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Multidrug Resistance Activity of Pseudomonas Aeruginosa from Urinary Tract Infected Patients in General and Private Hospitals, Amassoma

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Abstract

Pseudomonas aeruginosa is an agent of various infections in human globally. This research was undertaken between February and June, 2021, to determine the antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* to eight (8) different classes of antibiotics commonly prescribed in Nigeria hospitals. A total of 194 clinical samples which include 58 mid-stream urine, 63 wound and 73 ear samples were collected at random from General and Private (Tantua) hospitals, Amassoma community and immediately transferred to the Microbiology diagnostic laboratory, Niger Delta University for culture using Nutrient agar and Cetrimide agar. Pure isolates with 0.5 Mc Farland standard were characterised and identified using standard microbiology techniques. Antibiotics susceptibility was carried out with Mueller Hinton agar using Kirby buer and Agar diffusion method. Eighty {80} (41.2%) *Pseudomonas aeruginosa* isolated include; urine 20(25%), ear 24(30%) and wound 36(45%); with female having a higher prevalence of 44(55%) than the male 36(45%). All the *Pseudomonas aeruginosa* isolated was highly susceptible to imipenem(100%), levofloxacin (98.75%), gentamicin (98.75%), ceftazidine (56.5%), piperacillin tazobactam(52.5%), tetracycline (6.25%), co-trimoxazole (3.75%) and nitrofurantoin (0%). Findings showed that overall Multidrug Resistance (MDR) expressed by *Pseudomonas aeruginosa* was 98%, among which was 48.8% with Sulphamethoxazole trimethoprim, Tetracycline and Nitrofurantoin; more isolates were found in wound (45%), followed by ear (30%) and least in urine (25%) samples. In conclusion, imipenem (100%) was the most potent drug against *Pseudomonas aeruginosa* infection followed by gentamicin (98.75%) and levofloxacin (98.75%); these drugs are recommended for clinical use

