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Antimicrobial susceptibility Pattern and Incidence of Multi-drug Resistant *Pseudomonas aeruginosa* in Oyo StateAkinola Sikiru Ajibola¹, Tosan Amos Erhabor², Kevin Aghatise³, Christy Chinyere Fredrick³, Osakue Osareniro Eguagie³, Fatima Abba Kyari⁴, Alhaji Bukar^{*4}, Humphrey B Osadolor³Department of Medical Laboratory Science, Ministry of Health, Oyo State, Nigeria¹,Medical Laboratory Science Council of Nigeria, Abuja, Nigeria²,School of Postgraduate Studies and Research, Igbinedion University Okada, Edo State³,Department of Medical Laboratory Science, University of Maiduguri, Maiduguri, Nigeria⁴

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that causes severe invasive diseases in man. The frequency of infections caused by this pathogen is rising worldwide with multidrug-resistant (MDR) strains, resistant to several antibiotics. There is little knowledge of the incidence of multidrug-resistant *Pseudomonas aeruginosa* infections in Oyo state, Nigeria neither is there published data on molecular detection of *Pseudomonas aeruginosa* infection. The aim of this study was to determine the incidence and antimicrobial sensitivity pattern of the multidrug-resistant (MDR) *Pseudomonas aeruginosa* in patients attending tertiary health facilities in Oyo state, Nigeria, from June, 2016 to December, 2017. The antimicrobial susceptibility pattern using Kerby-Bauer disk diffusion method (with NCCLS \geq 6mm sensitivity) was employed. Majority of the 161 isolates were from wound 47(29.19%) followed by sputum 32(19.88%), burns 20 (12.45%) and urine 20(12.42%). Among all the 161 samples examined, 44(27.3%) contained *Pseudomonas aeruginosa*, of which 20(45.45%) were multidrug-resistant strains. Of the 20 multidrug-resistant *Pseudomonas aeruginosa* strains, Imipenem demonstrated highest sensitivity (85.0%) followed by Ciprotab (65%), Ceftazidime (60%), Ofloxacin (55%) and Ciprofloxacin (45%). Imipenem presented as the best antibiotic of choice for the treatment of multidrug-resistant *Pseudomonas aeruginosa* in Oyo State. There is urgent need for improved antibiotics policy and guidelines in Nigeria due to the increasing resistance pattern observed in *Pseudomonas aeruginosa* species. There is the

need for early molecular detection analysis in tertiary health facilities in order to reduce the emergence of multidrug-resistant *Pseudomonas aeruginosa* because resistivity is usually transmitted via plasmids; and there should be better infection control due to rapid dissemination of the strains.

Keywords: Antimicrobial susceptibility, Multi-drug Resistant, *Pseudomonas aeruginosa*, Oyo State

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that causes severe invasive diseases in patients. The frequency of infections caused by this pathogen is increasing and multidrug-resistant (MDR) strains, resistant to almost all available antimicrobials, are emerging in clinical settings, according to Rishi *et al.* (2013). *Pseudomonas aeruginosa* is a common pathogen in hospitals and particularly in intensive care units because of its ubiquitous nature, ability to survive in moist environments, and innate resistance to many antibiotics and antiseptics. It has become increasingly clear that resistance development in *P. aeruginosa* is multifactorial, with mutations in genes encoding porins, efflux pumps, penicillin-binding proteins, and chromosomal β -lactamase, all contributing to resistance to β -lactams, carbapenems, aminoglycosides, and fluoroquinolones. Strains of *Pseudomonas aeruginosa* are the cause of several diseases in nosocomial environments, predominantly pneumonia, bacteraemia, meningitis, urinary tract infections, as well as skin and soft-tissue infections. Due to the emergence of MDR pathogens, it is of utmost

importance to develop new antimicrobial drugs (Ali, *et al.*, 2015; Jena *et al.*, 2015). *Pseudomonas aeruginosa* has been characterized as one of the most versatile microbial organisms, with a wide span of habitats including soil, disinfectant solution and jet plane fuel. Low permeability of its outer membrane by a complex set of efflux pump systems and secretion of alginate during biofilm formation are major factors that allow the pathogen to become highly virulent and resistant to multiple antibiotic agents. Adding to these factors, other bacterial exoproducts such as lipopolysaccharides and elastase induce harmful pathogenesis resulting in tissue destruction (Harris *et al.*, 1999).

