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Antibiotic Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Clinical Samples in Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti State of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study, drafted the protocol and supervised the work. Authors OAI and ACO carried out the sample collection, isolation and antibiotics susceptibility testing. Authors ACO and JAB carried out the literature searches managed and analyzed the data. Authors OAI and JAB wrote the first and final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the prevalence of acquired multidrug resistance of *P. aeruginosa* among clinical samples obtained from patients attending Ekiti State University Teaching Hospital, Ado Ekiti, Ekiti State, Nigeria.
Place and Duration of Study: Ekiti State Teaching Hospital from January-March 2013.
Methodology: The isolates were characterized by standard cultural and biochemical tests and they were tested for their sensitivity to different antibiotics using disk diffusion method.
Results: A total of 192 clinical samples were collected from which 42 isolates of *P. aeruginosa*

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were obtained. Antibiogram profile showed that a total of 80.95% of the isolates were resistant to ceftriaxone and ceftizoxime respectively, 76.2% to augmentin, 73.8% to ceftazidime, 71.4% to nitrofurantoin, 47.6% to ofloxacin, 45.23% to gentamicin while ciprofloxacin had the lowest resistance of 42.86. Isolates from ear swabs had the highest resistance to 3rd generation cephalosporins, followed by isolates from urine while isolates from wound samples showed the lowest resistance.

Conclusion: There is a need to institute an effective antimicrobial resistance surveillance system that provides clinicians with up-to-date data on the prevalence and resistance pattern of commonly encountered pathogens like *P. aeruginosa* especially as nosocomial infection is concerned.

Keywords: Pseudomonas aeruginosa; nosocomial pathogen; clinical samples; antibiotics susceptibility; multidrug resistant- Pseudomonas aeruginosa.

1. INTRODUCTION

Antibiotic resistance is a worldwide problem and a threat to public health. New forms of antibiotic resistance emerge daily which cross international boundaries and spread with ease. *Pseudomonas aeruginosa* is an opportunistic pathogen notable as a leading cause of nosocomial infections responsible for at least 10% of all hospital acquired infections, ranking second among Gram-negative pathogens [1,2]. It is also associated with increased mortality and longer hospital stay mainly because of their high antibiotic resistance profile. *Pseudomonas aeruginosa* is a widespread pathogen in the hospital environment that frequently forms a permanent plaque (known as biofilm) on medical equipment and colonizes tissues of long-stay patients [3,4]. They majorly spread through hospital equipment and healthcare workers rather than person to person which pose as a threat to the hospital community [5]. The rapid increase of drug resistance in clinical isolates of this opportunistic human pathogen is of worldwide concern which is not only related to high morbidity and mortality but its significance influence on the economy. A number of studies have evaluated the resistance profile of *P. aeruginosa* in Nigeria, through assessment of clinical samples [6,7], however is paucity of information on the prevalence of *P. aeruginosa* in nosocomial infection in Ekiti State which necessitate this study. This study is aimed at determining the prevalence of *P. aeruginosa* among clinical samples obtained from Ekiti State University Teaching Hospital, Ado Ekiti and to elucidate the antibiogram profile of the isolates.

2. MATERIALS AND METHODS

2.1 Study Area and Period

The study area for this research work was Ekiti State Teaching Hospital, Ado-Ekiti, located in Ekiti central district. Clinical samples were collected between January-March 2013.

2.2 Study Population

The study populations were in-patients and out-patients attending the above named tertiary institution.

2.3 Sample Collection

A total number of 192 clinical samples were collected from the patients attending the above named tertiary institution. The various clinical specimens included in the study for the periods were wound, urine and ear swab/discharge.

2.4 Isolation of *Pseudomonas aeruginosa*

All samples received from the wards were cultured on selective media such as MacConkey and Cetrimide agar and incubated at a temperature of 37℃ for 24 h. Characterization and identification of *P. aeruginosa* was carried out using a combination of colonial morphology, Gram stain, motility tests, catalase and oxidase test and pigment production [8].

