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CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PROFILE OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL AND ENVIRONMENTAL SAMPLES

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ABSTRACT

Pseudomonas aeruginosa has emerged as a potential problematic Gram negative pathogen. The present study investigated the distribution of *P. aeruginosa* from the various clinical (wound and post-surgical wound) and environmental (water, industrial effluents and air) samples. The antibiotic resistance test was performed using the disc diffusion method according to NCCLS guidelines. Multidrug resistant genes among the isolates were characterized using PCR. 18 of the isolates with multidrug resistance showed least resistance to ceftazidime (23 %), gentamicin (20 %) and ciprofloxacin (31 %) from clinical isolates and 60, 38.5 and 60 % respectively from environmental isolates. Over 78 % of the isolates were polyvalent 1 strains, 17 % were polyvalent 2 and 5 % were polyvalent 3 strains. Polyvalent 1 were mostly strains from clinical samples while polyvalent 2 and 3 were strains from both (clinical and environmental) samples investigated. PCR analysis of multidrug resistant *P. aeruginosa* indicates that, PA-SS species specific signature sequences were present in all (100%) the isolates investigated. There was high emergence of antibiotic resistance in the environment and hospital investigated. Proper monitoring, regulating indiscriminate use of chemicals in the environment and restricting the use of antibiotics without susceptibility tests or prescription by a qualified physician should be adopted.

Contribution/Originality: This study documents the prevalence of drug resistant strains of *P. aeruginosa* isolated from clinical and environmental sources in Yola, Adamawa State., Nigeria.

1. INTRODUCTION

Pseudomonas aeruginosa belongs to a vast genus of obligate, aerobic, non-lactose fermenting, saprophytic, Gram-negative bacilli wide spread in natural environment such as soil, plant surfaces, fresh vegetables, sewage, waste water, sink, moist environment and river water [1]. This organism though endowed with weak pathogenic potential, has profound ability to survive on inert materials. Its minimal nutritional requirements, ability to grow in distilled water [2] high tolerance to a wide variety of physical conditions and relative resistance to several unrelated antimicrobial agents and antiseptics, contributed enormously to its ecological success and its role as an effective opportunistic pathogen.

P. aeruginosa resistance to a wide range of antibiotics is made possible by several mechanisms. The major mechanism of resistance to ß-lactam antibiotics is beta-lactamase production. The lactamase enzyme breaks the ß-lactam ring open, deactivating the molecules' (i.e the ß-lactam) antibacterial properties [3]. More than 340 β-

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lactamase enzymes have been detected to date. Although not completely understood, several factors have been identified as virulence determinants of *P. aeruginosa*. These include *rhl/las* otherwise known as quorum-sensing system [4] type III secretion system [5] multidrug efflux system [6] and biofilm forming system.

P. aeruginosa are increasingly developing resistance to "first-line" antibiotics, as well as the newer, expensive broader spectrum antibiotics employed in therapy [1] thereby further developing resistance to new class of drugs. In the present study, the molecular characterization and determination of antimicrobial resistance among *P. aeruginosa* isolates from clinical and environmental sources to some commonly used antibiotics in Yola, Adamawa State, Nigeria, was investigated. This is with a view to obtain baseline data for proper formulation of antibiotic use policies and guidelines to prevent antibiotics resistance, thus reducing morbidity and mortality rates due to *P. aeruginosa* infections.

2. MATERIALS AND METHODS

2.1. Study Design

Clinical samples were collected from Specialist Hospital, Federal Medical Centre and, Modibbo Adama University of Technology, Yola, and environmental samples from Faro Industry, Yola, Adamawa State, Nigeria. A total of 75 samples was collected and examined, comprising 15 environmental samples of water, air and industrial effluents, and clinical samples of wound infection and post-surgical wound patients respectively. Data such as age and sex of patients was taken to determine whether they have any association with the multidrug resistant pattern of *P. aeruginosa* infections.

