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Prevalence and Antibiotic Susceptibility of Carbapenem-Resistant *Pseudomonas aeruginosa* isolated from Clinical Specimens from Teaching Hospitals in Port Harcourt

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ABSTRACT

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) has become a serious public health challenge exhibiting multi-drug resistance against different antibiotics. This study aimed to investigate the prevalence and antibiotic susceptibility pattern of carbapenem-resistant *P. aeruginosa* isolated over the period of one year from different clinical specimens from the Rivers State University Teaching Hospital (RSUTH) and from the University of Port Harcourt Teaching Hospital (UPTH) both located in Port Harcourt Metropolis in Rivers State. Clinical specimens: urine, wound swabs, ear swabs and stool were collected from patients admitted to the Teaching Hospital using standard techniques. Isolation and identification of *P. aeruginosa* were performed using standard microbiological methods. Antimicrobial susceptibility testing, focusing on carbapenem resistance, was carried out using the Kirby-Bauer disk diffusion technique. The prevalence of *P. aeruginosa* recorded for specimens collected from the RSUTH and UPTH was 0-61.9% and 2.9-22.9% respectively. *P. aeruginosa* was more prevalent in wound specimens than in other specimens in both hospitals. Results of the prevalence of Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) in the ear, stool and wound specimens obtained from patients in RSUTH were 30.8%, 7.7% and 61.5% while 28.6%, 14.3%, 9.5% and 47.6% were observed for ear, stool, urine and wound specimens, respectively from UPTH. Results of the antibiotics susceptibility showed that the isolates displayed multi-antibiotic resistance (MAR) to most of the antibiotics used in this study. Despite the MAR index of 0.2-0.5 observed amongst the isolates, gentamycin, ciprofloxacin and ofloxacin displayed great antibiotic activity against the isolates. Thus, they are recommended for use in the control of infections caused by *P. aeruginosa* as highlighted in this study.

Keywords: Carbapenem-resistant *Pseudomonas aeruginosa*, antibiotics susceptibility

Introduction

Pseudomonas aeruginosa is a heterotrophic, motile, Gram-negative rod-shaped bacterium about 1–5 µm long and 0.5–1.0 µm wide. It is a facultative aerobe that uses both aerobic and anaerobic respiration to grow, with nitrate serving as the final electron acceptor (Prescott *et al.*, 2011). *P. aeruginosa* can also grow anaerobically with arginine and has poor fermentative skills, allowing for slow or no development. As a prototroph, the organism can thrive on a minimal basic growth media with a single source of carbon and energy and can use over 100 organic compounds as a source of carbon and/or energy **(**Lee *et al*., 2017**)**. *P. aeruginosa* develops well around 37°C. However, it can survive in a wide range of temperatures from 4 to 42 °C (Diggle and Whiteley, 2020). *P. aeruginosa* has the power to infect a wide range of hosts, including plants, nematodes, insects, and mammals. Infections in the lungs of people with cystic fibrosis (CF) frequently develop at a young age and advance to cause progressive lung function loss and ultimately mortality (Elborn, 2016). *P. aeruginosa* is renowned for developing robust biofilms that are highly resistant to antibiotics, disinfectants, and host defenses (Lee *et al*., 2017; Yan and Wu, 2019), impairing bacterial clearance and leading to the establishment of highly recalcitrant chronic infections that are major medical problems.

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Other *P. aeruginosa*-associated infections include chronic wound infection, chronic otitis media, chronic rhinosinusitis, catheter-associated urinary tract infection, and contact lens-related keratitis (Römling and Balsalobre, 2012; Lee *et al*., 2017).

The rise of antibiotic-resistant microorganisms is, without any doubt, a global health concern and *P. aeruginosa* has been identified as a key problem among notoriously multidrug-resistant (MDR) bacteria, posing a growing threat to world health and leading to a substantial increase in prevalence of nosocomial and chronic infections (Moradali *et al*., 2017). This is due to the bacteria's amazing ability to build resistance to a wide range of antimicrobials via a variety of molecular mechanisms, many of which are present in clinical isolates at the same time. Although each resistance mechanism is linked to a single class of antibiotics, resistance to each class of antibiotics is mediated by many mechanisms (Potron *et al*., 2015). Furthermore, each mechanism's contribution differs from country to country. Multi-drug resistance phenotypes in *P. aeruginosa* isolates have been linked to Outer Membrane Porin Drug (OprD) loss or reduced copy numbers, as well as over-production of active efflux pumps, AmpC b-lactamase, and extended-spectrum-lactamases (Moradali *et al*., 2017). There is a paucity of information on Carbapenem and antibiotics-resistant *P. aeruginosa* associated with medical specimens in Rivers State and Nigeria. Thus, the significance of the present study.

Materials and Method

Ethical Considerations

Ethical approval was sought and obtained from the Rivers State Hospital Management Board and the University of Port Harcourt Ethical Committee before the commencement of the research.

