Antibiotic Resistance In Clinical Isolates Of Pseudomonas Aeruginosa In Enugu And Abakaliki, Nigeria

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Abstract

The resistance of Pseudomonas aeruginosa isolates from pus samples obtained from wound infection patients in Enugu and Abakaliki were investigated. The paper disc diffusion technique was used to determine the sensitivity of amoxycillin, co-trimoxazole, streptomycin, gentamicin, chloramphenicol and ciprofloxacin to the isolates. The result of the study showed that, out of fifty pus samples screened, 34(64%) yielded P. aeruginosa. The highest resistance obtained was recorded for amoxycillin (88.2%), followed by co-trimoxazole(76.5%), streptomycin(67.6%), gentamicin(58.8%), chloramphenicol(58.8%) and ciprofloxacin(23.5%). This study heralds the presence of resistant strains of P. aeruginosa in the areas of study and recommends the use of ciprofloxacin for effective management of wound infections in Enugu and Abakaliki.

INTRODUCTION

Pseudomonas aeruginosa is a gram negative, asporogenous, obligate aerobic, motile and oxidase positive bacilli, usually found in the intestinal tract, water, soil and sewage ([1, 2]). Their pathogenic potentials unveil among individuals with compromised immune system ([3]). They are known to be one of the major causes of nosocomial pneumonia and spread mainly through hospital equipments and healthcare workers rather than person to person ([4, 5]). Their frequent contamination of ventilators and hospital equipments is attributed to the fact that they are resistant to temperature extremes and drying among others ([4]). The infection could be invasive or toxigenic ([6]).

P. aeruginosa is involved in the etiology of several diseases including endocarditis, meningitis, bronchopneumonia, ocular, burn and wound infections ([4, 7, 8]). Wound infection is one of the major causes of limb amputations in Nigerian children ([9]).

The greatest challenge facing the effective management of P. aeruginosa infection is multiple drug resistance ([10]). Emergence of drug resistant strains of P. aeruginosa has been reported in Ibadan and Ile Ife, Nigeria ([8, 11]). The implication of these emerging resistance in the successful treatment of infections caused by P. aeruginosa cannot be overemphasized. This study therefore aims at determining the resistant pattern of P. aeruginosa isolates from wound infection patients in Enugu and Abakaliki to commonly used antibiotics including Ciprofloxacin, Gentamicin, Co-trimoxazole, Streptomycin, Amoxycillin and Chloramphenicol

MATERIALS AND METHODS

ISOLATION OF TEST ORGANISM

A total of fifty pus samples were collected from wound infection patients at the National Orthopedic Hospital Enugu (NOHE) and Ebonyi State University Teaching Hospital (EBSUTH) Abakaliki between July and September 2006. Sixteen samples were obtained from NOHE while thirty four were collected from EBSUTH using sterile swab sticks. The samples were inoculated into nutrient agar media and incubated at 37 ° C for 24 hours. Subsequently the colonies were sub-cultured using the streak plate technique ([12]).

The isolates were identified using colony morphology, motility testing, Grams reaction and Biochemical tests as described by Cheesbrough ([1]) and Amadi and Ayogu, ([13]).

PREPARATION OF ANTIBIOTIC DISCS

The antibiotic disc used for the study were prepared according to the method described by Isu and Onyeagba ([12]).The antibiotics used were Chloramphenicol, Ciprofloxacin, Amoxycillin, Streptomycin, Gentamicin and Co-trimoxazole obtained from a pharmaceutical company in Abakaliki. All the antibiotics had the official registration number of the National Agency for Food and Drug

administration and Control (NAFDAC) Nigeria, with their expiration dates ranging from June 2008 to July 2010. The discs were impregnated with the antibiotics to obtain the following concentrations per disc, Ciprofloxacin (10µg), Streptomycin (30µg), Amoxycillin (30µg), Gentamicin (10µg), Chloramphenicol (30µg) and Co-trimoxazole (30µg).

