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Antibiogram Comparative Analysis of Multi-Drug Resistant Gram Negative Bacteria Isolated from Clinical Infections in a Tertiary Hospital

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Abstract

Antibiotic-resistant bacteria (ARB) strains have become a global health threat. This study aimed to determine the antibiotic susceptibility pattern of Klebsiella pneumoniae and Pseudomonas aeruginosa isolated from clinical specimens (urine and wound) collected from 60 patients attending Federal Medical Center, Yenagoa, Nigeria. A total of 60 specimens were analyzed by culture, Gram staining and biochemical tests. The bacterial isolates were tested against ten commonly used antibiotics using antibiotic disc diffusion method. Plasmid profile analysis was done on the multidrug resistant isolates. Out of the sixty specimens, thirty-five (35) isolates were obtained with the percentage occurrence of Klebsiella pneumoniae (45.7%) and Pseudomonas aeruginosa (54.3%). The precentage frequency distribution of the bacterial isolates from wound was 17(48.6%) comprising Klebsiella pneumoniae with 7(20%) and Pseudomonas aeruginosa with 10(28.6%). While for urine; 18(51.4%) with Klebsiella pneumoniae and Pseudomonas aeruginosa having 9(25.7%) respectively. The highest number of isolates was obtained in urine with 18(51.4%). Most of the organisms were relatively sensitive to gentamycin, augmentin, ciprofloxacin and tarivid while resistant to reflacine, streptomycin, ceporex, nalidixic acid and augmentin. The plasmid profile analysis revealed the presence of multiple plasmid band only in P. aeruginosa at a molecular weight of 9498bp while absence for Klebsiella pneumoniae. The elevated resistance level of Klebsiella pneumoniae could be a sign of self-medication or usage of antibiotics inappropriately, which should be discouraged in order to curtail the menace of antibiotic resistance.

1.0. Introduction

In the history of chemotherapy, the greatest success is the discovery of antibiotics and their subsequent development for the treatment of infectious diseases. Nonetheless, the indiscriminate and extensive use of antibiotics over the past 70 years has resulted in the selection of strains that are resistant to every antibiotic that has been made available to far. Resistance was noted in the late 1930s with the introduction of the first antimicrobial drugs, such as sulfonamides [1]. Antimicrobial resistance (AMR) has been identified to be caused by several reasons. One of the main causes of AMR is acknowledged to be human usage of antimicrobial medications, especially antibiotics. Antibiotics are among the most well-known medications to the general population. As of 2015, it was projected that over 35 billion daily doses of antibiotics were consumed worldwide [2].
Infections with multidrug-resistant (MDR) bacteria have emerged as a cause of public health threat all over the world at a terrifying rate because they are hard to treat since few or even no treatment

options remain. These MDR bacteria can be life threatening specially for critically ill and hospitalized patients. In some cases, health care providers have to use antibiotics that are more toxic for the patient. Due to the pacing advent of new resistance mechanisms and decrease in efficiency of treating common infectious diseases, it results in failure of microbial response to standard treatment, leading to prolonged illness, higher medical costs and an immense risk of death. Although the development of MDR is a natural phenomenon, the inappropriate use of antimicrobial drugs, inadequate sanitary conditions and poor infection prevention and control practices contribute to emergence of and encourage the further spread of MDR. To decrease the rise and spread of MDR, cooperative efforts are required globally to focus on areas which are susceptible to inappropriate use of antimicrobials. This study provides up-to-date information on the multi-drug resistant patterns of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from clinical infections.

Antimicrobial resistance has always been a global health concern challenging treatment of human infections caused by MDR bacterial pathogens. This problem has opened a wide range of research studies investigating the possible strategies to address the problem. Various strategies have been put forth to address AMR. Antimicrobial stewardship programs, which support and encourage the responsible use of antibiotics, are one efficient approach to give interventions [3]. In order to prevent AMR, the concept of "one health" has more traction in the fight against AMR. According to [4], “one health” is a strategy for enhancing public health by encouraging and fostering collaboration amongst diverse fields.

Pseudomonas aeruginosa and *Klebsiella pneumoniae*, both Gram-negative rods, have been implicated as major causes of nosocomial infections [5]. However, other organisms have been associated with hospitals and the community that negate treatment because they are resistant to multiple antibiotics [6, 7]. According to [8], *Pseudomonas aeruginosa* is the main bacterium responsible for cystic fibrosis (CF) lung infections. A persistent infection with this bacterium is resistant to antibiotic therapy, which worsens lung function and eventually causes death in CF patients. Furthermore, *P. aeruginosa* is linked to a higher death rate in patients with chronic obstructive pulmonary disease (COPD), accounting for more than 5% of infectious exacerbations in this population [9]. As reported by [10] and [11], *Klebsiella pneumoniae* has shown a significant level of resistance to a wide range of medications, including aminoglycosides, fluoroquinolones, and beta-lactam antibiotics. Due to this resistance, there is an increasing global issue with selecting appropriate antibiotics for hospital-acquired infections [1]. Among the most prevalent bacterial illnesses are urinary tract infections (UTIs), which are common infections that are widespread in both hospital and community settings, and there is a rising concern about the escalating antibiotic resistance associated with them. The widespread use of broad-spectrum antibiotics for UTI symptoms is linked to self-medication or ineffective empirical treatment of patients who visit clinics or emergency rooms, which promotes the fast development of multidrug-resistant bacteria [12, 13]. Inadequate and prolonged antimicrobial prophylaxis increases resistance to antimicrobial drugs. Indeed this is more evident in developing countries like Nigeria, where drugs including antibiotics can be purchased over the counter without a doctor’s prescription. In addition, antimicrobials are hawked and dispensed at street and market corners by anybody that has the means. This of course comes with its concomitant abuse due to lack of proper legislation. In recent years antibiotic resistance has become a significant human health issue. Multiple antimicrobial resistant bacteria are considered presently as a great global threat to public health. Thus, this study was carried out to investigate multi-drug resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from clinical infections.

2.0. Materials and Method

2.1. Study Area

The area of study was Federal Medical Centre (FMC), Yenagoa, Bayelsa State. FMC, Yenegoa is located at Hospital Road, Ovum, Yenagoa. It is at the Southern part of Nigeria at longitude

6.268198º E and latitude 4.938862ºN. It is a Federal Medical Centre that provides quality tertiary health services for patients.

2.2. Specimen Collection
A total of 60 clinical specimens which comprised 30 urine specimens and 30 wound specimens were collected from 60 patients attending FMC, Yenagoa. The Laboratory analysis was carried out in Microbiology Laboratory of Federal University Otuoke, Bayelsa State, West-Campus.

