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Frequency and Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Patients Attending University of Maiduguri Teaching Hospital

Askira M. Umoru[1*], Tijani Isa[2], Muhammad M. Ibrahim[2], T. M. Isyaka[3], I. Y. Ngoshe[2] and A. Aliyu[4]

[1]Department of Medical Laboratory Science, University of Maiduguri, Nigeria.
[2]Department of Microbiology, University of Maiduguri, Nigeria.
[3]Department of Medical Microbiology, University of Maiduguri, Nigeria.
[4]Department of Biological Sciences, Abubakar Tafawa Balewa University, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AMU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TI, MMI and TMI managed the analyses of the study. Authors IYN and AA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at determining the frequency of occurrence of *Pseudomonas aeruginosa* and the susceptibility pattern of isolates to antibiotics.
Place of Study: Department of Medical Microbiology, University of Maiduguri Teaching Hospital (UMTH), Nigeria.
Methodology: One hundred and thirty one (131) clinical specimens (comprising of urine, wound swab, ear swab, high vaginal swab and catheter swab) were collected from patients (female=80, male=51) attending UMTH and were screened for *P. aeruginosa* using standard microbiological and biochemical methods. Antimicrobial susceptibility of the isolates was determined by the disc diffusion assay.

**Corresponding author: E-mail: mohammedaskirau@gmail.com;*

Results: Our results showed that thirty six (36) patients were positive for *P. aeruginosa* with an occurrence rate of 27.5% with male patients having the highest occurrence rate of 55.6% compared to female patients with 44.4%. The result also showed that the occurrence rate was higher among patients within the age group of 31-40 years (25.0%) and the least was observed among those in age group of 41-50 years and 71-80 years (5.6% respectively). The distribution of *P. aeruginosa* in relation to sample types examined indicate that wound swab had the highest isolation rate of 38.9% followed by urine 27.8%, while the least were catheter tips (2.8%). The distribution of *P. aeruginosa* based on clinics and wards of the hospital showed that patients from general out patients departments (GOPD) had the highest occurrence rate of 27.8%, while the least was observed among those from female medical ward (2.8%). The antimicrobial susceptibility test revealed that Tetracycline and Cefuraxime had high activity against 42.0% of isolates while Ofloxacin recorded the highest resistance of 92.0%.

Conclusion: This study reveals an increased distribution rate for *P. aeruginosa* in the hospital environment, hence appropriate measures and proper identification techniques are required for surveillance and control.

Keywords: Pseudomonas aeruginosa; antibiotics patients; antimicrobial; resistance; pathogenic bacteria.

1. INTRODUCTION

Resistance in pathogenic bacteria against antibiotics has remained a challenge to our clinicians in the management of various infections [1]. *Pseudomonas aeruginosa* (*P. aeruginosa*) are aerobic, non-fermenting Gram-negative bacilli, which are most commonly involved in opportunistic infections usually in the nosocomial setting ([2,3,4]). *P. aeruginosa* was the predominant Gram-negative bacteria isolated from broncho-pulmonary infections and accounts for 17% of health care-associated pneumonia and late –onset ventilate associated pneumonia [5] and also accounts for significant cases of cystic fibrosis, (Pier 2000).

P. aeruginosa develops resistance against almost all antibiotics by various mechanisms including multi-drug resistance efflux pumps, biofilm formation, resistance genes such as (SHV, IMP, VIM), Aminoglycosides modifying enzymes, and mutations in various chromosomal genes [6]. Exposure to broad spectrum antibiotics and patient to patients spread have added to the rapid increase in the isolation of these resistant strains [7].