Specimen Collection

A total of five hundred and seventy-nine (579?) specimens comprising wound swabs (73 each), ear swabs (60 and 61), urine (83 each) and stools (73 each) were collected from patients in the Rivers State University Teaching Hospital (RSUTH) and the University of Port Harcourt Teaching Hospital (UPTH) both located in Port Harcourt Metropolis in Rivers State. Urine and faecal (stool) specimens were collected using the standard method of specimen collection on sterile biological specimen bottles as described by Cheesbrough (2006). Ear swabs and wound swabs were collected according to standard methods. Wound swabs were collected from patients as described in a previous study (Kassam *et al.,* 2017). Before obtaining the specimen, the wound was thoroughly cleaned with 60–120 mL of sterile normal saline to prevent contamination of the swab with skin bacteria, pus, or necrotic tissue.

The wound's surface was cleaned of excess saline using sterile gauze, and pus swabs were obtained by swabbing the wound's centre with a sterile swab. The swab sticks were inserted into its tube and transported to the Microbiology laboratory, at Rivers State University in an iced-pack container for immediate analysis.

Isolation of *Pseudomonas aeruginosa*

Swab specimens were cultured as described by previous studies (Abaza *et al.,* 2017). Swab sticks were swabbed onto the surface of aqueously prepared sterile cetrimide and nutrient agar plates, while stool and urine specimens were inoculated onto cetrimide and nutrient agar plates by the streak plate method (Cheesbrough, 2006). The inoculation was done in duplicates. After inoculation, plates were incubated at 37ºC for 24- 48 hours. After incubation, plates were read and those showing blue-green colonies on cetrimide agar were isolated by streaking on freshly prepared sterile cetrimide and nutrient agar plates and incubated at 37 ºC for 24 hours.

The isolates were purified by subculturing on freshly prepared nutrient agar continually until isolates were void of contaminating microorganisms. Pure *Pseudomonas* isolates were preserved frozen in bijou bottles containing 5mL sterile glycerol (10%). These pure isolates were used both for the identification process and other further tests.

Characterization and Identification of Isolates

The bacterial isolates were identified using standard methods (Cheesbrough, 2006; Prescott *et al.,* 2011). First, isolates were identified using colonial characters (colour, shape, size, texture and opacity of the colonies), morphological characters (gram stain and motility) and biochemical tests: catalase test, growth on blood agar, oxidase test, Methyl-red test, Voges Proskauer test, motility test, citrate utilization and sugar fermentation tests.

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Determination of Antibiotic Susceptibility of Isolates

The antibiotic susceptibility pattern of *P. aeruginosa* was carried out according to the Clinical Laboratory Standard Institute (CLSI, 2019). Thus, colonies of a 24-hour-old bacterial isolate was transferred into 4mL sterile normal saline and standardized to a 0.5 McFarland standardized using a UV-spectrophotometer (DV 8200). The standardized isolate was inoculated on freshly prepared Mueller-Hinton agar by swabbing the surface of the plate horizontally and vertically to ensure even distribution. Plates were allowed to dry before multi-discs containing antibiotics were carefully placed on the dried inoculated agar plates and incubated for 24 hours. The diameter of the zone of inhibition after incubation was read and interpreted according to the CLSI (2019) as resistant, susceptible and intermediate. The gram-negative disc consisted of the following; ceftazidime (30 µg), cefuroxime, gentamycin (30 µg), ofloxacin (5 µg), Augmentin (30 µg), cefixime (5 µg), nitrofurantoin (30 µg) and ciprofloxacin (5 µg).

Screening for Carbapenem Resistant *Pseudomonas aeruginosa*

This was carried out using imipenem antibiotics as described by Maharjan (2022) on Mueller-Hinton agar plates. The plates were interpreted according to the CLSI guideline where ≤ 1 MIC is susceptible while ≥ 4 MIC is resistant (Richter and Marchaim, 2016).

Multiple Antibiotic Resistant Index Calculation (MAR)

For this study, multiple antibiotic resistance is defined as the ability of an isolate to withstand three or more antibiotics (Osundiya *et al.*, 2013). The formula MAR = a/b, where a represents the number of antibiotics to which the test isolate displayed resistance and b represents the overall number of antibiotics to which the test isolate has been evaluated for susceptibility

Statistical Analysis

Descriptive statistics were conducted to determine the frequency of the Diameter (mm) of the zone of inhibition of the antibiotics and the prevalence of the isolates on the specimens. All analyses were done using SPSS (version 27).

Results

The percentage distribution of *P. aeruginosa* in the specimens collected from patients attending the hospitals is presented in Figure 1. Results showed that the percentage occurrence of *P. aeruginosa* in ear specimens, stool, wound and urine of specimens collected from patients attending RSUTH was 19%, 19%, 61.9% and 0% respectively, while the percentage distribution of *P. aeruginosa* isolated from ear, stool, wound and urine specimens collected from patients attending UPTH was 22.9%, 2.9%, 62.9% and 11.4% respectively. *P. aeruginosa* was most prevalent in wound specimens collected from both hospitals. The organism was not isolated from urine specimens collected from RSUTH but was isolated from a urine specimen collected from UPTH.