2.2. Bacteriological Identification

The samples were immediately transported to the Microbiology Laboratory, Federal Medical Center (FMC), Yola. At the lab, samples were inoculated primarily on to nutrient agar (NA) and Mac Conkey agar (MCA) and incubated at 37ºC for 24-48 h respectively [7]. The bacterial isolates were observed for morphological charactics such as colony morphology, pigment formation, mucoidness and positive oxidase test. *P. aeruginosa* isolates were confirmed by subculturing onto Cetrimide agar selective medium and incubating at 37ºC for 24 h.

2.3. Antimicrobial Resistance Testing

The modified Kirby Bauer filter paper disc diffusion method as described by National Committee for Clinical Laboratory Standards [8] was used to determine the antibiotic susceptibility or resistance pattern of the *P. aeruginosa* isolates. A suspension of the *P. aeruginosa* isolates (0.5 McFarland standard) in nutrient broth was uniformly spread inoculated onto Mueller-Hinton plates using sterile cotton swab and the plates allowed on the bench to dry for 15-20 minutes. Antibiotic discs impregnated with various MIC concentrations of antibiotics were placed on the culture plates and incubated at 37ºC for 18 h. After incubation, resistance or susceptibility to the antibiotics were clinically evaluated as [resistant (R), Intermediately resistant (I) or susceptible (S)] by measuring the size of the diameter of zone of inhibition around the isolate using Vanier caliper.

2.4. Serotyping of isolates

Thirteen unabsorbed *P. aeruginosa* antisera (1-13) based on the international antigen typing scheme types was used to characterize the isolates into their various serotypes. Three antisera pools, each containing different antisera, was used. Pool I contained antisera 01, 03, 08, 09 and 012. Pool II contained antisera 02, 010 and 011. Pool III contained antisera 04, 05, 06, 07 and 014. Each polyvalent antisera was used undiluted in the slide agglutination test method as previously described [9].

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2.5. PCR Detection of *Pseudomonas aeruginosa*

2.5.1. Extraction of DNA

DNA was extracted from isolates as described by Liu, et al. [10]. Briefly, a single colony was suspended in 20µL of lysis buffer containing 0.25% (v/v) Sodium Dodecyl Sulfate and 0.05N NaOH. After heating for 15 min at 95°C, 180µL of Milipore water was added and the lysis suspension was stored at -20°C.

2.5.2. Primers

The primers used are: PA-SS-F (5' GGGGGATCTTCGGACCTCA 3') and PA-SS-R (5' TCCTTAGAGTGCCCACCCG 3') as were designed by Spilker, et al. [11] to amplify only *P. aeruginosa* as it targeted species specific signature sequences.

2.5.3. PCR

Amplification of targeted DNA was carried out in 25µL reaction volume as described elsewhere [11]. Each reaction volume containing 2mM $MgCl_2$, 50mM Trizma (pH 8.3), 250µM (each) triphosphates, 0.4 µM of the primer, 0.5 U of *taq* polymerase and 2µL of whole-cell bacterial lysate was adjusted to 25µL using PCR-molecular biology grade water. Amplification was carried out in thermal cycler. After an initial denaturation for 2 min at 95°C, 25 cycles were completed, each consisting of 20 seconds at 94°C, 20 seconds at 58°C and 40 seconds at 72°C. A final extension of 1 min at 72°C was applied. Amplification products were electrophoresed in agarose gel in order to observe for the resistant gene bands.

3. STATISTICAL ANALYSIS

To compare resistance rates with demographic factors and between different bacterial isolates obtained, the mean, standard deviation as well as, Chi-square (χ^2) and ANOVA test were performed using SPSS 20 statistical software for windows and a probability value of 0.05 or less was considered to be significant.

