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RESEARCH PAPER

RESISTANCE PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM SELECTED TERTIARY HOSPITALS IN OSUN STATE, NIGERIA

Idowu J. Adeosun, Elijah K. Oladipo and Olubukola M. Oyawoye*

Department of Microbiology, Laboratory of Molecular Biology, Bioinformatics and Immunology, Adeleke University, P.M.B. 250, Ede, Osun State, Nigeria

**E-mail*: oyawoyeom@yahoo.com
Tel.: +234 08068202021.

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Pseudomonas aeruginosa **is the most important and ubiquitous pathogen of the Pseudomonas species which has high intrinsic resistance to antibiotics resulting to wide spectrum of opportunistic infections. The aim of this study was to determine the resistance pattern of *Pseudomonas aeruginosa* isolated from selected tertiary hospitals in Osun state, Nigeria. A total of 36 *Pseudomonas aeruginosa* isolates were obtained from 500 samples of blood, urine, wound, ear, eye swab and other collection sites that were routinely submitted to the diagnostic laboratories of the selected tertiary hospitals. Susceptibility to fifteen (15) antibiotics (Oxoid) was determined using the Kirby Bauer disk diffusion method. Rate of resistance to fluoroquinolones, monobactams, polymyxins, carbapenems, penicillins, phosphonic acid derivative and cephalosporins was found to be 43.51%, 41.67%, 50.00%, 27.77%, 78.70%, 63.89% and 28.70% respectively. The isolates were mostly susceptible to carbapenems, especially, imipenem with 72.22%. Highest resistance was to Penicillin (97.22%). The multiple antibiotic resistance (MAR) index revealed that 30 (83.33%) out of 36 isolates were multi-drug resistant. There were statistically significant differences between some group of antibiotics as a whole and the location sites with P<0.05. Increase in antibiotic resistance continues to be a problem amidst patients infected with *Pseudomonas aeruginosa* which can be attributed to increase in antibiotic misapplication, misuse and abuse. It is important that a consistent monitoring of antibiotic resistance be done as it will assist in the appropriate selection of empiric antibiotic treatment in the proper setting.**

Key words: *Pseudomonas aeruginosa*, Resistance pattern, Tertiary hospitals, Antibiotics, Clinical isolates.

INTRODUCTION

Pseudomonas species is an ubiquitous and diverse genus of Gram-negative bacteria that can be found in soil, water, decaying vegetation and animals [1]. The most important and ubiquitous pathogenic pseudomonas is *P. aeruginosa* (**Figure 1**). It is medically significant and has a high intrinsic resistance to antibiotics while causing wide spectrum of opportunistic infections [2]. Several potent derivatives including peptides showed efficacy against this gram-negative bacteria [3-7]. *P. aeruginosa* is best known for chronic lung infections among cystic fibrosis patients and is a cause of serious infections among immune-compromised cancer patients, burn patients, catheterized patients, and other hospitalized individuals [2]. *Pseudomonas aeruginosa* is responsible for 10-15% of the nosocomial infections worldwide [8].

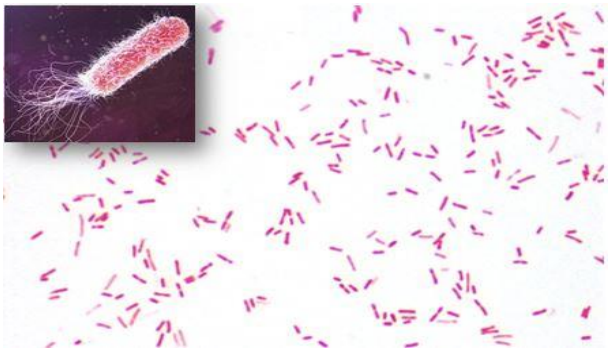

Fig. 1. *P. aeruginosa*, Gram (-) bacteria

Often, these infections are hard to treat due to the natural resistance of the species, as well as to its remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents. *P. aeruginosa* represents a phenomenon of antibiotic resistance and demonstrates practically all known enzymatic and mutational mechanisms of bacterial resistance. These mechanisms often exist simultaneously, thus conferring combined resistance to many strains [8].