The main anti-pseudomonal antimicrobial groups are Penicillin-ß-lactamase inhibitor combinations (Cefoperazone- Sulbactam, Piperacillin-Tazobactam), Cephalosporins (Cefoperazone, Ceftazidime), Monobactam (Aztreonam), Fluoroquinolone (Ciprofloxacin, Levofloxacin), Fosfomycin, Carbapenems (Meropenem, Imipenem, Doripenem) and Aminoglycosides (Amikacin, Gentamicin, Tobramycin, Netilmicin) [8,9]. However, the overuse and misuse of antimicrobials has often led bacteria to become resistant against antimicrobial agents [10]. Over-ambitious use of these broad-spectrum antibiotics is playing an important role in the increased incidence of infections due to multi-drug resistant (MDR) bacteria [1]. The MDR strains of pathogenic bacteria are emerging as an important cause of mortality and morbidity especially if not treated properly and timely [6].

The knowledge of the frequency and susceptibility pattern of the MDR *P. aeruginosa* is very important for effective treatment of infectious diseases of pseudomonal origin. Few studies regarding the frequency and susceptibility pattern of MDR *P. Aeruginosa* was conducted in the local set up so far. This study aimed to find out the frequency and antibiogram of *P. aeruginosa* in the clinical specimen received from patients attending the tertiary-care hospital in Maiduguri.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at the University of Maiduguri Teaching Hospital (UMTH), Maiduguri. Borno State Nigeria. Maiduguri is located in the north -eastern part of Nigeria, lies within latitude 11.15°N and longitude 30.05°E in the sudano-sahelian savanna zone with a dense population that are mostly farmers, fishermen, herdsmen, traders, civil servant and public servant. It is a city with a rich cultural heritage and a home to

the Kanem Borno Empire [11]. It has population of approximately 4,098,391 as at 2006 population census (www.borno.stategov.nig). The population of the study include patients attending the clinics and those admitted in the wards of the hospital (Outpatients & Inpatients).

2.2 Sample Collection

A total of 131 one hundred and thirty one clinical samples (urine, wound swab, ear swab, high vaginal swab and catheter tips) were collected from female (80) and male (51) patients of all ages attending University of Maiduguri Teaching Hospital Maiduguri, The samples were collected and transported to the laboratory for microbiological analysis.

2.3 Identification and Characterization of Bacterial Isolate

All samples were bacteriologically prepared and were cultured onto Blood agar and MacConkey agar and incubated aerobically at 37^0C for 24hrs. Suspected colonies were identified using colonial morphology, motility testing, Grams reaction and Biochemical test was performed as described by Cheesbrough [12].

2.4 Antibacterial Sensitivity Testing

Antibiotic sensitivity testing was used to determine the susceptibility of *P. aeruginosa* to a range of potential therapeutic agents. The test was performed using Kirby-Bauer test method [13]. Commercially available antibiotic discs (Oxoid Ltd, Bashing stoke, Hampshire, England) were used for the test to determine the drug sensitivity pattern. The test was performed by inoculating a colony of the test organism in nutrient broth and was incubated at 37°C for 3-5hrs until it reach 0.5 Mcfarland standard then the entire surface of the agar plates was flooded with the culture broth and excess was discarded. Antibiotic discs were placed over the media using dispenser and gently tap each antibiotic disc onto the surface of the agar with a sterile stick, and plates were incubated at 37°C.

The antibiotics used against the test bacteria were: Amoxicillin (10 µg), Ofloxacin (5 µg), Ampicillin (10 µg), Ceftriaxone (30 µg), Cefuraxime (30 µg), Amoxicillin (30 µg), Gentamycin (30 µg), Tetracycline (30 µg), Cotrimoxazole (30 µg) and Augmentin (30 µg). The diameter of zone of inhibition was measured in millimeters and isolates were scored as sensitive or resistant by comparing with standard charts as recommended by National committee for clinical laboratory standards [14].

3. RESULTS

One hundred and thirty one (131) clinical samples of urine (46), wound swab (27), high vaginal swab (27), catheter tips (3) and ear swab (28), were collected from 80 female and 51 male of patients attending University of Maiduguri Teaching Hospital. Thirty six (36) patients were positive for *P. aeruginosa* with a recovery rate of 27.5%. However, the result showed that male had the highest incidence rate of 20(55.6%) while female had 16(44.4%) (Table 1).

