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Antibiotic Susceptibility Profile of Clinical Isolate of Carbapenem-Resistant *Pseudomonas aeruginosa*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RCO, POA and OLN wrote the protocol. Authors IUP and IRI wrote the first draft of the manuscript. Authors ACN and IRI managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: Carbapenem-resistant *Pseudomonas aeruginosa*, are among the top tier of the list of antibiotic-resistant priority pathogens that pose the greatest threat to human health. In recent years, the rate of carbapenem resistance in *Pseudomonas aeruginosa* has increased worldwide and has become of great concern since it significantly restricts the therapeutic

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options for patients. Therefore this study was undertaken to determine the antibiotic susceptibility profile of the clinical isolate of Carbapenem-resistant *Pseudomonas aeruginosa.*

Methodology: A total of five hundred (500) clinical samples were collected from patient's attending Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State (AFEUTHA). The collected samples were analyzed for the presence of *Pseudomonas aeruginosa* using standard microbiological techniques for isolation and characterization of bacteria. Further strain confirmation was performed using VITEK 2 System. Phenotypic detection of Carbapenem-resistant *Pseudomonas aeruginosa* was performed using Modified Hodge testing. Antibiotic susceptibility was performed by employing Kirby-Bauer disk diffusion method and the results were interpreted using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints.

Results: The occurrence rate of *Pseudomonas aeruginosa* in clinical samples accounted for 119(23.8%) consisting of a high proportion from urine sample 81(27.4%) followed by wound swabs 13(25.5%), high vaginal swabs 17(20.7) while the least occurrence rate was observed against catheter tips 5(12.8%) and sputum 3(9.4%). Modified Hodge testing revealed 31(6.2%) carbapenem-resistant *Pseudomonas aeruginosa* comprising of high proportion of 24(8.1%) from urine samples followed by wound swab 5(9.8%) while Carbapenem-resistant *Pseudomonas aeruginosa* was absent in High Vaginal Swab recording 0(0.0%). Carbapenem-resistant *Pseudomonas aeruginosa* isolates were highly resistant to amoxicillin-clavulanic 100%, colistin 100%, tetracycline 100%, nitrofurantoin 70.8%, aztreonam 87.5% but were susceptible to nalixidic acid 50.0 %, ofloxacin 75.0%, and ciprofloxacin 100%.

Conclusion: As *in-vitro* susceptibility of carbapenem-resistant *Pseudomonas aeruginosa* isolates to ofloxacin and ciprofloxacin is known, their judicious utilization will accelerate a significant improvement in the patient's condition. As such, there is a substantial need for the evaluation of a wide spectrum and new therapies in different classes to counteract this imminent crisis of resistance among Carbapenem-resistant *Pseudomonas aeruginosa.*

Keywords: Carbapenem-resistant; Pseudomonas aeruginosa; susceptibility.

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacillus that is one of the leading causes of community-acquired infections in patients with chronic underlying diseases and hospital-acquired infections, such as pneumonia, urinary tract infections, and bloodstream infections (BSIs) [1,2]. Recently, it has been described as a pathogen that co-infects patients with COVID-19 [3,4]. The 2016 report by the European Center for Disease Control and Prevention (ECDC) on infections acquired in the ICU in 18 European countries showed that, in 2014, *P. aeruginosa* was the most common cause of ventilator-associated pneumonia and the fifth most prevalent in ICU-acquired BSI [5,6]. *P. aeruginosa* infections are associated with elevated disease burden and mortality rates in the absence of optimal treatment [6,7]. The *Pseudomonas aeruginosa* accessory genome is often composed of genes involved in virulence to human hosts and antimicrobial resistance, resulting in a high risk of mortality and a high rate of multidrug resistance [8].

Although beta-lactams are one of the most commonly used antimicrobial drug classes for *P. aeruginosa* infection, antipseudomonal beta-lactam drugs are limited because of the species' intrinsic resistance due to the interplay of chromosomal beta-lactamases, a low outer membrane permeability, and the constitutive expression of efflux pump systems [2]. The beta-lactam regimens for *P. aeruginosa* infection include antipseudomonal penicillins in combination with a beta-lactamase inhibitor, i.e., piperacillin–tazobactam and ticarcillin–clavulanic acid; antipseudomonal cephalosporins alone or in combination with beta-lactamase inhibitors, i.e., ceftazidime (with avibactam), ceftolozane (with tazobactam), cefoperazone (with sulbactam), and cefepime [2,9,10]; and carbapenems, i.e., imipenem and meropenem. Among those, carbapenems are the preferred choice against multidrug-resistant *P. aeruginosa.* In recent years, the rate of carbapenem resistance in *P. aeruginosa* has increased worldwide and has become of great concern since it significantly restricts the therapeutic options for patients.