Resistance to beta-lactam antibiotics is usually, but not exclusively, mediated by beta lactamases. *P. aeruginosa* produces a chromosomally encoded AmpC beta lactamase, which can hydrolyze antipseudomonal penicillins, aztreonam, and third-generation cephalosporins. The efflux pumps are an important mechanism of multidrug resistance, because they may confer resistance to quinolones, antipseudomonal penicillins, cephalosporins, and sometimes aminoglycosides [9].

Combating infections posed by this organism can mostly be done as a result of effective antimicrobial therapy. This involves administering of the right antibiotics to the infected patients. The high rate at which antibiotics are used all around the world in human therapy, animal therapy, and in livestock has given rise to the emergence of antibiotic-resistant isolates, leading to very detrimental problems in the affected individuals and in the community at large [10] as the treatment outcome of an infection is often affected by the presence of drug resistance in the infecting pathogen [11].

This study was therefore aimed at determining the resistance pattern of *Pseudomonas aeruginosa* isolated from selected tertiary hospitals in Osun state, Nigeria, so as to assist in the appropriate selection of empiric antibiotic treatment for infections caused by *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Study location

This study was carried out in some selected hospitals in Osun State, Nigeria. Osun state lies approximately on Latitude 40°N of the equator and Longitude 7.34°E of the Greenwich meridian and about 1,100 m above the sea level.

Collection of clinical isolates between September, 2018 and February, 2019

A total of 36 *Pseudomonas aeruginosa* isolates were obtained from 500 samples of urine, wound swab, ear swab, eye swab and other collection sites that are routinely submitted by both male and female patients to the diagnostic laboratories of Wesley Guild Hospital, Ilesha, State Specialist Hospital, Asubiaro, Osogbo, LAUTECH Teaching Hospital, Osogbo and OAUTHC, Ile-Ife. The isolates were transported at 4°C using ice packs to the Microbiology laboratory, Adeleke University, Ede, Osun state where they were processed immediately.

Conventional identification of Pseudomonas aeruginosa isolates

Pseudomonas aeruginosa isolates were identified using the conventional method [12]. This involved carrying out gram staining test, catalase test, urease test, oxidase test, coagulase test and some sugar fermentation tests on the pre-identified *Pseudomonas aeruginosa* colonies.

Antimicrobial susceptibility testing

Isolates confirmed as *Pseudomonas aeruginosa* were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084), according to the Clinical and Laboratory Standards Institute guidelines. Inoculated plates were incubated aerobically at 37°C for 18-24 hrs. Results were interpreted in accordance with criteria provided [13]. *Pseudomonas aeruginosa* Strain RH 815 (ATCC 10145) was used as a control which was tested on weekly basis. The tested antibiotics were Imipenem (10 μg/disk), Ertapenem (10 μg/disk), Meropenem (10 μg/disk), Cefepime (30 μg/disk), Ceftazidime (30 μg/disk), Penicillin (10 μg/disk), Ampicillin (30 μg/disk), Levofloxacin (10 μg/disk), Norfloxacin

(10 μg/disk), Fosfomycin (200 μg/disk), Ticarcillin (100 μg/disk), Moxifloxacin (5 μg/disk), Aztreonam (10 μg/disk), Colistin sulphate (10 μg/disk) and Vancomycin (10 μg/disk). Susceptibility of *Pseudomonas aeruginosa* isolates to each antimicrobial agent was measured and categorized as sensitive, intermediate or resistant.

Analysis and calculation of multiple antibiotic resistance (MAR) index

MAR index was calculated by following the method previously used [14]. Data were entered into Microsoft Excel (Microsoft Corp., Redmond, WA) and analyses were done using Epi-info v.7.0 Statistical analysis. Analysis of variance of data was done using the Statistical package for Social Science (SPSS) software, version 16.0 (SPSS Inc, Chicago, IL, USA, 2014). The values were presented as percentages, multiple comparisons was done using Turkey Post Hoc Test. P values ≤0.05 were considered statistically significant.

