IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 *Volume 3 issue 1 Jan, 2024* Research Article

Plasmid Profile of Multidrug Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* Isolated from Urine Specimens in South-South, Nigeria

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ABSTRACT

Urinary tract infections (UTIs) continue to be the most prevalent type of infections affecting all age groups worldwide. The primary objective of this study was to ascertain the plasmid profiles and multidrug resistance (MDR) pattern of *Staphylococcus aureus* and *Pseudomonas aeruginosa* among female Students at Federal University Otuoke Bayelsa State, Nigeria. A total of fifty (50) urine specimens were collected in sterile containers and conventional microbiological methods were used for analysis. Antibiotic susceptibility testing was conducted by standard laboratory guidelines. Among the 50 examined, 28(56%) exhibited significant bacteriuria, out of which 20 had cases of *S. aureus* and 8 cases of *P. aeruginosa*. Statistical analysis revealed no significant difference (P>0.05) in the frequency of occurrence between *S. aureus* and *P. aeruginosa*. *S. aureus* exhibited high resistance to norfloxacin, amoxil, and ampiclox but was susceptible to ciprofloxacin, gentamicin, streptomycin, and levofloxacin. While *P. aeruginosa* displayed high resistance to ampicillin, nalidixic acid, and augmentin showing susceptibility to tarivid, ciprofloxacin, and septrin. Notably, 18(64.29%) isolates were resistant to more than two distinct antibiotic classes. Within this group of MDR isolates, 35.72% were identified as *S. aureus* and 28.57% were *P. aeruginosa*. Following curing, both *S. aureus* and *P. aeruginosa* became sensitivity to majority of antibiotics to which they had previously exhibited resistance. However, *P. aeruginosa* remained resistant to azithromycin, cefpodoxime, and amoxicillin-clavulanic acid. *S. aureus* and *P. aeruginosa* exhibited the highest sensitivity of 25mm and 23mm zones of inhibition respectively for Cefotaxime. These isolates were found to carry plasmid for MDR at 9466bp for *S. aureus* and *P. aeruginosa* respectively suggesting plasmid involvement in drug resistance within the majority of UTIs. Consequently, it is imperative to promote proper hygiene among individuals, monitor antibiotic resistance patterns, and raise public awareness through enlightenment campaigns.

Keywords: Urinary tract infections, plasmid profile, *S. aureus*, *P. aeruginosa*, multidrug-resistant (MDR) bacteria.

Introduction

The urinary tract, also known as the urinary system, comprises structures and organs involved in the production, transportation, storage, and excretion of urine. When any part of this is infested by a pathogenic microorganism, it results in a urinary tract infection (UTI). The kidney, ureter, bladder, urethra, nerve cells, and other related components are included in these structures (Otajevwo, 2013). Common clinical indications of UTI include a burning sensation during urination (micturition), increased urination frequency or incontinence, discomfort in the lower back or abdomen, cloudy, dar, or occasionally bloody urine, and an unusual odour in the urine accompanied by fever. Nausea or vomiting may also occur, particularly in the chronic stage of the infection (NKUDIC, 2014).

Reports have indicated instances of illness and mortality associated with the rising incidence of uropathogen-caused urinary tract infections, leading to a rise in hospital admissions and nosocomial infection (Iacovelli *et al*., 2014). Pathogenic microbes responsible for UTIs can encompass bacteria, fungi, viruses, or parasites. Notably, it is commonly known that bacteria are the main uropathogens that affect the urinary system (Abdullahi *et al*., 2020).

More than 100 million people worldwide are estimated to contract urinary tract infections annually (Abdullahi *et al*., 2020). This figure places a significant burden on both nations and affected individuals (Akingbade *et al*., 2014). The economic impact of UTI-related hospitalizations is substantial, with an estimated annual cost of $2.8 billion (Simmering *et al*., 2017).

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The epidemiology of pathogenic UTI cases is considerably undergoing significant changes, resulting in loss of lives. The increase in antibiotic resistance and the emergence of multi-drug resistant (MDR) pathogens are linked to the widespread prescription of inadequate empirical antibiotic therapy, often without conducting antibiotic susceptibility testing, ultimately leading to ineffective UTI treatment (Adamus-Bialek *et al*., 2018).

