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Prevalence of Aminoglycoside Resistance in Clinical Isolates of *Pseudomonas aeruginosa.*

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Abstract: *Pseudomonasaeruginosa is one of the leading causes of nosocomial infections. Severe infections such as pneumonia or bacteremia are associated with high mortality rates and are often difficult to treat, as the usefulanti-pseudomonial agents are limited. Moreover, P. aeruginosa exhibits remarkable ability to acquire resistance to these agents, and so surveillance to keep abreast of information on susceptibility pattern is crucial. In this study 114 isolates from various specimens previously identified as Pseudomonasspp in LUTH Medical Microbiology Laboratory was collected and identified using oxidase test and Pseudomonas centrimide agar. After identification process 56 of the isolates were found to be P. aeruginosa. The resistance pattern of Pseudomonasaeruginosa to aminoglycosides isolated in LUTH was investigated by disc diffusion method. Amongst the aminoglycosides tested, kanamycin had the highest resistance rate of 71.4%, followed by netilimicin, gentamicin and amikacin showing resistance rate of 67.8%, 44.6% and 35.71 respectively. The result of this study revealed that the resistance rate is high.Surveillance studies are crucial in monitoring antimicrobial susceptibility patterns and selecting empirical treatment regimen. Therefore it is suggested that there is a need for correct, high dosing and combination therapy to minimize the risk of resistance development in cases of P. aeruginosa infections.*

Key words*: Pseudomonas aeruginosa, Aminoglycoside, Resistance, Surveillance, Prevalence*

I. Introduction

Aminoglycosides are compounds that are characterised by the presences of an aminocyclitol ring linked to amino-sugars in their structure. Those that are derived from bacteria of the Streptomyces genus are named with the suffix –mycin (e.g streptomycin, neomycin, tobramycin etc), whereas those that are derived from *Micromonospora* are named with the suffix –micin(eg: gentamicin, netilimicin and amikacin)(1; 2). Their bactericidal activity is attributed to the irreversible binding to ribosomes. They have a broad antimicrobial spectrum (3). They are active against aerobic and facultative aerobic Gram-negative bacilli and some Gram-positive bacteria. Aminoglycosides are not active against anaerobes and rikettsia, they are however bactericidal against bacteria by inhibiting protein synthesis, they achieve this by binding to the 16S rRNA and also by disrupting bacterial cell membrane integrity (4). Streptomycin was the first aminoglycoside to be identified and characterised by Selman Waksman in 1944 and In contrast to penicillin which was isolated from fungi, streptomycin was the first antimicrobial to be isolated from bacterial source. The discovery of streptomycin was a landmark in the history of antimicrobials, since it was the first effective treatment for tuberculosis, a disease that had caused tremendous human suffering for centuries (5). Aminoglycosides are essential in antipsedomonal chemotherapy implicated in the treatmentof a variety of infections. *Pseudomonasaeruginosa*is one of the most prevalent nosocomial pathogens associated with higher mortality rates and antibiotic costs. It can survive in different environments, including soil, plants and animals. It is also considered the most opportunistic human pathogen especially in immunocompromised patients and one of the top five pathogens of nosocomial diseases worldwide (6, 7). *Pseudomonas infection*s arecommonly reported in burns, urinary tract infection (UTI) and pulmonary diseases such as cystic fibrosis (CF). This diversity of *Pseudomonas* infection is due to the development of various adaptive mechanisms such as the nutritional and metabolic pathways besides the regulation of gene expression (8).*P. aeruginosa* usually enters body tissues through injuries, it attaches to tissue cells using specific attachment fimbriae. The most important virulence factor is exotoxin A (ADP ribosyltransferase), which blocks translocation in protein synthesis by inactivating the elongation factor eEF2. Theexoenzyme S (also an ADP ribosyltransferase) inactivates cytoskeletal proteins and GTP binding proteins in eukaryotic cells. Thecytotoxin damages cells creating transmemnbranepores (9). Despite these pathogenic determinants, infections are rare in immunocompetentindividuals; defective non-specific and specific immune defences are preconditioned for clinically manifested infections. Patients suffering from neutropenia are at high risk. The main infections are pneumonia in cystic fibrosis or in patients on respiratory equipment, infections of burn wounds, postoperative wound infections, chronic pyelonephritis, endocarditis in drug addicts, sepsis and malignant otitis external (1) and often resistant to many antibiotics (10). Gentamicin and certain other

aminoglycosides are frequently used for the treatment of such infection but strains of *Pseudomonas aeruginosa* and other bacterial species resistant to aminoglycoside are now been reported in Nigeria and this is probably because gentamicin and other aminoglycosides is traditionally considered in this environment as the first line drug against gram negative bacilli in the hospital setting.Fortunately this organism can be detected easily in the laboratory using simple routine media. However antimicrobial sensitivity testing needs to be done routinely and accurately because of the ease with which resistance develops to traditionally used antipseudomonal antibiotics.