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), are among the top tier of the WHO list of antibiotic-resistant "priority pathogens" that pose the greatest threat to human health [11,12].

Moreover, carbapenem-resistant *P. aeruginosa* (CRPA) frequently produced carbapenemase, with 62.7% of isolates having at least one carbapenemase gene [13]. Carbapenem-resistant *P. aeruginosa* has been implicated in various infections with high mortality rates has been reported in different region [14]. Generally, there are limited studies conducted on carbapenem-resistant *P. aeruginosa* in Africa. Therefore, the rapid and sensitive detection of susceptible profile of Carbapenem-resistant *P. aeruginosa* is required if appropriate therapy is to be administered.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Characterization of *P. aeruginosa*

Aseptically, a total of five hundred (500) clinical samples of human sputum (32), urine (296), high vaginal swab (82), wound swab (51) and catheter tip (39) were collected from Alex Ekwueme Federal University Teaching Hospital Abakaliki (AFEUTHA), Ebonyi State during the period of nine (9) months. All collected clinical samples were transported using Amies transport medium (bioMérieux, France) to the microbiological research laboratory for bacteriological analysis. The collected samples were analyzed for the presence of *Pseudomonas aeruginosa* by inoculating of each sample into a separate tube of sterile nutrient broth (bioMerieux, France) and incubated at 37 °C for 24 h. After overnight incubation, a loopful of the turbid broth culture was aseptically seeded by streaking on sterile solidified, Cetrimide agar (bioMérieux, France) and was incubated at 37 °C for 24h. Suspected *Pseudomonas aeruginosa* from positive cultures were identified by their characteristic appearance (color, consistency, shape) on the media. Each Greenish colonies were sub-cultured on sterilized solidified Nutrient agar (bioMérieux, France) and incubated at 37 °C for 24h for Gram staining reaction and biochemical testing profiles, using standard procedures [15]. Further strain confirmation was performed using VITEK 2 System (bioMerieux, France) [16].

2.2 Modified Hodge Testing

The test was done using cabapenem class of antibiotics by preparing 0.5 Mcfarland dilution of the test organism in 5ml of sterile water. Thereafter a sterile swab stick was used to streak the organism on already prepared Muller Hinton agar plates and allowed to dry for 3-5minute. Imipenem (IPM 10 µg) and meropenem (MEM 10 µg) (Oxoid, Uk) susceptibility disk in the centre of the test area. In a straight line streak the test organism from the edge of the disk to the edge of the plate and incubate at 37^0C for 24hrs based on CLSI breakpoints [17].

2.3 Antibiotic Sensitivity Testing

Antibiotic susceptibility was performed by employing Kirby Bauer disk diffusion method using sterilized Mueller-Hinton agar in accordance with the guidelines of Clinical and Laboratory Standards Institute [17]. Carbapenem-resistant *P. aeruginosa* suspension of the test isolate was prepared using 0.5 McFarland standards and seeded on solidified Mueller–Hinton agar. The plates were allowed to pre-diffuse for 5 minute. Thereafter, the following antibiotic: amoxicillin-clavulanic acid, amoxicillin (30 µg), azetronam (30 µg), cefoxitin (30 µg) ceftazidime (30 µg), ceftriaxone (30 µg), colistin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nalixidic acid (30 µg), nitrofurantoin (100 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), ticarcillin-clavulanic acid (85 µg) (Oxoid , Uk) were impregnated on the inoculated Mueller-Hinton (bioMérieux, France) agar plates and incubated at 37 °C for 24 hours. After overnight, the diameters of zones of inhibition were measured, and the results interpreted according to susceptible and resistant [17,18]

2.4 Determination of Multiple Antibiotics Resistance Index (MARI)

Multiple antibiotic resistance index (MARI) was calculated as MARI =“a/b**”,** where “a**”** represents the number of antibiotics to which the isolates were resistant and “b**”** represents the total number of antibiotics to which the isolate was exposed to [16,19].

3. RESULTS

3.1 Distribution of *Pseudomonas aeruginosa* in Clinical Samples

Overall occurrence rate of *Pseudomonas aeruginosa* accounted for 119(23.8%) consisting of high proportion from urine sample 81(27.4%) followed by Wound swabs 13(25.5%), HVS 17(20.7) while the least occurrence rate was observed against catheter tips 5(12.8%) and Sputum 3(9.4%) as presented in Table 1.

