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Multidrug-resistant *Pseudomonas aeruginosa* isolated from ear and wound swabs in some selected hospital laboratories in Sokoto Metropolis, Nigeria

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ABSTRACT

Objective: *Pseudomonas aeruginosa* is a metabolically versatile bacterium that can cause a wide range of severe opportunistic infections in patients with serious underlying conditions and can be found in most communities in Nigeria. The study was to determine the prevalence, resistance pattern and distribution of multiple drug resistant *P. aeruginosa* (MDR-PA) isolated from ear and wound specimens in patients attending Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital Sokoto.

Materials and Methods: A total of 150 samples were analysed by standard bacteriological methods. Screening for MDR-PA was carried out by antibiotic sensitivity testing using disc diffusion method with ceftazidime (30 µg), ofloxacin (5 µg), cefuroxime (30 µg), cloxacillin (30 µg), amoxicillin (30 µg), ceftriaxone (30 µg), imipenem (10 µg), gentamycin (10 µg) and colistin (10 µg) discs on Mueller Hinton agar.

Results: Of a total of 55 (36.7%) isolates of *P. aeruginosa* strains were obtained, 30 (54.5%) isolates were resistant to imipenem, 31 (56.3%) were resistant to ofloxacin, 44 (80.0%) to gentamycin, 53 (96.3%) to ceftazidime and cefuroxime, 50 (90.9%) to ceftrizone, 54 (98.1%) to cloxacillin and amoxycillin and lastly 15 (27.2%) to colistin. All the isolates were multi drug resistant, this probably due to improper use or over the counter purchase of antibiotics without professional oversight.

Conclusion: Almost all the isolates were resistant to all the antibiotics used including colistin. There is need to improve on the public health awareness on the indiscriminate use of antimicrobial agents as it is one of the major ways microbes develop resistance to them.

Keywords: Multiple drug resistant *Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is an aerobic Gram-negative rod-shaped opportunistic bacterium that can cause disease in plants and animals, including humans.[1] It has been known for many years to be a cause of serious wound and surgical infections but has often been regarded as a secondary or opportunistic invader rather than a cause of primary infection in healthy tissues, *P. aeruginosa* has now clearly emerged as a major nosocomial pathogen, especially in immunocompromised and debilitated patient.[2] It is a highly versatile microorganism capable of tolerating low oxygen conditions and can survive with low levels of nutrients and grow in temperatures ranging from 4°C to 42°C. These characteristics enable it to attach

Table 1: Prevalence of *Pseudomonas aeruginosa* isolates from ear and wound swabs from some selected hospitals.

Centre	Number of samples collected	Number of positive	Prevalence
Maryam Abacha Women and Children Hospital	58	20	34.5
Specialist Hospital Sokoto	92	35	38.0
Total	150	55	36.7

itself and survive on medical equipment and on other hospital surfaces which favor the beginning of infections in immunocompromised patients.[3]

This pathogen is associated with nosocomial and ventilator-associated pneumonia, cystic fibrosis (CF), meningitis, abscess, soft tissue infections, urinary tract infections, catheter-associated infections, corneal infections and conjunctival erythema.[1] In addition to acute infections, *P. aeruginosa* also causes debilitating chronic lung infections in immunocompromised patients and individuals receiving prolonged chemotherapy. It also causes high morbidity and mortality rates in patients with CF due to chronicity of infections that eventually result in pulmonary damage and respiratory insufficiency.[4] It can form biofilms on indwelling medical devices such as catheters and on native airways of the CF patients.[5] It also colonizes through disruption of the normal flora balance caused by the administration of broad-spectrum antibiotics or dysfunction of the immune system. *P. aeruginosa* is a nosocomial pathogen responsible for a wide range of infections that may present high rates of antimicrobial resistance.[6] Infections due to *P. aeruginosa* are difficult to eradicate due to their elevated intrinsic resistance as well as their capacity to acquire resistance to different antibiotics.[3] Low outer membrane permeability, an extensive efflux pump system, and its remarkable ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents, including β-lactams, aminoglycosides, and fluoroquinolones, enable *P. aeruginosa* to routinely develop multidrug resistance throughout the course of a treatment regimen.[7]

The prevalence of antibiotic-resistant *P. aeruginosa* is increasing, with up to 10% of global isolates found to be multidrug resistant. This represents a major treatment challenge, as it is the second leading cause of Gram-negative nosocomial infections.[8] Thus, *P. aeruginosa* represents a phenomenon of bacterial resistance since practically all known mechanisms of antimicrobial resistance have been associated with it.[7] The objective of the current study is to isolate and identify multidrug-resistant (MDR) *P. aeruginosa* from ear and wound swabs in Sokoto Metropolis.

MATERIALS AND METHODS

Study design

The study was a cross-sectional descriptive study.

Table 2: Antibiogram profile of *Pseudomonas aeruginosa* to various antibiotics.

