ANTIBIOTIC SUSCEPTIBILITY OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM PATIENTS IN A SPECIALIST HOSPITAL IN LAFIA, NIGERIA.

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ABSTRACT

Infection is one of the most frequent and severe complications in patients who have sustained wounds; and the bacterium Pseudomonas aeruginosa is an important etiological agent of wound infections. This study evaluated the antibiotic susceptibility of Ps. aeruginosa isolated from wounds of patients who attended Dalhatu Araf Specialist Hospital, Lafia, Nigeria. A total of 59 wound swab samples were collected between February and April, 2014. Twelve (12) Ps. aeruginosa isolates were obtained using standard bacteriological procedure. The isolates were tested for their susceptibility to a range of commonly used antimicrobials using the Kirby-Bauer disc diffusion method based on protocol of the United States of America's Clinical and Laboratory Standards Institute. The isolates were highly susceptible to ciprofloxacin (100%) and gentamicin (100%); and moderately susceptible to ofloxacin (50.0%) and ceftriaxone (58.3%). Low susceptibilities were observed to perfloxacin (41.7%), augmentin (33.3%), tetracycline (25.0%) and ampicillin (16.7%). All (100%) isolates were resistant to nitrofurantoin, cotrimoxazole and streptomycin. Multiple antibiotic resistance (MAR) was observed in all (100%) of the isolates tested. The MAR indices were 0.2 and above, an indication that all the isolates have been exposed to antibiotic selective pressure. In view of the foregoing observations, gentamicin, ciprofloxacin, ceftriaxone and ofloxacin may be useful in the treatment of wound infection caused by Pseudomonas in this study area. There is thus, a need to control antibiotic usage in this study area.

Keywords: Antibiotic Susceptibility; *Pseudomonas aeruginosa*, Multiple Antibiotic Resistance

INTRODUCTION

Wound infections have been regarded as the most common nosocomial infections and are associated with increased morbidity and mortality (Iroha *et al*., 2008; Christopher *et al.*, 2011). The development of wound infection depends on the integrity and protective function of the skin (Anupurba *et al.*, 2010). *Pseudomonas aeruginosa* is an opportunistic pathogen in burn and cystic fibrosis patients all over the world (Mirsalehian *et al.*, 2010), especially because of its natural resistance to many classes of antibiotics and also, for its potential virulence factors plus additional acquired resistance due to plasmids. It is also the most common gram negative bacterium associated with wound infections (Song *et al*., 2003; Lister *et al.*, 2009; Garba *et al.*, 2012). This pathogen is intrinsically resistant to most antibiotics such as β-lactams, quinolones, chloramphenicol, tetracyclines, macrolides, trimethoprim-sulfamethoxazole, and rifampin (Dundar *et al.*, 2008). Resistance in *Ps. aeruginosa* may be due to outer membrane modifications, production of extended-spectrum beta-lactamase and efflux pumps, which confers various levels of resistance to expanded spectrum cephalosporins, such as cefotaxime, ceftazidime, and aztreonam (Mirsalehian *et al.*, 2010; Tam *et al.*, 2010).

The wide spread use of antibiotics together with the length of the time over which they have been available have led to major problems of resistance pathogens in wound infections contributing to morbidity, and mortality (Nwachukwu *et al.*, 2009). However, this study was designed to isolate, identify and determine the susceptibility of *Ps. aeruginosa* from wounds of patients attending Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Sample Collection

A total of 59 wound swab samples were collected between February and April, 2014 from patients in Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria.

Isolation and Identification of *Pseudomonas aeruginosa*

The wound swab samples were cultured on chocolate and MacConkey agar plates which were incubated aerobically at 37°C for 24 h. The colonies that grew on chocolate and MacConkey agar were identified based on colonial morphology (the size, shape, colour, elevation, opacity, consistency, surface and pigmentation), Gram stain reaction and biochemical tests as described by Cheesbrough (2000).

Antibiotic Susceptibility Testing

The antibiotic susceptibility testing was carried out using Kirby-Bauer disc diffusion method as modified by Clinical and Laboratory Standards Institute (CLSI, 2007). Briefly four (4) colonies of the isolates were transferred into 5 ml of sterile normal saline in a tube such that the turbidity of the bacteria suspension was equivalent to 0.5 McFarland Standard. The sterile swab was moistened in the bacterial suspension and streaked on Mueller-Hinton Agar (MHA). The antibiotic discs were aseptically placed on the MHA plate previously inoculated with the isolate. The plates

were incubated at 37ºC for 24 h. The diameters of zones of inhibition were measured using meter rule and the result was interpreted in accordance with the susceptibility break-points (CLSI, 2007).

RESULTS AND DATA ANALYSES

Antibiotic Susceptibility

The antibiotic susceptibilities of the twelve strains of *Ps. aeruginosa* isolated from wound swabs of patients who attended the hospital are shown in Table 1. They were highly susceptible to ciprofloxacin (100%), gentamicin (100%), ofloxacin (50%) and ceftriaxone (58.3%); but less susceptible to perfloxacin (41.7%), augmentin (33.3%), tetracycline (25.0%) and ampicillin (16.7%). All (100%) isolates were resistant to nitrofurantoin, cotrimoxazole and streptomycin (indicate the percentage resistance).