The result of the Carbapenem Susceptibility test of *P. aeruginosa* is shown in Figure 2. Results showed that 60.7% of the isolates were resistant to carbapenem (Imipenem) antibiotics while only 39.3% were susceptible.

Results of the prevalence of CRPA across the specimens showed that the percentages recorded for ear, stool and wound specimens obtained from patients in RSUTH were 30.8%, 7.7% and 61.5% while the percentages recorded for ear, stool, urine and wound specimens from UPTH was 28.6%, 14.3%, 9.5% and 47.6%, respectively (Fig. 3).

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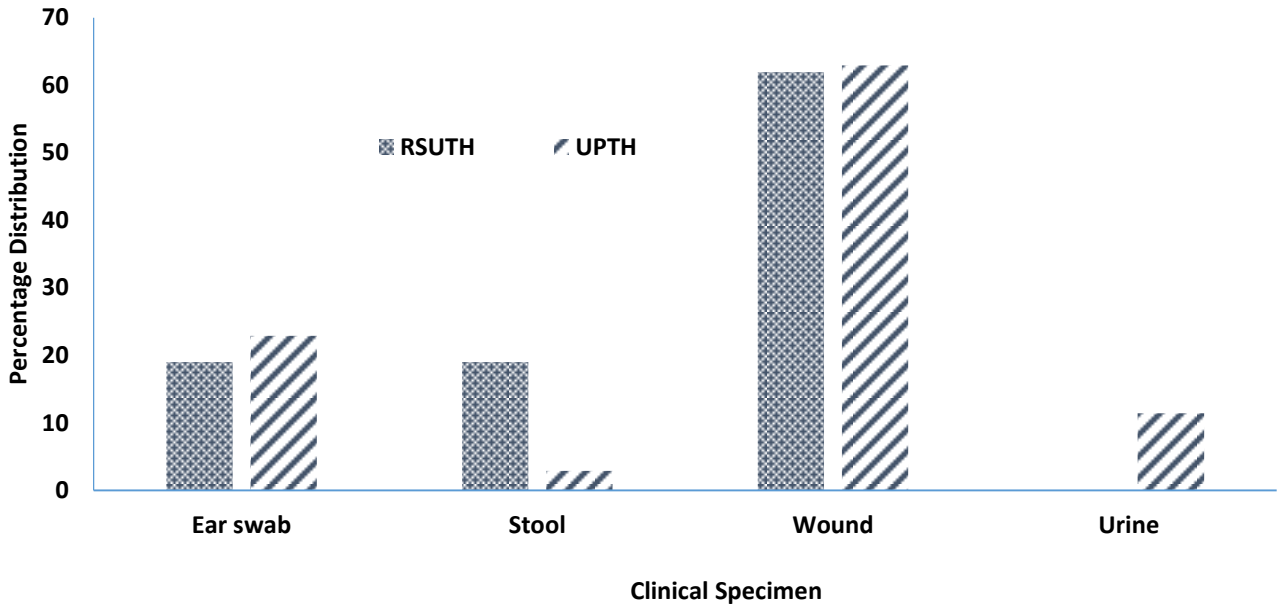

Fig. 1: Distribution (%) of *P. aeruginosa* in clinical specimens from Teaching Hospitals

Legend: RSUTH = Rivers State University Teachings Hospital; UPTH = University of Port Harcourt Teachings Hospital

