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Multi-Drug Resistant *Pseudomonas aeruginosa* Isolated from Hospitals in Onitsha, South-Eastern Nigeria

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ABSTRACT

Background: *The increasing trend of multi-drug resistant Pseudomonas aeruginosa implicated in most nosocomial infections in Nigeria has necessitated this present study; which investigated the resistance and susceptibility patterns of P. aeruginosa isolated from hospitals in Onitsha, Southeast Nigeria.* ***Methods****: A total of 22 clinical and environmental isolates of P. aeruginosa were recovered from 10 hospitals in Onitsha, Southeastern Nigeria and they comprised 3 (13.6%) isolates from hospital sinks, 2 (9.1%) from hospital mops, patients' table, trolleys, sphygmomanometer, laboratory work bench and cleaning buckets respectively and 1 (4.5%) from theatre bed, wound swab, nasal swab, nurses' tray, floor, disinfectant and ear swab respectively. Antimicrobial susceptibility testing for 11 antibiotics was performed by agar disk diffusion on Muller-Hinton agar plates and multiple antibiotic resistance index (MARI) was also determined.* ***Results****: Antibiogram categorized the 22 P. aeruginosa isolates into 5 different antibiotypes. Results showed that each isolate was resistant to ≥ 3 classes of antibiotics; 3 (13.6%) were resistant to 7 antibiotics; 11 (50%) were resistant to 8 antibiotics; 5 (22.7%) were resistant to 9 antibiotics and 3 (13.6%) were resistant to 10 antibiotics. Susceptibility testing showed that all the 22 isolates were multi-drug resistant being resistant to at least 3 classes of anti-pseudomonal drugs. P. aeruginosa had highest resistance rates to the cephalosporins (ceftazidime, cefuroxime, cefotaxime, cefepime) at 100%, followed by piperacillin (100%), amoxicillin-clavulanic acid (100%) and tetracycline (100%). The multi-drug resistance (MDR) rate was determined at 100%. Highest number of susceptible isolates was recorded for imipenem (100%) and amikacin (86.4%) respectively.* ***Conclusion:*** *The high resistance profiles observed in this study could limit available therapeutic options for infections caused by these multidrug resistant strains if they are not properly detected and reported by hospital laboratories.*

Keywords*: Antibiotics, Multidrug Resistance, Pseudomonas aeruginosa, Nosocomial Infection, Nigeria*

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INTRODUCTION

Pseudomonas aeruginosa is a non-fermentative Gram-negative bacteria widely distributed in nature and can survive on a wide variety of surfaces and in hospital environment, as the moist environment of most wards encourage bacterial growth.[1] Within the hospital, *P. aeruginosa* finds numerous reservoirs: disinfectants, respiratory equipment, food, sinks, taps, and mops. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated

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reservoirs, and by the ingestion of contaminated foods and water. *Pseudomonas aeruginosa* is responsible for about 10% - 20% of nosocomial infections such as bacteraemia and sepsis in intensive care unit (ICU), cystic fibrosis, pneumonia, urinary tract infections, burn infection and wound infections.[2] Patients who are hospitalized and have catheters or other medical devices within them may become infected when these devices are contaminated. Despite its high nutritional versatility, it resists high concentrations of salt, dyes, weak antiseptics, and many commonly used antibiotics. *Pseudomonas aeruginosa* is frequently resistant to many commonly used antibiotics. Multi-drug resistant (MDR) *P. aeruginosa* phenotype is defined as *P. aeruginosa* isolate that is resistant to one anti-microbial agent in three or more anti-pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides).[3] Broad-spectrum anti-pseudomonal drugs such as imipenem, ceftazidime, and amikacin have been recommended for treatments of infections caused by multi-drug resistant (MDR) *P. aeruginosa*. However, resistance to one or more of these anti-pseudomonal drugs during therapy has been widely observed.[1,2,3,4] United States records an estimate of 51,000 healthcare-associated *P. aeruginosa* infections each year with 6,000 (13%) of these being multidrug-resistant, and roughly 400 deaths per year attributed to these infections.[4] In Cairo, Egypt, all the isolates of *P. aeruginosa* in a recent study were found to be resistant to ampicillin, cloxaclllin and co-trimoxazole.[5] Reports have also shown increasing trends of MDR *P. aeruginosa* implicated in most nosocomial infections in Nigeria.[6] In Calabar, Nigeria, all the *P. aeruginosa* isolates recovered from a recent study showed a high profile multiple resistance against all drugs used especially ampicillin, tetracycline and co-trimoxazole.[7] This present study aimed at investigating the resistance patterns and susceptibilities of *P. aeruginosa* isolated from hospitals in Onitsha, Southeast Nigeria.

