)	62 (62.0)
Cephalosporin	Ceftazidime	26 (26.0)	4 (4.0)	70 (86.0)
	Cefepime	40 (40.0)	5 (5.0)	55 (55.0)
Fluoroquinolones	Ciprofloxacin	48 (48.0)	2 (2.0)	50 (50.0)
	Levofloxacin	46 (46.0)	3 (3.0)	51 (51.0)

Penicilins/β-lactamase inhibitors	Piperacillin/tazobactam	40 (60.0)	5 (5.0)	55 (35.0)
Monobactams	Aztreonam	32 (32.0)	10 (10)	58 (58.0)
Polymyxins	Colistin	80 (80.0)	0 (0)	20 (20.0)

Table 3 shows the prevalence of resistant types according to the source of the isolate and the different resistance patterns rates recorded. Out of the 38 isolates from the wound, 20 (62.5%) were MDR, and 18 (26.5%) were XDR. Urine isolates recorded 3 (9.4%) MDR and 26 (38.3%) were XDR. The highest number of isolates with MDR pattern was from wound 20(62.5%), followed by catheter tips 5% 15.6%), and the least was from ear swabs and HVS 2(6.3%) each. The highest number with XDR pattern was from Urine 26 (38.2%), followed by Wound 18(26.5%), and the least was from HVS 2(2.9%). Out of the 100 isolates of *P. aeruginosa*, XDR ranked highest 68(68.0%) and MDR was the lowest 32 (32.0%). There was no PDR isolates.

Table 3: Prevalence of Resistance Types According to the Source of Isolates

Source of Isolate	**MDR**	**XDR**	**Total**
Wound	20 (62.5)	18 (26.5)	38 (38.0)
Urine	3 (9.4)	26 (38.2)	29 (29.0)
Sputum	0 (0.0)	8 (11.8)	8 (8.0)
Catheter tip	5 (15.6)	6 (4.4)	11 (11.0)
Ear swab	2 (6.3)	8 (11.8)	10 (10.0)
High Vaginal Swab (HVS)	2 (6.3)	2 (2.9)	4 (4.0)
Total	**32 (32.0)**	**68 (68.0)**	**100 (100.0)**
	X^2= 16.63		

4. DISCUSSION

Pseudomonas aeruginosa continues to be a significant hospital-acquired organism known for its morbidity and mortality, especially in immunocompromised people and susceptible patients in intensive care units [21]. It is possible to isolate the bacterium from any clinical sample. In this work, majority of the isolates were mostly found in wounds and this is in line with what was reported in Ethiopia [1]. This may be as a result of contaminated surgical tools and the environmental proliferation of *P. aeruginosa* in healthcare facilities [1; 22].

Our research revealed that carbapenems, such as imipenem (68%), and meropenem (62.0%), had higher rates of resistance than cephalosporins and fluoroquinolones. The evolvement of carbapenemase-producing bacteria has restricted the use of carbapenems, which were once very effective anti-pseudomonal drugs [23]. Our findings were closely related to those reported in Mexico 70% and 54% [24] and 53% and 63% in India [25]. A lower resistance rate was recorded in Ethiopia 18% and 13% [1]. The higher resistance rate observed in our research may be attributable to prescription practices used in our clinical setting, inappropriate use of broad-spectrum antibiotics, and a special trait of *P. aeruginosa* that makes it susceptible to acquiring resistance such as low cell wall permeability, formation of inducible cephalosporinases and efflux pumps, and a poor affinity to the target sites [26]. Our study also showed higher resistance among imipenem as against meropenem. This is consistent with the study of Addis *etal* and Kateete*etal* [1; 23]. This might be due to differences in the chemical structures of the two drugs. Meropenem is more effective against *P. aeruginosa* because it penetrates the outer membrane porin-D (OprD) more quickly. However, due to its increased risk of membrane selection, imipenem has been less effective [1].

Theisolates encountered during this investigation displayed considerable cephalosporin resistance, especially to ceftazidime (70%), and cefepime (55%).This is in consonance with what was reported in other nations. In Uganda, 69% and 55% for ceftazidime and cefepime, 63% and 62% in Egypt, 65 and 55% in Mexico, 66% and 63% in India were reported [22; 23; 24; 26]. However, lower prevalence has been reported in Iran 35% and 38%, and in Ethiopia, 35% and 31% [1; 28]. Over-production of beta-lactamases in particular may be the cause of the increased resistance that has been observed in Nigeria and other nations. Improper prescription of cephalosporins causes the pathogen to undergo a genetic change.