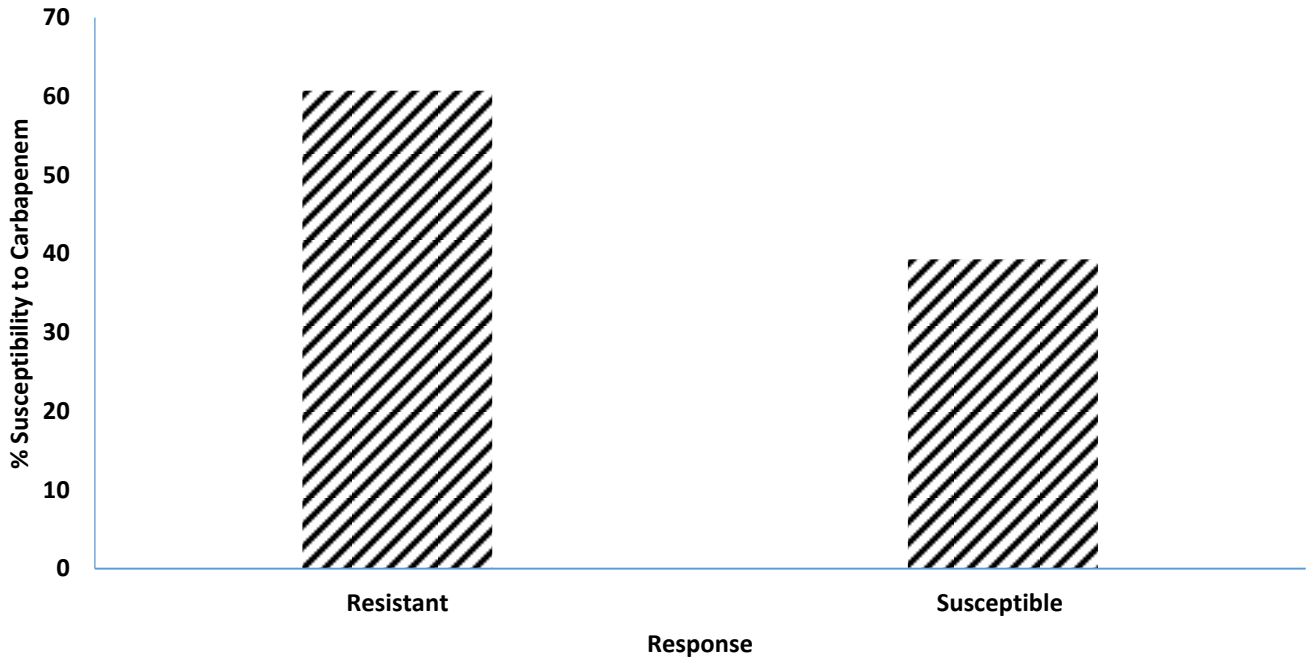

Fig. 2: Response (%) of *Pseudomonas aeruginosa* from clinical specimens to Carbapenem

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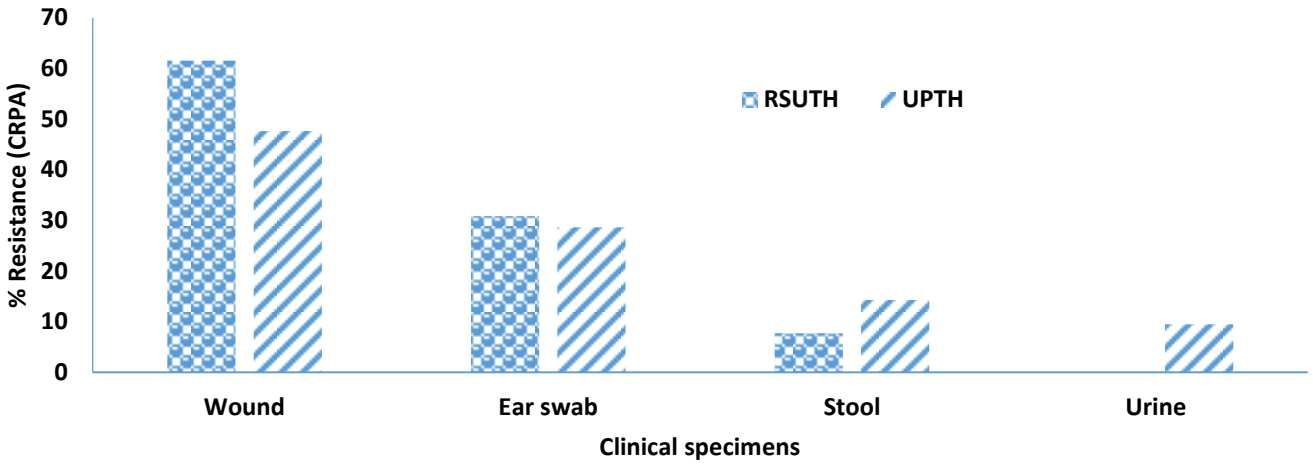

Fig. 3: Distribution of Carbapenem Resistance in *P. aeruginosa* across the specimen

Legend: RSUTH = Rivers State University Teachings Hospital; UPTH = University of Port Harcourt Teachings Hospital

Results of the percentage susceptibility pattern of *P. aeruginosa* isolates from different specimens of patients attending RSUTH and UPTH are presented in Figs. 4-8. Results showed that 57.1%, 71.4%, 28.6% and 81% of *P. aeruginosa* isolates from different specimens of patients in RSUTH were susceptible to gentamycin, ofloxacin, nitrofurantoin and ciprofloxacin. Ciprofloxacin susceptibility was the highest in this region followed by ofloxacin while susceptibility to gentamycin was higher than that recorded for nitrofurantoin. Percentage susceptibility of the isolates from different specimens in UPTH showed that 82.9%, 77.1% and 100% were susceptible to gentamycin, ofloxacin and ciprofloxacin, respectively. Ciprofloxacin was still the most effective followed by gentamycin and ofloxacin (Fig. 4).