Virulence factors for UTI caused by *S. aureus* and *P. aeruginosa* include specific surface-associated adhesions, toxin production, cell wall polysaccharides, motility organelles, and siderophores (Bien and Bozko, 2012). Sexual contact with an infected individual, gender (Dielubanza and Scheaffer, 2011), urinary catheter use (Gould *et al*., 2010), genetic predisposition, underlying medical conditions like diabetes, uncircumcised status (Morris and Wiswell, 2013), enlarged prostates (Lane and Takhar, 2011), and spinal cord injury from using catheter use to void in cases of urethral dysfunction (Eves and Rivera, 2010). Another known risk factor for UTIs is prior exposure. UTI risk is also increased by cultural customs and habits, as well as poor personal cleanliness. The leukocyte esterase test, which finds blood in urine (10 WBC/mm3), urinalysis, and the presence of nitrite in a urine sample which indicates a UTI are the main methods used to diagnose UTIs (Sobel and Kaye, 2014). However, the most popular and extensively utilized techniques for diagnosis are urine microscopy, culture, and sensitivity.

Antimicrobial resistance in pathogenic organisms is a global concern, with *S. aureus* and *P. aeruginosa receiving* special attention (Chakupurakal *et al*., 2011). It is imperative to intensify efforts to identify the agents responsible for UTIs in connection with their susceptibility to antibiotics to easily accessible medications. This will support the formulation of suitable prescription guidelines and the local community's choice of antimicrobial drugs. Multidrug-resistant bacteria that cause UTIs are a constant threat to societies (Reis *et al*., 2016). This problem is acknowledged by many as a significant health concern (Timothy *et al*., 2014). Microbial resistance among uropathogens has increased as a result of patients and healthcare providers' careless use and management of antimicrobial drugs, posing a serious health threat (Sharif *et al*., 2012).

The nature of infection is determined by factors such as age, individual health status, and the resistance profile of infecting species (Tong *et al*., 2015). Many instances of illness and renal abnormalities or failures have been attributed to *S. aureus* and *P. aeruginosa* infections among urology patients. In epidemiological studies, the determination of plasmid is of great importance. It is considered one of the early techniques based on DNA to access resistance patterns, frequency, and resistance's prospective state in the future concerning specific parameters (Nworie *et al*., 2013). Molecular identification of genes provides valuable information about the resistance mechanisms, the long-term effectiveness of bacteria in maintaining these traits, and a genotypic perspective on resistance. The study of plasmids and their transfer frequency sheds light on the extent of resistance transfer and the emergence of resistance in novel strains (Nworie *et al*., 2013). Hence, this study was aimed at determining the plasmid profile of multidrug-resistant *S. aureus* and *P. aeruginosa* isolated from urine specimens of female students of the Federal University Otuoke, Bayelsa State.

Materials and Methods

Ethical Approval

This study was carried out with ethical approval from the Ethic Review Committee (Human Research) of Federal University Otuoke. Prior to sampling, participants in this study gave their informed permission. Information obtained from research subjects was kept confidential.

Specimen Collection

Fifty (50) urine specimens were randomly collected from female students of Federal University Otuoke Bayelsa State, Nigeria.

Culturing of Specimen and Identification

The streak plating technique was used to aseptically inoculate the obtained specimens onto the bases of Mannitol salt agar (Oxoid, England) and cetrimide agar (Hi Media, India) plates and incubated at 37^{o}C for 24 hours. The culture plates were processed using standard microbiological procedures.

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Characterization and identification of isolates were carried out using a combination of colonial morphology, method (Dilnessa and Bitew, 2016) with slight modification was adopted for the Gram stain and biochemical tests (catalase, oxidase, urease, citrate, coagulase, indole, methyl red, and voges Proskauer to confirm the presence of *S. aureus* and *P. aeruginosa* (Amit *et al*., 2016; Forbes *et al*., 2007).