The distribution rate of *P. aeruginosa* was higher within the age group of 31- 40 yrs with 25% (9) followed by 11-20 yrs and 21-30 yrs with 16.7% (6). There was no incidents recorded for the age group of 81-90 yrs while, the least occurrence rate of *P. aeruginosa* was observed within the age groups of 41-50 yrs, 61–70 yrs, 71-80 yrs with 5.6% (2) (Table2).

The distribution of *P. aeruginosa* among the clinics and wards revealed that general out patients departments has the highest with 10(27.8%),followed by Pediatrics surgical ward 6(16.7%),male medical ward 5(13.9%) and ear nose and throat departments with 4(11.1%). The lowest occurrence rate was with the female medical ward with 1(2.8%) (Table3).

The antibiotics resistance profile to *P. aeruginosa* with relation to age of patients indicated that isolates were susceptible to Tetracycline and Cefuraxime with (42%) each and highly resistant Ofloxacin (92%), followed by Cotrimoxazole (81%) and ampicillin (78%) (Table 4).

Table 1. Isolation rate of *Pseudomonas aeruginosa* among clinical samples of patients attending University of Maiduguri Teaching Hospital

Test organisms (*P. aeruginosa)*	Male (%)	Female (%)	Total (%)
Positive	20(15.3)	16(12.2)	36(27.5)
Negative	31(23.7)	64(48.9)	95(72.5)
Total	51(39.0)	80(61.1)	131(100)

Table 2. Distribution of *Pseudomonas aeruginosa* in relation to the age and sample types of patients attending University of Maiduguri Teaching Hospital

Age group (years)	Urine		HVS	W/Swab		EAR/Swab		CT	Total
	M	F	F	M	F	M	F	M	
0-10	0	0	0	1	0	1	0	1	3
11-20	1	2	0	1	1	0	1	0	6
21-30	3	0	0	1	0	0	2	0	6
31-40	2	2	1	1	1	1	1	0	9
41-50	1	0	0	0	1	0	0	0	2
51-60	0	1	0	2	0	1	0	0	4
61-70	0	1	0	0	0	0	1	0	2
71-80	0	0	0	1	0	0	0	1	2
81-90	0	0	0	0	0	0	0	0	0
90 above	0	0	0	0	0	0	1	1	2
Total	7	6	1	7	3	3	6	3	36

HVS=High vaginal swab, W/S=Wound swab, E/S = Ear swab, CT= Catheter tips

Table 3.The distribution of *Pseudomonas aeruginosa*in respect to clinics and wards of the hospital

P. aeruginosa	GOPD	MOPD	POPD	ENT	A&E	G/E	MMW	FMW	SCBU	EPU	PSW	NHIS	Total
POS	10	1	0	4	2	2	5	1	0	2	6	3	36
NEG	24	9	6	10	13	9	4	2	2	2	3	10	94
Total	34	10	6	14	15	11	9	3	2	4	9	13	131

Key: = GOPD= General out patients department, MOPD= Medical out patients department, POPD= Pediatrics outpatient departments, ENT= Ear nose and throat, A$E= accident and emergency, MMW= male medical ward, FMW= female medical ward, SCBU= special care pediatrics unit, EPU= emergency pediatrics unit, PSW= pediatrics surgery ward, NHIS= national health insurance scheme

Table 4. Percentage of antibiotics resistance profile of *Pseudomonas aeruginosa* with relation to age of patients attending University of Maiduguri Teaching Hospital