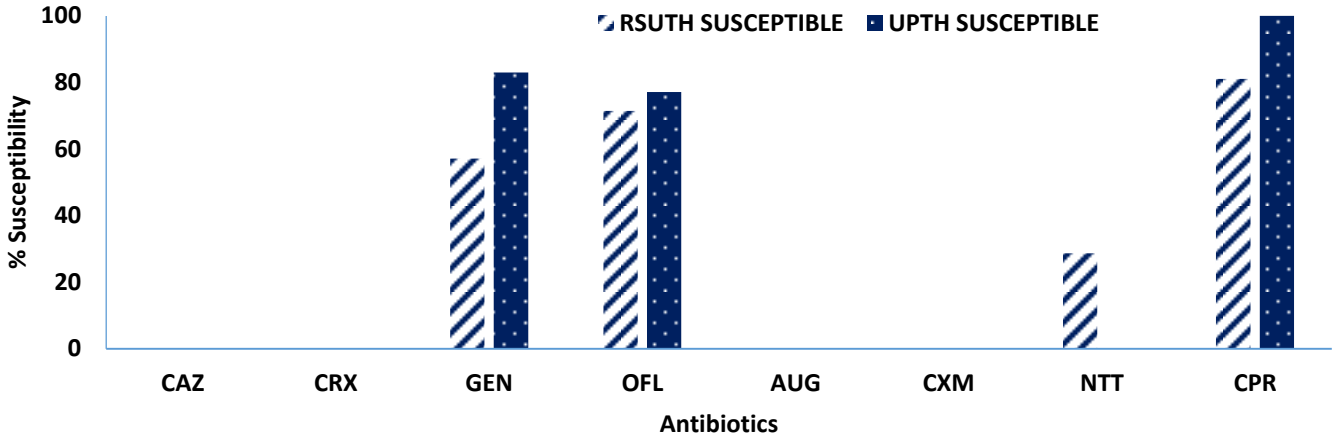

Fig. 4: Antibiotics Susceptibility Profile of *P. aeruginosa*

Keys: CAZ = ceftazidime; CRX = cefuroxime; GEN = gentamycin; ofl = ofloxacin; AUG = augmentin; CXM: cefixime; NIT = nitrofurantoin; CPR = ciprofloxacin

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Results of the percentage susceptibility of *P. aeruginosa* isolates from urine specimens showed that 25%, 75% and 100% of the isolates were susceptible to gentamycin, ofloxacin and ciprofloxacin. Results further showed that in order of effectiveness, ciprofloxacin was the most effective with 100% sensitivity against the *P. aeruginosa* isolates followed by ofloxacin (75%) and thirdly, gentamycin (25%) (Fig. 5).

Results of the percentage susceptibility of *P. aeruginosa* isolates from wound specimens from RSUTH and UPTH showed that 53.8%, 61.5%, 38.5% and 84.6% of isolates from RSUTH were susceptible to gentamycin, ofloxacin, nitrofurantoin and ciprofloxacin while 100%, 68.2% and 100% of *P. aeruginosa* from UPTH were susceptible to gentamycin, ofloxacin and ciprofloxacin, respectively. Results further showed that *P. aeruginosa* isolates from the wound specimens in UPTH were more susceptible to gentamycin and ciprofloxacin than ofloxacin (Fig. 6).

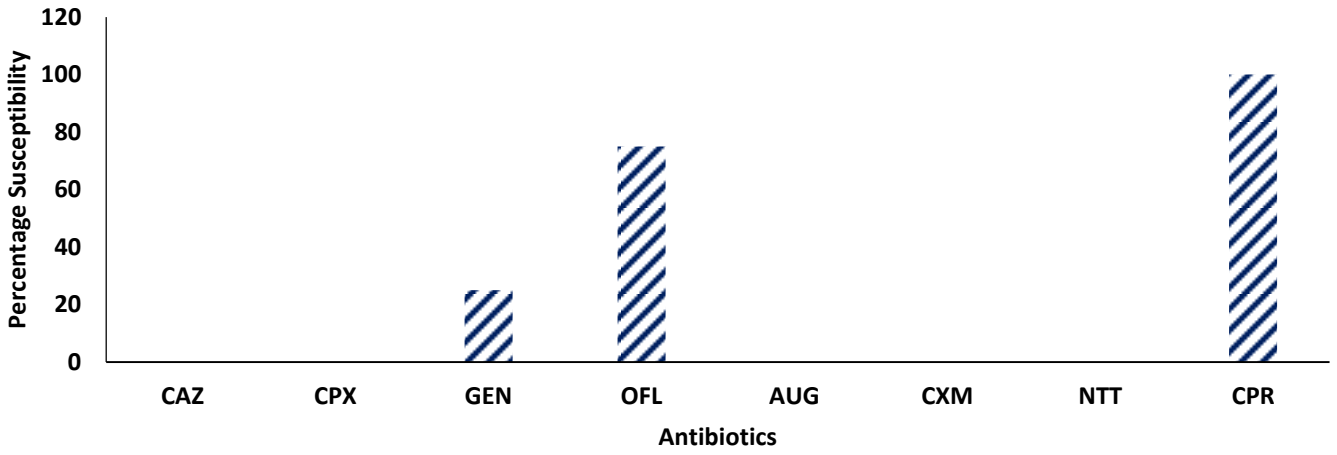

Fig. 5: Antibiotics Susceptibility Profile of *P. aeruginosa* isolated from Urine Specimen