Antibiotics	Sensitive (%)	Intermediate	resistant (%)
Imipenem	7 (12.7)	18 (32.7)	30 (54.5)
Ofloxacin	18 (32.7)	6 (10.9)	31 (56.3)
Gentamycin	6 (10.9)	5 (9.1)	44 (80.0)
Ceftazidime	0 (0)	2 (3.6)	53 (96.3)
Cefuroxime	1 (1.8)	1 (1.8)	53 (96.3)
Ceftrixone	2 (3.6)	3 (5.5)	50 (90.9)
Cloxacillin	0 (0)	1 (1.8)	54 (98.1)
Amoxicillin	0 (0)	1 (1.8)	54 (98.1)
Colistin	40 (72.7)	0 (0)	15 (27.2)

Study area

The study was conducted in Sokoto Metropolis. Sokoto state is located within the North-Western geopolitical zone of Nigeria, created on February 3, 1976. Population figures stand at 3,702,676 persons in an area of 33,776.89 km^2 of land. The population mainly consists of the Hausa/Fulani ethnic groups. The main occupation of the people is farming (during the rainy season and irrigation in the dry season) and animal husbandry.

Study population and study sites

The study was carried out among patients attending the two study centers, patients receiving care at Maryam Abacha Women and Children Hospital and Specialists Hospital Sokoto.

Ethical considerations

Ethical approvals for the research protocol were obtained from the ethical review committees of the two study sites; a written informed consent was also obtained from each of the participant.

Sample collection

A sterile swab stick was used to obtain the sample from the site of wound by first cleansing the wound with sterile saline to irrigate any purulent debris. The swab was then rotated over a 1 cm^2 area with sufficient pressure to express fluid from within the wound tissue. Ear samples were obtained after cleansing with normal saline. The swab was then inserted into the ear and rotated before placing back into the cultural sleeve.

Table 3: Distribution of multiple drug-resistant *Pseudomonas aeruginosa* between centers.

Center	Frequency	Percentage	χ^2	*P*-value
Maryam Abacha Women and Children Hospital	19	34.5	52.358	0.017
Specialist Hospital Sokoto	36	65.5		
Total	55	100.0		

P≤0.05 (significant)

Table 4: Distribution of multiple drug-resistant *Pseudomonas aeruginosa* by age groups.

Age	Frequency	Percentage	χ^2	*P*-value
1–10	1	1.8	232.697	0.02
11–20	15	27.3		
21–30	20	36.5		
31–40	11	20		
41–50	4	7.2		
51–60	4	7.2		
Total	55	100.0		

P≤0.05 (significant)

Table 5: Distribution of multiple drug-resistant *Pseudomonas aeruginosa* strain by gender.

Sex	Frequency	Percentage	χ^2	*P*-value
Male	29	52.7	56.825	0.075
Female	26	47.3		
Total	55	100.0		

P≥0.05 (not significant)

Table 6: Distribution of multiple drug-resistant *Pseudomonas aeruginosa* by patients' admission category.

Category	Frequency	Percentage	χ^2	*P*-value
Inpatient	24	43.6	57.410	0.049
Outpatient	31	56.4		
Total	55	100.0		

P≤0.05 (significant)

Table 7: Distribution of multiple drug-resistant *Pseudomonas aeruginosa* isolates from ear and wound swabs.

Sample type	Frequency	Percentage	χ^2	*P*-value
Wound	29	52.7	52.729	0.000
Ear	26	47.3		
Total	55	100.0		

P≤0.05 (significant)

Bacteriological isolation cultural and biochemical isolation of bacterial isolates

A total of 150 samples were cultured on MacConkey agar and incubated at 37°C for 24 h. The growth of pale-colored colonies on MacConkey agar was Gram stained to demonstrate Gram-negative single rods. Catalase test was done to differentiate *P. aeruginosa* which is catalase positive from other Gram-negative bacteria. Oxidase test was also done to confirm *P. aeruginosa* from other species of *Pseudomonads*.[9]

Antibiotic susceptibility testing

Standard inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18-h agar plate incubated at 37°C. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland (1–2 × 10^8 colony-forming unit/ml). It was then observed, using adequate light to visually compare the inoculum tube and the 0.5 McFarland standards against a card with a white background and contrasting black lines. Antibiogram was done in accordance with the Clinical and Laboratory Standard Institute (CLSI),[10] on Mueller-Hinton agar, using Gram-negative bacterial sensitivity discs. Antibiotic discs included ceftazidime 30 µg, ofloxacin 5 µg, cefuroxime 30 µg, cloxacillin 30 µg, amoxicillin 30 µg, ceftriaxone 30 µg, and gentamycin 10 µg (Rapid Labs, U.K), with imipenem 10 µg and colistin 10 µg (Oxoid, England).

Diameter of zone of inhibition was used to determine the susceptibility and resistance to the antibiotics in line with the CLSI M100 inhibition zone standard.[10] Diameter of zone of inhibition was used to determine susceptibility and resistance to the antibiotics in line with the CLSI M100 inhibition zone standard.[10] For test result validation and quality control, *P. aeruginosa* (ATCC® 27853) control strain was used. In this study, multidrug resistance is considered to be resistance to at least three antibiotics of different classes.