METHODS

Bacterial Isolates: A total of 22 clinical and environmental isolates of *P. aeruginosa* were recovered from various sites of intensive care unit (ICU), operation theatres (OT) and wards from 10 hospitals in Onitsha, Southeastern Nigeria within a period of nine months. All isolates recovered were Gram stained and subcultured on a selective media, Cetrimide agar (Oxoid Ltd Basingstoke, UK) to obtain pure cultures of the test organism. *P. aeruginosa* isolates were confirmed by standard biochemical methods and by conventional microbiological tests.[8]

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing of 22 isolates of *P. aeruginosa* against 11 antibiotics (Oxoid, UK), representing 8 classes of antimicrobial agents was performed by the Kirby Bauer disc diffusion method on Muller-Hinton agar plates and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[9] The antibiotics tested included (drug:concentrations in µg) piperacillin (PRL: 30 µg), cefuroxime (CXM: 30 µg), ceftazidime (CAZ: 30 µg), cefotaxime (CTX: 30 µg), cefepime (FEP: 30 µg), amikacin (AK: 30 µg), imipenem (IPM: 10 µg), ciprofloxacin (CIP: 30 µg), chloramphenicol (C:30µg), tetracycline (TE: 30 µg), and amoxicillin - clavulanic acid (AMC: 30 µg) (Oxoid Ltd Basingstoke, UK). After overnight incubation at 37^{o}C, the diameters of the zone of inhibition were measured and compared with zone diameter of the interpretative chart to determine the sensitivity of the isolates to antibiotics as per the CLSI guidelines.

Determination of multiple antibiotic resistance index: Multiple antibiotic resistance index (MARI) was determined by the method of Olayinka *et al*.[10] To determine resistant profile of these resistant isolates, MARI was evaluated using the following formula:

MAR Index (MARI) = A/B

Where: A = Number of antibiotics isolate is resistant to

B = Total number of antibiotics tested

MAR index higher than 0.3 is indication of wide use of the antibiotics in the originating environment of the isolate.[10]

Statistical analysis: Statistical analysis was carried out with the Statistical Package for Social Sciences (SPSS) version 16.0; and this was determined using Chi square tests at p-value < 0.05 and at a confidence interval of 95 %.

RESULTS

Pseudomonas aeruginosa was most commonly isolated from hospital sinks (13.6%), then hospital mops and cleaning buckets (9.1%) and least from theatre bed, nasal and hand swabs, floor, disinfectant, ear and wound swabs (4.5%) (Table 1). The antibiotic susceptibility patterns of 22 *P. aeruginosa* isolates using disk-diffusion methods were summarized in Table 2. *P. aeruginosa* had highest resistance rates to the cephalosporins (ceftazidime, cefuroxime, cefotaxime, cefepime) at a rate of 100%, and this was followed by piperacillin (100%), amoxicillin-clavulanic acid (100%) and tetracycline (100%). The least amount of resistance was observed in amikacin (13.6%) and no resistance was seen against imipenem (Table 2). The multi-drug resistance (MDR) rate was determined at 100%. Imipenem was the most effective drug against all *P. aeruginosa* isolates and showed maximum sensitivity (100%).

Amikacin was the second active drug being effective against 19 (86.4%) isolates and ciprofloxacin was the 3rd active drug against 12 (54.5%) isolates of *P. aeruginosa*. Choramphenicol was also active but only against 5 (22.7%) isolates.

The distribution of resistance among the 22 *P. aeruginosa* isolates against 11 antimicrobial drugs was also obtained (Table 2). Of the 22 MDR *P. aeruginosa* isolates, 3 (13.6%) isolates were resistant to 10 antibiotics used, 5 (22.7%) isolates were resistant to 9 and 11 antibiotics, and 3 (13.6%) isolates were resistant to 7 antibiotics. In addition, the environmental isolates of *P. aeruginosa* exhibited higher

antibiotic resistance than the clinical isolates (Table 2). There was a statistically significant difference between the rate of resistance and susceptibility in *P. aeruginosa* isolates obtained in this study at $p< 0.05$.

Antibiogram categorized the 22 *P. aeruginosa* isolates into 5 different antibiotypes, with (1-4) antibiotypes including 20 isolates being the most prevalent (Table 3). One environmental isolate and 2 clinical isolates were included in antibiotype (4) which had resistance to the highest number of antibiotics (10). In this study, 22 (100%) of the *P. aeruginosa* isolates were MDR (Table 3). Table 4 shows the Multiple Antibiotic Resistance Index (MARI) of the *P. aeruginosa* strains. Analysis of the MAR index showed that 3 (13.6 %) *P. aeruginosa* isolates had MAR index of 0.6 and above, with 0.9 as the highest MARI.

