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ANTIBIOTIC RESISTANCE PATTERN OF UROPATHOGENIC *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM A NIGERIAN HOSPITAL

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ABSTRACT

Bacterial resistance to antibiotics is a global problem which results in difficulty in treatment. Surveillance study should always be carried out in different geographical location to determine current effective antibiotics against bacterial infections. The purpose of this study therefore is to study prevalence of *Pseudomonas aeruginosa* in infections in a Nigerian hospital over a two months period and determine its resistance pattern. *Pseudomonas aeroginosa* strains were isolated from fifty five clinical samples comprising endocervical swab (12) and urine samples (43) of outpatients at Igbinedion University Teaching Hospital between April and May, 2010. Identification of *P. aeroginosa* strains were based on growth on selective agar media, oxidase and Gram's reactions. Antibiotic susceptibility testing of the *P. aeroginosa* strains was done against Ciprofloxacin, Doxycycline, Trimethroprim/Sulphamethazole, Chloramphenicol and Gentamicin using the disk agar diffusion method and Minimum Inhibitory Concentration (MIC) determination by macrodilution method. Twelve *P. aeroginosa* strains were isolated and identified. The observed percentages of resistance to Ciprofloxacin, Doxycycline, Trimethroprim/Sulphamethazole, Chloramphenicol and Gentamicin were 50%, 83%, 83%, 67%, and 75% respectively. The strains were highly resistant to Doxycycline, Gentamicin and Trimethroprim/Sulphamethazole while relatively susceptible to Ciprofloxacin. All the resistant strains exhibited very high MIC to Trimethroprim/Sulphamethazole and Gentamicin. Resistance of *P. aeroginosa* to antibiotics results in difficulties in treatment of urogenital infections caused by the organism. This study calls for strict control in the use of antibiotics for chemotherapy of pseudomonas infections to reduce the organism's resistance to commonly used antibiotics.

KEYWORDS: Resistance, Antibiotics, Uropathogens, Hospital-acquired Infections

INTRODUCTION

Urogenital infections are common infections that affect human urinary tract, especially those of women. The incidence of urinary tract infections, bacterial vaginosis and yeast vaginitis, is estimated to affect one billion women each year [1]. Urinary tract infection (UTI) remains a worldwide therapeutic problem, not only causing nosocomial disease but also community acquired infections [2]. The 2006-7 report by National Healthcare Safety Network (NHSN) at the Centers for Disease Control and Prevention (CDC) ranked *P. aeruginosa* as the sixth most common healthcare associated pathogen-causing infection. Also, it is typically found at even higher rank in studies focused on the intensive care unit (ICU). [3]

Therapeutic approaches to treatment of bacterial urogenital infections have remained essentially unchanged for many years. Current therapy involves long-term, low-dose antibiotic treatment which involves the active killing of bacteria that enter the bladder. However, *P. aeroginosa* is a highly prevalent opportunistic pathogen. It is currently one of the most frequent nosocomial pathogens and the infections due to this organism are often difficult to treat due to antibiotic resistance [3]. Drug resistance is a consequence of evolution and is a response to selection pressures affecting all living organisms. Individual organisms that are not as susceptible to the drug effects are capable of

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surviving drug treatment, and therefore have greater fitness than the more susceptible individuals.

Community and noseocomial multidrug resistant *P. aeruginosa* is an important health care problem worldwide that prolongs the duration of treatment, thereby increasing the cost of patient care. Considering the problem of *P. aeruginosa* infections and its multidrug antibiotic resistance, the present study has been carried out to determine current antibiotic resistance pattern among *P. aeruginosa* strains isolated from outpatients in a Nigerian hospital and to determine antibiotics that are effective against uropathogenic *P. aeroginosa*.

MATERIAL AND METHODS

Collection and analysis of samples

Fifty five samples comprising of 12 Endocervical Swab (ECS) and 43 urine samples were collected from outpatients at Igbinedion University Teaching Hospital, Okada, Nigeria by the staff of Medical Microbiology unit of the hospital between April and May, 2010.