RESULTS AND DISCUSSION

P. aeruginosa remains one of the leading nosocomial pathogens worldwide that cause severe infections in hospitalized patients. The factors which make this organism problematic are the inherent resistance to many classes of drugs, ability to acquire antibiotic resistance by mutation, and frequent involvement of this organism in serious infections. Table 1 shows the prevalence (36.7%) of *P. aeruginosa* out of the total of 150 samples isolated from ear and wound swabs

of patients attending Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital Sokoto. This is in agreement with findings from studies by Jombo *et al*.,[11] in Calabar, Nigeria, and Deji *et al*.,[12] in Lagos, Nigeria, with the prevalence of 31.8% and 29.0%, respectively. In contrast, Olayinka and Onile[13] reported a lower prevalence of 10.5% in Zaria, a much less humid zone in Nigeria.

Ullah *et al*.[14] reported 29.0% prevalence in Pakistan and a prevalence of 45.2% in India.[15] The observed differences in the isolation rates of *P. aeruginosa* may be due to some environmental and climatic conditions like humidity patterns in the two subregions.

Table 2 shows the antibiogram of *P. aeruginosa* isolates following exposure to different antibiotics. The highest resistance rate of the isolates was observed against cloxacillin (98.1%) and amoxicillin (98.1%), followed by ceftazidime (96.3%), cefuroxime (96.3%), ceftriaxone (90.9%), gentamycin (80.0%), ofloxacin (56.3%), and imipenem (54.5%). The least resistance was observed to colistin (27.2%), which remains the last resort in treatment against MDR *P. aeruginosa*. This is in contrast to a research by Zaheer *et al*.[16] who reported comparable resistance rates to ofloxacin (61.3%), cefepime (57.3%), ceftazidime (53.9%), and amikacin (53%) with colistin, polymyxin, and meropenem as the most effective. Ullah *et al*.[17] reported resistance to sulfamethoxazole (98.04%), amoxicillin (95.1%), imipenem (43.1%), cefoperazone (50.9%), and amikacin (53.9%). All the isolates subjected to antibiogram were found to be MDR *P. aeruginosa* (MDR-PA) (36.7%), an observation that may be due to repeated or improper use of antibiotics.

Table 3 shows the distribution of MDR *P. aeruginosa* in the study area. The prevalence of MDR-PA was higher in Specialist Hospital Sokoto with 65.5% compared to 34.5% prevalence observed among Maryam Abacha Hospital strains. This difference may be due to the status of the Specialist Hospital Sokoto with a larger patient referral service turnover than Maryam Abacha Women and Children Hospital with less number of patients. Consequently, more samples were obtained in that hospital than from Maryam Abacha Women and Children Hospital.

Table 4 shows the distribution of MDR *P. aeruginosa* strains among the age groups of the patients. The prevalence of MDR-PA was highest in the age range of 21–30 (36.5%) and lowest in 1–10 (1.8%). A study by Zaheer *et al*.[16] reported the highest prevalence in the age group of 10–19 years (22.2%).

Table 5 shows the distribution of MDR strains between genders. MDR-PA infection was marginally higher in males 52.7%, compared to 47.3% prevalence observed in females ($P > 0.05$), as earlier reported from a previous study.[16] Socially and culturally, males are more dominant and aggressive than females and are, therefore, more prone to drug abuse.

The distribution of MDR *P. aeruginosa* based on the type of patients visiting the hospitals is shown in Table 6. In this study, MDR-PA was higher among outpatients 56.4% than inpatients 43.6% (<0.05), indicating that there is a statistically significant difference in the occurrence of MDR-PA between patient categories. This difference may arise as most outpatients self-medicate and this increase the chances of drug abuse. The inpatients' drug intake is usually monitored and regulated by medical personnel and may, therefore, have accounted for the lower prevalence of MDR-PA among them.

Table 7 shows the frequency of recovery of MDR *P. aeruginosa* from patients' ear and wound swabs samples. Wound swabs accounted for 52.7% as against ear swabs (47.3%). Statistical analysis ($P < 0.05$) indicates a significant difference in the distribution of MDR-PA between sample types. This is in agreement with the findings of Olayinka and Onile[13] and Ogbolu *et al*.[18] who also reported high prevalence of MDR-PA of 41.3% and 41.9%, respectively, from their studies in Zaria, Nigeria. However, findings by Akinyola and Ako,[19] in Ile Ife, Nigeria, described a lower prevalence of 11.1% from wound samples. The high prevalence observed in wound may be attributed to the fact that *P. aeruginosa* is a nosocomial pathogen that can be acquired as a contaminant in wound during dressing. Anatomically, most wounds are exposed and most people tend to apply topical drugs to wounds without culture and sensitivity tests of causative bacteria, thereby increasing chances of the infecting bacteria to develop multiple drug resistance.

CONCLUSION

This study conclude that all the *P. aeruginosa* isolated are multi drug resistant, and 27.2% of the isolates are resistant to colistin which is the last resort drug in treatment of multi drug resistant *P. aeruginosa*.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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