Flagellins in *Pseudomonas aeruginosa* perform several functions during host infection. Apart from enabling motility, the flagellum of *P. aeruginosa* plays an indirect role in membrane permeabilization and surfactant protein-mediated bacterial clearance. Similarly, pili are involved during inflammation due to glycosylation in the interface between pili and host cells. Flagellins are classified into two types: Type-a (polymorphic glycosylated) and type-b (non-glycosylated) (Jena *et al.*, 2015).

The discovery of antibacterial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antibacterial agents. Therefore, the emergence of bacterial resistance to most of the commonly used antibiotics is of considerable medical significance (Bhatia and Narain, 2010). Antibiotic resistance genes in most bacteria are frequently found in extra chromosomal elements known as R-plasmids. *Pseudomonas aeruginosa* is naturally resistant to many of the widely used antibiotics, so chemotherapy is often difficult (Jena *et al.*, 2015).

Resistance is due to a resistance transfer plasmid (R-plasmid) which is a plasmid carrying gene encoding proteins that detoxify various antibiotics (Piekarska and Hanson, 2015). Antibiotic resistant bacteria are widespread. Several antibiotic resistant genes can be carried by a single R-plasmid or alternatively, a cell may

contain several R plasmids. In either case, the result is multiple resistances (Matthijs *et al.*, 2013). Infections with antibiotic resistant bacteria make the therapeutic options for infection treatment extremely difficult or virtually impossible in some instances (El-Astal, 2004). Therefore, the determination of antimicrobial susceptibility of clinical isolates is often crucial for optimum antimicrobial therapy of infected patients (Ozumba, 2005).

A high-density patients' population in frequent contact with health care staff and the attendant risk of cross-infection contributes to the spread of antibiotic-resistant microorganisms in the environment (Bataneh and Alrashed, 2007). Occurrence and prevalence of these resistant strains in the environment is, therefore common in developing countries.

The Gram-negative bacterium *Pseudomonas aeruginosa* is a ubiquitous aerobe that is present in water, soil and on plants (Banerjee and Stableforth, 2000). Naturally, this organism is endowed with weak pathogenic potentials. However, its profound ability to survive on inert materials, minimal nutritional requirement, tolerance to a wide variety of physical conditions and its relative resistance to several unrelated antimicrobial agents and antiseptics, contributes enormously to its ecological success and its role as an effective opportunistic pathogen (Gales *et al.*, 2012). The organism is pathogenic when introduced into areas devoid of normal defences (Al-Kobaisi, 2007) and infections are both invasive and toxigenic (Khatab *et al.*, 2015).

Pseudomonas aeruginosa has been incriminated in cases of meningitis, septicaemia, pneumonia, ocular and burn infections, hot tubs and whirlpool-associated folliculitis, osteomyelitis, cystic fibrosis-related lung infection, malignant external otitis and urinary tract infections with colonized patients being an important reservoir (Jena *et al.*, 2015). Cross-transmission from patient to patient may occur via the hands of the health care staff or through contaminated materials and reagents (Jena *et al.*, 2015).

However, it is believed that *Pseudomonas aeruginosa* is generally environmentally

acquired and that person-to-person spread occurs only rarely (Ruiz-Martínez *et al.*, 2011). As such, contaminated respiratory care in the infusions, cosmetics, dilute antiseptics, cleaning liquids, and even soaps have been reported as vehicles of transmission (Jena *et al.*, 2015).

Increase in antibiotic resistance level is now a global problem. *Pseudomonas aeruginosa* is naturally resistant to many of the widely used antibiotics, so chemotherapy is often difficult. Resistance is due to a resistance transfer plasmid (R-plasmid) which is a plasmid carrying genes encoding proteins that detoxify various antibiotics out of the cell. Low antibiotic susceptibility, which is a worrying characteristic, is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes e.g. mexAB-oprM, mexXY (Piekarska and Hanson, 2015), and low permeability of the bacterial cellular envelopes. Besides intrinsic resistance, *Pseudomonas aeruginosa* easily develops acquired resistance either by mutation in chromosomally-encoded genes, or by the horizontal gene transfer of antibiotic resistance determinants. Development of multidrug resistance by *Pseudomonas aeruginosa* isolates requires several different mutations and/or horizontal transfer of antibiotic resistance genes (Meletis and Bagkeri, 2013).

