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Research Article

Incidence of *Pseudomonas aeruginosa* Resistance in Clinical Isolates from Selected Hospitals in Oyo State, Nigeria

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ABSTRACT

Fifty clinical isolates of *Pseudomonas aeruginosa* were obtained from both in and out-patients of selected hospitals in Oyo State, Nigeria using standard procedure. Presumptive identification of the isolates was carried out using standard biochemical tests. according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics used in the study includes: Ciprotab, Colistin-sulphate, Meropenem, Ceftriaxone and Cefepime. 50 clinical isolates of *Pseudomonas aeruginosa* obtained, consisting of 48% male isolates and 52%female isolates. The percentage ratio of in-patient and out-patient examined were 32% and 68%. The percentage distribution of the administration class for medical and surgical was 34% and 66% respectively. The highest incidence of *Pseudomonas aeruginosa* was from patients that have undergone caesarean section (28%). Highest susceptibility was observed in Ciprotab (82%) Meropenem (64%) and Ceftraxone (46%). Highest number of resistance was observed against Cefepime and Colistin Sulphate while less than 5% were resistant to Ciprotab and Meropenem. Meropenemand ciprotab were the two classes of drugs that showed highest activity against *Pseudomonas aeruginosa*. Commonly used antibiotics must be continuously examined for its efficacy. There is therefore a need for consistent screening of microorganisms implicated with various infections characterization of their antimicrobial susceptibility pattern which will serve as a guide to clinicians in the selection of appropriate antimicrobial drug for empirical treatment of infections.

Keywords: *Pseudomonas aeruginosa*; Antibiotics; Susceptibility; Multi drug resistance; Clinical isolates; Clinical site

INTRODUCTION

Numerous bacteria are majorly involved in high death rate and morbidity; Prominent amongst is *Pseudomonas aeruginosa* [1]; this is achieved by the organism colonizing almost all form of human tissue, which makes them to cause various type of infections either acute or chronic e.g meningitis, septicemia (Peter et al.,). *Pseudomonas aeruginosa* is characterized as a gram-negative, monoflagellated, non-spore forming and rod-shaped bacterium, which often times is capable of causing diseases in mostly all tissues and organs of the body. It can persevere in both community and hospital settings which is as a result of its ability to thrive on very little nutritional requirements and survive under different physical conditions [2]. Majorly, patients in the hospitals, particularly, patients in intensive care units and those having burn, chronic diseases, catheterization and immune compromised individuals are often infected by *Pseudomonas aeruginosa* [3]. *Pseudomonas aeruginosa* is often found almost everywhere and it is an opportunistic disease-causing organism that affects morbidity, mortality and healthcare costs in hospitals and in the community [4]. According to information from the US Centres for Disease Control and Prevention and the National Nosocomial Infection Surveillance System, *P. aeruginosa* is the second most common cause of nosocomial pneumonia (17%), the fourth most common cause of surgical siteinfection (8%),

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the seventh most frequently isolated pathogen from the bloodstream (2%) the third most common cause of urinary tract infection (7%), and the fifth most common isolate (9%) generally from all sites[5,6] . Antibiotics used in the treatment of infections caused by *P. aeruginosa* infections include the Aminogylcosides (amikacin, tobramycin, gentamicin), Cephalosporins, third-generation (cefoperazone, cefsulodin, ceftazidime), Fluoroquinolones (ciprofloxacin, levofloxacin), cephalosporins, fourth-generation (cefepime, cefpirome, cefclidin), Monobactam (aztreonam), Extended spectrum penicillins (ticarcillin and/or ticarcillin-clavulanate, piperacillinand/or piperacillin azobactam, azlocillin), Polymyxin B/Colistin and Carbapenems (imipenem, meropenem, doripenem), [2]. However, *P. aeruginosa* has developed natural resistance to most of the antibiotics in these classes and are also developing resistance rapidly to other drugs during treatments, making treatment difficult and ineffective resulting into high rate of death. [2] has reported an increase in the antibiotic resistant rate of *P. aeruginosa* to the common antimicrobial drugs. *P. aeruginosa* infections are commonly life-threatening and uneasy to combat as it shows intrinsically high level of resistance to many antimicrobial drugs, thereby resulting in high rate of multi-drug resistance in health care settings [7]. Mechanisms of drug resistance in *P. aeruginosa* include the acquisition of resistance genes (e.g. those encoding beta-lactamase [8] and amino-glycoside modifying enzymes [9] through horizontal gene transfer and mutation of chromosomal genes [10]. *Pseudomonas aeruginosa* infected patients are subjected to severalfactors that may be associated with multidrug resistant microorganism's carriage such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admission [2]. Due to the emergence of antimicrobial resistance, the treatment formicrobial infection has become difficult, expensive and scarce [11]. There is therefore a need for consistent screening of microorganisms implicated with various infections and characterization of their antimicrobial susceptibility pattern which will serve as a guide to clinicians in the selection of appropriate antimicrobial drug for empirical treatment of infections. This experiment therefore aimed at determining the antibiotic susceptibility patterns of *Pseudomonas aeruginosa* from clinical isolates to frequently used antibiotics and determining the level of resistance of the isolated *Pseudomonas aeruginosa*.

