International Journal of Biomedical and Health Sciences
Vol. 8, No. 2, June 30, 2012
Printed in Nigeria

0794-4748/2012 $5.00 + 0.00

http://www.asopah.org

IJBHS 2012034/8208

Prevalence and antibiotic susceptibility patterns of *Pseudomonas aeruginosa* in Minna.North Central Nigeria

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(Received March 8, 2012; Accepted May 14, 2012)

ABSTRACT: *Pseudomonas aeruginosa* is an opportunistic pathogen causing nosocomial and community infections.Increasing infections and antibiotic susceptibility patterns of this bacteria is of great public health problem.This study was therefore carried out to determine the prevalence and antibiotic susceptibility patterns of *Pseudomonas aeruginosa* in a tertiary Hospital. Standard microbiological procedures were adopted in the isolation and identification of *Pseudomonas aeruginosa* using Blood agar,CLED, MacConkey agar,Cetrimide agar and liquid Nutrient broth.Invitro antibiotic susceptibility patterns were determined by the Bauer-Kerby method (Disc Diffusion test) using Mueller Hinton agar and inpregnated antibiotic discs. From the result of this study, the isolation rate of *Pseudomonas aeruginosa* infections in clinical specimens was 13.5% ($P<0.05$) with the highest occurance of 7.0% in urine followed by 4.7% in wound swabs. This study shows that there is no major clinical significance in the infection rate in males(50.3%) and females (49.7%) while the pattern of spread involve all the age groups. Co – morbility factors in these study reveals a 3.9% infection rate in High Blood Pressure and 2.0% in Diabetic patients while duration of infection and antibiotic history had a major significance as 53.6% were already on antibiotics before visiting the hospital ($P<0.05$).The susceptibility pattern showed that 85.5% of isolates were sensitive to levofloxacin and 65.5% to pefloxacin while their MICs showed lower concentrations.Result showed a higher prevalence in in-patients (50.7%) and the isolates from in-patients reveal higher resistance to all antibiotics tested than the isolates from out – patients, most especially Levofloxacin and Pefloxacin. The over all susceptibility data showed resistance rate of 63.2% to all antibiotics tested which calls for concern.

Keywords; *Pseudomonas aeruginosa*, Prevalence; Antibiotic; Susceptibility patterns.

Introduction

Microorganisms play a vital role in the health of humans and they exist either as normal flora or potential pathogens. In recent times, bacterial infection has become a major problem to health care world wide as diseases caused by these bacteria are major causes of death, disability, social and economic disruption for millions of people (1). Bacterial infections result from the interplay between those few pathogens and the defenses of the hosts they infect. The appearance and severity of the infection resulting from any pathogen depends on the ability of the pathogen to damage the host as well as the ability of the host to resist the pathogen (2). These organisms easily infect humans due to changes in the immune system, poor hygiene or abridging the skin integrity (3).

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Pseudomonas aeruginosa is a common inhabitant of the intestinal resident flora in about 10% of normal people (4).They are nutritionally and metabolically versatile and can adapt to a wide range of habitat. It is a frequent contaminant of ventilators, I.V. solutions and hospital equipments and exhibit marked resistance to soaps, dyes, and temperature extremes. The minimum nutritional requirements of this bacteria, its tolerance to a wide range of physical conditions, ability to utilize a wide range organic substances such as nitrogen and carbon coupled with its relative resistance to antibiotic agents contribute to its ecological success (4).

Pseudomonas aeruginosa is an effective opportunistic pathogen unlikely to cross healthy, intact anatomical barrier thus its infectiousness result from invasive medical procedures or weakened host defenses. These predisposing conditions include debilitating illness, immunosuppressant medication or intravenous injections (5).Once in the tissues, this bacteria expresses virulent factors including exotoxins and also causes endotoxic shock. *Pseudomonas aeruginosa* has been reported as a leading cause of nosocomial infection the most common which occur in compromised hosts with severe burns, neoplastic diseases and cystic fibrosis (6).