Keys: CAZ = ceftazidime; CRX = cefuroxime; GEN = gentamycin; ofl = ofloxacin; AUG = augmentin; CXM: cefixime; NIT = nitrofurantoin; CPR = ciprofloxacin

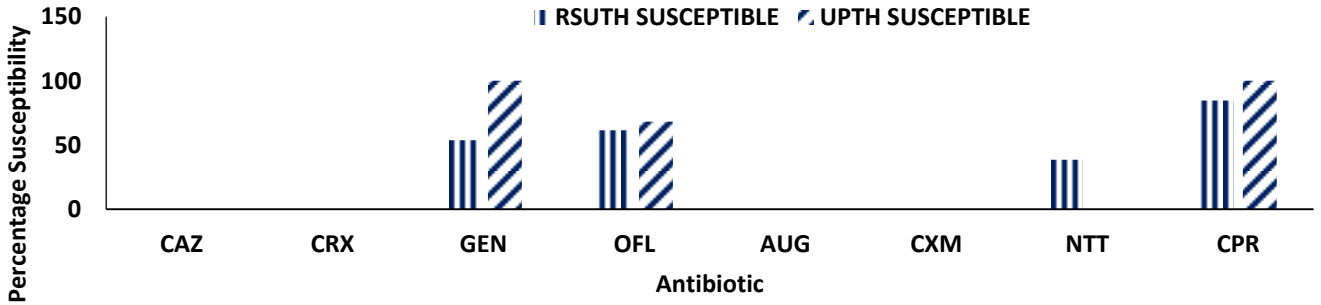

Fig. 6: Antibiotics Susceptibility Profile of *P. aeruginosa* isolated from wound specimen

Keys: CAZ = ceftazidime; CRX = cefuroxime; GEN = gentamycin; ofl = ofloxacin; AUG = augmentin; CXM: cefixime; NIT = nitrofurantoin; CPR = ciprofloxacin

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Results of the percentage susceptibility of *P. aeruginosa* isolates from ear specimens in RSUTH and UPTH showed that the percentage susceptibility to gentamycin, ofloxacin, nitrofurantoin and ciprofloxacin of isolates from wound specimens in RSUTH were 75, 75, 25 and 50%, respectively. While the percentage susceptibility of the isolates from ear specimens in UPTH were 62.5, 100 and 100% for gentamycin, ofloxacin and ciprofloxacin, respectively. Susceptibility to ofloxacin and ciprofloxacin was very high compared to the susceptibility to gentamycin (Fig. 7).

Results of the percentage susceptibility of *P. aeruginosa* isolates from wound specimens showed that 50, 100, and 100% of the isolates from the specimen obtained from RSUTH were susceptible to gentamycin, ofloxacin and ciprofloxacin, while 100% of the isolates from similar specimen obtained from UPTH was 100% susceptible to gentamycin, ofloxacin and ciprofloxacin, respectively (Fig. 8).

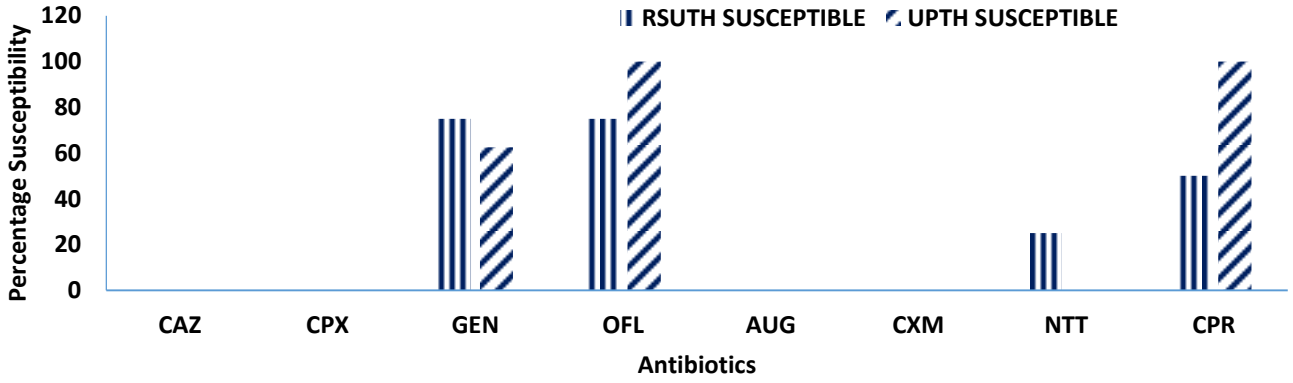

Fig. 7: Antibiotics Susceptibility Profile of *Pseudomonas aeruginosa* isolated from ear swab specimen