Mechanism of Action of Aminoglycoside

The mode of action of aminoglycoside can be grouped into two namely: uptake of aminoglycosides into the bacteria for the purpose of biological activity and the second is the activity that occurs within the cell.This is actualized when aminoglycosides binds to ribosome and inhibit protein synthesis (11). The method by which bacterial cell wall is penetrated by aminoglycoside occurs in three-phases, one of which is an energy independent step and the other two are energy dependent (12). In the energy independent step the aminoglycoside binds to the surface anionic compounds of the bacteria cell wall such as lipopolysaccharides, phospholipids and outer membrane proteins in Gram negatives and teichoic acids and phospholipids in Gram positives (12). This step is followed by the energy dependent phase I where small amounts of the aminoglycoside molecule cross the cytoplasmic membrane in a process that requires a threshold transmembrane potential generated by a membrane bound respiratory chain (12).

Finally the loss of membrane integrity triggers the energy dependent phase II. The damaged cytoplasmic membrane results in an accelerated rate of uptake of aminoglycoside molecules. The higher the amount of aminoglycoside, the more rapid is the onset of energy dependent phase II and the death of the bacteria cell (2).

Resistance Mechanism of *Pseudomonasaeruginosa* to Aminoglycosides

The mechanism of resistance of resistance recognised includes ribosome alteration, decreased permeability and inactivation of the drugs by aminoglycoside modifying enzymes (7)

Inactivation by Modifying Enzymes

In bacteria the resistance is often due to enzymatic inactivation by acetyltransferases, nucleotidyltranferases and phosphotransferases (1). These enzymes are common determinants of aminoglycoside resistance in *Pseudomonasaeruginosa*. Aminoglycoside resistant strains often emerge as a result of plasmid borne genes encoding aminoglycoside modifying enzymes (13); many of these genes are associated with transposon which aid in the rapid dissemination of drug resistance across species boundaries. The most common enzyme providing for aminoglycoside resistant in *Pseudomonasaeruginosa* is Aminoglycoside nucleotidyltransferase(adenylate)(ANT) (14). Aminoglycoside phosphoryltransferase (Phosphorylate) (APH) catalyse the transfer of aphosphate group to the aminoglycoside molecule while aminoglycoside acetyltransferase(acetylate)(AAC) catalyses acetylation of aminoglycosides and this can occur at 1,3.6' and 2'amino groups ad involves virtually all medically useful compounds (15)

Decrease Permeability/ Impermeability Resistance

Some strains of *Pseudomonasaeruginosa*and other gram-negative bacilli exhibit aminoglycoside resistance due to transport defect or membrane impermeabilization (6).This mechanism is likely chromosomally mediated and a result in cross-reactivity to all aminoglycosides. The outer membrane constitutes a semipermeable barrier to the uptake of antibiotics substrate molecules. Because uptake of small hydrophilic molecules such as b-lactams is restricted to a small portion of the outer membrane (namely the water-channels of porin proteins).The outer membrane limits the movement of such molecules into the cell and this is true for all gram-negative bacteria, but is especially true in the case *P. aeruginosa* which has an overall outer-membrane permeability that is approximately 12-100-fold lower than for example that of *E. coli* (16).

Efflux Pumps

The efflux pump transporterin *Pseudomonasaeruginosa* belongs to resistance nodulation division 'RND' family. It is composedof three parts, the transporter, the linker and the outer membrane pore that ensures that the extruded compound does not remain in the periplasm, hence, avoiding its return to the cytosol (17). In view of the fact that the majority of multidrug resistance pathogens expresses and overproduce efflux pumps that are responsible for expelling and extruding of the antibiotics from the cell, the new direction of other chemotherapeutics is the use of efflux pump inhibitors (EPIs). (15). Using EPIs with antibiotics can reduce the invasiveness of*Pseudomonasaeruginosa* besides its role in lowering the antibiotic minimal inhibitory concentration.

The aim of this study is therefore to investigate the Prevalence of Aminoglycoside Resistance in Clinical Isolates of *Pseudomonas aeruginosa*obtained from Lagos.