Ages	Antibiotics									
	AMP	OFX	AMX	CPX	CEF	TE	SXT	CN	CFX	AUG
0-10	11.1	19.4	2.8	16.6	8.3	5.6	11.1	22.2	5.6	2.8
11-20	13.8	13.8	0	11.1	8.3	2.8	19.4	8.3	5.6	8.3
21-30	25.0	8.3	2.8	2.8	5.6	5.6	2.8	8.3	5.6	2.8
31-40	5.6	2.8	8.3	5.6	16.6	5.6	8.3	2.8	5.6	8.3
41-50	2.8	8.3	11.1	5.6	5.6	2.8	2.8	8.3	5.6	2.8
51-60	5.6	16.6	5.6	0	2.8	0	8.3	5.6	0	5.6
61-70	0	8.3	2.8	5.6	8.3	5.6	2.8	0	5.6	13.9
71-80	5.6	0	13.8	8.3	5.6	2.8	11.1	5.6	5.6	0
81-90	2.8	8.3	2.8	5.6	8.3	5.6	5.6	5.6	0	2.8
91 above	5.6	5.6	8.3	2.8	5.6	5.6	8.3	5.6	2.8	8.3
Total	(78%)	(92%)	(58%)	(64%)	(75%)	(42%)	(81%)	(72%)	(42%)	(52%)

Key: AMX =Amoxicillin (10 µg), OFX =Ofloxacin (5 µg), AMP = Ampicillin (10 µg), CEF =Ceftriaxone (30 µg), CFX =cefuraxime (30 µg), CPX = Ciprofloxacin (30 µg), CN = Gentamycin (30 µg), TE = Tetracycline (30 µg), SXT = Cotrimoxazole (30 µg) and AUG = Augmentin (30 µg)

4. DISCUSSION

Although similar study has been carried out in the study area, the limitation lies in difference in study designs, numbers of samples, and examination techniques, but the factors that may influence the results are constant. The present study indicates that the percentage rate of isolation of *P. aeruginosa* in the studied area was 27.5%, with male leading with 15.3% and female having 12.2%. This is in contrast with the studies carried out by Okon et al. in Maiduguri, with low occurrence rate of 2.1%, and in Zaria with 10.5%c [15]. The results of this studies indicate that distribution rate of isolation was higher within the age group of 31- 40 with 9(25%) and least's incidence rate was among age group of 41-50, 61–70, 71-80 with 2(5.6%) each. The distribution of isolates varies with studies and clinical specimens. In Zaria; Olayinka et al. [15] reported

51.1% in urine, 41.3% in wound and 1.1% in sputum. In Ile-Ife, southwestern Nigeria, prevalence of 11.1% in open musculoskeletal injuries (Akinyele et al.), and in Ibadan, isolate rate of 16.8% with 41.9% and 39.35 from ear and wound swab respectively. [16]. However, the possibility of *P. aeruginosa* contamination of catheter tips cannot be ruled out. This is possible in hospital environment where strict hand washing procedure is not strictly adhered to and unhygienic procedure especially in the insertion of indwelling catheter may be a contributory factor.

The disruption of natural barriers because of insertion of intravascular medical devices, endotracheal tubes and urinary catheters also predispose patients to acquire nosocomial infection by this pathogen. The general out patients department (GOPD) had the highest percentage rate with 27.8%, followed by pediatrics surgical ward (PSW) 16.7%, male medical ward 13.9% and Ear nose and throat departments (ENT) with 11.1% while the lowest was the female medical ward (FMW) with 2.8%. In hospitalized patients, this organism colonizes with higher rates, particularly when the patient is under administration of broad-spectrum antibiotics which affects normal flora. Isolates recovered from patient on admission and GOPD since it is the starting point of patient on administration in the hospital. This observation affirmed the significant role of *P. aeruginosa* in nosocomial infection.

The antimicrobial susceptibility test shows that tetracycline and Cefuraxime exhibit high activity of 42% respectively while is resistant to Ofloxacillin and Cotrimoxazole with 92% and 81% respectively. The unique feature of *P. aeruginosa* isolates is the resistance to a variety of antibiotics, primarily attributed to low permeability of the cell wall, production of inducible cephalosporinase, active efflux and poor affinity for the target (DNA gyrase).

5. CONCLUSION

In conclusion, *P. aeruginosa* isolated in this study poses a serious clinical consequence in terms of patient management and infection control in hospital environment. We observed an increase in occurrence rate of *P. aeruginosa* in the study area, hence appropriate measures and proper identification techniques are required for routine surveillance and control of this pathogen.

ETHICAL APPROVAL

University of Maiduguri Teaching Hospital, Borno State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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