Complications include pneumonia, urinary tract infections, abscesses, otitis and corneal diseases. Pseudomonas septicemia can give rise to diseases and grave conditions such as endocarditis, meningitis and broncho pneumonias. Infection is especially virulent in premature infants and neonates while healthy people are subject to outbreaks of skin rashes, urinary tract infections and external ear infections.

Therefore,this study was carried out to determine the prevalence of *Pseudomonas aeruginosa* in patients with diverse infections, to know the predisposing factors associated with *Psudomonas aeruginosa* infections and to ascertain the patterns of antibiotic susceptibility.

Materials and Methods

A prospective Cross sectional and analytical study was carried out at IBB specialist hospital Minna, Nigeria. The subjects were recruited from patients with suspected cases of bacterial infections, who either attended the out-patient unit or were hospitalized (in-patients) from October 2009- March 2010. Ethical approval was granted by the ethics committee of the Hospital.

IBB Specialist hospital is a 1200 beds tertiary hospital which serves mainly the lower and middle class population of Niger state and its environs. The subjects were presenting with symptoms of Sexually transmitted diseases, Urinary tract infections, post operational infections, inflammatory diseases, meningitis etc.On enrolment, the following base line data were collected sociodermographic characteristics, co- morbidity factors, duration of illness and duration of previous and current antibiotics usage.

Clinical samples (Aspirate, wound swabs, sputum, blood, urine cerebrospinal fluid and semen), were collected from patients in accordance with standard procedures. Briefly the samples were collected in sterile containers and special procedures were required for samples like cerebrospinal fluid. The physicians or surgeons in the hospital did the collection by lumber puncture. Blood samples were aseptically collected by venipuncture into diphasic medium bottle containing 25ml broth.

Bacteriological Analysis

The specimens were cultured and sub – cultured on specialized media for isolation. The media used includes CLED, Nutrient Agar, Blood Agar, MacConkey Agar, Cetrimide Agar and liquid medium Nutrient broth. A standard Pseudomonas aeruginosa; NCTC 10662 was used as control. The agar plates were incubated aerobically at 37oc for 24 hours. After 24 hours incubation, the isolates were identified based on standard protocols . The identification of *Pseudomonas aeruginosa* from the isolates first observed on nutrient agar, the greenish pigmentation characteristics, further identification methods were the colonial morphology on various agar used and biochemical tests which include oxidase reaction, motility, urease activity, citrate utilization and sugar fermentations.

Antimicrobial Susceptibility Test

In vitro antibiotic sensitivity test to various antibiotics was performed by the Bauer – Kerby Method (Disc Diffusion Test Flooding Method using Mueller Hinton Agar (M173). Few colonies from pure culture of each of the

isolates were picked from the plate and emulsified in 5ml sterile nutrient broth, then incubated at 37^0c for 2 -3 hours (till light to moderate turbidity develops).

Agar plates were dried at 37^0c for 30min until when no visible moisture was observed from the surfaces. Using a sterile Pasteur pipette, 2mls of the diluted suspension was dispensed on the surface of the dried plates and the plates were tipped at different directions to wet the whole of its surface. Excess fluids were decanted and plates allowed to dry for about 20 minutes.

Without delay, the antibiotic discs of varying concentrations were applied aseptically on the surface of the inoculated plates with adequate spacing using sterile fine pointed forceps and pressed gently to ensure full contact with the medium according to CLSI 2005 criteria (7).The plates were then incubated at 37^0c for 24 hours.

The antibiotics used were: Gentamycin (10ug)(Abtek), Ciprofloxacin (5ug)(fidson & co), Pefloxacine (5ug)(fidson & co), Ofloxacin (30ug)(Nigerian German Chemicals), Ceftriazone (30ug)(Swipha), Augmentin (25ug)(Fidson & co), Amoxicillin (20ug)(Fidson & co), Levofloxacin (10ug)(Emzor pharma), Nitrofurantoin (300ug)(Fidson & co), Cotrimozxazole (30ug)(M & B) Tetracycline (30ug)(Abtek).