4. RESULTS

Result of the morphological characterization of *P. aeruginosa* isolates from the 75 [clinical (30) and environmental (45)] samples showed that 24 (32%) were *P. aeruginosa* isolates. Results of distribution frequencies of the organisms by sample showed that, 53.3% (16/30) *P. aeruginosa* isolates were from clinical samples, [10/15 (67%) were from wound and 6/15 (40 %) were from post-surgical wounds. For environmental samples (water, air and industrial effluent), a total frequency of 20 % (3), 0% (0) and 33.3% (5) respectively, of *P. aeruginosa* occurrence was recorded. Results also showed that while there were other bacterial isolates observed from the air samples investigated, no or 0 % *P. aeruginosa* was isolated (Table 1). Furthermore, the Chi-square statistical analysis shows that, there is significant difference in the prevalence of *P. aeruginosa* (P=0.002) isolated from the different sample sources.

Table-1. Distribution of *Pseudomonas aeruginosa* in clinical and environmental samples

Clinical Samples	No. of Positive Isolates (%)	Environmental Samples	No. of Positive Isolates (%)
Wound (n=15)	10 (67)	Water (n=15)	3 (20)
Post-surgical wound (n=15)	6 (40)	Air (n=15)	0 (0)
		Industrial Effluents (n=15)	5 (33.3)
Total	**16 (53.3)**		**8 (17.7)**

χ^2 =16.789, DF= 4, P = 0.002
Key: χ^2 = Chi-square, DF= Degrees of freedom, P = Probability value

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Results of antimicrobial resistant patterns of the different isolates against some commonly prescribed antibiotics showed that, out of the 16 positive isolates recovered from clinical samples, 13 were Multi-Drug Resistant (MDR) with 3 (23.1%) resistant to ceftazidime, 4 (31%) resistant to gentamicin and 5 (38.5%) resistant to ciprofloxacin. Results also showed that, all the isolates (100%) from the clinical samples investigated were resistant to ofloxacin, amoxicillin/clavulanate and cotrimoxazole. Furthermore, all the isolates (100 %) obtained from post-surgical wounds were resistant to cefuroxime, nitrofurantoin, streptomycin and ampicillin but only 6 (75 %) and 7 (88 %) from wound isolates showed resistance respectively (Table 2).

Table-2. Antimicrobial resistance profile of *Pseudomonas aeruginosa* isolated from clinical samples

	Wound (n=8)			Post-surgical wound (n=5)			Total (n=13)		
Antimicrobial (μg)	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Ceftazidime 30	6(75)	0(0)	2(25)	4(80)	0(0)	1(20)	10(76.9)	0(0)	3(23.1)
Cefuroxime 30	2(25)	0(0)	6(75)	0(0)	0(0)	5(100)	2(15)	0(0)	11(85)
Gentamicin 10	3(37.5)	3(37.5)	2(25)	3(60)	0(0)	2(40)	6(46)	3(23)	4(31)
Ciprofloxacin 5	4(50)	1(12)	3(38)	3(60)	0(0)	2(40)	7(53.8)	1(7.7)	5(38.5)
Ofloxacin 5	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)	13(100)
Amoxicillin 30	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)	13(100)
Nitrofurantoin 300	0(0)	2(25)	6(75)	0(0)	0(0)	5(100)	0(0)	2(15)	11(85)
Ampicillin 10	0(0)	1(12)	7(88)	0(0)	0(0)	5(100)	0(0)	1(7.7)	12(92.3)
Streptomycin 30	2(25)	0(0)	6(75)	0(0)	0(0)	5(100)	2(15)	0(0)	11(85)
Cephalexin 10	0(0)	1(12)	7(88)	0(0)	1(20)	4(80)	0(0)	2(15)	11(85)
Nalidixic acid 30	2(25)	0(0)	6(75)	1(20)	1(20)	3(60)	3(23)	1(7.7)	9(69.3)
Cotrimoxazole 30	0(0)	0(0)	8(100)	0(0)	0(0)	5(100)	0(0)	0(0)	13(100)

KEY: %= Values in parenthesis, S= Susceptible, I= Intermediately susceptible and R= Resistant.