2.5 Standardization of Inoculum

Four pure colonies of each isolate on a 24 h plate culture were randomly selected and inoculated into 2 mL of sterile peptone water broth in bijou bottles. This was incubated at 37℃ for 6 h and the turbidity was adjusted by serial dilution in phosphate buffer saline (pH 7. 2) to match an opacity tube containing 0. 5 mL of 1% barium chloride in 1% suplhuric acid (a Mc Farlands 0.5 bariumsulphate standard containing 10^5 cfu/mL of the inoculums). One milliliter (1 mL) of the culture dilution (bacteria suspension) was transferred into a well dried surface of diagnostic sensitivity test agar (DST) medium and titled to

spread evenly over the entire surface of the agar plate. The excess fluid was drained off and dried within 5 min multi-antibiotic discs were then placed of the surface of the inoculated plate and incubated aerobically at 37℃ for 18 to 24 h (over-night). The diameter of the zone of inhibition was measured in millimeter. The result of each antimicrobial agent tested was reported as susceptible or resistant when the test organism was compared with antibiotics chart.

2.6 Antibiotics Susceptibility Test

Antibiotics susceptibility testing and interpretation was performed using the disc diffusion method of the modified Kirby-Bauer technique according to Clinical Laboratory Standards Institute guidelines [9]. *P. aeruginosa* ATCC 27853 was used as control strain. Sensitivity to the following antibiotics was determined augmentin (amoxicillin/clavulanate) (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ceftizoxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5µg) and nitrofurantoin (300 µg). Isolates was considered multidrug resistant (MDR) if they showed resistance to 3 or more classes of the tested antibiotics.

2.7 Ethical Consideration

The ethical clearance for this research was given by Ekiti State Teaching Hospital (EKSTH) ethical committee after due processes. Before the collection of the samples, information regarding the study was explained to the subjects. Oral and written consent for participation in the study were also obtained.

3. RESULTS

In the present study a total of 192 samples were collected from which 42 isolates of *P. aeruginosa* were obtained (Table 1). Of these, 140 samples were collected from female while 52 samples were collected from male patients. The antibiotic resistance of *P. aeruginosa* isolated against different groups of antibiotics was represented in Table 2. The organism showed varying resistance pattern to the antibiotics tested such as 3rd generation cephalosporin (ceftriaxone, ceftizoxime, and ceftazidime), augmentin, and nitrofurantoin. The percentage resistance to cephaphalosporin ranged between 73.8 to 80.5% Ceftriaxone and ceftizoxime had the total resistance of 80.95%, ceftazidime, 73.8%, augmentin, 76.2%, nitrofurantoin, 71.4%, ofloxacin, 47.6%, gentamicin 45.23% and ciprofloxacin with the lowest resistance rate of 42.86%. Isolates from ear swabs had the highest resistance to 3rd generation cephalosporins, followed by isolates from urine while isolates from wound samples showed the lowest resistance. 50% of the isolates from wound samples exhibited resistance against nitrofurantoin while 89.5% and 80% of isolates from urine and ear swabs respectively exhibited resistance against the same tested antibiotic. Table 3 shows the resistance pattern of *P. aeruginosa* isolates from the various clinical samples. 36 strains were resistant at least 2 antibiotics. It was found 24 different patterns of resistance among strains tested. The number of patterns of resistance to strains isolated from wound – 13, ear – 1, urine – 10.

Table 1. The distribution of various clinical samples examined in EKSUTH

Type of samples examined	No of sample examined	Male	Female
Urine	95	25	70
Wounds	85	25	60
Ear swabs	12	2	10
Total	192	52	140

4. DISCUSSION

In our study, a high prevalence of *Pseudomonas* infections was found in the female patients than their male counterparts similar to a study in the northern part of Nigeria [10]. This may either be due to anatomical predisposition or urolithical mucosal adherence to mucopolysaccharide lining or other host factors. The distribution of isolates differs with studies and clinical specimens. In Jos, Jombo et al. [10] reported 4.6% in urine, while in Zaria, Olayinka et al. [11] reported 51.1% in urine and 41.3% in wound and 1.1% in sputum. 80% of the of the isolates from our study (data not shown) were recovered from in-patient justifying the role of this organism in nosocomial infection. The current study also demonstrated that *P. aeruginosa* strains isolated from ear swabs and urine samples were more resistant to tested antimicrobial agent which may have resulted from the fact that the strains isolated from these specimens have been subjected to the selective actions of both disinfectants or possibly an effect of a prolonged hospitalization and the application of medical equipment (airway, catheters etc).