After overnight incubation, the diameters of zones of inhibition were measured using millimeter ruler. The susceptible and resistant inhibitory zone diameter breakpoint used throughout the study was $\geq$ 1.5mm indicating sensitivity and $\leq$ 1.5mm indicating resistance based on CLSI 2006 recommendation (CLSI 2006) sensitivity pattern was compared with standard *Pseudomonas aeruginosa* (NCTC 10662). Different antibiotic discs were obtained from the following companies: - Fidson & Co, Nigeria German Chemicals, Glaxo, Swipha, Abtex and Oxiod (uk).

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of selected Isolates (5 multi resistant and 5 sensitive isolates) was determined using broth dilution method (8). Antibiotics used were ciprofloxacin (500mg) pefloxacine (500), ofloxacine (500mg), ceftriaxone (1000mg), Augmentin (500mg) Amoxicillin(500mg), levofloxacin (500mg), nitrofurantoin (300ug) cotrimoxazole (480mg), tetracycline(250mg).

Stock solutions of the antibiotics were prepared by dissolving the powder of each into 10ml sterile buffered distilled water. Dissolution of the antibiotics at the desired concentrations was prepared in sterile nutrient broth. The preparation and dilution of antibiotics were all done on the day of use. Data were captured and analyzed on statistical package for social science (SPSS for windows version 15.0.) at P=0.05

Results

A total of 511 clinical samples were processed. 373 (73%) yielded bacterial growth of which 69 (13.5%) were identified as *Pseudomonas aeruginosa* .There was considerable variation in the infection of *Pseudomonas aeruginosa* as classified by the clinical sources. Urine and wound swabs recorded the highest figures 36 (7%) and 24 (4.7%) respectively, 4(0.8%) each were obtained from blood culture and sputum while the least was found in pleural fluids1 (0.2%). (Table 1).

Table 2 shows the sociodemographic features as regards *Pseudomonas aeruginosa* infections. There was no significant difference between the male 35 (50.3%) and female 34 (49.67%) (P>0.05) while *Pseudomonas aeruginosa* infection was prevalent between the age group 31 – 40years 18 (26%). The sociodemographic study further reveals that Civil Servants 25(36%) had the highest rate of *Pseudomonas aeruginosa* infections with less than one month duration of infection 43(62%).Table 2 also shows the risk factors associate with *Pseudomonas aeruginosa* infections. Results from this table shows that co-mobility such as high blood pressure and diabetes mellitus have no major clinical significance on Pseudomonas aeruginosa infection (P>0.05) rather the antibiotic history had a major significance as 37 (53.6%) of patients with Pseudomonas aeruginosa infection were previously on antibiotic treatment before visiting the Hospital. 21 (30.4%) were currently on antibiotic treatment while 11 (16%) were not on any antibiotic treatment (P>0.05).

Table3 shows the antimicrobial susceptibilities of *Pseudomonas aeruginosa* isolates. Several differences in antimicrobial susceptibilities indexed by body sites were observed. Only Levofloxacin and Pefloxacin (both fluroqunolones) had the least resistance; 10 (14.5%) and 24 (34.8%) respectively out of all the antimicrobial agents tested. The highest resistance were seen in tetracycline 69 (100%), Amoxicillin 67 (97.1%) and Cotrimoxazole 66 (95.7%).