METHODS

Collection of clinical isolates

Fifty (50) clinical isolates of *Pseudomonas aeruginosa* were obtained from the Department of Medical Microbiology, University College Hospital (UCH) Ibadan, Oyo State. Samples were taken from various infection sites, from CS, right and left sides of the thigh, legs, left side of the head, left hand and right, they were transported to Adeleke University's microbiology laboratory and stored in the ultra-low freezer until when needed. The isolates were identified were grown on centrimide agar and standard biochemical tests were carried on them to confirm they were *Pseudomonas aeruginosa*.

Antibiotics used: FEP, CT (ceftriaxone), MEM (meropenem), CRO(ciprotab) and CPT (cefepine) were used being the, antibiotics without studies investigating resistance.

Antibiotic susceptibility test (Agar diffusion method)

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disk diffusion method as described by Jorgensen [12]. 24-hour old broth culture of the *Pseudomonas aeruginosa* was standardized. A sterile swab stick was inserted into the standardized inoculums and drained to remove excess inoculum load and swabbed on the surface of the Mueller-Hinton agar and was allowed to dry after which, antibiotics impregnated discs of known concentration: were carefully placed on the Mueller-Hinton agar using sterile forceps and then incubated at 37°C for 24 hours. Zones of inhibition were measured and interpreted as resistant, intermediate and susceptible following the Clinical Laboratory Standard Institute (CLSI,) guidelines.

Statistical analysis

ANOVA was carried out to assess the significance of the means of the diameter of the zones of inhibition of the antimicrobial agents tested on the clinical isolates of *P. aeruginosa*. The p-value was less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Sex distribution and percentage class of patient distribution of the study population are shown in Figures 1 and 2.

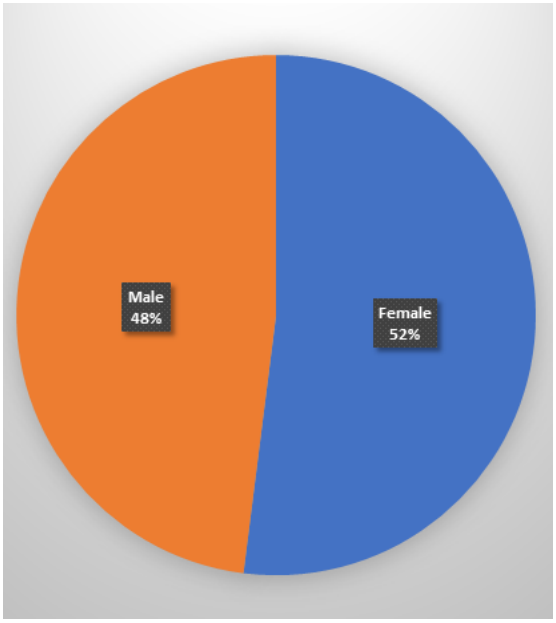

Figure 1: Sex distribution of the study population.

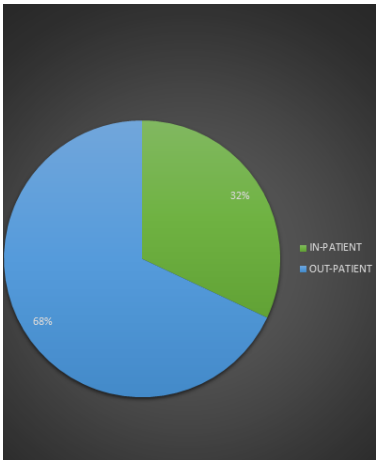

Figure 2: Percentage class of patient distribution.

Percentage occurrence of *P. aeruginosa* in the clinical samples is shown in Figure 3. Percentage distribution of the administration class of patients can be seen in Figure 3. Tables 1 and 2 show Percentage distribution of antibiotic susceptibility of *P. aeruginosa* and ANOVA for the resistance of *Pseudomonas aeruginosa* from the sites to different antibiotics *respectively*.