ANTIBIOTIC SENSITIVITY SCREENING

Overnight old cultures of the respective isolates, adjusted to McFarland 0.5 ($_1$) were used for the sensitivity testing. The Kirby-Bauer modified disc diffusion technique ($_1$) was used to determine the sensitivity of the antibiotics. The discs were evenly distributed on the sensitivity agar in such a way that they were 15mm from the edge of the plate and 25mm from one disc to another. Each disc was slightly pressed down to ensure its sufficient contact with the agar media. The plates were then incubated at 37 ° C for 24 hours. The inhibition zone diameter (IZD) were subsequently measured using meter rule. The diameter of the disc (6mm) were subtracted from the total inhibition zone diameter of each antibiotic disc to obtain the final IZD. Resistance were regarded as IZD between 0 –5mm while sensitivity was considered IZD above 5mm ($_{14}$).

RESULTS

ISOLATION AND IDENTIFICATION OF ISOLATES

Out of the fifty pus samples obtained from patients in Enugu and Abakaliki, 34 (68%) yielded Pseudomonas aeruginosa (Table 1).

SENSITIVITY PATTERN OF ANTIBIOTICS ON ISOLATES

The isolates had their highest resistance against amoxycillin (88.2%) and the least against Ciprofloxacin (23.5%) (Table 2,3). Seven isolates (20.6%) were 100% resistant to all the antibiotics screened in this study (Tabe 3). It was also observed that 79% of the isolates were multi-drug resistant.

Figure 1

Table 1: Frequency of isolation of from different sources

Source	No. of samples	No. of samples yielding *P. aeruginosa*
EBSUTH Abakaliki	16	12 (75%)
NOH Enugu	34	22 (64.7%)
Total	50	34 (68%)

Key: EBSUTH - Ebonyi State University Teaching Hospital
NOH - National Orthopedic Hospital

Figure 2

Table 2: Inhibition zone diameter (Izd) of antibiotic against different isolates of

Isolates	Antibiogram						
	Cip.	Cotr.	Strep.	Gent.	Chlor.	Amox.	PR
1.	S**	R	R	R	S*	R	66.7
2.	S**	R	R	R	S*	R	66.7
3.	S**	R	R	R	R	R	83.3
4.	R	R	R	R	R	R	100
5.	S**	R	R	R	S*	R	66.7
6.	R	R	R	R	R	R	100
7.	S**	R	R	R	R	R	83.3
8.	S*	S*	S*	S*	S*	R	16.7
9.	R	R	R	R	R	R	100
10.	R	R	R	R	R	R	100
11.	S**	S*	R	R	S*	R	50
12.	S**	R	R	S*	R	R	66.7
13.	S*	R	R	S**	R	S*	50
14.	S**	R	R	R	R	R	83.3
15.	S**	S***	S**	S**	S***	S**	0
16.	S**	S***	S**	S*	S**	S**	0
17.	S*	R	R	R	R	R	83.3
18.	S**	S*	S**	S*	S*	R	16.7
19.	R	R	R	R	R	R	100
20.	S*	S***	S**	S**	S**	S*	0
21.	S**	R	R	R	R	R	83.3
22.	S**	S*	S**	S*	S**	R	16.7
23.	R	R	R	R	R	R	100
24.	S**	R	R	R	R	R	83.3
25.	R	R	R	R	R	R	100
26.	S**	R	R	R	R	R	83.3
27.	S*	R	R	S*	R	R	66.7
28.	S**	S*	S**	S**	S*	R	16.7
29.	S**	R	R	R	S*	R	66.7
30.	S*	R	R	S**	S**	R	50
31.	S**	R	S**	S**	R	R	50
32.	R	R	S*	S*	R	R	66.7
33.	S**	R	S**	S*	R	R	50
34.	S**	R	S*	S*	S*	R	33.3