Table 1: Isolates of *Pseudomonas aeruginosa* from 511 clinical specimens

Source(s)	No of Samples	*P. aeruginosa* Isolates	Percentage (%)
Urine	225	36	7.00
Blood	41	4	0.80
Wound	132	24	4.70
Sputum	54	4	0.80
Fluids	10	1	0.20
Semen	15	-	-
CSF	4	-	-
Total	511	69	13.5%

Table 2: Sociodemographic characteristics of patients

Variable(s)	Total no of samples (511)	No of *P. aeruginosa* isolates (69)
Gender		
Male	286 (46)	35 (6.8)
Female	225 (44)	34 (6.7)
Age (Range)		
≤ 20	74 (14.5)	11 (2.2)
21 – 30	105 (20.5)	11 (2.2)
31 – 40	122 (23.9)	18 (3.5)
41 – 50	112 (21.9)	14 (2.7)
50 +	98 (19.2)	15 (2.9)
Occupation		
Civil Servants	266 (52.0)	25 (4.9)
Farmer	45 (8.8)	4 (0.78)
Trader	80 (15.7)	19 (3.7)
Artisans	22 (4.3)	4 (0.78)
Others (e.g. students)	98 19.2)	17 (3.3)
Duration of infection		
≤ 1	311(60.9)	43(8.4)
≥ 2	200(39.1)	26(5.1)
Co- morbidity		
Diabetes Mellitus	168 (32.9)	10 (2.0)
Renal Failure	54 (10.6)	5 (9.8)
Liver Disease	44 (8.6)	5 (9.8)
High blood pressure	112 (21.9)	20 (3.9)
HIV/AIDS	56 (11.0)	3 (0.60)
Others	77 (15.0)	26 (5.1)
History of antibiotics		
Currently	211(41.3)	21 (4.1)
Previously	202(39.5)	37 (7.2)
None	98(19.2)	11 (2.2)

Thirty five 35 (50.7%) strains of *Pseudomonas aeruginosa* were obtained from in - patients while 34 (49.3%) were from out - patents. It was observed that strains from in-patients were found to be more resistant to

Levofloxacin (20%) when compared to out – patients (9%). Similarly, Ofloxacin resistance was 51% and 29% for in-patients and out-patients respectively P>0.05). (Table 4).

Table 5 Shows that Levofloxacin sensitive and resistant isolates of *Pseudomonas aeruginosa* were sensitive to lower concentration of the antibiotics (0.078uglml and 0.62ug/ml) respectively while other Fluoroquinolones (Ciprofloxacin, Ofloxacin and Pefloxacin) had relatively higher concentrations. Amoxylcillin, Tetracycline and Cotrimoxazole sensitive and resistant isolates were found to be generally resistant to these antibiotics even at higher concentrations.

It could be observed from table 5 that the Levofloxacin resistant Pseudomonas aeruginosa (LERPA) were all resistant to Amoxylcillin, tetracycline and Cotrimoxazole even at concentrations 16 folds higher than their respective MICs. Whereas other resistant isolates other than iprofloxacin, Ofloxacin and Pefloxacin resistant isolates were sensitive to a moderately higher MICs

Table 3: Antibiotic susceptibility pattern in 69 *P*seudomonas *aeruginosa* isolates

Antibiotics	Total Sensitive (%)	Total Resistant (%)
Ciprofloxacin	41 (59.4)	28 (40.6)
Pefloxacin	45 (65.2)	24 (34.8)
Augmentin	21 (30.4)	48 (69.6)
Ceftriazone	28 (40.6)	41 (59.4)
Gentamycin	13 (18.8)	56 (81.2)
Amoxicillin	2 (2.9)	67 (97.1)
Tetracycline	O (-)	69 (100)
Nitrofurantoin	26 (37.7)	43 (62.3)
Cotrimoxazole	3 (4.3)	66 (95.7)
Ofloxacin	41 (59.4)	28 (40.6)
Levofloxacin	56 (85.5)	10 (14.5)

Table 4: Comparison between antibiotic resistant patterns: In-Patients and Out–Patients