Table 1. Distribution of *Pseudomonas aeruginosa* in clinical sample isolated from patients at AFEUTHA

Clinical Sample	No. of sample	*Pseudomonas aeruginosa* (%)
Sputum	32	3(9.4)
Urine	296	81(27.4)
HVS	82	17(20.7)
Wound swabs	51	13(25.5)
Catheter tips	39	5(12.8)
Total	**500**	**119(23.8)**

Key: HVS– High Vaginal Swab

Table 2. Distribution of carbapenem-resistant *Pseudomonas aeruginosa* in clinical samples isolated from patients at AFEUTHA

Clinical Sample	No. of sample	*Pseudomonas aeruginosa* (%)	CPR (%)	CPS (%)
Sputum	32	3(9.4)	0(0.0)	3(9.4)
Urine	296	81(27.4)	24(8.1)	57(19.3)
HVS	82	17(20.7)	0(0.0)	17(20.7)
Wound swab	51	13(25.5)	5(9.8)	8(15.7)
Catheter tip	39	5(12.8)	2(5.1)	3(7.7)
Total	**500**	**119(23.8)**	**31(6.2)**	**88(17.6)**

Key: HVS-High Vaginal Swab, CPR-Carbapenem Resistant, CPS- Carbapenem Susceptible

Table 3. Antibiotic susceptibility profile of carbapenem-resistant *Pseudomonas aeruginosa* from patients at AFEUTHA

Antibiotics (µg)	Urine (n=24)		Wound swab (n=5)		Catheter tip (n=2)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amoxicillin CA (20/10)	24(100)	0(0.0)	5(100)	0(0.0)	2(100)	0(0.0)
Amoxicillin (30)	24(100)	0(0.0)	5(100)	0(0.0)	2(100)	0(0.0)
Azetronam (30)	21(87.5)	3(12.5)	4(80)	1(20)	2(100)	0(0.0)
Ceftriaxone(30)	24(100)	0(0.0)	5(100)	0(0.0)	1(50)	1(50)
Cefoxitin (30)	24(100)	0(0.0)	5(100)	0(0.0)	2(100)	0(0.0)
Colistin (10)	24(100)	0(0.0)	5(100)	0(0.0)	2(100)	0(0.0)
Ciprofloxacin (5)	0(0.0)	24(100)	0(0.0)	5(100)	0(0.0)	2(100)
Ofloxacin (5)	6(25)	18(75)	2(40)	3(60)	0(0.0)	2(100)
Nalixidic acid (30)	24(100)	0(0.0)	3(60)	2(40)	1(50)	1(50)
Nitrofurantoin (100)	17(70.8)	7(29.2)	5(100)	0(0.0)	1(50)	1(50)
Tetracycline (30)	24(100)	0(0.0)	5(100)	0(0.0)	2(100)	0(0.0)
Trimethoprim-Sulfamethoxazole (25)	24(100)	0(0.0)	3(60)	2(40)	2(100)	0(0.0)
Ticarcillin-clavulanic acid (85)	24(100)	0(0.0)	5(100)	0(0.0)	2(100)	0(0.0)

Key: CA- Clavulanic Acid, n=Number of isolate, R-Resistance, S-Susceptible

Table 4. Multiple Antibiotic Resistant Index (MARI) of carbapenem-resistant *Pseudomonas aeruginosa* in clinical sample from patients at AFEUTHA

Clinical Sample	Multiple Antibiotic Resistant Index(MARI)
Sputum	NIL
Urine	0.7
HVS	NIL
Wound swab	0.7
Catheter tip	0.6

Key: HVS-High Vaginal Swab

3.2 Distribution of Carbapenem-Resistant *Pseudomonas aeruginosa*

In Table 2, overall detection rate of Carbapenem-resistant *Pseudomonas aeruginosa* was 31(6.2%) comprising of high proportion 24 (8.1%) from Urine sample followed by Wound swab 5(9.8%) while CR *Pseudomonas aeruginosa* was absence in High Vaginal Swab recording 0(0.0%). Carbapenem-Susceptible *Pseudomonas aeruginosa* accounted 88(17.6%).