Table 3 showed the resistance profile of *P. aeruginosa* from environmental (water and Faro industrial effluents) samples. Results showed that only 20 % of the isolates were resistant to ceftazidime and 60 % were resistant to gentamicin and ciprofloxacin respectively. For water samples, results showed that the isolates were 100% resistant to the prescribed antibiotics tested except for ceftazidime where the isolates showed (00.0%) resistance. Results from Faro industrial effluents showed 100 % resistance to cefuroxine, ofloxacin, amoxicillin/clavulanate, nitrofurantoin, ampicillin, cephalexin, nalidixic acid and cotrimoxazole by the *P. aerugenosa* isolates. In contrast, the results showed ceftazidime to be very effective among the environmental isolates with only 20 % (1 isolate) resistance. Result also revealed that, there was no significant difference in resistance to the various antibiotics used on the isolates studied ($P > 0.05$)

Table-3. Antimicrobial resistance profile of Pseudomonas aeruginosa isolated from environmental samples

	Water (n=1)			Industrial effluent (n=4)			Total (n=5)		
Antimicrobial (μg)	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Ceftazidime 30	1(100)	0(0)	0(0)	3(80)	0(0)	1(20)	4(80)	0(0)	1(20)
Cefuroxime 30	0(0)	0(0)	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(100)
Gentamicin 10	0(0)	0(0)	1(100)	0(0)	2(50)	2(50)	0(0)	2(40)	3(60)
Ciprofloxacin 5	0(0)	0(0)	1(100)	1(25)	1(25)	2(50)	1(20)	1(20)	3(60)
Ofloxacin 5	0(0)	0(0)	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(100)
Amoxicillin 30	0(0)	0(0)	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(100)
Nitrofurantoin 300	0(0)	0(0)	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(100)
Ampicillin 10	0(0)	0(0)	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(100)
Streptomycin 30	0(0)	0(0)	1(100)	0(0)	1(25)	3(75)	0(0)	1(20)	4(80)
Cephalexin 10	0(0)	0(0)	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(100)
Nalidixic acid 30	0(0)	0(0)	1(100)	1(20)	1(20)	4(100)	0(0)	0(0)	5(100)
Cotrimoxazole 30	0(0)	0(0)	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)	5(100)

KEY: %= Values in parenthesis, S= Susceptible, I= Intermediately susceptible and R= Resistant

Comparison ($P>0.05$) of the percentage of resistant *P. aeruginosa* isolates between the clinical (wound and post-surgical wounds) and environmental (water and Faro industrial effluents) samples is shown in Table 4. Results

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showed that, *P. aeruginosa* isolated from environmental samples exhibited higher percentage resistance than those from clinical. Also, results among the clinical isolates showed 100 % resistance to ofloxacin, amoxicillin/clavulanate and cotrimoxazole and 31 and 38.5 % resistance to gentamicin and ciprofloxacin respectively. However, for environmental isolates, result showed that while only 1 (20 %), isolate was resistant to ceftazidime 60 % resistance was recorded against gentamicin.

Table-4. Percentage of resistance to antibiotics of Pseudomonas aeruginosa isolates from clinical and environmental samples

	Source of Sample								
	Clinical (n=13)			Environmental (n=5)			Total (n=18)		
Antimicrobial (µg)	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Ceftazidime 30	10(76.9)	0(0)	3(23.1)	4(80)	0(0)	1(20)	14(78)	0(0)	4(22)
Cefuroxime 30	2(15)	0(0)	11(85)	0(0)	0(0)	5(100)	2(11)	0(0)	16(89)
Gentamicin 10	6(46)	3(23)	4(31)	0(0)	2(40)	3(60)	6(33)	5(28)	7(39)
Ciprofloxacin 5	7(53.8)	1(7.7)	5(38.5)	1(20)	1(20)	3(60)	8(44.4)	2(11.2)	8(44.4)
Ofloxacin 5	0(0)	0(0)	13(100)	0(0)	0(0)	5(100)	0(s0)	0(0)	18(100)
Amoxicillin 30	0(0)	0(0)	13(100)	0(0)	0(0)	5(100)	0(0)	0(0)	18(100)
Nitrofurantoin 300	0(0)	2(15)	11(85)	0(0)	0(0)	5(100)	0(0)	2(11)	16(89)
Ampicillin 10	0(0)	1(7.7)	12(92.3)	0(0)	0(0)	5(100)	0(0)	1(6)	17(94)
Streptomycin 30	2(15)	0(0)	11(85)	0(0)	1(20)	4(80)	2(11)	1(6)	15(83)
Cephalexin 10	0(0)	2(15)	11(85)	0(0)	0(0)	5(100)	0(0)	2(6)	15(83)
Nalidixic acid 30	3(23.1)	1(7.7)	9(69.2)	0(0)	0(0)	5(100)	3(16)	1(6)	14(78)
Cotrimoxazole 30	0(0)	0(0)	13(100)	0(0)	0(0)	5(100)	0(0)	0(0)	18(100)