Hypermutation favours the selection of mutation-driven antibiotic resistance in *Pseudomonas aeruginosa* strains producing chronic infections, whereas the clustering of several different antibiotic resistance genes in integrons favours the concerted acquisition of antibiotic resistance determinants. Some recent studies have shown that phenotypic resistance associated with biofilm formation or to the emergence of small-colony-variants may be important in the response of *Pseudomonas aeruginosa* populations to antibiotic treatment (Workentine *et al.*, 2013).

Pseudomonas aeruginosa has become a serious life-threatening problem due to the limited therapeutic options. Increasing rates of resistant and multidrug-resistant (MDR) *P. aeruginosa* in clinical settings constitute a major public health threat (Khattab *et al.*, 2015). Despite the ever

growing of multidrug resistant *Pseudomonas aeruginosa* infections in Oyo State, Nigeria, there is no available documented molecular detection study which represents extensive picture of *Pseudomonas aeruginosa* infection in the States due to absence of molecular diagnostic methods and restricted sampling areas.

Numerous reports have documented the incidence and isolated transmission events of multidrug-resistant *Pseudomonas aeruginosa* strains, but knowledge of the genes associated with the organisms remains limited. Strains of *P. aeruginosa* are the cause of several diseases in nosocomial environments, predominantly; pneumonia, bacteraemia, meningitis, urinary tract infections, as well as skin and soft-tissue infections. The aim of the study was to determine the incidence, molecular detection of multi-drug resistant *Pseudomonas aeruginosa* and the antimicrobial susceptibility pattern of multi-drug resistant *Pseudomonas aeruginosa* in Oyo state.

Methodology and Study Area

Oyo State is situated in tropical rain forest zone in the latitude 8⁰⁰'0"N and longitude 4⁰⁰'0"E. It covers about 27,249 square kilometres of landmass and it is bounded in the South by Ogun State, North by Kwara State, West partly by Ogun State and Republic of Benin, while in the East, it is bounded by Osun State. Oyo State consists of 33 local governments. The State is homogenous with a population of about 6.5 million estimation (NPC, 2006). Subject's information such as gender, site, type of infection and type of medication used prior to the hospital consultation was obtained. Drug susceptibility testing was carried out using Kirby-Bauer disc diffusion antibiotics method with NCCLS (≥ 6 mm), while each isolate was identified using standard microbiological techniques. Patients who received antibiotic for at least one week without improvement are included and those received antibiotic with improvement excluded. The study was carried out in two government hospitals, Adeoyo Maternity Teaching Hospital Ibadan and State Hospital, Ogbomoso. One hundred and sixty-one (161) samples were obtained within that period. Urine, Wound Swab, Eye Swab, Ears

Swab, Sputum, Blood and Aspirate samples were aseptically collected with sterile swabs and containers.

Bacteriological procedures

Specimens were aseptically inoculated onto Blood, Chocolate, Ceftrimide, MacConkey and Nutrient agars, then incubated aerobically at 37°C for 24 hours and observed for colonial growth.

Identification of isolates

Isolates were identified by their colonial morphology, Gram reaction, motility, oxidase positivity, pigment production and growth at 42°C (Cheesbrough, 2002). Urine samples were mixed thoroughly by inverting the containers several times. Using a sterile wire loop, the samples were inoculated on Blood agar, MacConkey chocolate and Ceftrimide agar plates. The plates were incubated at 37°C for 24 hrs. Distinct colonies were sub-cultured on nutrient agar repeatedly to obtain pure cultures. The isolates were stored on nutrient agar slants for further use. While swab sticks from wounds, ears and eyes were inoculated into tubes of nutrient broth and incubated at 37°C for 24 hrs. Ten-fold serial dilutions of the culture broth were prepared. Diluents were plated out on MacConkey agar using the spread plate method. The plates were incubated at 37°C for 24 hrs. Different colonies were further purified to obtain pure cultures (Kootallur *et al.*, 2011). All isolates were Gram stained and examined microscopically. Biochemical tests were carried out based on Gram reactions (Cheesbrough, 2002). **For selective growth of *Pseudomonas aeruginosa***, 0.2% of Cadmium Sulphate (CdSO₄) in Nutrient agar makes this selective medium only for *Pseudomonas aeruginosa* which grow on this media with the secretion of a variety of pigments, while other *Pseudomonas species* do not thrive in the presence of 0.2% CdSO₄ medium (Cheesbrough, 2002).