RESULTS

Distribution of Pseudomonas aeruginosa isolated from selected hospitals in Osun state

A total of 500 samples of urine, wound swab, ear swab, eye swab and other sample types from the diagnostic laboratories of the selected hospitals were received between September, 2018 and February, 2019. Overall, *Pseudomonas aeruginosa* was cultured from 36(7.20%) of the 500 samples including 12 (33.33%) from urine, 12 (33.33%) from wound swab, 2(5.55%) from sputum, 8(22.22%) from ear swab, 2(5.55%) from eye swab and 1(2.77%) from blood. *Pseudomonas aeruginosa* infected patients consisted of 18 males (50.00%) and 18 females (50.00%). The percentage ratio of in-patient and out-patient examined were 66.67% and 33.33% respectively. The percentage distribution of the admission class for medical and surgical was 58.33% and 41.67% respectively. The highest incidence of *Pseudomonas aeruginosa* was from patients with urine and wound swab infections (33.33%) each. Maximum number of cases occurred in age group less than 50 years (n= 20, 55.55%).

Antimicrobial susceptibility testing

Table 1 shows the antibiotic susceptibility profile of studied *Pseudomonas aeruginosa* isolates. According to the *in vitro* antibiotic susceptibility testing (AST), imipenem was the most effective antibiotic against *Pseudomonas aeruginosa* isolates (72.22% isolates were susceptible) and penicillin was the least effective antibiotics (97.22% isolates were resistant). Susceptibility rates for the carbapenem group of antibiotics which included imipenem, ertapenem and meropenem were 72.22%, 19.44% and 38.89% respectively and the susceptibility rates of other antibiotics tested including levofloxacin, colistin sulphate, aztreonam, vancomycin, ceftazidime, ticarcillin, cefepime, penicillin, moxifloxacin, ampicillin, fosfomycin and norfloxacin found to be 13.89%, 0.00%, 30.56%, 0.00%, 50.00%, 33.33%, 38.89%, 0.00%, 33.33%, 0.00%, 5.56% and 44.44% respectively.

Multiple antibiotic resistances (MAR) index

Results of multiple antibiotic resistances (MAR) index of *Pseudomonas aeruginosa* isolates are shown in **Table 2**. In total, 30 (83.33%) of 36 *Pseudomonas aeruginosa* isolates were identified as multi-drug resistant (MDR), having MAR index greater than 0.2.

Statistical analysis results

There were no statistically significant differences ($P > 0.05$) between the groups of antibiotics tested on *Pseudomonas aeruginosa* and the anatomical sites as determined by one-way ANOVA, however, there were significant differences between some groups of antibiotics as a whole as found in TIC ($F(3, 34) = 6.033$, $P = 0.002$), CAZ ($F(3, 34) = 8.349$, $P = 0.000$), FEP ($F(3, 34) = 7.289$, $P = 0.001$), ATM ($F(2, 34) = 2.951$, $P = 0.046$), ETP ($F(3, 34) = 5.013$, $P = 0.006$), MEM ($F(3, 34) = 8.906$, $P = 0.000$) and the location sites. Results from multiple comparisons of the significant means using Turkey post hoc test showed statistically significant differences between OAUTHC and other locations ($P < 0.05$).

DISCUSSION

Antimicrobial resistance in pathogens such as *P. aeruginosa* has resulted in increased morbidity and mortality from treatment failures and increased health care cost as treatment of these infections with first line antibiotics are becoming more difficult [14]. Results from this study showed that *P. aeruginosa* was mostly found in urine and wound swab with (33.33%) each. *P. aeruginosa* has been previously reported [15] as

Table 1. Antibiotic susceptibility profile of the *Pseudomonas aeruginosa* isolates

Antibiotics class	Antibiotics	Percentage (%) response of isolates to antibiotics		
		No. of susceptible isolates	No. of intermediate isolates	No. of resistant isolates
Carbapenems	Meropenem (10 μg)	14 (38.89)	10(27.78)	12(33.33)
	Ertapanem (10 μg)	7(19.44)	12(33.33)	17(47.22)
	Imipenem (10 μg)	26(72.22)	9(25.00)	1(2.78)
Fluoroquinolones	Levofloxacin (10 μg)	5(13.89)	14(38.89)	17(47.22)
	Norfloxacin (10 μg)	16(44.44)	5(13.89)	15(41.67)
	Moxifloxacin (5 μg)	12(33.33)	9(25.00)	15(41.67)
Polymyxins	Colistin sulphate (10 μg)	0(0.00)	18(50.00)	18(50.00)
Monobactams	Aztreonam (10 μg)	11(30.56)	10(27.78)	15(41.67)
Glycopeptides	Vancomycin (10 μg)	0(0.00)	6(16.67)	30(83.33)
Cephalosporins	Ceftazidime (30 μg)	18(50.00)	3(8.33)	15(41.67)
	Cefepime (30 μg)	14(38.89)	6(16.67)	16(44.44)
Penicillins	Ticarcillin (100 μg)	12(33.33)	8(22.22)	16(44.44)
	Penicillin (10 μg)	0(0.00)	1(2.78)	35(97.22)
	Ampicillin (30 μg)	0(0.00)	2(5.56)	34(94.44)
Phosphonic acid derivative	Fosfomycin (200 μg)	2(5.56)	11(30.56)	23(63.89)