Keys: CAZ = ceftazidime; CRX = cefuroxime; GEN = gentamycin; ofl = ofloxacin; AUG = augmentin; CXM: cefixime; NIT = nitrofurantoin; CPR = ciprofloxacin

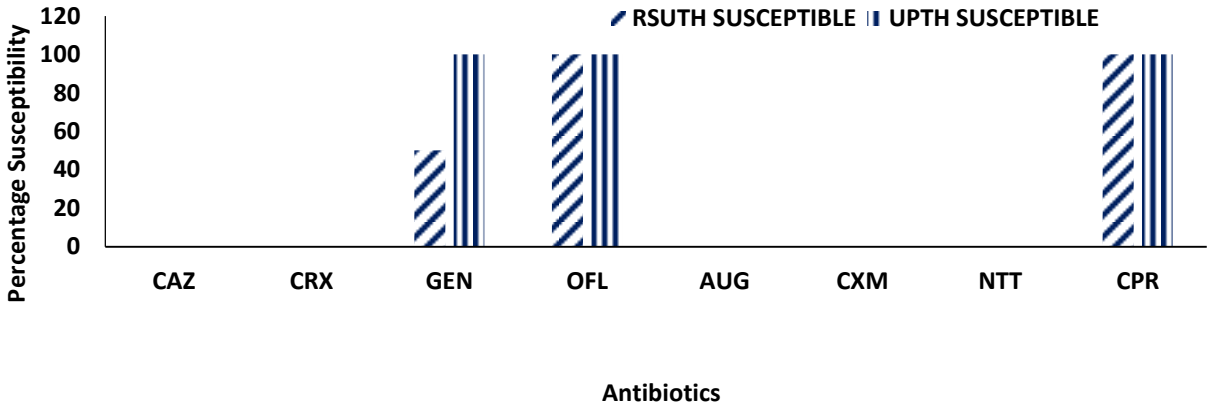

Fig. 8: Antibiotics Susceptibility Profile of *P. aeruginosa* isolated from stool specimen

Keys: CAZ = ceftazidime; CRX = cefuroxime; GEN = gentamycin; ofl = ofloxacin; AUG = augmentin; CXM: cefixime; NIT = nitrofurantoin; CPR = ciprofloxacin

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Results of the multiple antibiotic-resistant index (MAR index) of *P. aeruginosa* isolates from the different hospitals are presented in Table 1. Results showed that 40% of *P. aeruginosa* isolates from UPTH specimens have a MAR index of 0.2. There were no isolates within the MAR index of 0.3 and 0.4 while at a higher MAR index of 0.5, 21 isolates of *P. aeruginosa* from specimens in RSUTH were recorded. Furthermore, 48.6% of *P. aeruginosa* isolates had a MAR index of 0.6.

Table 1: MAR Indices and Percentage Resistance of the Bacterial Isolates

MAR Index	*P. aeruginosa* RSUTH	*P. aeruginosa* UPTH
0.2	0(0.00)	14 (40)
0.3	0(0.00)	0(0.00)
0.4	0(0.00)	0(0.00)
0.5	13 (61.9)	0(0.00)
0.6	0(0.00)	17 (48.6)
0.7	0(0.00)	0(0.00)

Discussion

Carbapenem-resistant *P. aeruginosa* (CRPA) have been regarded as critical pathogens (priority 1) by the World Health Organization due to the severity of the infections they cause, how frequently they are resistant to antibiotics currently available, and whether longer hospital stays are necessary for treatment, as well as the mode of transmission (animal to human, and from human to human) (WHO, 2012). The level of carbapenem resistance amongst the *P. aeruginosa* isolates in the present study was very high. More so, the prevalence of carbapenem resistant *P. aeruginosa* in the various specimens and hospitals varied. The total CRPA recorded in RSUTH was 38.2% while a higher prevalent rate of 61.8% was recorded in specimens from UPTH. This disparity could be attributed to the number of *P. aeruginosa* isolated from specimens from the different hospital specimens as well as the resistant strains in the various specimen.