Antibiotics	In–Patients Resistant	Out–Patients Resistant
Ciprofloxacin	19 (54%)	9 (26%)
Pefloxacin	17 (49%)	7 (21%)
Augmentin	29 (83%)	19 (56%)
Ceftriazone	23 (66%)	17 (50%)
Gentamycin	33 (94%)	33 (97%)
Amoxicillin	34 (97%)	33 (97%)
Tetracycline	35 (100%)	34 (100%)
Nitrofurantion	22 (63%)	22 (65%)
Cotrimoxazole	34 (97%)	32 (94%)
Ofloxacin	18 (51%)	10 (29%)
Levofloxacin	7 (20%)	3 (9%)

TABLE 5: Minimum Inhibitory Concentration (MIC, µg/ml) results of used antibiotics against selected multiresistant and sensitive isolates of *Pseudomonas aeruginosa*

Antibiotics	**Mean MIC (μg/ml) of sensitive isolates**	**Mean MIC (μg/ml) of multiresistant isolates**
Ciprofloxacin	0.15	1.25
Pefloxacin	0.15	1.25
Augmentin	1.25	2.50
Ceftriazone	1.25	2.50
Gentamycin	1.25	5.0
Amoxicillin	5.0	10.0
Tetracycline	10.0	10.0
Nitrofurantoin	0.62	2.5
Cotrimoxazole	5.0	10.0
Ofloxacin	0.15	1.25
Levofloxacin	0.078	0.62

Discussion

Pseudomonas aeroginosa due to its flare to exist in varied environment is a leading cause of both nosocomial and community acquired infections. Such existence and corresponding infections is of public health concern because of increased rate of morbidity and mortality (9).Emergence of antibiotic resistance lead to persistence of infection and subsequently high cost of treatment (10).In-vitro susceptibility testing is crucial to assess the resistance pattern in any specific location and for each individual antimicrobial agent (11).

In this study, a prevalence rate of *Pseudomonas aeroginosa* infection of 13.5% from various clinical symptoms was established.This agrees with the study of Diani (12) that reported a prevalence rate of 16.8% in Ibadan.The risk factors associated with *Pseudomonas aeruginosa* infection includes its ability to thrieve in extreme environments which include disinfectants and antiseptics routinely used in hospitals and homes,ability of this bacteria to colonize surgical equipments which inadvertently transfered to unsuspecting patients thereby causing surgical infecttions,the length of stay in the hospital,type of surgical procedures and lack of awareness of aseptic techniques amongst nurses and auxillary staffs (13).

Isolation of *Pseudomonas aeruginosa* was more in patients with Urinary tract Infection (7.0%) followed by those with wound infection (4.0%) which is lower than that obtained in a survey carried out where they had 8.5-10.5% of all nosocomial infections followed by surgical wounds and burns infection(14). The occurance of this bacteria in Urinary Tract Infection and wounds may have been due to poor hygienic practice by the individual that allowed the penetration of this agent.

This study established the fact that majority of *Pseudomonas aeruginosa* infection were found in In- patients(50.4%). This is supported by the work of Morizon and Wenzel (15) that *Pseudomonas aeruginosa* infection is responsible for an increasing proportion of infection acquired in modern Hospital setting due to the lenght of stay and debilitating condition of the patients.

The pattern of spread of *Pseudomonas aeruginosa* infections involves all the age groups. infections caused by *Pseudomonas aeruginosa* is not specific or limited to any age group due to the fact that this bacteria is widely distributed in nature and has reservour host, its minimal nutritional requirements and its tolerance to a wide range of physical conditions (16).

In this study, co-morbidity factors in Pseudomonas aeruginosa infection in high blood pressure is (3.9)% and (2%) in diabetes mellitus. In diabetic patients, there is immunosuppresion and this bacteria has the potential to cause bactereamia which is indicated with the prevalence of 0.8% isolation of Pseudomonas aeruginosa from blood cultures. (17). It can be postulated that the exotoxins produced by these organisms while infecting the host can induce high blood pressure.(16).