*According to Clinical and Laboratory Standard Institute [13]

Table 2. Multiple antibiotic resistance (MAR) index of *Pseudomonas aeruginosa* isolates

Isolate code	Name of species	Antibiotic resistance pattern	MAR index
[a]P1	*Pseudomonas aeruginosa*	AMP, CT, P, TIC	0.27
[a]P2		AMP, ATM, CAZ, FEP, CT, ETP, FOS, LEV, MEM, MXF, NOR, P, TIC, VA	0.93
[a]P3		AMP, P, VA	0.20
[a]P4		AMP, ATM, CAZ, FEP, CT, FOS,LEV, MEM, P, TIC, VA	0.73
[a]P5		AMP, P, VA	0.20
[a]P6		ATM, CAZ, FEP, CT, ETP, LEV, MXF, NOR	0.53
[a]P7		AMP, ATM, CAZ, FEP, CT, ETP, FOS, MXF, NOR, P, TIC, VA	0.80
[a]P8		AMP, ETP, FOS, LEV, P, VA	0.40
[a]P9		AMP, FOS, P, VA	0.27
[a]P10		AMP, ETP, FOS, P, VA	0.30
[a]P11		AMP, ATM, CT, ETP, FOS, LEV, MXF, NOR, P, VA	0.67
[a]P12		AMP, CT, LEV, MXF, NOR, P, TIC, VA	0.53
[a]P13		AMP, FEP, FOS, LEV, MXF, NOR, P, VA	0.53
[a]P14		AMP, CT, FOS, LEV, MXF, NOR, P, TIC, VA	0.60
[a]P15		AMP, P, VA	0.20
[a]P16		AMP, CT, FOS, LEV, MXF, NOR, P, VA	0.53
[a]P17		AMP, P, VA	0.20
[a]P18		AMP, FEP, ETP, LEV, MXF, NOR, P, TIC, VA	0.60
[a]P19		AMP, ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P, TIC, VA	0.80
[a]P20		AMP, ATM, CT, P, VA	0.33
[a]P21		AMP, ATM, CAZ, FEP, CT, FOS, IPM, LEV, MEM, NOR, P, TIC	0.80
[a]P22		AMP, ATM, CAZ, FEP, CT, ETP, MEM, P, TIC	0.60
[a]P23		CAZ, FEP, CT, ETP, FOS, MEM, P	0.47
[b]P1		AMP, ATM, CAZ, P, VA	0.33
[b]P2		AMP, CT, FOS, P, VA	0.33
[b]P3		AMP, CT, ETP, FOS, LEV, MXF, P, VA	0.53
[b]P4		AMP, CT, FOS, P, VA	0.33
[b]P5		AMP, P, VA	0.20
[c]P1		AMP, FOS, P, VA	0.27
[d]P1		AMP, CAZ, FEP, CT, ETP, FOS, LEV, MEM, MXF, NOR, P, TIC	0.80

[d]P2		AMP, ATM, CAZ, FEP, ETP, FOS, MEM, P, TIC, VA	0.67
[d]P3		AMP, ATM, CAZ, FEP, ETP, FOS, LEV, MEM, MXF, NOR, P, TIC, VA	0.87
[d]P4		AMP, ATM, CAZ, FEP, ETP, FOS, MEM, P, TIC, VA	0.67
[d]P5		AMP, ATM, CAZ, FEP, ETP, FOS, LEV, MEM, MXF, NOR, P, TIC, VA	0.86
[d]P6		AMP, ATM, CAZ, FEP, ETP, FOS, LEV, MEM, MXF, NOR, P, TIC, VA	0.86