Table 1: Distribution of *P. aeruginosa* isolates in both environmental and clinical specimens

Type of Specimen	Total No. of specimen	*P. aeruginosa* Isolates [No. (%)]
Theatre bed	9	1 (4.5)
Sink	12	3 (13.6)
Patients bed	10	0 (0)
Mops	12	2 (9.1)
Hands swab	10	0 (0)
Nasal swab	10	1 (4.5)
Nurses' tray	10	1 (4.5)
Floor	11	1 (4.5)
Disinfectant	9	1 (4.5)
Patients' table	10	2 (9.1)
Trolley	10	2 (9.1)
Sphygmomanometer	7	2 (9.1)
Water tap	10	0 (0)
Buckets	11	2 (9.1)
Lab work bench	9	2 (9.1)
Ear swab	1	1 (4.5)
Wound swab	1	1 (4.5)
Total	152	22 (100)

DISCUSSION

This study showed that *Pseudomonas aeruginosa* was isolated from a nasal swab, a wound and ear swab samples as well as from environmental sites such as sinks, cleaning mops, buckets and others like trolley, and floor. This is in line with the reports by Olayinka *et al.*[10], Akingbade *et al.*,[11] Nworie *et al.*[12] and Haleem *et al.*[13]. This study also recorded several resistances to anti-pseudomonal drugs such as the penicillins, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems antibiotics that have been shown by many studies to be active against MDR bacteria including *P. aeruginosa.*[11,12,13,14,15] The susceptibility pattern also revealed that 22 of the *P. aeruginosa* isolates were 100% resistant to all the extended spectrum cephalosporins (ceftazidime, cefuroxime, cefotaxime and cefepime), piperacillin, amoxycillin-clavulanic acid and tetracycline but showed 77.3% resistance to chloramphenicols. These findings corroborate results of Haleem *et al.*[13], Al-Yasseen *et al.*[14] and Mahmoud *et al.*[15].

Twenty-two (22) of the *P. aeruginosa* isolates were 100 % sensitive to imipenem, while 19 (86.4 %) isolates were sensitive to amikacin and 12 (54.5 %) isolates were sensitive to ciprofloxacin. These findings are in line with the report by Olayinka *et al.*[10] in North Central Nigeria; Gad *et al.*[16] and Mahmoud *et al.*[15] both in Egypt. These earlier reports showed that amikacin and imipenem were the most effective drugs against *P. aeruginosa.*[10,15,16] The study carried out in Southwestern Nigeria by Odumosu *et al.*[17] revealed the susceptibility pattern of 98.1% and 92.6% of *P. aeruginosa* isolates to colistin and imipenem respectively. The high susceptibility pattern of these drugs could be associated to less drug abuse by the population being that the cost of these antibiotics prevents patient's self-medication. However, recent studies by Ranjbar *et al.*[18] and Hamze *et al.*[19] in Lebanon revealed a high resistance of 97.5% and 33.3% of *P. aeruginosa* to imipenem respectively, thus, demonstrating the evolution of imipenem-resistant strains of *P. aeruginosa.* Nevertheless, imipenem remain a potent anti-pseudomonal antimicrobial agent in Nigeria contrary to other report outside Nigeria where high imipenem resistance is prevalent.[17]

This study revealed a moderate activity of ciprofloxacin against *P. aeruginosa* (54.5%). A similar result of 54.5% susceptibility to ciprofloxacin was also reported by Zulfiqar *et al.*[20] The studies carried out by Olayinka *et al.*,[10] Akingbade *et al.*,[11] Nworie *et al.*,[12] Odumosu *et al.*,[17] Odusanya[21] and Nikbin *et al.*[22] revealed a high sensitivity of 66.7%, 84.6%, 90.2%, 64.8%, 64.5% and 83.3% to ciprofloxacin respectively. The high susceptibility pattern of these drugs could be associated to less drug abuse by the population. This study revealed a resistance of 45.5% to ciprofloxacin. This concurs with the study from Haleem *et al.*[13]. Increasing resistance to this broad-spectrum antibiotic might be as a result of monotherapy on the part of clinicians or as a result of selective pressure due to its frequent use in Nigeria.[23]

High resistance patterns were exhibited by most of the *P. aeruginosa* isolates obtained in this work and antibiotype categorized the 22 *P. aeruginosa* isolates in this study into 5 different antibiotypes with antibiotype 4 showing the highest resistance pattern to 10 antibiotics. Antibiogram is a sensitive phenotypic marker, however it has the disadvantage of being non reproducible in many instances due to the exchange of resistance (R)-factor among the isolates.[23]

A high multiple antibiotic resistances were observed among the clinical and environmental isolates of *P. aeruginosa*. This study revealed that a high prevalence of MDR *P. aeruginosa* (100%) was detected in Onitsha. Similar pattern of multi-drug resistance have been reported in Lagos by Odusanya,[21] in Zaria, Northeastern Nigeria by Olayinka *et al.*,[10] in Calabar by Jombo *et al.*,[7] and in Southwest Nigeria