II. Materials And Method

STUDY DESIGN

Bacterial Isolates

A total of 114 isolates were collected from various clinical specimens of patients treated at the Lagos University Teaching Hospital (LUTH) between June and September 2012. They were identified by oxidase testing and morphology on *Pseudomonas*centrimide agar

Materials

1. Media

A. MacConkey Agar (BIOTECH) which was used to subculture the isolates for oxidase test
B. Muller Hinton Agar (OXOID) was used for sensitivity testing
C. Nutrient Agar (OXOID) slants were used to store the organism before identification and sensitivity testing.
D. *Pseudomonas*Cetrimide Agar (OXOID) CMO579 for final identification of *Pseudomonasaeruginosa*Isolates

2. Four oxoid (Basing-stroke, Hamshire, England) Antimicrobial susceptibility discs were used and they included;

Amikacin	30mcg
Gentamicin	30mcg
Kanamycin	30mcg
Netilimicin	30mcg

3. Oxidase Strips: These strips wereused to identify oxidise positive isolates

Methods

Isolation and Identification

*P. aeruginosa*isolates were carefully identified by proper laboratory methods. All gram stained negative bacilli that were oxidase positive were further identified for growth on *Pseudomonas*Cetrimide Agar base medium. The isolates were stored on nutrient Agar slant.Oxidase detection strips MB0266A (MICROBACT) was used and these strips are impregnated with NNNN' tetra-methyl-p- phenylenediaminedihydrochloride for the detection of bacteria cytochrome oxidase enzyme.The colonies was touched with oxidase detection strip and observed for up to 5 seconds. The appearance of a blue/violet colour indicates a positive reaction.

Antibiotics Susceptibility Testing

The susceptibility of all strains of *P. aeruginosa*was tested using the disc diffusion method on Muller Hinton medium (OXOID) as described by Bauce*r et al.,* 1966 (18). The isolates were subculture from nutrient agar slant into*Pseudomonas*centrimide Agar base medium and then incubated at 37^0C for 24 hours.Four colonies of each pure isolates were emulsified in test tube containing 5ml of sterile normal saline. A swab stick was dipped into the suspension and the swab is turned against the side of the tube to remove excess fluid and then streaked across the surface of the Muller Hinton Agar. The inoculated plates were allowed for 3-5 minutes to dry. The antibiotic discs were aseptically placed on the surface of the inoculated plates with a sterile forceps and press gently to ensure even contact with the medium. The plates were incubated at 37^0C for 18-24 hours and the zones of inhibition of growth were measured. Interpretation of result was done using the zone size interpretive chart for clinical laboratory standard institute 2007 (19).

III. Result

A total of 56 isolates of *Pseudomonasaeruginosa* were identified from 114 clinal isolates collected from the Department of Medical Microbiology and Parasitology, Idiraba of Lagos University Teaching Hospital. The age of patients ranged from 0-70 years, amongst which 39.29% and 60.71% of this isolates were from male and female population respectively.

Table 1 and Fig 1 below shows that the susceptibility of strains of *Pseudomonasaeruginosa* to Gentamicin, Amikacin, Netilimicin and Kanamycin were 51.79%, 57.14%, 28.57% and 25% respectively. It also shows that 44.6%, 5.71%, 67.85% and 71.4% of the isolates were resistance to Gentamicin, Amikacin, Netilimicin and Kanamycin respectively.

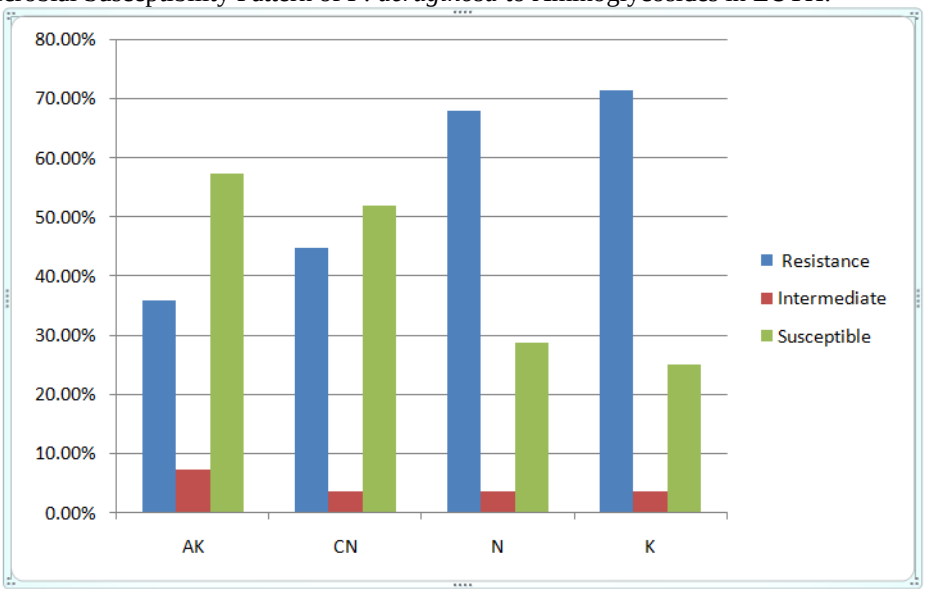
Table 1: Antimicrobial susceptibility pattern of P. *aeruginosa* to aminoglycoside