2.3. Ethical clearance
Ethical clearance for conducting this project was sought from the Hospital's Ethical Committee before commencement of the research project. No problem was envisaged with ethical clearance since there are no manipulations in the human subjects that were involved.

2.4. Inclusion criteria
Patients who are not on any antibiotic treatment within the last 14 days.

2.5. Exclusive criteria
Patients currently on antibiotic treatment within the last 14 days.

2.6. Isolation of organisms
The specimens were cultured on MacConkey agar for *K. pneumoniae* and Centrimide agar for *P. aeruginosa* and incubated overnight at 37ºC. The isolates were sub-cultured on nutrient agar to obtain pure culture.

2.7. Identification of isolates
The isolates were identified by Gram staining and biochemical tests such as oxidase test, indole test, citrate test and motility test [14].

2.8. Antibacterial sensitivity testing
The disc diffusion method according to the modified Kirby-Bauer technique with Clinical Laboratory Standards Institutes guidelines [15] was used. The disc diffusion method was performed using Mueller-Hinton Agar (Hi Flown Biotech, UK). The inoculum was prepared using nutrient broth (HI Media, India), the broth was incubated at 35°C until it achieves turbidity and the turbidity was adjusted to 0.5 McFarland standard. Inoculation on Mueller-Hinton Agar was done by dipping a sterile cotton swab into the standardized bacterial suspension and streaked. Using a sterile forceps, the Gram-negative antibiotic discs containing ten (10) different antibiotics; gentamycin (10µg), augmentin (30µg), ciprofloxacin (10µg), septrin (30µg), streptomycin (30µg), ceporex (30µg), tarivid (10µg), nalidixic acid (20µg), ampicillin(10µg), and reflacine (10µg) were placed on the surfaces of the inoculated plates. The plates were incubated at 35°C and observed for zones of inhibition afterwards. The diameter of zones of inhibition were measured in mm and compared with zone diameter interpretative chart, with the test results classed as Sensitive (S), Intermediate (I) or Resistant (R) using the Clinical Laboratory Standard Institutes guidelines [15]. In cases where isolates demonstrated resistance to three or more of the tested drugs, they were classified as multidrug resistant [16].

2.9. Plasmid Extraction
Extraction of plasmid from bacteria isolates was carried out using TENS-miniprep method [17]. An overnight bacterial culture on agar plates was harvested using a sterile inoculating loop and transferred into 100µl of nutrient broth medium. Each Eppendorf tubes (5) were vortexed using a vortex machine (Sci Finetech vortex mixer microfield) at high speed to resuspend the cells

completely. 300µl of TENS buffer was added and the tubes were mixed by inverting them 3-5 times until the mixture became sticky ensuring to prevent chromosomal DNA degradation which may affect the plasmid DNA. 150µl of 3.0m sodium acetate (pH 5.2) was added into the tubes respectively that contains the isolates harvested cells, all the tubes were vortex to mix the cells and the reagents completely. After vortexing, the tubes were spun using microcentrifuge (Beckman Coulter Microfuge) for 5 minutes at the highest revolution per minute (14,000rpm) in order to pellet the cell debris and chromosomal DNA. After spinning, the supernatant was transferred into a fresh labeled sterile Eppendorf tube, where it was mixed with 900µl of 100% ethanol (absolute) which has been pre-cooled to -20C. Then spun for two (2) minutes at 1000rpm to precipitate the plasmid DNA (white pellet is observed) from the supernatant. The supernatant was discarded; the pellet was rinsed twice with 500µl of 70% ethanol, mixed by vortexing and spun for two (2) minutes at 14,000rpm. The supernatant was discarded by decanting, blotted and dried for 3hours at a safe and sterile atmosphere. The plasmids extracted were packed in a Ziploc bag and was kept inside the freezer for further use.

2.10. Agarose Gel Electrophoresis (AGE)

The plasmid and DNA were analyzed by gel electrophoresis. Agarose gel (1.5%) was prepared by dissolving 1.5g agarose in 100ml of 1 X TBE (Tris borate EDTA) buffer. The slurry was heated on an electric cooker to dissolve the agarose and the solution was allowed to cool to about 50^0C. Two (2) drops of ethidium bromide (EtBr) as an intercalating agent was added to the solution and was gently swirl for even mixture. The solution was carefully poured into the horizontal gel casting tray of which two (2) combs has been inserted prior to the pouring and the gel was left to set at room temperature. The combs were carefully removed and 4µl each of the plasmid samples was mixed with 1µl of the loading dye giving 5µl each for nineteen (19) samples. The DNA ladder was loaded in the first well and the samples were loaded using a micropipette into each well starting from the second well. The gel was submerged in the electrophoresis tank and 0.5 X TBE buffer was poured into the tank the buffer covers the surface of the gel. The electrodes were connected to the power source and run at 80volts for 45 minutes. Plasmids were visualized on ultraviolet (UV-Transilluminator) and the bands were photographed using gel documentation. Twelve (12) bands were pictured from the 19 samples ran using gel documentation.

2.11. Plasmid Curing with Acridine Orange

Plasmid curing was carried out by treatment with acridine orange. After the gel documentation, the two (2) bacterial isolates that produced bands were subjected to curing. The preserved bacterial isolates were subcultured by streaking on nutrient agar plates and incubated at 37^0C for 24 hours. The overnight bacteria culture were harvested in 1ml of lysogeny broth, each labeled and incubated at 37^0C for 24 hours. 85ml of nutrient agar was prepared into a conical flask and it was supplemented with 0.043g of acridine orange. The solution was carefully mixed by swirling and reaction (change in colour) was observed. The overnight broth culture was vortexed for 1 minute to mix completely and the micro-centrifuge was used for spinning at 10,000rpm for 5 minutes to pellet cell debris. After spinning, the supernatant was discarded by decanting leaving the cell debris. 1ml of the acridine orange broth was suspended in each of the Eppendorf tubes, mixed by vortexing and each tube was wrapped with aluminum foil because acridine orange is light sensitive. The tubes were incubated at 37^0C for 24 hours in a rotary incubator.

2.12. Application of Antibiotics Sensitivity Disc

The two (2) isolates in acridine orange broth incubated in a shaking/rotary incubator was brought out and each was swabbed with a sterile swab stick on Mueller-Hinton agar plates. Antibiotic

susceptibility testing was performed using Kirby-Bauer disc diffusion method according to the recommended standard of Clinical Laboratory Standard Institute [18]. The eight (8) different discs (Rapids Labs International) contained the following antibiotics for Gram negative: gentamicin CN (10µg), azithromycin AZM (15µg), chloramphenicol C (30µg), cefpodoxime CPD (10µg), amoxicillin-clavulanic acid AUG (30µg), cefotaxime CTX (30µg), ciprofloxacin CIP (5µg), tetracycline TE 30µg. The results were recorded after 24 hours of incubation and the diameter of the inhibitory zone surrounding each disc was measured and interpreted as sensitive, intermediate and resistant using CLSI guideline [19, 20].