Antibiotics Susceptibility Testing

Antibiotic susceptibility was determined on Mueller Hinton agar (Hi Flown Biotech, UK) using the disc diffusion method according to the modified Kirby-Bauer technique with Clinical and Laboratory Standard Institute (CLSI, 2012). The bacterial inoculum was generated using nutrient broth (HI Media, India) and the turbidity was adjusted to 0.5 McFarland standard. A sterile swab stick dipped in the solution was used to uniformly cover freshly prepared Muller Hinton Agar. Using sterile forceps, a Gram positive antibiotic disc containing ten (10) different antibiotics for *S. aureus* was used; ciprofloxacin (10µg), norfloxacin (10µg), gentamicin (10µg), amoxil (20µg), streptomycin (30µg), rifampicin (20µg), erythromycin (30µg), chloramphenicol (30µg), ampiclox (20µg), levofloxacin (20µg).

All isolated *P. aeruginosa* strains were also tested for their sensitivity using the Gram negative disc containing (10) different antibiotics: tarivid (10µg), reflacine (10µg), ciprofloxacin (10µg), augumentin (30µg), gentamicin (10µg), streptomycin (30µg), ceporex (30µg), nalidixic acid (20µg), septrin (30µg), and ampicillin(10µg). After incubation, the diameter of zones of inhibition were measured in mmand compared with zone diameter interpretative chart, with the test results classed as Sensitive (S), Intermediate, or Resistant (R) using the Clinical Laboratory Standard Institutes guidelines (CLSI, 2012). In cases where isolates demonstrated resistance to three or more of the tested drugs, they were classified as multidrug resistant (Akingbade *et al*., 2014).

Plasmid Extraction

The TENS-miniprep approach was used to extract plasmids from bacterial isolates (Zhou *et al*., 1990). A sterile inoculating loop was used to collect an overnight bacterial culture on agar plates and transfer it into 100µl of nutrient broth medium.

Each Eppendorf tube (5) was vortexed at high speed using a vortex machine (Sci Finetech vortex mixer microfield) to thoroughly resuspend the cells. 300µl of TENS buffer was added, and the tubes were inverted 3-5 times until the mixture became sticky, preventing chromosomal DNA degradation that may harm the plasmid DNA. 150µl of 3.0m sodium acetate (pH 5.2) was added to the tubes containing the isolated collected cells, and all of the tubes were vortexed to mix the cells and reagents. Following vortexing, the tubes were spun for 5 minutes at the maximum revolution per minute (14,000 rpm) on a microcentrifuge (Beckman Coulter Microfuge) to pellet the cell debris and chromosomal DNA. Following spinning, the supernatant was transferred to a newly labeled sterile Eppendorf tube and mixed with 900l of pre-cooled to -20^{o}C 100% ethanol (absolute). The supernatant was then spun for 2 minutes at 1,000 rpm to precipitate the plasmid DNA (a white pellet was noticed). The supernatant was discarded, and the pellet was washed twice with 500µl of 70% ethanol, vortexed, and spun at 14,000rpm for 2 minutes. The supernatant was decanted, blotted, and dried for 3 hours in a safe and sterile atmosphere. The isolated plasmids were stored in a Ziploc bag in the freezer for future use.

Agarose Gel Electrophoresis

The plasmid and DNA were analyzed by gel electrophoresis. Agarose gel (1.5%) was prepared by dissolving 1.5g agarose in 100 ml of 1X TBE (Tris borate EDTA) buffer. The slurry was heated in an electric cooker to dissolve the agarose and the solution was allowed to cool to about 50°C. Two drops of ethidium bromide (EtBr) as an intercalating agent were added to the solution and gently swirled for an even mixture. The solution was carefully poured into the horizontal gel casting tray of which two combs were inserted before 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 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 0.5X 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 80 volts for 45 minutes.

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Plasmids were visualized on ultraviolet (UV-Transilluminator) and the bands were photographed using gel documentation. Twelve bands were pictured from the 28 samples run using gel documentation.