3.3 Antibiotic Susceptibility Profile of Carbapenem-Resistant *Pseudomonas aeruginosa*

Carbapenem-resistant *Pseudomonas aeruginosa* isolated from urine samples were highly resistant to amoxicillin-clavulanic 100%, colistin 100%, tetracycline 100%, nitrofurantoin 70.8%, azetronam 87.5% but were susceptible to ofloxacin 75.0 %, and ciprofloxacin 100% as shown in Table 3. Antibiotic resistant profile of Carbapenem-resistant *Pseudomonas aeruginosa* isolate from wound swab samples revealed 80%, 60.0%, 100% resistant to Azetronam, Trimethoprim-Sulfamethoxazole and Ticarcillin-clavulanic acid respectively but were 40.0%, 60.0% and 100% sensitive to Nalixidic acid, Ofloxacin and Ciprofloxacin as shown in Table 3. Carbapenem resistant *Pseudomonas aeruginosa* isolates in Table 3 demonstrated resistant to Amoxicillin 100%, Tetracycline 100%, Colistin 100%, Ceftriaxone 50.0% but were susceptible to Nalixidic acid 50.0%, Nitrofurantoin 50.0%, Ciprofloxacin 100% and Ofloxacin 100%. Carbapenem-resistant *Pseudomonas aeruginosa* demonstrated multidrug resistant with MARI value of 0.7, 0.6 and 0.7 from Urine, Catheter tip and Wound swab respectively as shown in Table 4.

4. DISCUSSION

From the result, *P. aeruginosa* accounted for 23.8% of all the isolates. Although there seem to be geographical differences in the proportions between the species earlier identified in other study, this observation is not parallel with report in Zaria were 10.5% and in North-eastern Nigeria 2.1% was reported [20,21], but strongly agrees with previous report of higher prevalence rate of 32.1% and 20.3% as published by Rajat et al. [22] and Javiya et al. [23] in Ahmadabad and Gujarat, India, 31.7% in Ethopia [24] and other studies that reported their presence in clinical samples in Germany and Tehran, Iran [25] respectively. It's worth noting that, the prevalence of *P. aeruginosa* isolates varied with clinical conditions and specimens while comparison of epidemiological data of enterobacteria as in this study might be difficult as there are other variables that influence the outcome of results such as, clinical specimens received for examination, studied population, type of hospitals and geographical locations.

In this study, the prevalence rate of carbapenem resistant *P. aeruginosa* 17.6 % reiterate with a finding from Uganda were low prevalence rate of 7.4% [26] slightly comparable to this our study was reported. Also carbapenemase production among CR- *P. aeruginosa* has been recently highlighted in the multi-national ERACEPA Surveillance Program were 807 CR-PA collected over 2019–2021 from 17 centres in 12 countries, only 33% tested carbapenemase-positive phenotypically (using the mCIM method) [27,28]. In Africa, studies conducted on carbapenemase-producing *P. aeruginosa* are limited. The few reports available are from northern Africa and mainly from Egypt [29,30]. In northern Africa, the prevalence ranges from 0 to 96% [31]. From this study, the potential source of colonization could be both hospital and community. There are several key mechanisms of carbapenem resistance in *P. aeruginosa* which may the World Health Organization (WHO) designated carbapenem-resistant *P. aeruginosa* a priority 1 or ''critical'' pathogen in substantial need of new therapies to counteract this imminent public health crisis of resistance [32].

Antimicrobial susceptibility profiles of CR- *P. aeruginosa* isolates indicated a high level of resistance to most of the antibiotics studied. The unique feature of *P. aeruginosa* isolates is their ability to exhibit resistance to a variety of antibiotics, Amoxicillin CA and Amoxicillin were not an exception as the isolate demonstrated 100 % resistance. Similarly, Abdelrahman et al. [33] confirmed *P. aeruginosa* isolates were resistant to Amoxicillin and Amoxyclav (100%). Ahmad et al. [34] in Pakistan reported resistance to Amoxicillin and Amoxyclav was 73.4% and 67.7% respectively while similar reported in this study is in line with the pattern in earlier studies in Abakaliki, Nigeria [35] and in Malaysia [36]. The established fact, is that, the irrational and inappropriate use of antibiotics is responsible for the development of resistance against *P. aeruginosa* to routinely used antipseudomonal antibiotics. Additionally, among these porins,

OprD is involved in antibiotic uptake. It contains the binding sites for, a class of β-lactam inhibitors antibiotics, and absence of *OprD* in *P. aeruginosa* increases the resistance to this class of antibiotic [37] as observed in this study.