Key: MEM = Meropenem 10 μg, ETP= Ertapanem 10 μg, IPM = Imipenem 10 μg, LEV = Levofloxacin 10 μg, CT = Colistin sulphate 10 μg, ATM = Aztreonam 10 μg, VA = Vancomycin 10 μg, CAZ = Ceftazidime 30 μg, TIC = Ticarcillin 100 μg, FEP = Cefepime 30 μg, P = Penicillin 10 μg, MXF = Moxifloxacin 5 μg, AMP = Ampicillin 30 μg, FOS = Fosfomycin 200 μg, NOR = Norfloxacin 10 μg. [a]Isolates collected from Wesley Guild Hospital, Ilesha, [b]Isolates collected from LAUTECH, Osogbo, [c]Isolates collected from Asubiaro, Osogbo, [d]Isolates collected from OAUTHC, Ife.

the third most common organism implicated with urine infections and causing nosocomial urinary tract infections, and the fifth most common isolate overall from all sites. High percentage of resistant strains of *P. aeruginosa* present in urine samples which are capable of causing dreaded complications if not treated with an antibiotic effective against it as obtained in this study corroborates the results obtained from a previous study [16] where the isolation rate of *P. aeruginosa* from urine samples was 32 and recorded as the third most common urinary isolate after *Escherichia coli* and *Klebsiella* species during the study period.

From this study, higher percentage of *Pseudomonas aeruginosa* isolates demonstrated resistance to a large number of the antibiotics tested. A similar observation of a high rate of increase in the resistance of *P. aeruginosa* has been observed in ICU isolates in the USA in a study [17]. Due to the very broad spectrum of activity of the carbapenem group of antibiotics, some carbapenems such as imipenem were very effective against *P. aeruginosa*.

In this study, the resistance rate of *Pseudomonas aeruginosa* isolates to imipenem was relatively low and accounted for 1(2.78%) isolate compared to other carbapenems tested which corroborates the results obtained in a previous reported study [18]. Therefore, imipenem still remain a beneficial antibiotic for the treatment of infections caused by *P. aeruginosa*.

In a previous report from the study [19] in Saudi Arabia, the prevalence of imipenem resistance was 9.2% which was also quite low. However, in a report from another study [20] in Croatia, higher rate of imipenem resistance was reported where resistance ranged from 10.2% to 31.6%. It was opined that geographical variation in the resistance rates of *P. aeruginosa* may be related to the antibiotic prescribing habits in different parts of the world [18].

However, the least effective antibiotics to *P. aeruginosa* from this study was found to be penicillin with 97.22% resistance. This is in tandem with the study [21] who reviewed the current approach to antimicrobial therapy for *P. aeruginosa* and observed an increased rate of 37.00% resistance to penicillin.

From a study on antibiotic resistance in clinical isolates of *Pseudomonas aeruginosa* in other states aside Osun State in Nigeria, particularly Enugu and Abakaliki states [22], the presence of resistant strains of *Pseudomonas aeruginosa* in the areas of study was observed specifically in isolates obtained from pus samples obtained from wound infection patients. Their findings revealed that the highest resistance of *Pseudomonas aeruginosa* was to Amoxicillin (88.20%) which is a widely used antibiotics that belongs to the penicillin group of drugs. This is in tandem with the result obtained from the current study carried out in Osun state, a south western state in Nigeria.

Results from this study also showed very high percentage of multidrug resistant clinical isolates of *P. aeruginosa* with 83.33%. It was opined that *P. aeruginosa* is the most common multidrug-resistant (MDR) gram-negative pathogen causing pneumonia in hospitalized patients [21]. The accumulation of antibiotic resistance and cross-resistance between antibiotics and the appearance of multidrug-resistant (MDR) forms of *P. aeruginosa* consistently results from increasing resistance of *P. aeruginosa* to a wide array of antibiotics, as a result of disproportionate administration of antibiotics [22]. *In-vitro* sensitivity of antibiotics is an important factor that should be seriously considered in selecting the antimicrobial agents

for treatment of an infection [23].

CONCLUSION

Increase in antibiotic resistance continues to be a problem amidst patients infected with *Pseudomonas aeruginosa* which can be most likely attributed to increase in antibiotic use, hence, posing difficult therapeutic challenge and ultimately leading to clinical failure, however, imipenem still remain a beneficial antibiotic for treatment of infections caused by *P. aeruginosa*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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