Antimicrobial susceptibility testing with antibiotic was assessed using the Kirby-Bauer disc diffusion method and zones of inhibition on Mueller-Hinton agar medium using NCCLS \geq 6mm sensitivity (NCCLS, 2004). The following antibiotics were used Ampicillin (30µg), Augmentin (30µg), Cefixime (5µg), Gentamycin

(10µg), Nitrofurantoin (30µg), Ofloxacin (5µg), Cefotaxime (30µg), Cefuroxime (30µg), Mipenem (10µg), Ciprofloxacin (5µg), Ciprotab (10µg) and Ranicef (5µg). Pure cultures of isolates were inoculated in nutrient broth and incubated at 37°C for 24hrs. The growth was standardized by diluting the culture with normal saline to match the turbidity of 0.5 McFarland standards.

A volume (0.1 ml) of the cultured isolate was spread on the surface of Mueller-Hinton agar using sterile wire loop of each of the cultures and allowed to dry. The antibiotic disks were placed carefully to make good contact with the agar surface using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30 min to allow for diffusion of the antibiotics and were incubated at 37°C for 24 hrs. Results were recorded by measuring the zone of inhibition and comparing with the NCCLS susceptibility testing. *Pseudomonas aeruginosa*, ATCC27853 was used as positive control and *E. coli* ATCC 35218 was used as Negative control (NCCLS, 2004).

DNA Isolation and Profiling

Plasmid isolation was conducted using a highly pure isolation kit (Zyppy Plasmid Miniprep Kit). Electrophoresis of the DNA was carried out on a 0.8% agarose gel in a 1X concentration of Tris-Borate-EDTA (TBE) buffer at a constant voltage of 100V, 60°C for about 1 hr 30 m. After electrophoresis, the gel was photographed under UV illumination. The approximate molecular weights of the primers were determined using marker with known molecular weight ladder as standards (Nikbin *et al.*, 2012).

DNA Extraction and Amplification

The stock culture was streaked on Luria Bertani (LB) agar and grown overnight. Afterwards, total genomic DNA was extracted by dispensing 200 µl of 5 % Chelex in a tube. A loopful of the overnight grown colony of bacterium from LB agar was then inoculated into the solution. The mixture was then boiled at 100°C for 10 min and centrifuge at 13Rev/ min. Five microliter of the supernatant which consist of the extracted DNA was then used as template with 2 mM MgCl₂, 0.8 mM dNTPs, 0.2 µl of primer 1 and primer 2 and

1× PCR buffer. DNA amplification was done. PCR products were then separated and visualized on 1 % agarose gel electrophoresis to confirm amplification (Nikbin *et al.*, 2012). A single colony of *P. aeruginosa* isolate was inoculated into 10 ml of nutrient broth. After incubation at 37°C for 24 hrs, 0.2 ml of bacterial culture was inoculated into 10 ml of fresh nutrient broth and incubated at an elevated temperature of 45°C for 24hrs with shaking at 100 rpm. More so, several dilutions were performed up to 10⁻⁷ dilutions, then 0.1 ml of the last three dilutions were spread on plates of nutrient agar which contain different antibiotics and incubated at 37°C for 24hrs. Subsequently, the results were recorded by the loss of ability of the tested bacteria to survive on the medium which contains the antibacterial agents. Resistance curing was conducted on multidrug resistant isolates. This was done to determine whether the gene coding for resistance is carried in the chromosomes or plasmids. Plasmid being an extra-chromosomal DNA molecule is eliminated from host bacteria after exposure to elevated temperature.