In this current study, colistin (100%) resistance in *P. aeruginosa* reiterate with a study In Thailand Hospital, where eighteen clinical specimens of Colistin resistant *P. aeruginosa* were isolated from various sites during a 10-year period [38]. This pattern of resistant may result from overexpression of MexXY-OprM multidrug efflux system in *P. aeruginosa* or additionally, mutations in the two-component regulatory systems of PhoPQ and PmrAB promoted modification of aminoarabinose addition to lipid A, leading to enhanced polymyxin resistance in *P. aeruginosa* [39-41].

Some *P. aeruginosa* clinical isolates overproduce β-lactamases caused by mutations in a β-lactamase inducible gene ampC, which greatly increased the resistance to cephalosporins [42]. Moreover, inactivating mutations in the ampD gene, which encodes a cytosolic N-acetyl-anhydromuramil-l-alanine amidase may acts as a repressor of ampC expression, resulting in hyperproduction of β-lactamases in *P. aeruginosa* [43]. The above genotypic interaction may result in *in-vitro* resistance of CR-*P. aeruginosa* to Cefoxitin 50 %- 100% from different samples collected from AE-FUTHA. The clinical implication is that there is a need for evaluation of the efficacy of cephalosporin in the treatment of pseudomonal infections to prevent treatment failure, a scenario that is often common in the management of pseudomonal infections.

As noted, 100% resistance of *P. aeruginosa* to tetracycline may result from the inducible cytoplasmic membrane protein Tet (=TET) which mediates the efflux of tetracycline [28,44] in *P. aeruginosa.* CR-*P. aeruginosa* 100% resistance to tetracyclines in our study conforms with the result obtained by Ferguson et al. [45]; Al-Khafaji. [46] who found that all isolates were 100% resistance to tetracycline while Abd El-Baky et al. [47] reported 50 % resistant to tigecyline in the same class. The resistance pattern of tetracycline in this study may be attributable to factors like exposure to the sub-lethal dose of antibiotics by the studied population. However, due to increase pattern of resistance noted in this study, the loss of porins such as OprD may represent an effective barrier for drug entry into the cell, a reduction in drug accumulation in the periplasmic space can also be achieved through active export by membrane-bound efflux pumps.

The most disturbing pattern observed in this study was the multiple Antibiotic resistance exhibited by all the isolates showing multidrug resistance with MARI values of 0.7, 0.6 and 0.7 from Urine, Catheter tip and Wound swabs respectively. The isolate were non-susceptibility to at least one antibiotic in at least three classes for which *P. aeruginosa* susceptibility is generally expected. Patient's populations at greatest risk of acquiring MDR-*P. aeruginosa* infections include most likely those admitted to the Surgical outpatient department, Intensive Care Unit, etc., in the preceding year, immunocompromised patients and those with chronic pulmonary disease.

Although this strain of carbapenem-resistant *P. aeruginosa* did not portray difficult to- treat resistance (DTR) as proposed in 2018 and defined as non-susceptibility to all of the following: piperacillin–tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem–cilastatin, ciprofloxacin, and levofloxacin [32,48,49]. This raises hope that ciprofloxacin and ofloxacin have good potential for the treatment of *Pseudomonas* infections. The use of this antibiotic agent is strongly recommended when the susceptibility of the implicated MDR *P. aeruginosa* isolate is known, and there is a significant improvement in the patient's condition but it's also worrisome as only two drugs from the flouroquinolone class were effective in this study. As such, there is a substantial need for the evaluation of wide spectrum and new therapies in different classes to counteract this imminent crisis of resistance among Carbapenem-resistant *P. aeruginosa.*

5. CONCLUSION

This study reports the prevalence of carbapenem-resistant *P. aeruginosa* thus, the clinical implication is that there is a need for evaluation of the efficacy of some antibiotics agent in the treatment of carbapenem-resistant *Pseudomonas* infections in order to prevent treatment failure, a scenario that is often common in management of carbapenem-resistant *Pseudomonas* infections. As *in-vitro* susceptibility of MDR carbapenem-resistant *P. aeruginosa* isolates to ofloxacin and ciprofloxacin is known, their judicious utilization will accelerate

a significant improvement in the patient's condition. Further studies should provide epidemiological information on carbapenemase genotype and other resistant determinants.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

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

ETHICAL CLEARANCE

The protocol for this study was conveyed with Ethical clearance number SMOH/ERC/043/21 which was gotten from the research and ethics committee of Ministry of Health Ebonyi State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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