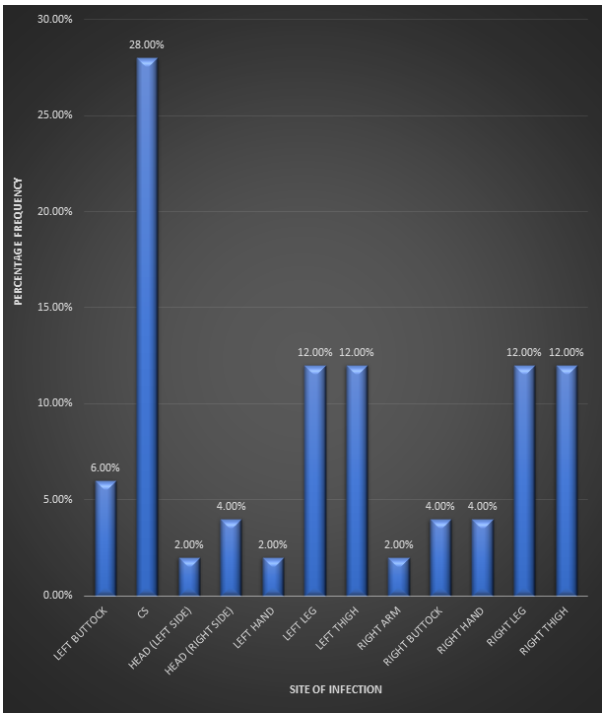

Figure 3: Percentage occurrence of *P. aeruginosa* in clinical samples.

Sex distribution of the study population showed that *P. aeruginosa* occurred more amongst the females (52%) than the males (48%) (Figure 1). Most of them belonged to older age group of 31- 40 years (38.3%) and elderly age group of 41 years (10%). This could be resulting from decrease in immunity, prolonged stay in the hospital and other associated co-morbidities in these age groups.

Table 1: Percentage distribution of antibiotic susceptibility of *Pseudomonas aeruginosa*.

Antibiotics (disc potency)	Frequency (%)			
	Resistance	Intermediate	Susceptibility	Total
FEP	50 (100)	0 (0)	0 (0)	50 (100%)
CPT	1 (2)	8 (16)	41 (82)	50 (100%)
MEM	2 (4)	16 (32)	32 (64)	50 (100%)
CRO	27 (54)	0 (0)	23 (46)	50 (100%)
CT	50 (100)	0 (0)	0(0)	50 (100%)

A study carried out by Sheetal and Preeti [13] showed (29.00%) of patients were aged between 31-45 years. Results obtained from this study revealed that sex-wise, female patients (52%) constituted a larger group in the study. In contrast, Patel et al., [14] reported an increased incidence in male sex (59.3%) as well as a higher prevalence rate among elderly 61-80 years (43.92%). Similarly, according to Mohanasoundaram [15], the highest prevalence of Pseudomonas infection was found in 31-40 years age group. Variation in the distribution of specimens of *P. aeruginosa* with each hospital can be as a result of the facilities each hospital has and the different environment associated withit. The maximum number of *P. aeruginosa* were isolated from CS samples (28%) because of the fact that they are the most populated among those exposed to the infection in this study, followed by both the right and left sides of the thigh (12%) and leg (12%). The least number were isolated from the leftside of the head (2%), left hand (2%) and right arm (2%) because they are the least populated in this study (Figure 3). Highest number of resistances was observed against FEP and CT. *P. aeruginosa* is intrinsically resistant to several antibiotics including Cefepime (FEP) and Colistin sulphate (CT) because of low permeability of its outer membrane, the constitutive expression of various efflux pumps, and the production of antibiotic inactivating enzymes [16]. More than 50% of the isolates were found resistant to CRO. Less than 5% were resistant to CPT and MEM (Table 1) and CPT and MEM had the highest activity (30.00 mm) while FEP had the least activity (0.00 mm) against *P. aeruginosa* by the mean measured. However, a single factor Oneway ANOVA revealed no statistically significant difference between the antibiotics used against the sites of infection (Table 2). One prominent observation in this study was that all the *P. aeruginosa* isolates were sensitive to ciprotab also known as ciprofloxacin which belong to a group of antibiotic called Quinolone. This may be

due to the restricted use of ciprotab in the hospital used in this study [17]. This corroborates the report of Shenoy et al., [18]. However, report from El-Halfawy et al., [17] and Al-Kabis (2011) revealed varying degrees of resistance to ciprotab in recent years. 82% sensitivity was observed to Ciprotab followed by meropenem (64% sensitivity). Resistance to ciprofloxacin is an emerging menace in different part of the world. It was observed that the resistance rate of ciprofloxacin was 16.5%, whilst in Saudi Arabia, a resistance of 50% was recorded [14].