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Introduction

Microorganisms can be seen everywhere in the environment such as sewage, farmland, water farms soil, oil polluted areas, health centres, hospitals, maternity homes. *Pseudomonas aeruginosa* is one of the microorganisms that have the ability of survival especially in human host; it can be isolated and proliferate in can drinks, tablets, syrups, lotions, solutions, detergents, industrial production water, environments, analgesics, eye drops, ophthalmic washing solutions, eye lens water, marine and fresh water, in river basin, maternity homes, pharmaceutical industries, industrial production sites. *Pseudomonas aeruginosa* is virulent due to its production of toxins (due to production of Type 3 Secretion System T3SS), lipopolysacharides, lipolipids, flagellae, with ability to deplete the normal functions of the immune system such as cytokines, interleukins, interferons. It is a gram negative bacterium, obligate aerobe due to possession of cytochrome oxidase enzyme with capsule formation and can deplete the normal flora of the host body such lactobacillus. The organism is associated with wound infection and a known causative agent of nosocomial infection among hospital workers, it causes blood stream infection (septicaemia and bacteremia) which could lead to anaemic conditions; the organisms has the ability to cause intestinal abnormalities, cartilage and bone dysfunctions encephalitis, meningitis, conjunctivitis, pulmonary infections, bowel irritations, epidermal scaling, otitis media, lower and upper respiratory infections, bladder and pyelonephritis infections Ali *et al*., 2015. Predisposing factors to *Pseudomonas aeruginosa* infections in patients with impaired immune system include tuberculosis, transplant, insertion of biomedical foreign devises, tumorigenesis, elderly and children are at risk of this infection Mahmoud *et al*., 2013. *Pseudomonas aeruginosa* can grow on various media including general purpose (Nutrient), enriched (blood and chocolate), selective (cetrimide) and can move from one place to another with ability to produce pigments such as pyocyanin (blue); pyoverdin (yellow), pyorubin (red), pyoverdine (yellow) due to iron depletion, pyomelanin (brown pigment). Various classes of antibiotics include Aminoglycoside eg Gentamycin; Carbapenems eg Imipenem, meropenem; Cephalosporins eg Ceftazidime; Fluoroquinolones eg Ciprofloxacin and Levofloxacin; Penicillin eg Ticarcillin-clavulanic acid and Piperacillin-tazobactam; Monobactams eg aztreonam. Multidrug resistant *Pseudomonas aeruginosa* can be interpreted as an expression of resistance by this microbe to two or more classes of test antibacterial agents such as Penicillins and Fluoroquinolones or Aminoglycoside eg Gentamycin, the later may not be crucial here since it is not considered as the major treatment of *Pseudomonas aeruginosa* infection. French National Technical Committee for Nosocomial infections explained Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) as a *Pseudomonas aeruginosa* that resist the drug treatments of one or two classes of the antibiotics such as Cephalosporins eg Ceftazidime, Penicillins eg Ticarcillin and Carbapenem eg Imipenem either partially or wholly Hassuna *et al.*, 2015. *Pseudomonas aeruginosa* can survive everywhere due to some intrinsic factors such as efflux pump, capsule and plasmids possession, lipopolysacharides, lipids layers, beta lactamase enzyme. The infection caused by *Pseudomonas aeruginosa* could be fatal and resist chemotherapy, because the organism can survive in harsh environmental conditions such as high humidity, hence can resist several classes of antibiotics. Antibiotics can be produced from natural, synthetic and semisynthetic products such as plants extracts, microorganisms, combination of plant and chemicals, or basically chemical components. Antibiotics could mount their action on microorganisms as cidal or static hence called bactericidal and bacteriostatic antibiotics of which the formal is killing and the later is inhibition of microbial growth Arikekpar, 2016. Various antibiotics have their ways of expressing their actions on microorganisms; these include prevention of protein synthesis, nucleic acid synthesis, attacks on the cell walls peptidoglycan example is penicillin; interference with synthesis of protein by stopping the action of 30S ribosomal subunits eg Tetracycline; nucleic acid synthesis eg Ciprofloxacin. Antimicrobial susceptibility testing is an important tool to evaluate the potency of antibiotic drugs and the target microorganism

response to that drug Jorgensen *et al*., 2015. The aim of this study is to determine the antimicrobial susceptibility patterns of the clinical isolates of *Pseudomonas aeruginosa* to eight(8) different classes of antibiotics commonly prescribed in Nigerian hospitals.

Materials and Methods

Study Area and Population

Bayelsa state (capital Yenagoa) is a one of the states in southern Nigeria in Niger Delta region, in-between the neighbouring states of Delta and Rivers; it was created in the year 1996, with a population of 1.8 million inhabitants going by 2006 Nigeria Government Census estimation. There are eight (8) Local Government areas; these are: Sagbama, Brass, Yenagoa, Ogbia, Ekeremor, southern Ijaw, Nembe Local Government area with three (3) senatorial districts (Bayelsa State Government, 2022).

This study was carried out in Amassoma, a town located in Southern Ijaw Local Government 6,970 with a population and the hospitals in which the study was carried out include General hospital and Tantua memorial hospital. General hospital is a Government facility and it is a 60 bed hospitals and Tantua Memorial Hospital, a private facility and commonly visited than any other private hospitals located in Amassoma.

Ethical Considerations

Consent was sought from the Managing Directors and the participated subjects in the study hospitals, permission was given to collect the samples..

Sample collection

A total number of 194 {92 female; 102 males}clinical samples which includes 58 mid-stream urine samples, 63 wound samples and 73 ear samples were collected from the medical laboratory unit of General hospital and Tantua memorial hospital in Amassoma, Bayelsa state.