Plasmid Curing with Acridine Orange

Plasmid curing was carried out by treatment with acridine orange. After the gel documentation, two (2) bacterial isolates that produced bands were subjected to curing. The preserved bacterial isolates were subcultured by streaking on nutrient agar and incubated at 37ºC for 24 hours. The overnight bacteria cultures were harvested in 1ml of lysogeny broth each, labeled, and incubated at 37ºC 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 a reaction (color change) 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 37ºC for 24 hours in a rotary incubator.

Application of Antibiotics Sensitivity Disc

Two isolates in acridine orange broth cultured in a rotary incubator were pulled out and swabbed on Mueller-Hinton Agar plates with a sterile swab stick. The Kirby-Bauer disc diffusion method was used to evaluate antibiotic susceptibility according to the Clinical and Laboratory Standard Institute (CLSI) established standard. The eight (8) different discs (Maxicare Medical Lab) included the following antibiotics for Gram-positive: gentamicin CN (10µg), azithromycin AZM (15µg), chloramphenicol CH (30g), cefpodoxime CPD (10µg), amoxicillin-clavulanic acid AUG (30µg), cefotaxime CTX (30µg), ciprofloxacin CPX (10µg), tetracycline TE (30µg).

The findings 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 guidelines (CLSI, 2015; CLSI, 2010).

Statistical Analysis

The data obtained were subjected to the Analysis of Variance (ANOVA) test. This is to determine the significant difference at a 95% confidence interval. Antibiotic resistance of isolates was described using an anti-biogram profile (CLSI, 2012).

Results

In this study, a total of 50 urine specimens were examined, from which twenty-eight isolates were obtained. The percentage of individuals infected was 28(56%). The distribution of bacterial isolates is presented in Table 1, with 20 (71.4%) being *S. aureus* and 8(28.6%) *P. aeruginosa*.

Table 1: Percentage Distribution of *S. aureus* and *P. aeruginosa* among Female Students of Federal University Otuoke, Bayelsa State Nigeria

Isolates	Infected (%)	Non-infected (%)	Total
Staphylococcus aureus	20 (71.43)	30 (41.67)	50
Pseudomonas aeruginosa	8 (28.57)	42 (58.33)	50
Total	28 (100)	72 (100)	100

Figure 1 shows the frequency occurrence of the bacterial isolates. *S. aureus* exhibited the highest frequency of occurrence with 20 (71.4%) while *P. aeruginosa* had the lowest count at 8 (28.6%).

The statistical analysis indicated P=1.0 and P>0.05, suggesting that there is no significant difference in the frequency of occurrence of the bacterial isolates.

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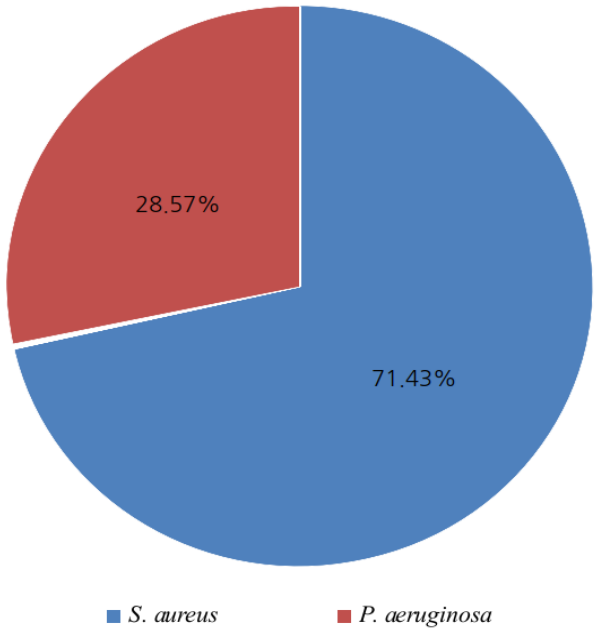

Figure 1: Percentage of Occurrence of Bacteria Isolates

The age distribution of female students of Federal University Otuoke with urinary tract infections is depicted in Figure 2**.** The highest number of UTI cases was observed within the age group 18-25 years with 15(53.6%) while the lowest was noted in the age group 26-33years accounting for 13(46.4%).