Table 2. Antibiotic resistance of *P. aeruginosa* isolated from different clinical samples in EKSUTH against different groups of antibiotics

Groups of antibiotics	Wounds (n=18)	Urine (n=19)	Ear swabs (n=5)	Total % of resistance
	R (%)	R (%)	R (%)	
3rd generation cephalosporin				
CRO	11 (61.1)	18 (94.7)	5 (100)	34 (80.95)
CXM	13 (72.2)	17 (89.5)	4 (80)	34 (80.95)
CAZ	12 (66.7)	14 (73.7)	5 (100)	31 (73.8)
Fluoroquinolones				
OFL	7 (38.9)	10 (52.6)	3 (60)	20 (47.6)
CPR	9 (50)	7 (36.8)	2 (40)	18 (42.86
Aminoglycosides				
GEN	5 (27.8)	11 (57.9)	3 (60)	19(45.23)
Penicillin				
AUG	12 (66.7)	17 (89.5)	3 (60)	32 (76.2)
Nitrofurantoins	9 (50)	17 (89.5)	4 (80)	30 (71.4)

Key: CRO = Ceftriaxone, CXM=Ceftizoxime, OFL = Ofloxacin, AUG = Augmentin, GEN = Gentamicin, NIT = Nitrofurantoin, CPR = Ciprofloxacin, CAZ = Ceftazidim, (00) = Numbers in brackets are percentage values, (n) = Total number of isolates in a sample

Table 3. Showing the resistance pattern of *P. aeruginosa* from the various clinical samples

Samples	Resistance pattern	No of organism showing resistance pattern	No of patterns
Wounds	CRO, AUG	1	
	CXM, NIT	1	
	CRO, AUG, NIT	1	
	CRO, CXM, AUG, CAZ	2	
	CXM, NIT, CPR, CAZ	1	
	CRO, CXM, AUG, CPR,CAZ	1	
	CRO, CXM, OFL, AUG, CAZ	1	
	CRO, CXM, AUG, NIT, CPR	1	13
	CRO, CXM, OFL, AUG, CPR, CAZ	1	
	CXM, OFL, GEN, NIT, CPR, CAZ	1	
	CRO, CXM, GEN, NIT, CPR, CAZ	1	
	CXM, OFL, AUG, GEN, NIT, CPR, CAZ	1	
	CRO, CXM, OFL, AUG, GEN, NIT, CPR, CAZ	2	
	Sub-total	15	
Ear swabs	CRO, CXM, OFL,AUG, GEN, NIT,CPR, CAZ	2	1
	Sub-total	2	
Urine	CXM, NIT	1	
	CRO, CXM, OFL, AUG	1	
	CRO, CXM,AUG, NIT	3	
	CRO, OFL,AUG, NIT, CAZ	1	
	CRO, CXM,GEN, NIT, CAZ	1	10
	CRO, CXM, AUG, NIT, CAZ	2	
	CRO, CXM, AUG, GEN, CAZ	1	
	CRO, OFL, AUG, GEN, NIT,CAZ	1	
	CRO, CXM,AUG, GEN, NIT, CAZ	1	
	CRO, CXM, OFL,AUG, GEN, NIT, CPR, CAZ	7	
	Sub-total	19	
	Grand-total	36	24

Key: CRO = Ceftriaxone, CXM=Ceftizoxime, OFL = Ofloxacin, AUG = Augmentin, GEN = Gentamicin, NIT = Nitrofurantoin, CPR = Ciprofloxacin, CAZ = Ceftazidime

Pseudomonas aeruginosa isolates showed 80.5% and 73.8% resistance to ceftizoxime and ceftzazidime respectively while all the isolates exhibited different resistance patterns to all the classes of antibiotics. Similarly, Wassef et al. [12] reported 43.9% *Pseudomonas aeruginosa* isolates were ceftazidime resistant in a lab based surveillance of multidrug resistant *Pseudomonas aeruginosa* in Cairo University Hospitals, Egypt. Selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains. In a related study, Ojo-Bola and Oluyege [13] reported that *P. aeruginosa* isolates exhibited 100% resistant to all the conventional antibiotics tested except 94.3% and 97% resistant to sparfloxacin and ceftazidime respectively. Our study also shows that the percentage of the MDR in the *P. aeruginosa* strains had increased over the years compared to previous studies [14].