KEY: %= Values in parenthesis, S= Susceptible, I= Intermediate and R= Resistance

Results of serotypic characterization of the antibiotic resistant *P. aeruginosa* showed that 2 (11%) out of the 18 isolates were non typeable and 78 % of the isolates were predominantly the polyvalent 1 serotype prevalent among the typeable isolates (Table 5). Results also showed that, the polyvalent 1 serotypes occurred more frequently among the clinical (wound and post-surgical wounds) samples than the environmental (water and Faro industrial effluents) samples. Furthermore, the results of Chi-square statistical analysis revealed that there was high significant difference in *P. aeruginosa* strain distribution among the three polyvalent serotypes used (P=0.000).

Table-5. Distribution of samples that are tested positive for *Pseudomonas aeruginosa* serogroup (n=18)

S/N	*P. aeruginosa* Antisera	No. of positive isolates	% of positive isolates
1.	Polyvalent 1 (01, 03, 08, 09 and 012)	14	78
2.	Polyvalent 2 (02, 010 and 011)	3	17
3.	Polyvalent 3 (04, 05, 06, 07 and 014)	1	5
	Total	**18**	**100**

χ^2 =15.889, DF= 4, P = 0.000

Key: χ^2 = Chi-square, DF= Degrees of freedom, P = Probability value

PCR was applied to determine the presence of PA-SS gene among multidrug resistant *P. aeruginosa* strains in the clinical and environmental isolates studied. Results showed that 100 % of the *P. aeruginosa* isolates carried the species specific PA-SS gene (Figs. 1 and 2). Fig. 1 showed the PCR profiles of the *P. aeruginosa* strains isolated from clinical (wound and post-surgical wound infections) and Fig. 2 showed the PCR profiles of isolates from environmental (water and Faro industrial effluents) samples. Eighteen clusters were generated and analyzed for MDR, lanes 1-8 were from the wound samples while lanes 9-13 were samples from post-surgical wounds (Fig. 1). Fig. 2 showed isolates from environmental samples; lane 1 was from the water sample and lanes 2-5 were samples from Industrial effluents. In all the clusters, the isolates showed 100 % similarity, and each of them had formed one real clone. The detected gene bands produced same gene size of 956 bp in the agarose gel after electrophoresis (Figs. 1 and 2).