The samples were microbiologically examined for colonial morphology and lactose fermenting ability to exclude lactose fermenters. Non lactose fermenters were selected and Gram stained.

Identification of Pseudomonas aeroginosa strains

The following biochemical tests were done to confirm the identities of the isolates. Oxidase test was done by making a smear of the suspected organism on a filter paper coated with oxidase reagent. The production of a purple coloration within 10 seconds is an oxidase positive reaction. Gram negative, non-lactose fermenters, oxidase positive isolates were further grown on cetrimide agar to confirm the presence of *P. aeroginosa* strains. Cetrimide agar mixed with glycerol was sterilized for 15 minutes at 121^{0}C. The mixture was poured into bottles and cooled in slant form. The suspected *P. aeroginosa* strains were inoculated on cetrimide agar slant and incubated at 37^{0}C for 24 h. Change of colour of the cetrimide agar from blue to green after incubation confirms the presence of *P. aeroginosa*

Antibiotic susceptibility tests

The susceptibility of the bacteria to different antibiotics was evaluated by disk diffusion method and the results interpreted according to a breakpoint standard indicated by National Committee for Clinical Laboratory Standard (NCCLS, USA), using standard antibiotic discs (Oxoid, UK). Eighteen hour old broth culture of *P. aeroginosa* strains of approximately 10^8 cfu/ml which is equivalents to 0.5 MacFarland standards was inoculated onto solidified and sterilized Muller Hinton agar by spread plate method. Five different standard antibiotic disks: Ciprofloxacin (5 µg), Doxycycline (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg) and Trimethoprim/Sulphamethoxazole (TMP/SMX) (1.25/23.75 µg) were placed firmly at least 2 cm apart on the agar plates using a sterile forceps. The plates were kept on the bench for 30 min to allow diffusion of the antimicrobials. They were incubated for 24 h at 37^{o}C and the resultant inhibition zones were measured (in diameter) and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

All isolates that exhibited gross resistance to Ciprofloxacin, Gentamicin and TMP/SMX were assayed for their MIC to the antibiotics. Stock solution of Ciprofloxacin (Bal Pharma, China), Gentamicin (Greenfield, China) and TMP/SMX (Celtech Pharma S.A., Spain) were prepared to concentration of 50 µg/ml, 100 µg/ml and 4000 µg/ml respectively. The MIC of the antibiotics to the bacterial strains was determined by broth dilution method. Briefly, 1 ml of stock antibiotic solution was added to 9 ml of nutrient broth thereby reducing the concentration by 1/10. Four serial dilutions (2-fold) of each resulting solution were done. One hundred microliter of 24 h old bacterial culture of approximately 10^8 cfu/ml which is equivalent to McFarland standard 0.5 was inoculated into each antibiotics/medium mixture and incubated at 37^{o}C for 24 h. The tubes were examined for growth. The least concentration at which no bacterial growth can be discerned is selected as the MIC.

RESULTS

Twelve *P. aeroginosa* strains were isolated from urogenital tract samples comprising seven urine samples and five endocervical swab (Table 1). The identified *P. aeroginosa* strains were Gram negative, presented pale colonies on McConkey agar media. They grew and changed Cetrimide agar from blue to green and the strains were all oxidase positive (Table I). These characteristics confirm the bacterial strains as *P. aeroginosa*

Antibiotic susceptibility tests were performed on all *P. aeroginosa* strains by disc diffusion. The strains were relatively sensitive to ciprofloxacin (50%) while high resistance of 75%, 83%, 67% and 83% were observed in gentamicin, doxycycline, chloramphenicol and Trimethoprim/Sulphamethoxazole (TMP/SMX)

respectively. Each strain had varied overall resistance pattern that ranges between 20% and 100%. *Pseudomonas aeroginosa* OKO 005, 007, 008, 012 were resistant to all tested antibiotics while *P. aeroginosa* OKO 002 was only resistant to one out of the five tested antibiotics. (Table II, Fig I and Fig II).