Although other environmental factors including specimen type could also be the reason for disparity in the prevalence observed between the two hospitals (Moglad, 2019). More so, the CRPA was more prevalent in wound specimens obtained from both hospitals while the least CRPA isolates were recorded in stool specimen obtained from UPTH. The prevalence of CRPA in the present study is higher than the prevalence of 20.5% reported in clinical isolates in Lagos State Teaching Hospital (Ettu *et al.*, 2021). Although in their study, they reported an increase from 4.1% in 2007 and 5.9% in 2013 to 20.5% in 2021. Thus, the rate of CRPA in hospitals is actually increasing and this is a problem especially since Carbapenem antibiotics are known as last resort for *P. aeruginosa* infections (Çiçek *et al.*, 2021). When it comes to treating both gram-negative and gram-positive bacterial infections, including the treatment for *P. aeruginosa*, Carbapenem, a β-lactam antibiotic, is very effective (Fernando *et al.*, 2021). Hu *et al.*, (2008) in their study reported a prevalence of 41.3% of CRPA in intestinal specimens while 20% and 18% were reported in Turkey and India (Nishu *et al.*, 2019; Kalayci and Aktas, 2021), although these values are high, they are lower than the present study.

Resistance to carbapenem antibiotics has been linked to different factors including the presence of Carbapenem resistant genes, efflux pump, modification of drug target sites as well as other environmental and anthropogenic factors (incomplete dosage, indiscriminate use of the drug). However, a recent study has reported that resistance to Carbapenem may not be due to the presence of Carbapenem-resistant genes but other factors (Reyes *et al.*, 2023). Thus, using antibiotics haphazardly in terms of dosage and treatment days, using low-quality medicines, and writing unnecessary prescriptions may all contribute to carbapenem resistance (Tesalona *et al.*, 2017).

According to research, humans are primarily responsible for the emergence and spread of carbapenem resistance. Meletis, (2016) opined that human-related factors: inappropriate antibiotic prescriptions combined with uncontrolled consumer

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access to antibiotics in many nations with poorly enforced sales regulations, lack of infection prevention controls in healthcare facilities after carbapenem resistance has emerged, and the use of sub-therapeutic antibiotic doses in agricultural sector has contributed to the development of CRPA.

The response to antibiotic susceptibility by *P. aeruginosa* isolates varied greatly with respect to the tested antibiotics and ciprofloxacin was recorded as the most effective antibiotics in isolates from UPTH and RSUTH. *P. aeruginosa* isolates exhibited multi-drug resistance (MDR) by being resistant to more than three antibiotics. In the present study, the isolates were all resistant to ceftazidime, cefuroxime, augmentin, and cefixime. Fazzeli *et al.* (2012) have also reported very high resistance of *P. aeruginosa* to ceftazidime antibiotics while Mohammad *et al.* (2008) reported 75% resistance to ceftazidime. The isolates of *P. aeruginosa* from urine specimens despite being highly resistant to penicillin antibiotics were highly susceptible to ciprofloxacin and ofloxacin with only 25% being susceptible to gentamycin. For isolates from ear swabs, nitrofurantoin recorded 25% sensitivity against the isolates. Generally, ciprofloxacin antibiotics, ofloxacin and gentamycin showed high antibiotics sensitivity against these isolates. Thus, they were more potent and effective in inhibiting most of the *P. aeruginosa* isolates in the present study. Zarei *et al.* (2018) reported that imipenem, meropenem, ciprofloxacin and gentamycin had low effectiveness against the *P. aeruginosa* isolates. This is contrary to the present study which showed high potency of ciprofloxacin and gentamycin antibiotics to the isolates. Previous studies have also reported high levels of ciprofloxacin resistance by *P. aeruginosa* isolates (Dou *et al.*, 2017; Mobaraki *et al.*, 2018). In a previous study, it was reported that the susceptibility of *P. aeruginosa* isolates to ciprofloxacin and gentamycin was very good, thus, the drugs were recommended as the drug of choice for empirical use against *P. aeruginosa* isolates (Gebreegziabher, 2020).

Amongst the contributing factors affecting the increased antibiotic resistance in clinical isolates, prior usage of not only carbapenem but also aminoglycosides has been identified as a risk factor for intestinal CRPA infections, though there is a lack of specific evidence for aminoglycoside-induced CRPA (Hu *et al*., 2020). A metagenomics analysis of 50 studies published between 2010 and 2014 investigated antimicrobial-resistant *P. aeruginosa* and reported varying levels of resistance, with high levels of gentamicin resistance (Zarei *et al.*, 2018). According to Gonçalves *et al*. (2017), 73.9% of *P. aeruginosa* were multi-drug resistant and 43.9% were carbapenem resistant. Furthermore, high-level resistance to gentamicin, ciprofloxacin, and imipenem, with no resistance to colistin, was reported in *P. aeruginosa* strains isolated from burn patients in a burn centre in Ahvaz, Iran (Khosravi *et al.*, 2017).

In conclusion, this study has highlighted the prevalence of Carbapenem-resistant *P. aeruginosa* in clinical specimens (urine, wound, ear swab and stool) with the wound specimen recording the highest prevalence. More so, the prevalence of CRPA was very high and findings showed that these isolates exhibited multi-drug resistance. Thus, the urgent need for enhanced infection control measures, antibiotics stewardship programs and continued monitoring to curb the spread of multi-drug resistant isolates is highly recommended.

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