Antimicrobial Agents	No of Isolates Tested	No. (%) resistant	No (%) Intermediate susceptible	No (%) Susceptible)
Amikacin	56	20 (35.71)	4 (7.10)	32 (57.14)
Gentamicin	56	25 (44.60)	2 (3.57)	29 (51.79)
Netilimicin	56	38(67.85)	2(3.57)	16(28.57)
Kanamycin	56	40(71.40)	2(3.57)	14(25.00)

Table 2: Ratio of Susceptible: Intermediate Susceptible: Resistance

Antimicrobial Agent	Ratio
Amikacin	16:2:10
Gentamicin	15:1:12
Netilimicin	8:1:19
Kanamycin	7:1:20

Fig 1: Antimicrobial Susceptibility Pattern of *P. aeruginosa* to Aminoglycosides in LUTH.



KEY
CN: Gentamicin
AK: Amikacin
N: Netilimicin
K: Kanamycin

IV. Discussion

This study shows that only 44.6% of all isolates was resistant to gentamicin which is considered as the first line of drug against gram negative bacilli in the hospital setting in Nigeria; this shows an increase to what was reported in Lagos University by Oduyebo*et al*., 1997(20). The increase in gentamicin resistant in this study might be due to misuse and abuse of these drugs in the environment and the availability of these drugs over the counter. 57% of the isolates were found to be sensitive to Amikacin. This is not worthy since it is often used as an alternative drug to deal with gentamicin resistant *P. aeruginosa*.It was observed that most isolates was resistant to Gentanmicin was also resistant to Amikacin therefore it will be advised that antimicrobial susceptibility test should be done before treating *Pseudomonasaeruginosa* infections or to treat serious *P.aeruginosa* infections with a combination of antibacterial agents. Although synergistic interactions is an important aspect for some drug combination (e.gtrimethoprimsulfamethoxazole), the primary focus of combination therapy against *P.aeruginosa* is preventing the emergence of resistances. The combination of antipseudomonal beta-lactam with an aminoglycoside has often been the treatment of choice for this pathogen.

Over 71.4% of the isolates were resistant to kanamycin (the highest rate in this research) and this resistance could be as a result of the mechanism listedabove.This resistance might also be as a result of the abuse of this drug and as such should not be considered as the first line drug for the treatment of *P.aeruginosa* infections in the environment.

Isolates of *Pseudomonasaeruginosa* resistant to aminoglycosides are frequently recovered in the hospital setting. Studies have shown that these organisms are related to the amount of aminoglycosides used in a particular institution (21).Aminoglycoside resistant can be mediated by mutations reducing the binding of aminoglycoside to the ribosome by enzymatic modification or by membrane impermeability and most resistance in clinical isolates of *P. aeruginosa* is due to enzymatic modification and membrane impermeability.

V. Conclusion

This study shows that*Pseudomonas aeruginosa* infections are highly prevalence in LUTH as fifty six*P. aeruginosa* isolates were identified between June and September 2012. Thesesisolates showed a high resistance rate against aminoglycoside tested.

Recommemendation

This study highlights the need to establish an antimicrobial resistance surveillance network for *P.aeruginosa* to monitor the trend of resistance in Nigeria. Resistance of allaminoglycosides among *P.aeruginosa* is clearly on the increase. This can be combated by all physicians if they can be obliged to prescribe antimicrobial agents more deliberately following antimicrobial susceptibility testing.In order to overcome the worrisome development of resistance of *Pseudomonasaeruginosa* to aminoglycoside resistance, continued national surveillance program are crucial.

The methods used for detecting antibiotic resistance should be continuously validated and resources should be made available for continued research on antibiotics resistance and development of new antimicrobial agents.

Hospitals have to ensure that appropriate infection controlpractices are implemented to limit continued spread of resistant microbes.Finally, as with any agent, the prudent use of aminoglycoside and the use of effective infection controlpractices can go a long way to limiting the development and spread of aminoglycosides resistance, ensuring that the3se agents continue to find a place in the treatment of *P.aeruginosa* infections

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