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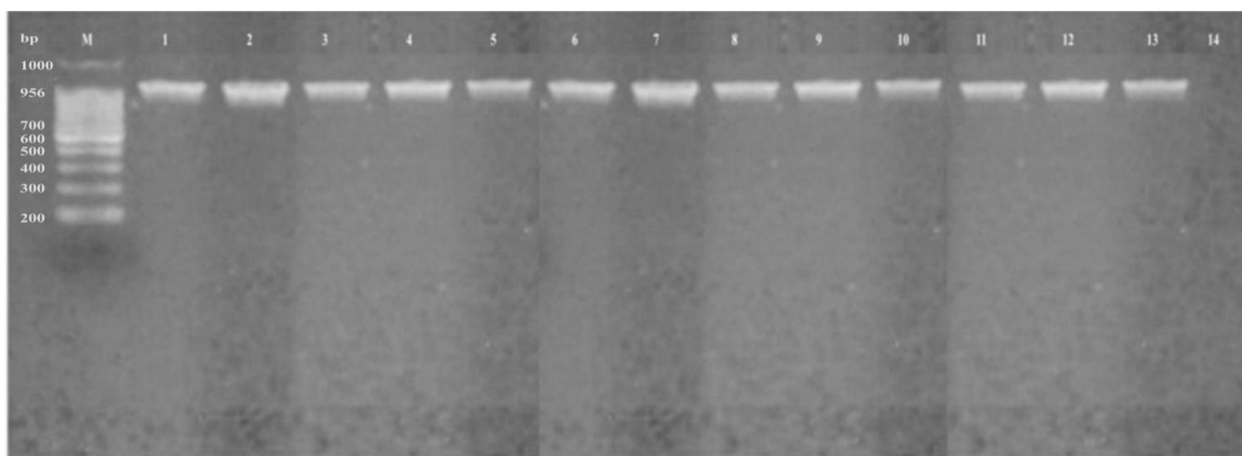

Fig-1. Agarose gel electrophoresis analysis of PCR amplification using PA-SS pair primers, extracted from *P. aeruginosa* strains isolated from clinical samples

KEY; Lane M: DNA molecular size marker (100 bp ladder), lane 1-8: Wound samples, lane 9-13: Post-surgical wound samples and lane 14: Negative control

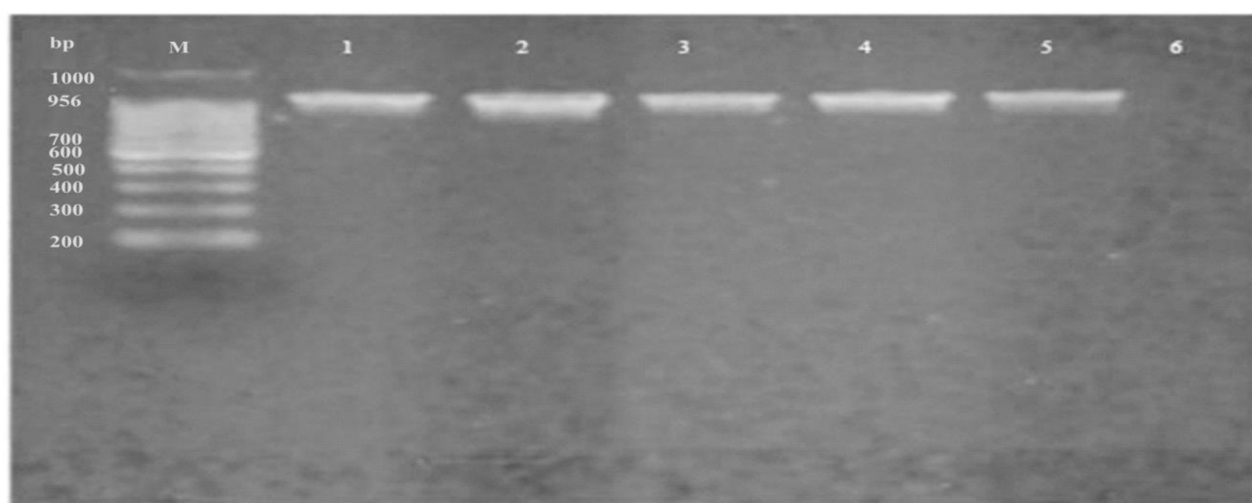

Fig-2. Agarose gel electrophoresis analysis of PCR amplification using PA-SS pair primers, extracted from *P. aeruginosa* strains isolated from the environmental samples

KEY; Lane M: DNA molecular size marker (100 bp ladder), lane 1: Water sample, lane 2-5: Industrial effluents samples, 6: Negative control

5. DISCUSSION

The high distribution of *P. aeruginosa* obtained in this study particularly from clinical sources is led by the widespread use of antibiotics, especially in the treatment of post-surgical wound patients and also the indiscriminate use of antibiotics in the community. The resistance profile of *P. aeruginosa* is changing, as evidenced by increasing occurrence of antibiotic resistance among the *P. aeruginosa* populations [1]. The wound is considered one of the major health problems in the world, and infection is one of the most frequent and severe complications in patients who have sustained wounds. This is because wounds have large exposed areas of dead tissue free of any defenses and, therefore, are ideal sites for infection by *P. aeruginosa* as it affects immunocompromised patients and patients with a pre-existing disease or other predisposing conditions.