Results

A total of 161 samples were collected for this cross-sectional study in Oyo state. Table 1 shows the distribution of the specimens' sources. Wound samples were highest in frequency 47/161 followed by Sputum samples 32/161 and

CSF has the lowest frequency 06/161. Table 2 shows the types of microbial isolates from the samples; *Staphylococcus aureus* 62(38.51%), *Pseudomonas aeruginosa* 44(27.33%), *Proteus spp* 21(13.04%), *Klebsiella spp* 18(11.18%), *Escherichia coli* 15(9.32%) and *Candida albicans* 01(0.62%). Table 3 shows the distribution of the MDR- *Pseudomonas aeruginosa* isolates in different clinical samples. Wound samples were highest with 10 (50%) followed by sputum 4 (20%) and ear, urine, burns 2 (10%) each respectively. Table 4 shows the antimicrobial susceptibility of MDR- *Pseudomonas aeruginosa* isolates of the participants in Oyo state. Table 5 shows the age and gender distribution of the 44 individuals with *Pseudomonas aeruginosa* infection of which 20 among with MDR- *Pseudomonas aeruginosa* in Oyo State. There are 32 males (15 with MDR *P aeruginosa*) and 12 females (5 with *P. aeruginosa*). Table 6 shows the number of MDR *Pseudomonas aeruginosa* isolates sensitive/resistance against the Ten (10) antibiotics. It shows the number isolates that shows sensitivity or resistance to each of the antimicrobials. More isolates showed sensitivity to Imipenem 85% than to other antibiotics. More isolates showed resistance to Augmentin (95%) than to other antibiotics. The pattern of resistance to Cefuroxime and Cefixime by all the isolates is similar (only 3 isolates are sensitive to Cefuroxime and Cefixime).

Table 1: Clinical Samples Collected

Clinical Samples	Oyo State
Wound	47
Sputum	32
Otitis media	12
Burn	20
HVS (PID)	12
Urine (UTI)	20
Blood	12
CSF	06
Total	161

Table 2: Identities of Microbial Isolates

Isolates	Number of Isolate Positive (%)	Number of Isolate Negative (%)
Klebsiella spp	18 (11.1)	143 (88.8)
E. coli	15 (9.3)	146 (90.0)
S. aureus	62 (38.0)	99 (61.5)
Proteus spp	21 (13.0)	140 (86.9)
C. albicans	1 (0.6)	160 (99.4)
N. gonorrhoea	0(0)	161 (100)
Ps. Aeruginosa	44 (27.3)	117 (72.9)

Table 3: Distribution of the MDR- *Pseudomonas Aeruginosa* Isolates in different Clinical Samples

Sample	Positive Isolate	Percentage (%)
Wound	10	50
Sputum	4	20
Ear (otitis media)	2	10
Burn	2	10
HVS	0	0
Urine	2	10
Blood	0	0
CSF	0	0
Total	20	100

Key: HVS = High Vaginal Swab;

CSF = Cerebrospinal Fluid.

Table 4: Antimicrobial Susceptibility of MDR-*Pseudomonas aeruginosa* Isolates of the Participants in Oyo State

Antibiotics (µg/ml) Isolates	CAZ (30)	CRX (30)	GEN (10)	CXM (5)	OFL (5)	AUG (10)	CPR (5)	CPT (10)	RNF (5)	MIP (10)
1	R	R	R	R	R	R	R	S	R	S
2	S	R	R	R	R	R	R	R	R	S
3	S	R	S	R	S	R	S	S	R	S
4	R	S	R	R	R	R	S	S	R	S
5	R	R	R	R	R	R	R	S	R	S
6	S	R	R	R	R	R	R	R	R	S
7	R	R	R	R	S	R	R	S	R	R
8	S	R	R	R	R	R	R	R	R	S
9	S	R	R	R	R	R	R	R	R	S
10	R	R	R	R	R	R	R	R	R	S
11	R	R	R	R	R	R	R	S	R	S
12	S	R	R	S	S	R	S	S	R	S
13	S	R	R	R	S	R	S	S	R	S
14	S	S	S	R	S	R	S	S	S	S
15	R	R	S	R	S	R	R	R	S	S
17	R	R	R	R	S	R	S	R	R	R
18	S	R	S	R	S	R	R	S	R	S
19	S	S	S	S	S	R	R	S	S	S
20	R	R	R	R	S	R	S	S	R	S