Materials

Growth media, Glass wares and Equipment and Reagents used

Cetrimide Agar, Simon Citrate Agar, Nutrient Agar and Mueller-Hinton Agar (Hi-Media, India)

Glass slides, Beakers, Test tubes, Wide-mouthed glass bottles, McCartney bottles, Measuring cylinders, Sterile petri dishes, Pipettes.

Autoclave (B.Bran Sci and InstCompany England), Incubator (Baird and Tat lock ltd, Essex light), Hot air oven (Baird and Tat lock ltd, Essex light) and Refrigerator Tripple beam balance (W& TA ltd, Birming England). 0.5% McFarland standard, gram staining, Kovacs reagent tryptone water and oxidase regents. Sterile urine bottles, Sterile swab stick, Spatula, Wire loop, Forceps, Syringes, Test tube plugs and rack, Sterile swab sticks, Sterile distilled water, Bunsen burner, Markers, Napkins, Antiseptics, and Masking tape.

Antibiotics

Antibiotis used include: Levofloxacin(5µg); Gentamicin(10µg); Nitrofurantoin (300µg); Ceftazidine (30µg); Imipenem (10 µg); Co-trimoxazole(25µg); Tetracycline (30µg); Piperacillintazobactam (110µg); Sulphamethox-azoletrimethoprim (25µg), they are products of Oxoid, United Kingdom

Methods

Isolation, Characterization and Identification of the Isolates

Growth media Nutrient and Cetrimide agar media were prepared according to the manufacturers' direction under aseptic condition and used immediately. Twenty (20 ml) portion of both sterile prepared Nutrient agar and Cetrimide agar melted, sterilized, allowed to cool were poured aseptically into sterile petri dishes. Sterile swab sticks carefully used to streak aseptically prepared media to give well discrete colonies after incubating at 37^0c for 24 h and 48 h. Bluish-green, green and light yellow colour were observed. Pure culture was achieved through sub culture and then identified on the basis of colony morphology, gram staining, citrate, indole, oxidase and lactose fermentation procedures. Antibiotics susceptibility testing was carried out on the pure standardised isolates with 0.5 McFarland turbidity standard, which is (1.5×10^8 CFU/ml) using Mueller Hinton agar with Kirby Bauer (1966) and agar diffusion method. Eight (8) different single Oxoid antibiotic discs were placed on each agar plate and pressed lightly onto the surface aseptically using a sterile forceps

constantly flamed and cooled. Inoculated plates were incubated at a temperature of 37°c for 24 hours and observed for growth; zones of inhibition was measured to the nearest millimetre with a rule and recorded. The result was interpreted using the CLSI zone diameter interpretative standards (NCCLS, 2011)

Table 1: Age and gender distribution of clinical samples

Sample type						
Sex (age in years)	Wound		Ear		Urine	
	M	F	M	F	M	F
≤10	2	3	5	7	-	1
11-20	5	3	11	8	2	5
21-30	21	09	15	7	7	25
31-40	11	5	10	1	2	10
≥41	3	1	4	5	3	2
Total	42	21	45	28	15	43

Table 2: Gender distribtion of *P.aeruginosa* from clinical samples

Sex	No. of samples	No. of subject infected (%)
Male	102	36 (35%)
Female	92	44 (48%)
Total	194	80(83%)

Table 3: Distribution of *P.aeruginosa* isolated from the clinical sample

Type	**No. of sample**	**No.(%) of bacterial isolate**
Urine sample	58	20(33%)
Wound sample	63	36(60%)
Ear sample	73	24(32%)
Total	**194**	**80(41.2%)**