Results from this study agrees with results of the studies carried out by Anupurba., et al. [7] which showed that *P. aeruginosa* was isolated in 32 % of the isolates investigated. A similar study from ABU Zaria, Nigeria showed that *P. aeruginosa* was the most frequent pathogen isolated from wound samples, accounting for 36 % of the total number of the organisms [1]. Concerning the distribution of *P. aeruginosa* in the environment, lower frequencies were found which is similar with the previous studies in Egypt [12] and in Irag [13]. Nevertheless, similar results in Nigeria [14] were also obtained.

Results obtained in this study may be an indication that *P. aeruginosa* in the examined hospital is endemic. According to Olayinka et al. [5], most patients going in for major surgery tend to get catheterized, so most isolates

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of *P. aeruginosa* were obtained from urine which eventually contaminates the wounds in hospital-based cases. The results also indicated high prevalence (67 %) of *P. aeruginosa* in wound samples obtained from male patients. This may likely be facilitated by the vigorous activities the males are involved in, which can lead to contamination of the wound by *P. aeruginosa* obtained from the environment. Moreover, *P. aeruginosa* is one of the bacteria that commonly contaminates wounds and develops biofilm [2]. The results of this study therefore, represent a major public health hazard for both the hospitals and community acquired infection especially for surgical wound contamination.

The resistance profile of *P. aeruginosa* isolates to the 12 antimicrobials tested *in vitro* were relatively high compared to the sensitivity pattern to different anti pseudomonal drugs reported worldwide [7]. Such high antimicrobial resistance is probably promoted by the selective pressure exerted on bacteria aggravated by non-adherence to hospital antibiotic policy, and excessive and indiscriminate use of broad-spectrum antibiotics [2].

Result of this study indicated that ceftazidime, gentamicin and ciprofloxacin recorded lower resistance rates among the clinical and environmental isolates (Table 4). Similarly, earlier studies have reported high susceptibility of *P. aeruginosa* against cefuroxime, nalidixic acid and streptomycin [1, 15].

This study provides important baseline data on current antimicrobial resistance profiles of clinical and environmental isolates of *P. aeruginosa* in Yola, Nigeria. The different antibiotic resistance patterns observed in the isolates indicated that the organism uses several mechanisms for resistance simultaneously, and that all isolates do not necessarily use the same mechanisms for resistance to particular classes of antibiotics as earlier reported [1]. Further, isolates that were resistant to one class of antibiotics were also resistant to at least one other class.

Findings with regard to antimicrobial resistance suggests that cefuroxime, ofloxacin, amoxicillin/clavulanate, nitrofurantoin, ampicillin, streptomycin, cephalexin, nalidixic acid and cotrimoxazole should not be considered effective agents for the treatment of *P. aeruginosa* wound infections in the hospital setting because of the high resistance rates observed in this study. The 23.1 % and 20 % rate of resistance to ceftazidime reported here contrasts with the 41 % resistance rate obtained in America [7] or with the 28-31 % rates in Europe [16]. Resistance to ciprofloxacin in this study was 38.5 and 60 %, compared to 26.8 % in Latin America [7] and 10-32 % in Europe [17] In contrast, resistance to gentamicin was higher (31 % and 60 %), but this rate was lower than in Latin America [7] or Europe [16].