The MIC of gentamicin, ciprofloxacin and TMP/SMX to some selected resistant *P. aeroginosa* strains were determined to discover the extent of resistance by broth macrodilution. All the tested strains exhibited very high resistance to the tested antibiotics with very high MIC. All tested strains had an MIC of ≥2.5 µg/ml to ciprofloxacin, ≥10 µg/ml to gentamicin and > 400 µg/ml to TMP/SMX (Table III).

TABLE I: Identification of *Pseudomonas aeroginosa* strains by different reactions.

Strains	Source	Gram staining	Oxidase test	Colour on McConkey	Colour on Cetrimide
Pseudomonas aeroginosa OKO 001	Endocervical Swab	+	+	Pale	green
Pseudomonas aeroginosa OKO 002	Endocervical Swab	+	+	Pale	green
Pseudomonas aeroginosa OKO 003	Urine	+	+	Pale	green
Pseudomonas aeroginosa OKO 004	Urine	+	+	Pale	green
Pseudomonas aeroginosa OKO 005	Urine	+	+	Pale	green
Pseudomonas aeroginosa OKO 006	Urine	+	+	Pale	green
Pseudomonas aeroginosa OKO 007	Endocervical Swab	+	+	Pale	green
Pseudomonas aeroginosa OKO 008	Endocervical Swab	+	+	Pale	green
Pseudomonas aeroginosa OKO 009	Endocervical Swab	+	+	Pale	green
Pseudomonas aeroginosa OKO 010	Urine	+	+	Pale	green
Pseudomonas aeroginosa OKO 011	Urine	+	+	Pale	green
Pseudomonas aeroginosa OKO 012	Urine	+	+	Pale	green

TABLE II: Antibiotic resistance pattern of *Pseudomonas aeroginosa* strains

Bacterial Strain	Ciprofloxacin, B.P.<15	Doxycycline B.P. <14	Chloramphenicol B.P.<12	Gentamicin B.P.<12	TMP-SMX B.P. <10
P. aeroginosa OKO 001	22 mm S	0 mm R	0 mm R	16 mm S	14 mm S
P. aeroginosa OKO 002	22 mm S	14 mm S	12 mm S	22 mm S	0 mm R
P. aeroginosa OKO 003	21 mm S	11 mm R	14 mm S	0 mm R	12 mm S
P. aeroginosa OKO 004	22 mm S	10 mm R	11 mm R	0 mm R	0 mm R
P. aeroginosa OKO 005	0 mm R	0 mm R	0 mm R	0 mm R	0 mm R
P. aeroginosa OKO 006	0 mm R	14 mm S	0 mm R	0 mm R	0 mm R
P. aeroginosa OKO 007	0 mm R	0 mm R	0 mm R	0 mm R	0 mm R
P. aeroginosa OKO 008	0 mm R	0 mm R	0 mm R	0 mm R	0 mm R
P. aeroginosa OKO 009	0 mm R	0 mm R	12 mm S	0 mm R	0 mm R
P. aeroginosa OKO 010	22 mm S	0 mm R	22 mm S	26 mm S	0 mm R
P. aeroginosa OKO 011	30 mm S	13 mm R	0 mm R	0 mm R	0 mm R
P. aeroginosa OKO 012	0 mm R	0 mm R	0 mm R	0 mm R	0 mm R