2.13. Statistical Analysis

The data obtained was subjected to analysis of variance (AVOVA) test to determine the significant difference at 95% confidence limit.

3.0. Result

The result of this study revealed the presence of *K. pneumoniae* and *P. aeruginosa* isolates from wound and urine. Out of the sixty specimens, thirty-five (35) isolates were obtained, the occurrence of the isolates were *K. pneumoniae* (45.7%) and *P. aeruginosa* (54.3%). The percentage distribution of bacterial isolates from wound was 17(48.6%) comprising *K. pneumoniae* with 7(20%) and *P. aeruginosa* with 10(28.6%). While for urine; 18(51.4%) with *K. pneumoniae* and *P. aeruginosa* having 9(25.7%) respectively. The highest number of isolates was obtained in urine with 18(51.4%). The distribution of the bacterial isolates from wound and urine by age showed age group 16-25 years with 17(48.6%) had the highest number of isolates while the least number was within the age group 56-65years with 3(8.6%) as shown in Table 1. No significant difference; P>0.05 between the distribution of bacteria isolates from wound and urine was seen.

Table 1: Distribution of bacterial isolates from wound and urine specimens of patients in Federal Medical Centre, Yenagoa by age

Age interval (years)	Number of bacterial isolates from wound	Number of bacterial isolates from urine	Total number of isolates
16-25	5	12	17
26-35	3	1	4
36-45	3	4	7
46-55	4	0	4
56-65	2	1	3
Total	17	18	35

Table 2 shows the frequency distribution of *K. pneumoniae* and *P. aureginosa* from wound and urine specimen. The frequency distribution of bacterial isolates from wound was *K. pneumoniae* 7(20%) and *P. aeruginosa* 10(28.6%), whereas from urine, *K. pneumoniae* and *P. aeruginosa* were 9(25.7%) respectively.

Table 2: Frequency distribution of *K. pneumoniae* and *P. aureginosa* from wound and urine specimens

Organisms	Number of isolates from wound (%)	Number of isolates from urine (%)	Total number of isolates (%)
K. pneumoniae	7(20.0)	9(25.7)	16(45.7)

P. aeruginosa	10(28.6)	9(255.7)	19(54.3)
Total	17(48.6)	18(51.4)	35(100)

The percentage distribution of *K. pneumoniae* and *P. aeruginosa* isolates from wound and urine is as shown in Figure 1. The percentage distribution of bacterial isolates from wound was *K. pneumoniae* 7(20%) and *P. aeruginosa* 10(28.6%). While for urine, *K. pneumoniae* and *P. aeruginosa* had 9(25.7%) respectively.

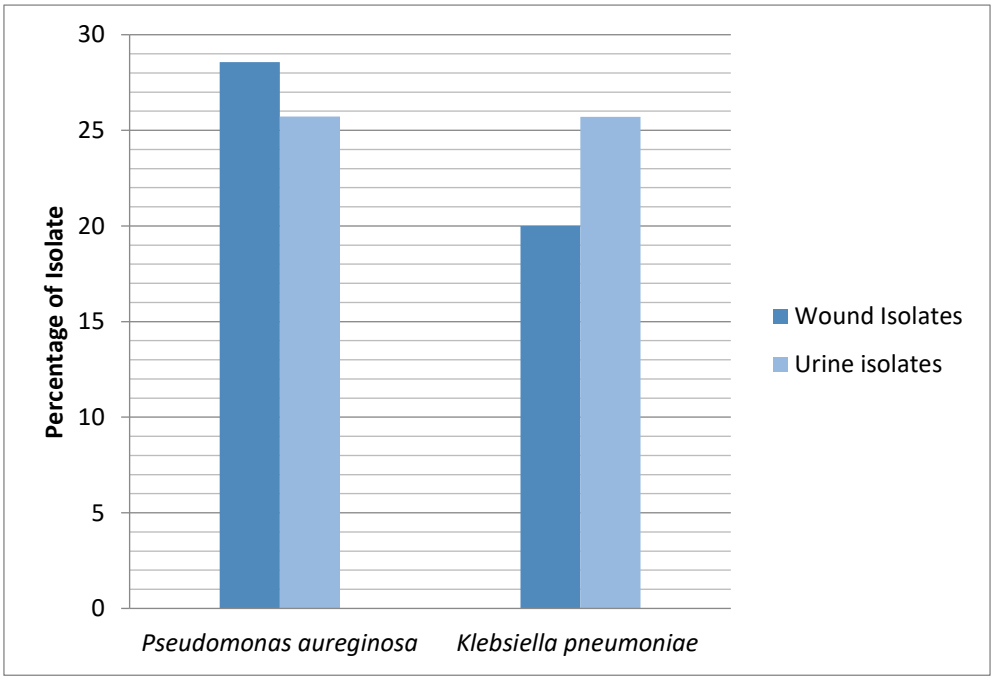

Figure 1: Percentage distribution of *K. pneumoniae* and *P. aeruginosa* isolates from wound and urine.

Figures 2a and **2b** show the antibiogram pattern of *P. aeruginosa* isolates from urine and wound respectively. The organism was sensitive to gentamycin, ciprofloxacin and tarivid in both urine and wound specimen. Resistance was observed with augumentin, septrin, ampicillin, ceporex and nalidixic acid for both urine and wound specimen. There is no significant difference between the antibiogram of *P. aeruginosa* isolates from urine and wound at $P>0.05$.