Antibiotic Resistance Phenotypes

The antibiotic resistance phenotypes of the *Ps. aeruginosa* isolates are shown in Table 2. The commonest resistant phenotypes were: COT-STR-TET (16.7%), STR-PN-AUG-PFX-OFX-TET-COT (16.7%) and AMX-AUG-OFX-PFX-STR-COT-CRO-TET (16.7%).

Multiple Antibiotic Resistance (MAR) and MAR Indices

Multiple antibiotic resistance, defined as resistance to at least two antibiotics was observed in all (100%) of the isolates. The Multiple Antibiotic Resistance Index (MARI) of *Ps. aeruginosa* isolates are shown in Table 3. The most common MARI were 0.3, 0.7 and 0.8 observed in 3(25.0%) of the isolates. When MAR index is greater than or equal to 0.2, it indicates that the organism originates from an area where antibiotics are freely available and misused (Krumpermann, 1983).

Table 1: Antibiotic Susceptibility of *Pseudomonas aeruginosa* isolated from wound swabs of patients

Antibiotic	Disc Potency (µg)	No. (%) Susceptibility
Ampicillin (PN)	30	2(16.7)
Augmentin (AUG)	30	4(33.3)
Nitrofurantoin (NIT)	30	0(0.0)
Ciprofloxacin (CPX)	10	12(100.0)
Ofloxacin (OFX)	10	6(50.0)
Perfloxacin (PFX)	10	5(41.7)
Cotrimoxazole (COT)	30	0(0.0)
Gentamicin (GEN)	10	12(100.0)
Ceftriaxone (CRO)	10	7(58.3)
Tetracycline (TET)	30	3(25.0)
Streptomycin (STR)	30	0(0.0)

Table 2: Antibiotic resistance phenotypes of *P. aeruginosa* isolated from wound swabs of patients

Antibiotic Resistance phenotype	No (%) Resistance phenotypes of isolates
PN-TET-COT	1(8.3)
COT-STR-TET	2(16.7)
PN-AUG-STR-COT	1(8.3)
PN-STR-COT-TET	1(8.3)
PN-AUG-STR-OFX-PFX-COT	1(8.3)
PN-AUG-CRO-STR-PFX-COT-TET	1(8.3)
STR-PN-AUG-PFX-OFX-TET-COT	2(16.7)
AMX-AUG-OFX-PFX-STR-COT-CRO-TET	2(16.7)
PN-AUG-CRO-STR-PFX-COT-TET-OFL	1(8.3)

PN = Ampicillin; TET = Tetracycline; COT = Co-trimoxazole; STR = Streptomycin; AUG = Augmentin; OFX = Ofloxacin; PFX = Perfloxacin; GEN = Gentamicin; CRO = Ceftriaxone; CPX = Ciprofloxacin; NIT = Nitrofurantoin.

Table 3: Multiple Antibiotics Resistance (MAR) Indices of *Pseudomonas aeruginosa* isolated from wound swabs of patients.

No. of antibiotics resistant to (a)	No. of antibiotics tested (b)	No. (%) of MAR isolates	*MAR index (a/b)
8	10	3(25.0)	0.8
7	10	3(25.0)	0.7
6	10	1(8.3)	0.6
4	10	2(16.7)	0.4
3	10	3(25.0)	0.3
2	10	2(16.7)	0.2

*MAR= Resistance to at least two antibiotics

DISCUSSION

Wound infection is one of the most frequent and severe complications in patients who have sustained wounds (Zogyani *et al.,* 2002). *Pseudomonas aeruginosa* is a bacterium which is a common etiological agent of wound infections, especially of thermal burns. This is because burns have large exposed areas of dead tissue free of any defenses and therefore, are ideal sites for infection by bacteria from the environment or normal microbiota.

The low susceptibilities of *Ps. aeruginosa* isolates in this study to streptomycin, nitrofurantoin, co-trimoxozole, ampicillin, tetracycline, augmentin and perfloxacin were not surprising. This could be as a result of misuse, overuse and inclusion of antibiotics in animal feed; also most of these agents are orally administered which can be easily abused (Garba *et al*., 2012). The low susceptibilities of *Ps. aeruginosa* observed in this study is not different from those of previous studies reported elsewhere (Iheanyi *et al.*, 2009; Mirsalehian *et al.,* 2010; Tam *et al.*, 2010; Garba *et al.,* 2012). Resistance in *Ps. aeruginosa* may be due to outer membrane modifications, production of extended-spectrum beta-lactamase and efflux pumps, which confers various levels of resistance (Mirsalehian *et al.,* 2010; Tam *et al.,* 2010).

The high susceptibilities observed in this study to ciprofloxacin, gentamicin, ceftriaxone, and ofloxacin, although expected, were in contrast with previous studies (Garba *et al*., 2012) but is similar to another study by Iheanyi *et al*. (2009) Most of these antibiotics are administered by injection and because of the discomfort and pains of the injection it cannot be easily abused. The *Ps. aeruginosa* isolates from this study were distributed into different resistant phenotypes and the commonest in this study implies that the organism have previous exposure to the antibiotics suspected to may have been abused within the location.

CONCLUSION

The *Ps. aeruginosa* isolates were highly susceptible to gentamicin, ciprofloxacin, ceftriaxone and ofloxacin; and these antibiotics may be useful in the treatment of wound infection with *Pseudomonas* in this location In view of the observation that the isolates originate from an area of high antibiotic selective pressure, it is imperative to educate the public about the consequences of irrational antibiotic use.

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