Note: S-Susceptibility; R-Resistance

Fig I: Percentage Antibiotic Resistance Pattern of different Pseudomonas strains

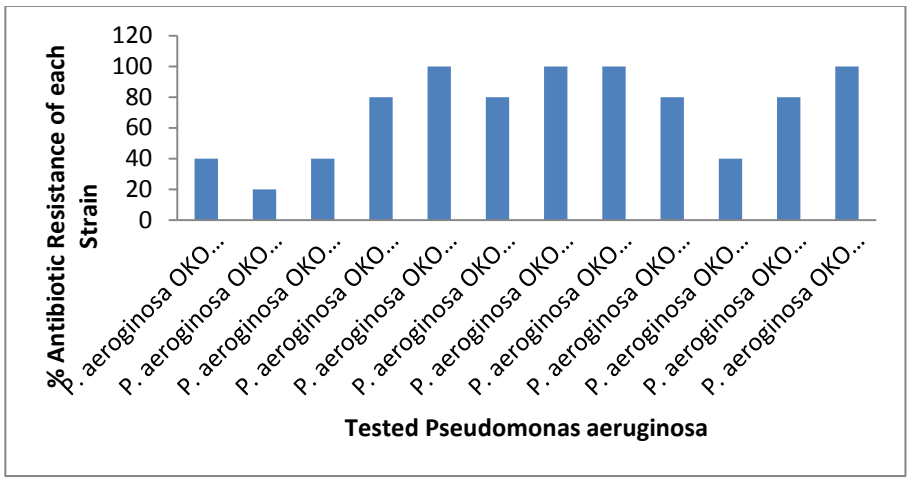

Fig II: Percentage Resistance observed in all Pseudomonas strains to Tested Antibiotics

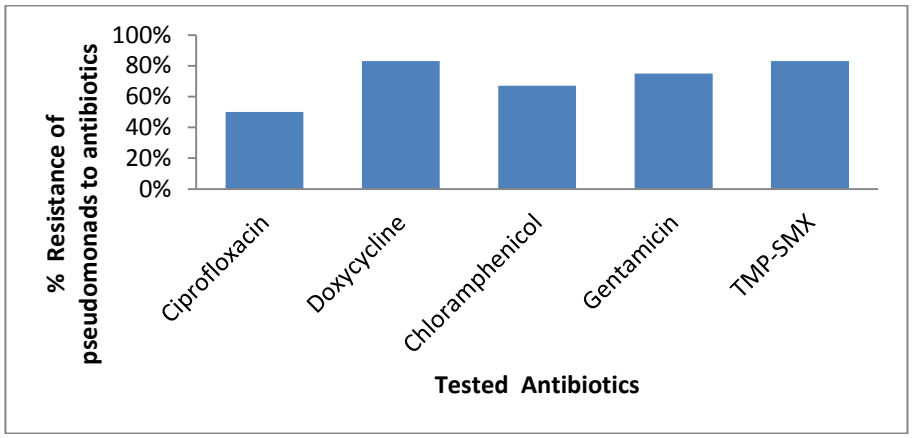

TABLE III: Minimum Inhibitory Concentration of three antibiotics to selected resistant *Pseudomonas aeroginosa* strains

P. aeroginosa strains	MIC
Ciprofloxacin B.P. 0.25 µg/ml	
P. aeroginosa OKO 005	5 µg/ml R
P. aeroginosa OKO 006	> 5 µg/ml R
P. aeroginosa OKO 007	> 5 µg/ml R
P. aeroginosa OKO 008	2.5 µg/ml R
P. aeroginosa OKO 009	> 5 µg/ml R
P. aeroginosa OKO 012	2.5 µg/ml R
Gentamicin B.P. 1 µg/ml	
P. aeroginosa OKO 003	> 10 µg/ml R
P. aeroginosa OKO 004	> 10 µg/ml R
P. aeroginosa OKO 005	> 10 µg/ml R
P. aeroginosa OKO 006	> 10 µg/ml R
P. aeroginosa OKO 007	> 10 µg/ml R
P. aeroginosa OKO 008	> 10 µg/ml R
P. aeroginosa OKO 009	> 10 µg/ml R
P. aeroginosa OKO 011	> 10 µg/ml R
P. aeroginosa OKO 012	10 µg/ml R
TMP/SMX B.P. 32 µg/ml	
P. aeroginosa OKO 002	> 400 µg/ml R
P. aeroginosa OKO 004	> 400 µg/ml R
P. aeroginosa OKO 005	> 400 µg/ml R
P. aeroginosa OKO 006	> 400 µg/ml R
P. aeroginosa OKO 007	> 400 µg/ml R
P. aeroginosa OKO 008	> 400 µg/ml R
P. aeroginosa OKO 009	> 400 µg/ml R
P. aeroginosa OKO 010	> 400 µg/ml R
P. aeroginosa OKO 011	> 400 µg/ml R
P. aeroginosa OKO 012	> 400 µg/ml R

CONCLUSION

In conclusion, there is need for clinicians to imbibe the practice of laboratory based diagnosis before initiating antimalarial treatment, particularly among children in whom data on safety profile of antimalarial medications are limited.