Table 2: Distribution of *P. aeruginosa* isolates in both environmental and clinical specimens

Antibiotic Class	Type of Antibiotic	Disk Content (µg)	Sensitive No. (%)	Resistant No. (%)
Cephalosporins	Ceftazidime	(30 µg)	0 (0)	22 (100)
	Cefuroxime	(30 µg)	0 (0)	22 (100)
	Cefotaxime	(30 µg)	0 (0)	22 (100)
	Cefepime	(30 µg)	0 (0)	22 (100)
Aminoglycosides	Amikacin	(30 µg)	19 (86.4)	3 (13.6)
Carbapenems	Imipenem	(10 µg)	22 (100)	0 (0)
Quinolones	Ciprofloxacin	(5 µg)	12 (54.5)	10 (45.5)
Tetracycline	Tetracycline	(30 µg)	0 (0)	22 (100)
ESP	Piperacillin	(30 µg)	0 (0)	22 (100)
Phenicols	Chloramphenicol	(30 µg)	5 (22.7)	17 (77.3)
β-lactamase Inhibitor	AMC	(30 µg)	0 (0)	22 (100)

* **ESP** = Extended- spectrum Penicillin
AMC = Amoxicillin-Clavulanic acid

Table 3: Group of *P. aeruginosa* isolates having the same antibiotype

Profile	No. of Antibiotics the isolates are resistant to	No. of Isolates	Resistance Pattern (100% resistance)
R1	7	3	Piperacillin-amoxicillin/clavulanic acid-ceftazidime-cefepime-cefuroxime-cefotaxime-tetracycline
R2	8	9	Piperacillin-amoxicillin/clavulanic acid-ceftazidime-cefepime-cefuroxime-cefotaxime-tetracycline-chloramphenicol
R3	9	5	Piperacillin-amoxicillin/clavulanic acid-ceftazidime-cefepime-cefuroxime-cefotaxime-tetracycline-chloramphenicol-ciprofloxacin
R4	10	3	Piperacillin-amoxicillin/clavulanic acid-ceftazidime-cefepime-cefuroxime-cefotaxime-tetracycline-chloramphenicol-ciprofloxacin-amikacin
R5	8	2	Piperacillin-amoxicillin/clavulanic acid-ceftazidime-cefepime-cefuroxime-cefotaxime-tetracycline-ciprofloxacin
Total		22	

Table 4. Results of Multiple Antibiotic Resistance Index (MARI)

MAR Index	No (%) of Isolates
0.6	3 (13.6)
0.7	11 (50.0)
0.8	2 (9.1)
0.9	6 (27.3)

by Akingbade *et al.,*[11] where majority of the isolates exhibited multi-drug resistance pattern as obtainable in this study. Furthermore, studies in Pakistan, Egypt and Iran observed that most of the *P. aeruginosa* strains isolated were MDR.[16, 18, 20] Contrary to this study, Zahra and Moniri[24] in Iran detected lower levels as 30% of their isolates were MDR. This high rate of MDR could be as a result of unrestricted use of antibiotics by the population who were mainly traders and depend much on self-medication. Multi-drug resistant *P. aeruginosa* develop resistance by various mechanisms like multidrug resistance efflux pumps, production of β-lactamases, aminoglycoside modifying enzymes, and decrease outer membrane permeability; and these properties of the organism contributes to the virulent nature of *P. aeruginosa* inclusive of its multidrug resistant nature as was previously reported [15, 25, 26]. Prompt and accurate detection of MDR bacteria from clinical samples is critical to proper patient care – since these measures will provide sound epidemiological data that will guide antimicrobial therapy during treatment. This measure will also help to nip in the bud, the emergence and spread of drug-resistant bacteria in both the community and hospital environment.

CONCLUSION

In conclusion, this study showed the presence of multi-drug resistant *P. aeruginosa* from hospitals in Onitsha with a high multi-drug resistant index. It also showed that there is intense antibiotic use probably both from doctors and patients, (as antibiotics can be obtained in Nigeria without a prescription), which is a well identified risk factor for the emergence of drug resistant strains in this environment. High antibiotic resistance to third generation cephalosporins as well as β-lactam inhibitors as obtained in this study is very disturbing as these drugs are mainly used against Gram-negative resistant microorganisms. However, imipenem could be the best drug of choice for the treatment of MDR *P. aeruginosa* in southeastern Nigeria especially in Onitsha. Improper use and prescription of drugs without prior susceptibility testing should be discouraged while good antibiotic surveillance system should be set up in this part of the country to ensure proper monitoring and control of drug resistant *P. aeruginosa.* This will also help to guide clinicians for appropriate therapy and in the management of *Pseudomonas aeruginosa* infection.

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