Ciprofloxacin, Nitrofurantoin and Nalidixic acid are considered potent agents in the treatment of infections caused by multi-resistant *P. aeruginosa* [7]. In this study ceftaxidime, followed by gentamicin, were the less resistant antibiotics ($P < 0.05$). This fact reflects the importance of controlling the use of these antibiotics in the hospital setting to prevent the emergence of cephalosporin and aminoglycoside-resistant strains [1]. Further, ciprofloxacin and streptomycin were both weakly effective against these resistant isolates. However, the low number of strains resistant to ceftaxidime in this study suggests that this drug can be considered effective against *P. aeruginosa* infections, but the use of ceftaxidime and other ß-lactams must be monitored, as these antibiotics induce selective pressure on ß-lactamase-producing strains, resulting in the development of resistance in the hospital environment [17]. Thus, routine microbiological surveillance and careful *in vitro* testing prior to antibiotic use and strict adherence to hospital antibiotic policy may help in the prevention and treatment of multi-drug resistant *P. aeruginosa* in wound infection.

While the prevalence of *P. aeruginosa* serotype varies from one hospital to another and from one country to another, polyvalent 2 (O2, O10 and O11) and polyvalent 3 (O4, O5, O6, O7 and O14) were often the most prevalent serotypes reported in previous studies [15, 18]. It is worthy of note that the prevalence of *P. aeruginosa* serotypes reported in previous studies was obtained either from multiple infectious sites or from a single hospital or country. Although the prevalence of serotypes is different among the countries and investigator sites, the overall rates of prevalence of the most common *P. aeruginosa* serotypes observed in this study was polyvalent 1(O1, O3, O8, O9 and O12) (Table 5). According to Rodriguez, et al. [18] polyvalent 1 (O12) strains emerged as the predominant

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serotype in clinical settings and outbreaks. The O12 exhibits high levels of resistance to various classes of antibiotics due to horizontal transfer and genetic recombination of lipopolysaccharide (LPS) biosynthesis genes originating from MDR taxonomy.

In this study, all the resistant isolates (n=18) investigated showed polyvalent 1 serotype to be the most prevalent (78%) serotypes (Fig. 1). There is however high significant difference between the three polyvalent when compared (χ^2 = 30.500; P = 0.000) (Table 5). This result agrees with those previously reported [15, 18]. In the study of Rodriguez, et al. [18] approximately 11% of cases failed to detect serotype because of self-agglutinating or non-agglutinating strains and the samples were classified as "not typeable". But in this study, the proportion is less than the previously reported. With PCR, the 18 MDR isolates were typed into different cluster profiles; the clusters consisted of only the resistant isolates from both (clinical and environmental) sources. The isolates showed a high genetic similarity. PA-SS genes were detected in all (100%) the isolates in the study. It is postulated that most antibiotic resistance in *P. aeruginosa* are mostly chromosomal. However, isolates containing the resistance genes were not susceptible to at least 12 different antibiotics. The presence of these resistance genes might have accounted for the multi-drug resistance observed in this study. Results obtained in this study further highlights the necessity to focus on tracing the source of infections for control of nosocomial infections and also the need for design of strategies to diminish the nonspecific use of broad spectrum antibiotics in the hospitals.

6. CONCLUSION

The hospital environment and healthcare personnel could serve as potential reservoirs of *P. aeruginosa* in the study locality. Furthermore, excessive use and disposing of antibiotics and chemicals contributes to the emergence of antibiotic resistance in the environment and hospital. The obtained results may help in prevention and control strategies of *P. aeruginosa* in both hospital and the community. Multi-drug resistant *P. aeruginosa* is emerging as a public health hazard in Adamawa State. Despite the strain diversity among clinical and environmental samples in the present study, PCR determination of PA-SS gene have also demonstrated a high level of genetic similarity in the *P. aeruginosa* strains investigated. Currently, the treatment of *P. aeruginosa* infections is based on combination antibiotic therapy that traditionally includes ß-lactam agents and aminoglycosides, in addition to this; treatment with third generation cephalosporin (ceftazidime) has offered new perspectives. The development of effective vaccine against *P. aeruginosa* is necessary in the modern world.

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