DISCUSSION

Pseudomonas aeroginosa is a common opportunistic human pathogen. It is the commonest Gram-negative bacteria found in nosocomial and community acquired infections. It has been isolated from various body fluids such as sputum, urine, wounds, eye or ear swabs and from blood [4]. It is also one of the bacteria responsible for urinary tract infections. In this study, 12 bacterial strains identified as *P. aeroginosa* were isolated from urine and endocervical swab. Ayeni *et al*, [5] also reported isolation of *P. aeroginosa* from patients with urinary tract infections.

The *P. aeroginosa* strains isolated in this study exhibited very high resistant to tested antibiotics. Bacterial isolates from urinary tract infections cases are usually characterized by steady increase in their level of resistance to commonly used antimicrobials including ampicillin, trimethoprim-sulphamethoxazole (TMP-SMX) or co-trimoxazole and the quinolones with most uropathogens being multidrug resistant [6]. The accumulation of multiple resistance mechanisms in clinical isolates of *P.*

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aeruginosa has resulted in strains that are resistance to all available antibiotics. This pandrug resistance, which means resistant to all antimicrobial agents, together with high attributable mortality, has thrust *P. aeruginosa* into the spotlight as an emerging superbug.

The organisms isolated in this study are multidrug resistant because most of the isolates were resistant to at least 2 different classes of antibiotics. This trend is similar to another study carried out in another part of Africa where >68% of all isolates were resistant to 2 or more antimicrobial [7]. Many factors contribute to occurrence of multi-drug resistant uropathogens in Africa including misuse of antibiotics, counterfeit drugs, shortfall in infection control, public health and also, the fact that many of the resistance genetic determinants are plasmid borne. Plasmid mediates resistance to several other classes of antibiotics and transmission to other microorganisms [8].

The tested *P. aeroginosa* strains were highly resistant to gentamcin and TMP/SMX as observed by the high MIC values. The high rate of resistance to TMP-SMX (co-trimoxazole) by *P. aeroginosa* strains found in this study is very disturbing. This combination is widely used for urogenital infections in Nigeria because of its low cost compared with other antibiotics. It is also commonly used as prophylaxis for HIV infected patients who have a high occurrence in Nigeria. This may account for a possible higher selective pressure on the agent therefore, accounting for increased rate of resistance. Ayeni *et al*, [5] also reported an alarming trend in the resistance of uropathogens isolated from Nigeria to TMP/SMX (Cotrimoxazole). Huovinen *et al*.[9] observed a clear trend in the resistance to TMP-SMX with strains isolated in the developing world being more often resistant than the strains isolated in the developed countries. The clinical significance of this finding is that resistance to co-trimoxazole has been associated with concurrent resistance to other antibiotics resulting in multi-drug resistant uropathogens [10].

Another striking feature found in this study was increased resistance to gentamicin. Various workers have also reported the resistance of *P. aeruginosa* strains to gentamicin [11]. Ayeni *et al*, [5] also reported 60% resistance of *P. aeroginosa* strains to gentamicin. *Pseudomonas aeruginosa* is currently one of the most frequent nosocomial pathogen and the infections due to this organism are often difficult to treat due to antibiotic resistance [12]. The mechanisms of resistance to antibiotics include reduced cell wall permeability, production of chromosomal and plasmid mediated ß-lactamases, aminoglycoside-modifying enzymes and an active multidrug efflux mechanism [8, 13]

In spite of the use of potent antibiotics, high mortality still exists for *P. aeruginosa* infections. Multidrug resistant *P. aeruginosa* is an important health care problem worldwide. From this study, *P. aeroginosa* strains are relatively susceptible to ciprofloxacin while highly resistant to other tested antibiotics. Therefore, ciprofloxacin can be use for treatment of urogenital infections caused by susceptible *P. aeroginosa* strains while the observed resistance to TMP/SMX and gentamicin makes the two antibiotics unsuitable for treatment of urogenital infections caused by *P. aeroginosa* strains.

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