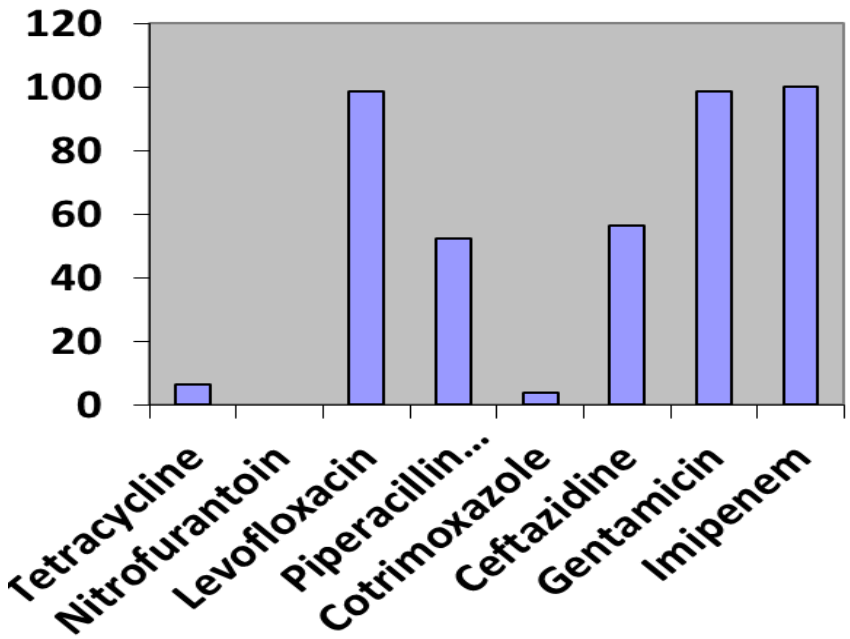

Figure 1: Antibiotic susceptibility profile of *Pseudomonas aeruginosa* to the various antibiotics on a graph chart

Table 4: Multi-Drug resistance pattern of *P.aeruginosa isolates*

Antibiotic Resistance Combination No.	Antibiotics	No. Of Isolate
R1	F	6(7.5%)
R2	TET+F	4(5%)
R3	TET+SXT	4(5%)
R4	F+SXT	4(5%)
R5	SXT+TET+F	39(48.75%)
R6	SXT+TET+F+CAZ	4(5%)
R7	SXT+TET+F+TZP	8(10%)
R8	TET+F+SXT+TZP+CAZ	8(10%)
R9	CAZ+TET+SXT+CN+F+LEV	1(1.25%)
Total		78(97.5%)

KEYS F=Nitrofurantoin; TET= Tetracycline; SXT=Sulphamethoxazole trimethoprim; CAZ=Ceftazidine; TZP=Piperacillintazobactam; CN=Gentamicin; LEV: Levofloxacin

Discussion, Conclusion and Recommendation

Pseudomonas aeruginosa can be difficult to treat with some available antibiotics in Nigeria hospitals because of inherent factors such as lipopolysaccharides, lipolipids, pigmentation and formation of biofilms. In this study female had a higher percentage prevalence of 48 than their male counterparts of 35; this is in concordance with the work done by Olugbue et al. (2018) with 42% female and 34% male, but lower than Ahmad et al. (2017) which reported 55% and 40% for female and male respectively; this could be attributed to the number of population examined and the geographical locations. Wound sample had the highest Pseudomonas aeruginosa isolates 36(60%) of 20 from male and 16 female, followed by ear 24(32%) and the least is urine 20 (33%) as depicted in table 3 above; this result agrees with the findings of Basak et al., 2012 and stated that Pseudomonas aeruginosa contribute more in wound infection than urinary tract and ear infections. Pseudomonas aeruginosa expressed multidrug resistance (MDR) ability in many antibiotics used in this study and it is estimated altogether to be 98% of which Nitrofurantoin had 1 (1.25%) resistant isolate; 4 (5%) isolates were resistant to Tetracycline and Nitrofurantoin; 4(5%) to Tetracycline, Sulphamethoxazole trimethoprim,; 4(5%) to Nitrofurantion Sulphamethoxazole trimethoprim; 35 (48.8%) Sulphamethoxazole trimethoprim, Tetracycline, Nitrofurantoin; 4 (5%)Sulphamethoxazole trimethoprim, Tetracycline, Nitrofurantoin, Ceftazidine; 8(10%) to Sulphamethoxazole trimethoprim, Tetracycline, Nitrofurantoin,Piperacillin tazobactam; 8(10%) Tetracycline, Nitrofurantoin, Sulphamethoxazole trimethoprim, Piperacillin tazobactam, Ceftazidine; finally one isolate showed resistance to Ceftazidine, Tetracycline, Sulphamethoxazole trimethoprim, Gentamycin, Nitrofurantoin and Levofloxacin. Nmema et al. (2013) stated that Multidrug Resistance (MDR) among Pseudomonas aeruginosa strains has been a tremendous health challenge especially in Nigeria hospitals where the potency of antibiotics is being challenged by many microorganisms due to their intrinsic possessions.