Statistical analysis revealed that there is no significant difference in the age distribution of female students as P=0.85, confirming P>0.05. *S. aureus* was the most prevalent isolate among the age groups accounting for 20(71%) while *P. aeruginosa* with 8(28.6%) had the lowest.

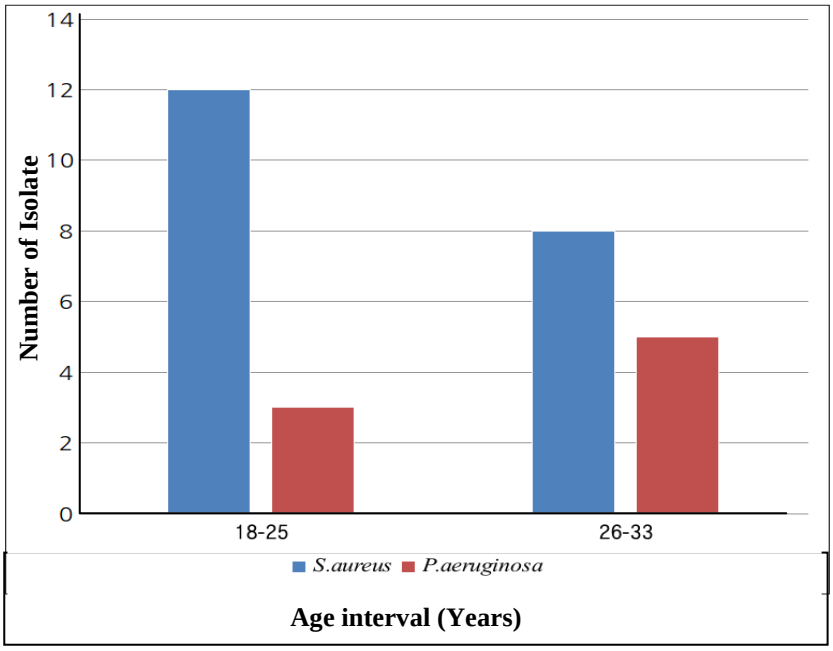

Figure 2: Age Distribution of Female Students with UTI

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The antibiotic susceptibility pattern of *S. aureus* is presented in Figure 3. *S. aureus* demonstrated its highest sensitivity to ciprofloxacin, gentamicin, streptomycin, and levofloxacin. Conversely, it exhibited the greatest resistance to norfloxacin, Amoxil, and ampiclox. Statistical analysis, with P=0.99 (P>0.05), indicates that there is no statistical difference in the antibiotics susceptibility profile of *S. aureus* among female students with UTI at Federal University Otuoke.

In the Figure 4 illustration, *P. aeruginosa* showed susceptibility to tarivid, ciprofloxacin, and septrin, while it exhibited resistance to augmentin, nalidixic acid, and ampicillin. The statistical analysis, yielding P=1.00 (P>0.05), indicates that there is no significant difference in the antibiotic susceptibility profile of *P. aeruginosa* among female students with UTI at Federal University Otuoke.