5. CONCLUSION

This study indicates a prevalence of *P. aeruginosa* isolates resistance to all the antibiotics tested which pose a great threat and such that if not properly checked may cause outbreaks within the population as well as increase morbidity and mortality in patients underlying diseases.

6. RECOMMENDATIONS

Rigorous monitoring of the MDR in *P. aeruginosa*, the restriction of the inappropriate use of antimicrobial agents and adherence to infection control practices should be emphasized in order to delay the emergence of clinically significant *P. aeruginosa*. The development and the application of antimicrobial usage policies along with the aid of the Hospital Infection control committee will decrease the appearance and the spread of the nosocomial infection epidemics.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Souli M, Galani I, Giamarellou H. Emergence of extensively drug-resistant and pandrug-resistant gram-negative bacilli in europe. Euro Surveill. 2008; 13(47):1-11.
2. Talbot GH, Bradley J, Edwards JE, Gilbert D, Scheld M, Barlett JG. Antimicrobial availability task force of the infectious diseases society of America: Bad bugs need drugs: An update on the development pipeline from the antimicrobial availability task force of the infectious diseases society of America. Clin. Infect. Dis. 2006;42:657-668.
3. Ashfaque H. Growth in biofilm enhances potential to form new biofilm by *Pseudomonas aeruginosa*. Life Science Journal. 2013;10(6s).
4. Revdiwala S, Bhaumesh MR. Summaiy M. Characterization of bacterial etiologic agents of biofilm formation in medical devices in critical care setup. Critical Care Research and Practice. 2012;945805.
5. Anaissie EJ, Penzak SR, Dignani MC. The hospital water supply as a source of nosocomial infection: A plea for action. Arch. Intern. Med. 2002;162(13):1483-1492.
6. Daini OA, Charles-Onyeaghala CG. Plasmid- mediated aminoglycoside resistance of clinical isolates of *Pseudomonas aeruginosa*. Global Advanced Research Journal of Microbiology. 2012;1(4):052-056.
7. Smith S, Ganiyu O, John R, Fowora M, Akinsinde K, Odeigah P. Antimicrobial resistance and molecular typing of *Pseudomonas aeruginosa* isolated from surgical wounds in Lagos, Nigeria. Acta Medica Iranica. 2012;50:433-438.
8. Cheesebrough M. District laboratory practice in Tropical countries. E.C.B.S Cambridge University Press, 2nd ed, 2002; 97-182.
9. Clinical and Laboratory Standards Institute. Performance standards for Aantimicrobial susceptibility testing; twenty-first informational supplement. M100-S21. 2011;31(1).
10. Jombo GT, Jonah P, Ayeni JA. Multiple resistant *Pseudomonas aeruginosa* in contemporary medical practice: Findings from urinary isolates at a Nigerian

University Teaching Hospital. Niger J Physiol Sci. 2008;23(1-2):105-9.

11. Olayinka AT, Olayinka BO, Onile BA. Prevalence of multi-drug resistant (MDR) *Pseudomonas aeruginosa* isolates in surgical units of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria: An indication for effective control measures. Annals of African Medicine. 2008;1:13-16.
12. Wassef M, Mahallawy HE, Zafer MM, Ghaith D, Hamid RA. Lab based surveillance of multidrug R resistant *Pseudomonas aeruginosa* in Cairo University Hospitals, Egypt. J. Microbiol. Exp. 2015;2(2):00039.
13. Ojo- Bola, Oluyege O. Antibiotics resistance of bacteria associated with pneumonia in HIV/AIDS patients in Nigeria. American Journal of Infectious Diseases and Microbiology. 2014;6:138-144.
14. Obritsch MD, Fish DN, MacLaren R, Jung R. National surveillance of antimicrobial resistance in *Pseudomonas aeruginosa* isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrob. Agents Chemother. 2004;48(12):4606-10.

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