Minimal resistance of *P. aeruginosa* to Meropenem (4.00%) as observed in this study corroborates the result of a study carried out in Saudi Arabia by Bukharie and Mowafi, (2010) who reported 5.00% resistance to Meropenem. This could be as a result of minimal use of the antibiotics as it is not readily available over the counter to be purchased and it is quite expensive which makes it unaffordable for most patients. It therefore has not been misused or overused, hence, minimal resistance to it. This, however is contrary to the result obtained in a study carried out by Khan and Faiz, [14] reported 30.6% resistance of *Pseudomonas aeruginosa* to Meropenem. The reason for the high resistance to meropenem is that the drug is commonly used in the settings they studied. Ciprotab and Meropenem proved to be the most effective drugs for routine use among the *P. aeruginosa* strains investigated in this study. According to an earlier study reported from Kathmandu, Nepal, ciprotab had 82% sensitivity also, while meropenem had 70.3% sensitivity among the *P. aeruginosa* strains examined. High resistance to ciprotab was reported in a study carried out by Mohanasoundaram [15]. Similarly, higher rates of resistance to meropenem (40.5%) had been reported in a study done in North Kerala, India (Patel et al.,). A high resistance (100% resistance) to Cefepime was observed in this study which corroborates the results of a study carried out by Li [19] where 92% resistance to cefepime was observed.

Table 2: ANOVA for the resistance of *Pseudomonas aeruginosa* from the sites to different antibiotics. **Abbreviation:**FEP: Cefepime; CPT: Ciprotab; MEM: Meropenem; CRO: Ceftriaxone; CT: Colistin-Sulphate.

Site of infection	Antibiotics				
	FEP	CPT	MEM	CRO	CT
Buttock	0.00 ± 0.00a	26.50 ± 2.53a	20.00 ± 2.74a	9.75 ± 2.59a	2.00 ± 1.08a
Ceaserian Section	0.00 ± 0.00a	24.00 ± 0.90a	20.31 ± 1.92a	8.15 ± 1.70a	2.00 ± 0.56a
Left head	0.00 ± 0.00a	27.50 ± 2.50a	19.50 ± 3.50a	8.50 ± 4.50a	2.50 ± 2.50a
Right head	0.00 ± 0.00a	17.50 ± 0.50a	21.00 ± 0.00a	5.00 ± 1.00a	2.00 ± 1.00a
Left hand	0.00 ± 0.00a	24.00 ± 0.00a	19.00 ± 0.00a	5.00 ± 0.00a	0.00 ± 0.00a
Left leg	0.00 ± 0.00a	22.00 ± 1.84a	19.50 ± 0.67a	5.17 ± 2.29a	1.17 ± 0.75a
Left thigh	0.00 ± 0.00a	23.00 ± 1.32a	19.50 ± 1.86a	9.00 ± 2.92a	2.50 ± 0.85a
Right arm	0.00 ± 0.00a	27.00 ± 0.00a	19.00 ± 0.00a	5.00 ± 0.00a	1.00 ± 0.00a
Right buttock	0.00 ± 0.00a	23.50 ± 0.50a	18.50 ± 2.50a	14.00 ± 9.00a	0.00 ± 0.00a
Right hand	0.00 ± 0.00a	30.00 ± 0.00a	20.00 ± 0.00a	30.00 ± 0.00a	5.00 ± 0.00a
Right leg	0.00 ± 0.00a	21.00 ± 4.37a	22.33 ± 1.38a	7.83 ± 1.97a	2.67 ± 0.76a
Right thigh	0.00 ± 0.00a	23.30 ± 0.75a	20.00 ± 0.65a	8.14 ± 0.91a	1.94 ± 0.29a

Shenoy et al., [18] had reported similar rate of resistance to colistin sulphate (54.66%). Relatively low meropenem resistance (11.5%) was observed in isolates of *P. aeruginosa* among in-patients in a study carried out by Al-tawiq, [20] which is in tandem with the result obtained from this study. *P aeruginosa* strains in this study exhibited a high rate of resistance to the third-generation cephalosporin drug-ceftriaxone (64%). Lesser rate of resistance to ceftriaxone (40%) had been reported in another study from Andhra Pradesh, Canada [21].

According to a study carried out by Nwankwo [22] in Kano, Nigeria, a more similar rate of *P. aeruginosa* resistance (97.7%) was observed to cefepime. The highest resistance rate as observed in this study is to Cefepime as 100% resistance was recorded to *P. aeruginosa* isolates. This implies that Cefepime might not be able to combat infections caused by this

organism, hence, it should no longer be administered as a treatment regimen for *P. aeruginosa* infections.

CONCLUSION

From this study, Ciprotab was the most susceptible antimicrobial drug. Hence, it can be used in the treatment of Pseudomonas infections. However, Colistin sulphate and cefepime were found to be the most resistant drugs possibly due to their indiscriminate use during treatment of *P. aeruginosa* infections.

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