Key: R: Resistant CAZ: Cefazidime CRX: Cefuroxime GEN: Gentamycin
 S: Sensitive CXM: Cefixime OFL: Ofloxacin AUG: Augmentin
 CPR: Ciprofloxacin CPT: Cipro tab RNF: Ranicef MIP: Imipenem

Table 5: Age and Gender Distribution of the 44 Individuals with *Pseudomonas aeruginosa* Infection and the 20 among with MDR- *Pseudomonas Aeruginosa* in Oyo State [32 Males (15 with MDR) and 12 Females (5 with MDR)]

Age	Males		Female		Total	
	Participants (%)	Participant with MDR Strains (%)	Participants (%)	Participant with MDR Strains (%)	Participants (%)	Participant with MDR Strains (%)
< 10	2(6.25)	0(0)	0(0)	0(0)	2(4.54)	0(0)
10 – 19	6(18.75)	2(12.5)	4(33.33)	1(20)	10(22.7)	4(19.04)
20 – 29	4(12.5)	2(12.5)	1(8.33)	0(0)	5(25.0)	2(9.52)
30 – 39	2(6.25)	0(0)	0(0)	0(0)	2(4.54)	0(0)
40 – 49	2(6.25)	1(6.25)	1(8.33)	0(0)	3(6.8)	1(4.76)
50 – 59	10(31.25)	5(31.25)	4(53.33)	3(60)	14(31.82)	8(38.08)
>60	6(18.75)	5(31.25)	2(16.67)	1(20)	8(18.17)	6(28.56)
Total	32(66.7)	15(33.3)	12(70.6)	5(29.4)	44(100)	20(45.45)

Key: MDR = Multidrug Resistant

Table 6: The Number of MDR *Pseudomonas Aeruginosa* Isolates Sensitive/Resistance against the Ten (10) Antibiotics

Antibiotics	N (%)
Ceftazidine	
Resistant	8(40)
Sensitive	12(60)
Cefuroxime	
Resistant	17(85)
Sensitive	3(15)
Gentamycin	
Resistant	13(65)
Sensitive	7(35)
Cefixime	
Resistant	17(85)
Sensitive	3(15)
Ofloxacin	
Resistant	9(45)
Sensitive	11(55)
Augmentin	
Resistant	19(95)
Sensitive	1(5)
Ciprofloxacin	
Resistant	11(55)
Sensitive	9(45)
Ciprotab	
Resistant	7(35)
Sensitive	13(65)
Ranicef	
Resistant	17(85)
Sensitive	3(15)
Imipenem	
Resistant	3(15)
Sensitive	17(85)

Discussion and Conclusion

Antibiotic resistance is a worldwide problem of major importance due to the detection and isolation in some countries of multi- drug resistant (resistant to three or more classes of antimicrobials) micro-organisms. Multidrug resistant-*Pseudomonas aeruginosa* causes

therapeutic problems and at the same time posing infection control issues in many hospitals. Numerous studies have highlighted the link between multidrug resistance with increased morbidity and mortality, increased length of hospital stay and higher hospital costs (Aloush *et al.*, 2006).

Clinical samples including urine, wound, sputum, ear, CSF, blood and HVS were examined for the presence of multidrug resistant *Pseudomonas aeruginosa* which showed that there was higher prevalence of *Pseudomonas aeruginosa* in wounds (50%) than the other samples. Our result is in agreement with the findings of Rajput *et al.* (2008) who reported that *Pseudomonas aeruginosa* was the most frequent of isolates obtained from burn wounds.

The isolates harvested were tested for resistance to different antibiotics which showed some degree of resistance to all the antibiotics. This agrees with the result of Manikandan *et al.* (2013) which reported multidrug resistance on bacteria isolated from UTI. This is also in line with the result of Uchenna (2015) who reported multidrug resistance by Gram Negative bacteria (Bataneh and Alrashed, 2007). The evaluation of resistance of wounds from Oyo. These are in keeping with the results of the studies conducted by Okesola and Oni (2012) in Southwestern, Nigeria.

The MDR-*Pseudomonas aeruginosa* isolate showed its highest resistance to Augmentin (95%) and least resistance to Mipenem among the tested antibiotics. The result obtained agreed with the work of Gales *et al.* (2012) who reported presence of Multidrug resistance *Pseudomonas aeruginosa* against various antibiotics. Manikandan *et al.* (2013) reported that the overuse of antibiotics has become the major factor for the emergency and dissemination of multi-antibiotics resistance strains of several bacteria. In these regards *Pseudomonas aeruginosa* antibiotics resistance was raised for both intrinsic and acquired resistance.