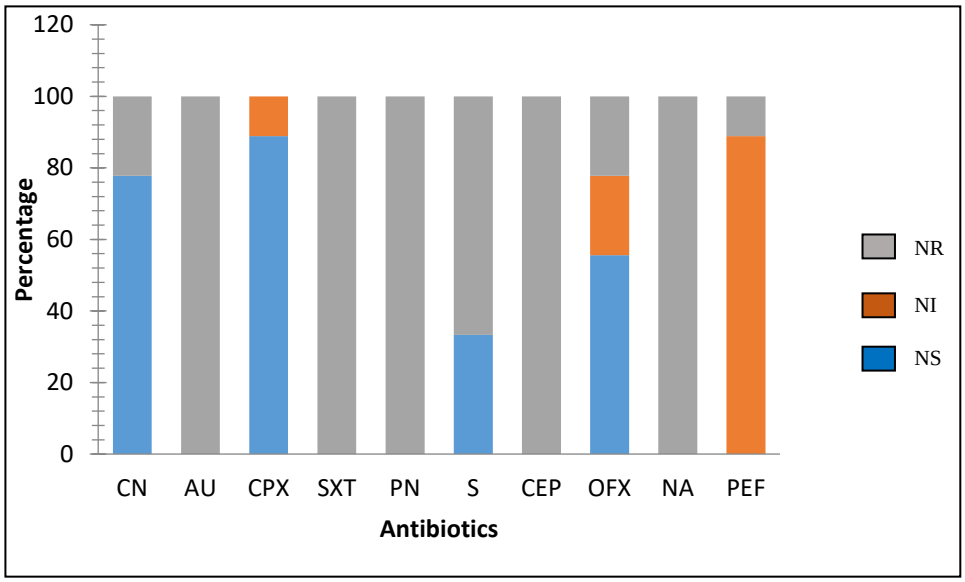

Figure 2a: Antibiogram pattern of *P. aeruginosa* isolates from urine

Key: CN-gentamycin, AU- augmentin, CPX-ciprofloxacin, SXT-septrin, PN-ampicillin, S- Streptomycin, CEP-ceporex, OFX-tarvid, NA- nalidixic acid and PEF-reflacine.
NS: number of sensitive, NI: number of intermediate, NR: number of resistant.

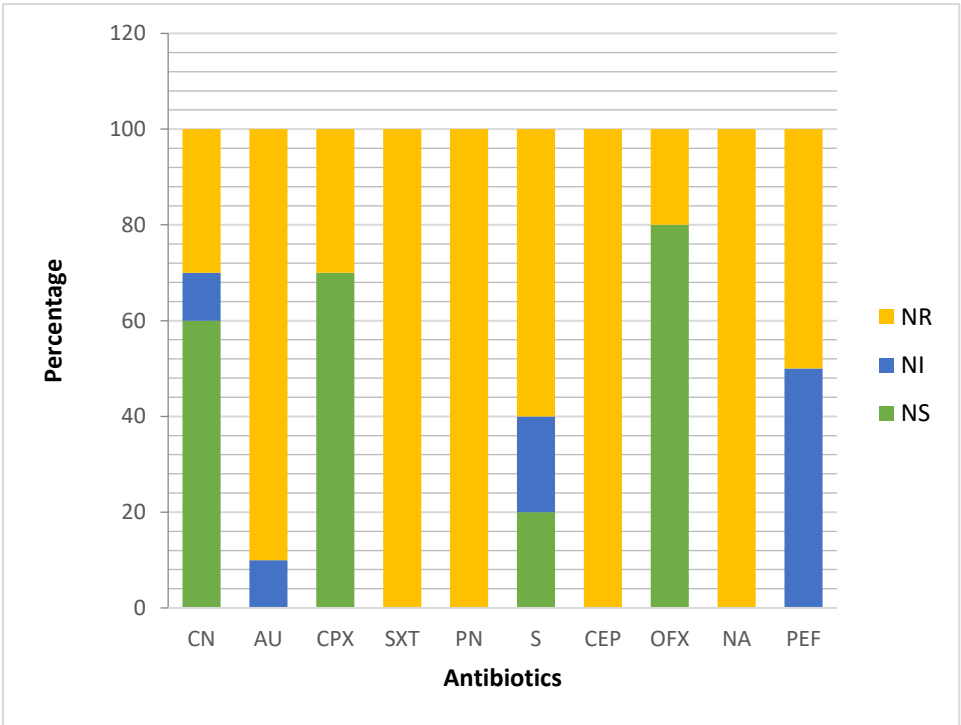

Figure 2b: Antibiogram pattern of *P. aeruginosa* isolates from wound.

Key: CN-gentamycin, AU- augmentin, CPX-ciprofloxacin, SXT-septrin, PN-ampicillin, S- Streptomycin, CEP-ceporex, OFX-tarvid, NA- nalidixic acid and PEF-reflacine.
NS: number of sensitive, NI: number of intermediate, NR: number of resistant.

Figures 3a and **3b** show the antibiogram pattern of *K. pneumoniae* isolates from wound and urine specimens respectively. The organism was sensitive to augmentin and tarivid in both urine and

wound specimen. *K. pneumoniae* was resistant to septrin, ampicillin, streptomycin, ceporex and nalidixic acid for urine while in wound, it was resistant to ciprofloxacin, septrin, ceporex, nalidixic acid and reflacine. There is no significant difference between the antibiogram pattern of *K. pneumoniae* isolates from urine and wound specimen at P>0.05.

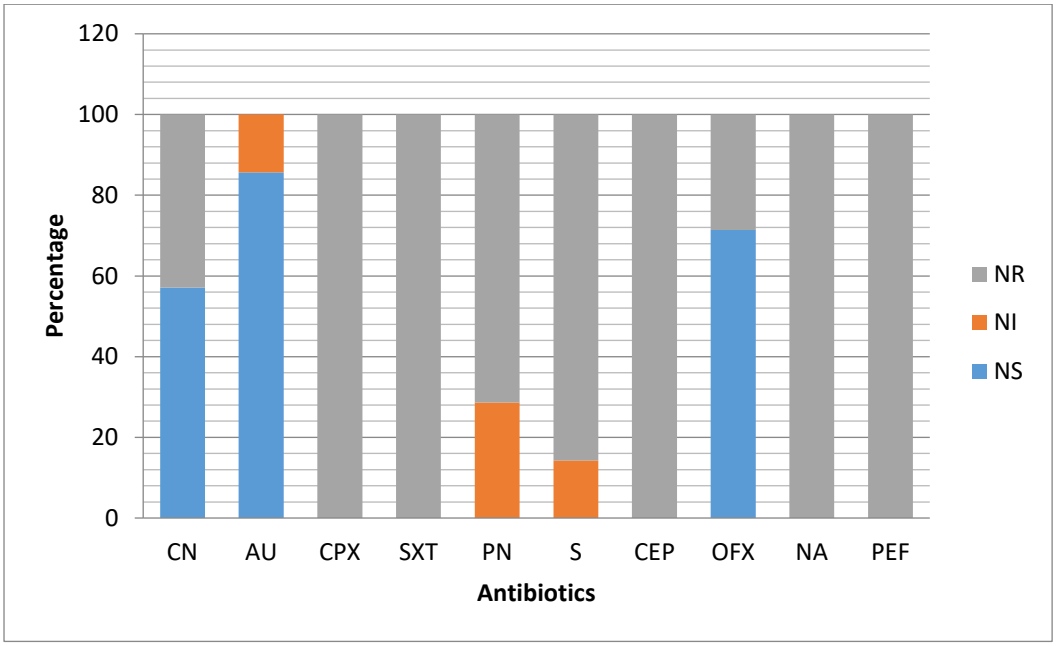

Figure 3a: Antibiogram pattern of *K. pneumoniae* isolates from wound

Key: CN-gentamycin, AU- augmentin, CPX-ciprofloxacin, SXT-septrin, PN-ampicillin, S- Streptomycin, CEP-ceporex, OFX-tarvid, NA- nalidixic acid and PEF-reflacine.
NS: number of sensitive, NI: number of intermediate, NR: number of resistant.