Key: 0-5mm Resistant (R), 6-15mm Sensitive (S*), 16-25mm Sensitive (S**), 26-35mm Sensitive(S***), PR- Percentage resistance

Figure 3

Table 3: Summary of the resistance pattern of isolates to different antibiotics

	Cotr.	Strep.	Cip.	Gent.	Chlor.	Amox.
%	76.5	67.6	23.5	58.8	58.8	88.2

Key:
Cotr. - Co-trimoxazole
Strp. - Streptomycin
Cip. - Ciprofloxacin
Gent. - Gentamicin
Chlor. - Chloramphenicol
Amox. - Amoxycillin

DISCUSSION

Microbial infection is one of the major serious complications

in wound patients. The result of this study showed that over 65% of the pus samples collected from Enugu and Abakaliki yielded Pseudomonas aeruginosa (Table 1). This goes to confirm that P. aeruginosa is a major factor in the etiology of wound infection. Previous studies had reported isolation of P. aeruginosa from pus samples obtained from post operative wound infections ([15]) and open fractures([8]).

Ciprofloxacin has been stated to be the most potent drug available for the treatment of P. aeruginosa infections ([16]). This report is in conformity with the result of this study in which ciprofloxacin recorded the least resistance (23.5%) to P. aeruginosa isolates from wound infection patients (Tables 2,3). Similar reduced resistance of P. aeruginosa to ciprofloxacin has been reported in Jamaica(19.6%) (Brown and Izundu,2004), Latin America (28.6%),([18]), Ilorin Nigeria (24.7%) ([19]) and in Kuala Lumpur, Malaysia (11.3%) ([20]). It is undoubtable that at the present time, ciprofloxacin is the most effective antibiotics against P. aeruginosa involved in wound infection relative to most other commonly used drugs. The comparatively high cost of ciprofloxacin may have reduced the rate of misuse. It is hoped however, that it will be spared- at least for a reasonable time, from gross abuse and under-storage which ultimately culminates to resistance.

Gentamicin, like ciprofloxacin is one of the drugs generally considered to be effective in the treatment of infections caused by resistant strains of P. aeruginosa. This study however revealed that 58.8% of P. aeruginosa isolates were resistant to gentamicin (Table 2,3). This level of resistance is quite high compared with previous reports in which 40.2% and 12.9% resistant strains were respectively reported in Ilorin, Nigeria ([19]) and Kuala Lumpur, Malaysia ([20]). The finding in this study calls for caution in the use of gentamicin as first line drug for the treatment of P. aeruginosa infection .

The 67.6% resistance of P. aeruginosa isolates in this study to streptomycin is high compared with the report of Fadeyi and co-workers ([19]) in Ilorin, Nigeria, in which a resistance rate of 36% was identified. The exact reason for this wide variation in resistance was not immediately apparent.

Furthermore, the high rate of resistance to chloramphenicol (58.8%), amoxycillin (88.2%) and co-trimoxazole (76.5%) as recorded in this study appears to corroborate a previous suggestion ([17]) that these three drugs should no longer be considered effective for the treatment of P. aeruginosa wound infection.

Seventy nine percent of P. aeruginosa strains isolated in this study were resistant to more than one antibiotic. This type of multiple resistance is one of the greatest challenges facing the clinician in the management of infections ([21]).

The relatively high resistance of P. aeruginosa isolates to commonly used antibiotics as recorded in this study gives course for worry, especially in the developing nations were most of these antibiotics still serve as first line drugs. The inordinate accessibility of antibiotics in shops and open markets as well as consumption of drugs without proper medical prescription- a common practice in resource poor countries, is probably an important factor worthy of consideration, if any success in the fight against microbial resistance to drugs is anticipated. Routine sensitivity screening of antibiotics before prescription is suggested. Also, the urgent need for health systems in the developing nations to strategize on appropriate drug administration channel based on their peculiar circumstances is a compelling necessity.

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