The multidrug resistance often reflects not one but a combination of resistance mechanisms. Efflux pumps are common components of multidrug resistant *Pseudomonas* isolates and they prevent accumulation of antibacterial drugs within the bacterium extruding the drugs from cell before they have the opportunity to achieve an adequate concentration at the site of action.

The age- gender distribution showed that male patients were more affected than the female in the study. High prevalence of multidrug resistant infections occurred in age group 50 – 59 years, 8 (38.08%) males 5(31.25%) and female 3(60%)

and least age group <10 years.

The antimicrobial susceptibility pattern of multidrug resistance *Pseudomonas aeruginosa* isolates showed more resistant to first- and second-generation antibiotics such as Augmentin, Cefuroxime, Cefixime and Ranicef. Members of Quinolones family also exhibited high susceptibility pattern. Ciprotab and Ofloxacin least susceptibility in the group.

The carbapenem group demonstrated better susceptibility than Mipenem. The pattern demonstrated by Mipenem may not be surprising, as it is a new drug in armanametarium, that is seldom prescribed or abuse in the community. Similar pattern had been reported in the studies in Enugu and Abakaliki, Nigeria by Okeke *et al.* (2000). The difference in the resistance pattern may be attributed to factors like exposure to antibiotics, studied population and type of clinical specimen examined.

Pseudomonas aeruginosa produces many resistant and virulence factors, whose expression is arranged by different systems. A recent study by Alhazmi, 2015, reveals *Pseudomonas aeruginosa* is most frequent pathogens that formed many of these resistant genes (GyrA, GyrB, ParC, MexB, MexA, SHV, TEM, and Virulence factors.

In this study, an overall prevalence rate of *Pseudomonas aeruginosa* associated infection was 27.3%. The preponderance of this pathogen nosocomial infections is no longer in doubt, while in Pakistan, Zaman *et al.* (2013) found isolation rate of 33% in hospitalized patients, higher than what was obtained in this work. The difference may be due to geographical location.

Out of the 44 *Pseudomonas aeruginosa* isolates in this study 20 were multi-drug resistant. Multidrug-resistant *Pseudomonas aeruginosa* have previously been reported among patients with diabetic wounds in Irrua, Nigeria (Isibor *et al.*, 2015). Smith *et al.* (2012) however recorded a higher prevalence (60%) for multidrug-resistant *Pseudomonas aeruginosa* isolated from surgical wounds in Lagos, Nigeria. Detection and isolation of MDR *Pseudomonas aeruginosa* has traditionally relied on phenotypic methods. This is the most accurate standard when dealing with typical isolates of *Pseudomonas*

aeruginosa. Moreover, biochemical testing takes long time to perform and requires more manpower, both for setup and for evaluation. Molecular methods have been reported to be superior to the phenotypic.

Conclusion

The molecular detection of MDR- *Pseudomonas aeruginosa* is very important as majority of the isolates exhibited multidrug resistance, which posed a great clinical and environmental consequence, especially with patient management. Having *Pseudomonas* infections and infections control approach in hospital environment due to rapid dissemination of the strains is vital. It is recommended that there should be focus on early detection using molecular method drug resistance mechanisms for all micro-organisms especially MDR- *Pseudomonas aeruginosa* to avoid having untreatable infections and superbugs. This could play a major role in the epidemiology of antibiotic resistance in Nigeria and could also be responsible for prolong treatment of bacterial infection and the emergence of emerging and re-emerging infectious diseases. There should be continuous monitoring of antimicrobial susceptibility pattern and strict adherence to infection prevention guidelines which are essential to prevent proliferation of multidrug resistant *Pseudomonas aeruginosa*. There is need to develop nationwide antibiotic policy and guidelines for the increasing resistance pattern. Local antibiogram database is also needed as this will improve the knowledge of antimicrobial resistance pattern in Oyo state and this will also help to improve treatment strategies based on unit specific data.

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Conflict of Interest:

The authors have no conflict of interest.

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