The use of antibiotics is very vital in the treatment of bacterial infections. In this study, the patients that has previousely used antibiotics before reporting to the hospital was 39.9%. This is very remarkable in that this group had 7.2% *Pseudomonas aeruginosa* isolated. The implication of this is that they will present with high antibiotic resistance.

The antimicrobial activity against isolates showed a varied susceptibility patterns. In this study, it was established that the 4-amino quinolones showed high sensitivity patterns.Ciprofloxacin and Ofloxacin showed 40.6% resistance each while Levofloxacin and Pefloxacin showed 10% and 24% respectively. Odugbemi*(*18) documented a 100% sensitivity of *Pseudomonas aeruginosa* to Ciprofloxacin in Lagos whereas Oni(19) and Diani (12) reported 15% resistance respectively in Ibadan while Ozumba(20) reported a 37.9% resistance of Pseudomonas aeruginosa to Ciprofloxcin. The moderate resistance recorded in this study by these quinolones may be due to spontaneous chromosomal mutations in the target site of these antibiotics, topoisomerase IV or DNA gyrase or by the induction of a multidrug efflux pump (21).

Augumentin and Amoxycillin recorded 69.6% and 97.1% depicting a high resistance level. These could be due to the fact that these B-lactam drugs are effective against gram postive bacteria while Pseudomonas aeruginosa is a gram negative bacteria. The lower resistance recorded with Augmentin could be due to a combination of two broad spectrum penicillins.

The high level of resistance to third generation Cephalosporin (Ceftriazone 59%) could be due to the presence of B-lactamase and Extended Spectrum Beta-Lactamases (ESBLS) although they were not sought for in this study (22).

Tetracycline recorded a 100% resistance majorly because of its mechanism of action. It is majorly used against Gram Positive Bacteria and some enteric bacteria.The level of resistance was significantly high than that reported by Kavlowsky in their studies where a surveillance of 65 laboratories in the United States from 1998-2001 found 90% of isolates of Pseudomonas aeruginosa to be susceptible to these antibiotics.(23)

The difference between this study and that of Kavlowsky (23) may be due to the potency of the drugs, as adequate storage patterns may not be followed, such that the drugs may have been exposed to high temperatures and secondly, self medication is widely practiced in Nigeria, such that the patients have taken these drugs prior to reporting to the hospital.

The susceptibility of Pseudomonas aeruginosa strains according to hospitalized patients (in-patients) and out-patients indicate that the isolate from the in-patients were more resistant to the antibiotics than the out-patients. This conforms with previous study (24) that strains from in-patients are usually more resistant than the strains from out-patient. The factor that may influence this trend can be attributed to either Pseudomonas aeruginosa strains were circulating within hospital environment or were being acquired from health workers that may be healthy carriers. Because of the increasing multiresistance strains in many hospitals, emperical usage of antibiotics is either abandoned or restricted in order to take the developing resistance rates under control (10). If emperical therapy must be used against this organism, it may require the initial use of two or more agents, until susceptibility testing results are known. Combination will suffice only for empirical therapy; they have not been shown to definitely reduce the development of resistance against modern B – lactams, and there is risk that this approach could encourage resistance to both agents (25). In this study, comparision have been made by using minimum inhibitory concentration (MICs) since it seems to be the most valid system when measuring antibiotic effects than concentration value (26). The high resistance rate of most antibiotics used was found to correlate with high MICs of the respective antibiotics.

Conclusion

The infection cause by *Pseudomonas aeroginosa* is wide spread in various clinical conditions, and presents high resistant pattern with a variety of antibiotics. Thus continuous evaluation of these infectious agents is essential to reduce the financial burden and productive hours on the patients. This involve continuous and accurate monitoring of infection processes in our hospitals, continuous education of all health care professionals in basic infection control procedures and policies. New information on antimicrobial resistance is recommended to increase detection and identification of infection, follow up study conducted to measure the improvement in the use of aseptic techniques by both physicians and nurses and indiscriminate use of antibiotics completely discouraged

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