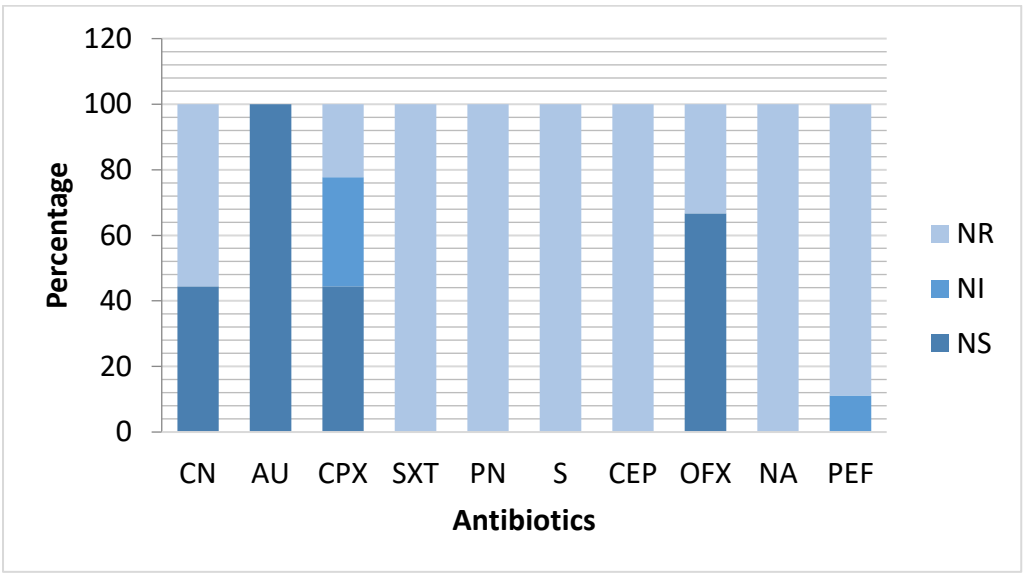

Figure 3b: Antibibiogram pattern of *K. pneumoniae* isolates from urine

Key: CN-gentamycin, AU- augmentin, CPX-ciprofloxacin, SXT-septrin, PN-ampicillin, S- Streptomycin, CEP-ceporex, OFX-tarvid, NA- nalidixic acid and PEF-reflacine.
NS: number of sensitive, NI: number of intermediate, NR: number of resistant.

The antibiotic susceptibility test of multidrug resistant *K. pneumoniae* and *P. aeruginosa* before and after curing using plasmid profiling is as indicated in **Tables 3a and 3b** below. After curing, the majority of antibiotics to which the *K. pneumoniae* had previously exhibited resistance still showed resistance while for *P. aeruginosa;* there was sensitivity to the previously resistant antibiotics except for azithromycin.

Table 3a: Antibiotics Susceptibility test before curing.

Organisms	CN	AZM	C	CPD	AUG	CTX	CIP	TE
K. pneumoniae	-	12	10	-	-	-	13	10
P. aeruginosa	12	-	11	14	12	13	15	16

All measurements are in millimeter (mm). CN - gentamicin, AZM- azithromycin,
C - chloramphenicol, CPD- cefpodoxime, AUG- amoxicillin-clavulanic acid, CTX- cefotaxime, CIP - ciprofloxacin, TE - tetracycline

Table 3b: Antibiotics susceptibility test after curing

Organisms	CN	AZM	C	CPD	AUG	CTX	CIP	TE
K. pneumoniae	-	-	-	-	-	-	-	-
P. aeruginosa	17	-	19	16	15	25	23	17

All measurements are in millimeter (mm). CN - gentamicin, AZM - azithromycin,
C-chloramphenicol, CPD - cefpodoxime, AUG - amoxicillin-clavulanic acid, CTX - cefotaxime, CIP - ciprofloxacin, TE- tetracycline

In **Table 4**, The highest sensitivity were seen with cefotaxime and ciprofloxacin, exhibiting the zones of inhibition of 25mm and 23mm respectively for *P. aeruginosa* .

Table 4: Interpretation of Susceptibility test before and after Plasmid curing

Antibiotics	Susceptibility test before curing						Susceptibility test after curing					
	K. pneumoniae			*P. aeruginosa*			*K. pneumoniae*			*P. aeruginosa*		
	S	I	R	S	I	R	S	I	R	S	I	R
Gentamicin			0			12			0	17		
Azithromycin			12			0			0			0
Chloramphenicol			10			11			0	19		
Cefpodoxime			0			14			0	16		
Amoxicillin-clavulanic acid			0			12			0	15		
Cefotaxime			0			13			0	25		
Ciprofloxacin			13			15			0	23		
Tetracycline			10			16			0	17		

Key: S- Sensitive, I-Intermediate, R-Resistant

The Plasmid profile of *K. pneumoniae* and *P. aeruginosa* from wound and urine is illustrated in **Figure 4**. The plasmid analysis indicates the presence of multidrug-resistant *P. aeruginosa* isolates harbor multiple plasmid DNA with a molecular weight of 9498kbp while there was the absence of plasmid in *K. pneumoniae.*

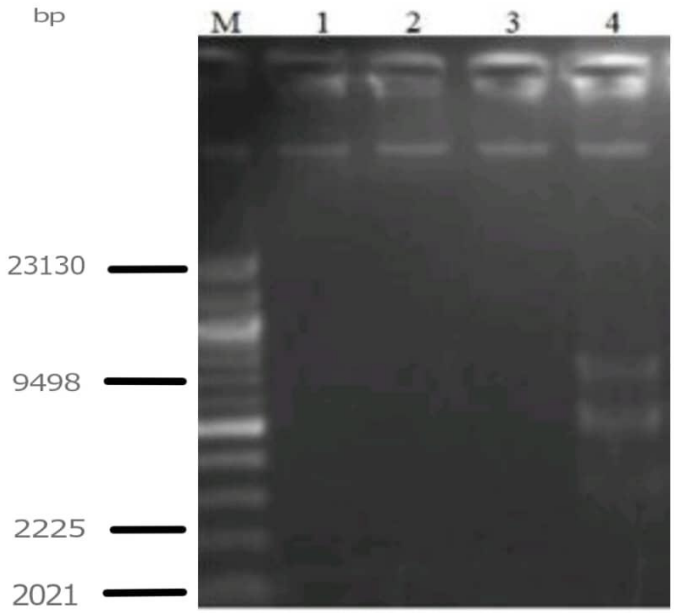

Figure 4: Molecular weight of plasmid DNA on an agarose gel stained with ethidium bromide. Lane (M) marker DNA - 23130bp DNA ladder, lanes 1, 2, and 3: no plasmid band of *K. pneumoniae*, lane 4 multiple plasmid DNA bands of multidrug drug-resistant *P. aeruginosa*.