This present study also showed that combination drug penicillin/ beta-lactamase inhibitor, Piperacillin-tazobactam recorded 55% resistance and was better than carbapenems, and cephalosporins.

The fluoroquinolones, ciprofloxacin (50.0%) and levofloxacin (51.0%) were more effective than cephalosporins. The result is consistent with what was reported in Uganda 64% and India 67% for ciprofloxacin [23; 24] but at variance with what was reported in Ethiopia 18% and 24% for ciprofloxacin

and levofloxacin respectively. This disparity may be caused by the diverse sample sizes, various study settings, and vast geographical differences [1].

Aminoglycoside was more potent than cephalosporins, fluoroquinolones, and carbapenem. Amikacin was 44.0% and Gentamicin 40.0%.Our findings were comparable to what was reported in Mexico (58.0% and 52%, in Uganda 31% and 69%) but higher than what was reported in Ethiopia [1], which was 2% and 7%. The effectiveness of this drug might be because of lower prescription practice in our setting.

In this study, colistin was the most potent drug for *P. aeruginosa* infection despite having an overall resistance rate of 20%. The nephrotoxicity and neurotoxicity of this drug deterred clinicians from using it in the past. It is a reserved drug used to treat confirmed or suspected infections caused by MDR pathogens [1]. Our colistin-resistance rate of 20% was consistent with the 23% reported in Egypt [27] but higher than the 6% and 9% reported in Ethiopia and Iran respectively [1; 28]. The discrepancy may be caused by methodological differences and/or the presence of colistin-resistant bacteria as a result of improper colistin use in veterinary medicine, where it has been frequently utilized to promote growth in animal husbandry [29]. Since no alternative antibiotics may be utilized, the rise of colistin-resistant *P. aeruginosa* strains is extremely worrisome and poses a severe global problem.

In this investigation, the prevalence of MDR and XDR Pseudomonas *aeruginosa* isolates was 32% and 68%, respectively. There was no PDR in this study. The definition of the acronyms was done according to the accepted global report for *P. aeruginosa* (18) except for Fosfomycin that was not included because of the absence of susceptibility breakpoints for fosfomycin against *P. aeruginosa*. Saderi and Owlia [28] reported 54.5% and 33% for MDR and XDR and there was no PDR recorded among 88 clinical isolates in Iran whereas Addis and his colleagues [1], reported 23%, 9%, and 2% for MDR, XDR, and PDR respectively in Ethiopia. There are few published data on multi-drug resistance using a proper definition of MDR. However, a high and lower prevalence of MDR was reported in many countries [29]. It is possible that the MDR's could have spread from healthcare workers and from patients to patients also, the organism being of nosocomial origin. This were mainly wound isolates. Perhaps the transfer of resistance strains could possibly happened through hands of healthcare workers during wound dressing or from beddings. Variations in prevalence have been also due to inappropriate use of antimicrobials, and geographical variations. There have been national and international efforts to address the prevalence of global antimicrobial resistance threats. Such efforts include funding and regulations to support antimicrobial policy and program development, incentive drug development to treat resistant pathogens, and efforts to strengthen existing health programs [30]

5. CONCLUSION

Multidrug resistance is becoming a severe problem in hospital settings, increasing the incidence of nosocomial infections as its prevalence rate rises and spreads globally. To identify trends in resistance,

the sensitivity pattern should be periodically monitored over time. Results of tests for antibiotic susceptibility should be used to guide treatment of bacterial infections. Antimicrobials work differently depending on where they are used, hence it is essential to replicate these studies by carrying out the researches in other national and international locations.Further studies will require checking alongside for this resistance strains in the health workers and hospital environments to ascertain the source of spread.

AUTHORS' CONTRIBUTIONS

'Author 1' designed the study, wrote the protocol and wrote the first draft of the manuscript. 'Author 2' performed the statistical analysis and edited for publication, 'Author 3' managed the analyses of the study and 'author 4' managed the literature searches. All authors read and approved the final manuscript.

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