Antibiotics susceptibility test was carried out on the confirmed strains of *P. aeruginosa* using the following antibiotic disc; tetracycline, nitrofurantoin, levofloxacin, ceftazidine, piperacillintazobactam, gentamicin, imipenem and co-trimoxazole.

In this study, highest resistance was seen in 80(100%) strains to nitrofurantoin followed by 69(86.25%) to co-trimoxazole, 65(81.25%) strains to tetracycline, 19(23.75%) to piperacillintazobactam, 13(16.25%) to cefatzidine and the least resistance was exhibited by levofloxacin and gentamicin 98.75% as shown in Table 4 & 5.

From this study, the most effective antimicrobial agent suitable for *P.aeruginosa* is Imipenem with the percentage susceptibility of 100% which is in

accordance with a study carried out by Chika *et al* .(2017) who reported Imipenem as the most potent. The study carried out in South-western Nigeria by Odumosu *et al*,.(2012) revealed the susceptibility pattern of 92.6% of *P. aeruginosa* to Imipenem. The high susceptibility pattern of these drugs could be related to less drug abuse by the population as the cost of these antibiotics prevents patients' self-medication.

Levofloxacin gave the second highest percentage susceptibility of 98.75%. Sensitivity to Quinolone antibiotic was 88.5% with Levofloxacin also reported by Rajiv *et al.,* (2017).); Levofloxacin 83.3% Ahmad *et al*. (2017) and Gentamicin sensitivity was 98.75% in contrast to Samad *et al.*, (2017) which reported 74.65%. This variation may due to increase inappropriate use of gentamycin among the study population thereby reducing the sensitivity of *P. aeruginosa.*

Ceftazidine in this study had 56.25% in contrast to 43.70% sensitivity and Piperacillintazobactamexihibited in comparison to 64.5% Piperacillin + Tazobactam reported by Sharma *et al*..,(2016) .

Taking resistance to two or more classes of antibiotics as Multi drug resistance, it was detected that among the 80 isolates, 72(90%)isolates were Multi drug resistant (MDR) strains. 8 (10%) was resistant to two (2) antibiotics and 64(80%) were resistant to three (3) or more antibiotics.

Conclusion

In this study, Imipenem was the most potent antibiotic against *Pseudomonas aeruginosa*

infection with 100% susceptibility and Nitrofurantoin was the least susceptible with 0% as such

should not be prescribed by the physicians. Also in the absence of imipenem, levofloxacin and

gentamicin should be prescribed based on these findings. Researches should be carried out at

intervals in Nigeria to study the pattern of *Pseudomonas aeruginosa* susceptibility to mostly

prescribed antibiotics in Nigeria hospitals for effective surveillance of its resistance.

Recommendation

Imipenem, levofloxacin and gentamicin are the choice drugs for optimal management of infections caused by *P. aeruginosa*.

Cogent reasons should accompany the use of these drugs in order to prevent further spread of antimicrobial resistance among *P. aeruginosa* strains that can lead to occurrence of multi drug resistance.

Unsystematic use of antibiotics in agriculture and veterinary practice should not be allowed

Patient's compliance to medications especially antibiotics should be encouraged.

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