3.1. Discussion

Wound is one of the most common causes of hospitalization. Patients with open wound are at high risk for nosocomial infections with opportunistic environmental bacteria [21]. As such, prevention of bacterial transmission from hospital environments to patients or directly from one patient to another especially MDR strains, can decrease morbidity and mortality in burns [22]. In healthcare settings, microbial contamination of wounds is a major issue particularly in surgical practice where the site of a sterile procedure may become contaminated and subsequently infected, potentially leading to the onset of illness [23].

Urinary tract infection (UTI) remains a significant healthcare challenge on a global scale representing a leading cause of mortality despite the array of therapeutic options available for its management. Notably, the treatment of urinary tract infections acquired in community settings faces a growing threat from antimicrobial resistance. The current investigation unveiled the detection of *K. pneumoniae* (45.7%) and *P. aeruginosa* (54.3%) in individuals presenting with urinary tract and wound infections. The prevalence of these identified bacteria in urinary tract infections was found

to be 18 out of 30 cases (60%) while in wound infections, it was 17 out of 30 cases (56.7%). Within the age range of 16 to 25 years, microbial colonization of the urinary system and wound infection were noted.

Our results are comparable to the study conducted in Southern Uganda by [24] where they found that the age group of 10-19 years exhibited the highest infection rates, although our study noted the most affected age group is slightly older. In the case of urinary tract infection (UTI), the incidence of *K. pneumoniae* and *P. aeruginosa* within the age group could be attributed to suboptimal hygiene practices. Conversely, for wound infections, factors such as inadequate hygiene practices, unhealthy lifestyle choices and nutritional deficiencies may contribute to the observed patterns. Notably, our study observed age group of 56-65 years displayed the least incidence of wound infections. In contrast, [25] reported individuals >60 years as the most affected, suggesting a discrepancy with our findings. The lower infection rate observed in our age group may be associated with a greater emphasis on avoiding contamination and adherence to practicing good hygiene, thereby reducing the risk of cross-infection and endogenous infection. While our findings align with the results of [26], it is essential to acknowledge that these observations may not necessarily imply a direct relationship between age and susceptibility to wound infections.

K. pneumoniae and *P. aeruginosa* were identified as predominant pathogens in urinary tract infections, accounting for 9(25.7%) of cases. This result is in line with a 2009 study by [27] where *P. aeruginosa* and *Klebsiella* spp. were similarly reported as the predominant bacteria in urinary tract infections respectively. Additionally, [28] documented *K. pneumoniae* as the predominant bacteria in UTIs, corroborating our findings. In contrast, a study encompassing a diverse range of uropathogens indicated the presence of *Klebsiella* spp. and *P. aeruginosa* in urine cultures from patients exhibiting clinical symptoms of urinary tract infections, with *E. coli* identified as the primary pathogen, followed by *Klebsiella* spp. and *P. aeruginosa* [29]. These collective observations underscore the consistency of microbial patterns in urinary tract infections across different studies, providing valuable insights into the prevalence and distribution of specific bacterial strains associated with this clinical condition.

P. aeruginosa emerged as the predominant bacterium in wound infections with a prevalence rate of 33.3% (10/30) in our study. This finding is consistent with the work conducted by [30] in Abia state, Nigeria where a comparable prevalence rate of 32.9% was reported. Additionally, a study in the United Arab Emirates (U.A.E) documented a microbiological examination of 67 surgical wound patients where 35.6% were diagnosed with *P. aeruginosa* infection, although this rate exceeded our observed prevalence [31]. Disparities in prevalence may be attributed to variations in study populations and hygienic practices. Furthermore, a global study highlighted Nigeria's isolation rate of 30.6% for *P. aeruginosa* in surgical wound infections [32] while the median frequency of *P. aeruginosa* isolation for the entire African region was documented at 23% [33]. The elevated prevalence of *P. aeruginosa* in our investigation may be attributed to its common role as an opportunistic pathogen as noted in studies by [34] and [35]. Additionally, *P. aeruginosa* virulence and adaptability to hospital environments have been well-documented [36]. However, our findings further highlights the prevalence of *P. aeruginosa* in wound infections within diverse geographic and emphasizing the importance of vigilance in infection control measures within healthcare settings.

The bacterial isolates exhibited sensitivity to gentamycin, augmentin, ciprofloxacin and tarivid. A finding consistent with the study conducted by [37]. However, *K. pneumoniae* demonstrated notable multidrug-resistant (MDR) resistance, aligning with the findings reported by [38] and a study in Saudi Arabia which documented high resistance rates (≥ 60%) for *K. pneumoniae* against various tested antibiotics [39]. In comparison with *P. aeruginosa*, *K. pneumoniae* demonstrated elevated

resistance levels especially in wound infections in our study. The elevated resistance observed in *K. pneumoniae* raises concerns about potential self-medication practices or inappropriate antibiotic usage by patients, suggesting uncontrolled access to these antimicrobial agents. This may be indicative of a lack of effective policy enforcement regarding antibiotic sales, contributing to widespread availability through unregulated outlets. The substantial antimicrobial resistance reported underscores the importance of minimizing antibiotic use by patients, urging clinicians to exercise caution in prescribing antibiotics and considering combined therapies when deemed necessary for the prevention and treatment of bacterial or hospital-acquired infections particularly in vulnerable patient populations. Moreover, our study suggests a potential association between the genetic profiles of *P. aeruginosa* and *K. pneumoniae* isolated from clinical specimens given their similar antibiotic susceptibility patterns. This observation could further contribute to the exploration of the interconnectedness between these bacterial strains.

The multiple antibiotics-resistant *K. pneumoniae* and *P. aeruginosa* were subjected to curing procedures. Before curing, the multidrug-resistant *K. pneumoniae* and *P. aeruginosa* isolates were resistant. The resistance found in *P. aeruginosa* could be due to a combination of different antibiotic resistance genes leading to a wide range of multidrug resistance [40] or possibly modifications of target sites the drugs due to mutation, biofilm-mediation, and development of multidrug-tolerant persister cells [41]. After curing, *P. aeruginosa* became sensitive to most antibiotics, displaying the highest sensitivity, particularly to cefotaxime and ciprofloxacin with zones of inhibition measuring 25mm and 23mm respectively. In contrast, *K. pneumoniae* retained resistance to all antibiotics after the curing process. Multidrug-resistant isolates of *K. pneumoniae* were found to lack plasmids while *P. aeruginosa* exhibited plasmids with a molecular weight of 9498 base pairs as determined by plasmid analysis. The observed resistance in *K. pneumoniae* possibly plasmid-related, demonstrates the association between plasmids and various forms of drug resistance. Plasmid has been linked as primary carriers of antimicrobial resistance genes in multidrug *K. pneumoniae,* and are nearly present in all antimicrobial-resistant *K. pneumoniae* as reported by [42] and [43]. These findings align with the research conducted by [44] and [45], further contributing to the role of plasmids in mediating antibiotic resistance. [46] stated that infections caused by *K. pneumoniae* are through gene or plasmid transfer. Therefore, the resistance in *K. pneumoniae* among the patients with urinary tract infections in this study could also be attributed to the presence of chromosomal and plasmid-encoded antimicrobial resistance genes [47].

4.0. Conclusion

The outcomes of this study highlights the presence of antibiotic-resistant bacteria within hospital settings, posing potential challenges such as therapeutic complexities, prolonged hospitalization, heightened mortality and morbidity rates and escalated treatment costs. Consequently, hospital infection control authorities are tasked with striking a delicate balance between ensuring the availability of safe and efficacious medications. In response to the emergence of antibiotic resistance, we advocate for the development of novel antimicrobial drugs and the exploration of combination therapies to effectively counter the evolving landscape of resistant pathogens. Additionally, there is a critical need for the establishment of comprehensive guidelines for the systematic monitoring and recording of antibiotic resistance cases on a global scale. This proactive approach could be essential for understanding of the dynamics of antibiotic resistance, guiding therapeutic strategies and ultimately mitigating the adverse impacts of resistant bacterial strains in healthcare settings.

References

[1] Davies, J.C and Davies, D. (2010). Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev. 74:417–433.
[2] Klein, E.Y., Van Boeckel, T.P, Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H. and Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci., 115(15):3463-3470.
[3] Kaki, R., Elligsen, M. and Walker, S. (2011). Impact of antimicrobial stewardship in critical care: a systematic review. J Antimicrob Chemother. 66:1223–30.

[4] World Health Organization, (2018).Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017-2018. World Health Organization: Geneva, Switzerland, ISBN 978-92-4-151506-1.

[5] Aernan P. T. and Umeh E.U. (2016). Molecular Characterization of Extended-Spectrum Beta Lactamase (ESBLs) genes in Pseudomonas aeruginosa from Pregnant Women Attending a Tertiary Health Care Centre in Makurdi, Central Nigeria. International Journal of Scientific and Research Publications; 6(10): 19-24.

[6] World Health Organization, (2014). Antimicrobial resistance: global report on surveillance 2014.World Health Organization; 2014. http://www.who.int/drugresistance/documents/surveillancereport/en/

[7] Julia, D., Brandon, R., Monique, W.R, Benjamin, H., Tobias, B., Sehaam, K. and Wesaal, K. (2022). Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. International Journal of Hygiene and Environmental Health; 244, 114006.
[8] Lyczak, J.B., Cannon, C.L. and Pier, G.B. (2002). Lung infections associated with cystic fibrosis. Clin Microbiol Rev. 15:194-222.

[9] Murphy, T.F. (2009). Pseudomonas aeruginosa in adults with chronic obstructive pulmonary disease. Curr Opin Pulm Med, 15:138-142.

[10] Fair, R. J. and Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st Century. Perspect. Med. Chem. 6 25–64. 10.4137/PMC.S14459
[11] Dsouza R., Pinto N. A., Hwang I., Cho Y., Yong D., Choi J., Lee, K. and Chong, Y. (2017). Panel strain of Klebsiella pneumoniae for beta-lactam antibiotic evaluation: their phenotypic and genotypic characterization. PeerJ 5:e2896. 10.7717/peerj.2896
[12] Kiguba, R., Karamagi, C., Bird, S.M. (2016). Extensive antibiotic prescription rate among hospitalized patients in Uganda: But with frequent missed-dose days. J. Antimicrob. Chemother. 71:1697–1706.
[13] Omulo, S., Thumbi, S.M., Njenga, M.K., Call, D.R. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: What can be done better? Antimicrob. Resist. Infect. Control, 4, 1.
[14] Cheesebrough, M. (2006). District Laboratory Practice in Tropical Countries: Part Cambridge University Press, U.K; 134-135.
[15] Clinical and Laboratory Standard Institute. (2012). Performance standards for antimicrobial susceptibility testing, Clinical and Laboratory Standard Institute. CLSI document, Wayne, PA. M100–S124.
[16] Akingbade, O., Balagun, S., Ojo, D., Akinduti, P., Okerentugba, P.O., Nwanze, J.C. and Okonkwo, O. I. (2014). Resistant plasmid profile analysis of multi-drug resistant Escherichia coli isolated from urinary tract infections in Abeokuta, Nigeria. African Health Sciences, 14(4):821–828.

[17] Zhou, C., Y. Yang, and A.Y, Yong, 1990. 'Mini-prep' in ten minutes. Biotechniques 8(2): 172–17.
[18] Clinical and Laboratory Standards Institute, (2020). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.
[19]Clinical and Laboratory Standard Institute, (2010). Performance Standards for Antimicrobial Susceptibility Testing, Twentieth informational Supplement. CLSI document, Wayne, PA. M100–S20. vol 30:1
[20]Clinical and Laboratory Standard Institute, (2015). Performance Standards for Antimicrobial Susceptibility Testing, Twenty-fifth informational Supplement. CLSI document, Wayne, PA. M100–S25.
[21] Azimi, L., Motevallian, A., Ebrahimzadeh, N. A., Asghari, B., and Lari, A.R. (2011). Nosocomial infections in burned patients in Motahari Hospital, Tehran, Iran. Dermatol Res Pract: 436952.

[22] Lanini, S., D'Arezzo, S., Puro, V., Martini, L., Imperi, F., Piselli, P., Montanaro, M., Paoletti, S., Visca, P. and Ippolito, G. (2011). Molecular epidemiology of a Pseudomonas aeruginosa hospital outbreak driven by a contaminated disinfectant-soapdispenser. PLoS One. 6(2):17064.

[23] Kemebradikumo, P., Beleudanyo, G.F. and Oluwatoyosi, O. (2013). Current Microbial Isolates from Wound Swabs, Their Culture and Sensitivity Pattern at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria. Tropical Medicine and Health 41(2):49-53.
[24] Maltezou, H.C and Giamarellou, H. (2012). Community-acquired methicillin-resistant Staphylococcus aureus infections. International Antimicrobial Agents Journal, (27), 87–96.
[25] Azzah, S.A (2022). Bacteriological profile of wound swab and their antibiogram pattern in a tertiary care hospital, Saudi Arabia. Saudi Medical Journal, 43(12):1373- 1382.
[26] Sanusi, A., Bello, M. , Muhammad, N. B and Mohammed, A. G. (2019). Antibiogram profile of Pseudomonas aeruginosa isolated from wounds of patients attending some selected hospitals in Sokoto metropolis, Nigeria. GSC Biological and Pharmaceutical Sciences, 09(02):032–043.

[27] Aboderin, O.A., Abdu, A., Odetoyinbo, B.W and Lamikanra, A. (2009). Antimicrobial resistance in Escherichia coli strains from urinary tract infections. National Medical Association 101: 1268-1273.

[28] Emmanuel, E.B., Mbah, M., Akpan, S.S., Ikpi, E.E., Alaribe, A.A.A. (2023). Prevalence of Symptomatic Bacteriuria and Associated Risk Factors among Patients Attending Major Hospitals in Calabar, Nigeria. medRxiv 2023.09.26.23296138.
[29] Oluwafemi, T.T., Akinbodewa, A.A, Ogunleye, A. and Adejumo, O.A. (2018). Urinary tract infections and antibiotic sensitivity pattern of uropathogens in a tertiary hospital in South West, Nigeria. Sahel Medical Journal; 21:18-22.
[30] Nwachukwu, N.C, Orji, F.A. and Okike, U.M. (2009). Antibiotic Susceptibility Patterns of Bacterial Isolates from Surgical Wounds in Abia State University Teaching Hospital (ABSUTH), Aba - Nigeria, Research Journal of Medicine and Medical Sciences, 4(2):575-579.
[31] Al-Akayley M. (1999). Surgical wound infections in United Arab Emirates. Journal of Clinical Medicine, (20), 861- 870.
[32] Omoyibo, E.E., Oladele, A.O., Ibrahim, M.H., Adekunle, O.T. (2018). Antibiotic susceptibility of wound swab isolates in a tertiary hospital in Southwest Nigeria. Ann. Afr. Med. 17:110–116.
[33] Phan, S., Feng, C.H., Huang, R., Lee, Z.X., Moua, Y., Phung, O.J., Lenhard, J.R. (2023). Relative Abundance and Detection of Pseudomonas aeruginosa from Chronic Wound Infections Globally. Microorganisms. 11(5):1210.
[34] Balarjishvili, N.S.H, Kvachadze, L.I, Kutateladze, M.I, Meskhi T.S.H., Pataridze, T.K, Berishvili, T.A, Tevdoradze, E.S.H. (2015). New Virulent Bacteriophages Active against Multiresistant Pseudomonas aeruginosa Strains. Prikl Biokhim Mikrobiol.51(6):600-609.

[35] Okpukpu,E.N Wemedo,S.A.and Sampson,T. (2022). Prevalence and Antibiotics Susceptibility Profile of Pseudomonas aeruginosa Associated with Wound Infections in Port Harcourt, Nigeria. Acta Scientific Clinical Case Reports; 3, 9.
[36] Coffin SE and Zaoutis TE. (2011). Infection Control, Hospital Epidemiology and Patient Safety. Journal of Infectious Disease of Clinical North American, (19), 647-665.
[37] Aly, M. and Balkhy, H.H. (2012) "The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries," Antimicrobial Resistance and Infection Control, 1(26).

[38] Kebbeh, A., Dsane-Aidoo, P., Sanyang, K. Darboe, S.M.K., Fofana, N., Ameme, D., Sanyang, A.M., Darboe, K.S., Darboe, S., Sanneh, B., Kenu, E. and Anto, F. (2023). Antibiotics susceptibility patterns of uropathogenic bacteria: a cross-sectional analytic study at Kanifing General Hospital, The Gambia. BMC Infect Dis 23, 723.
[39] Yahaya, A. and Hamza, M.A.E. (2023). Prevalence and antimicrobial susceptibility pattern of bacterial uropathogens among adult patients in Madinah, Saudi Arabia. BMC Infectious Diseases 23,582.
[40] Tran, H.A., Vu,T.N.B., Trinh, S.T., Tran, D.L, Pham, H.M., Ngo, T.H.H., Nguyen, M.T., Tran, N.D., Pham, D.T., Dang, D.A., Shibayama, K., Suzuki, M., Yoshida, L.M., Trinh. H.S., Le, V.T., Vu, P.T., Luu, T.V.N., Bañuls, A.L., Trinh, K.L., Tran, V.A., Tran, H.H. and van Doorn, H.R. (2021). Resistance mechanisms and genetic relatedness among carbapenem-resistant Pseudomonas aeruginosa isolates from three major hospitals in Hanoi, Vietnam (2011-15). JAC Antimicrob Resist. 27;3(3):dlab103.
[41] Zheng, P., Renee, R., Bernard, R. G., Tong-Jun, L. and Zhenyu, C. (2019). Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies, Biotechnology Advances, 37(1): 177-192
[42] Pitout, J.D.D., Nordmann, P. and Poirel, L. (2015). Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominace. Antimicrob. Agents Chemother, 59:5873-5884.
[43] Navon-Venezie, S., Kondratyeva, K. and Carattoli, A. (2017). Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol. Rev. 41:252-275.
[44] Ramirez, M.S.; Iriarte, A.; Reyes-Lamothe, R.; Sherratt, D.J.; Tolmasky, M.E. (2019). Small Klebsiella pneumoniae Plasmids: Neglected Contributors to Antibiotic Resistance. Front. Microbiol. 10, 2182.

[45] Miyoung, L. and Tae-Jin, C. (2021). Antimicrobial Resistance Caused by KPC-2 Encoded by Promiscuous Plasmids of the Klebsiella pneumoniae ST307 Strain. Ann Lab Med 41(1): 86-94.

[46] Wyres, K.L. and Holt, K.E. (2018). Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr OpMicrobiology. 45:131–9.

[47] Li, Y., Kumar, S., Zhang, L., Wu, H. and Wu H. (2023). Characteristics of antibiotic resistance mechanisms and genes of Klebsiella pneumoniae. 12;18(1):20230707.