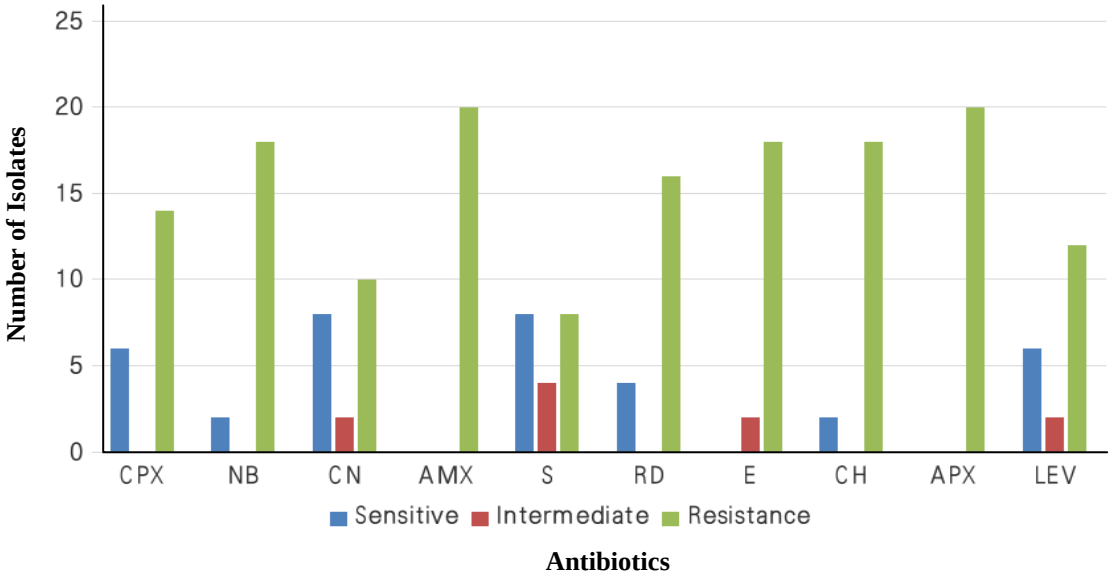

Figure 3: Antibiotics Susceptibility of *Staphylococcus aureus*

Key: CPX= Ciprofloxacin, NB= Norfloxacin, CN= Gentamicin, AMX= Amoxil, S=Streptomycin, RD=Rifampicin, E=Erythromycin, CH=Chloramphenicol, APX=Ampiclox , LEV=Levofloxacin.

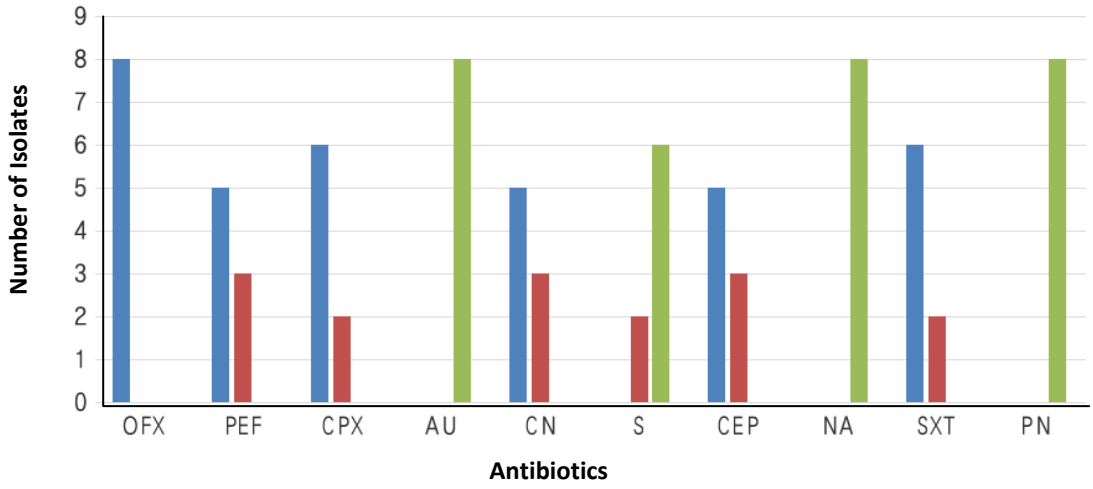

Figure 4: Antibiotics Susceptibility of *Pseudomonas aeruginosa*

Key: OFX=Tarivid, PEF=Reflacine, CPX= Ciprofloxacin, AU=Augmentin, CN= Gentamicin, S=Streptomycin, CEP=Ceporex, NA= Nalidixic, SXT=Septrin and PN=Ampicillin.

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Tables 2a and 2b below show the susceptibility pattern of the multidrug *Staphylococcus aureus* before and after curing. Before curing, *Staphylococcus aureus* was resistant to all the antibiotic. *Pseudomonas aeruginosa* also showed resistance to all the antibiotics except for tetracycline. After curing, the majority of antibiotics to which the bacteria had previously exhibited high resistance, were effective against both *Staphylococcus aureus* and *P. aeruginosa* except for azithromycin, cefpodoxime, and amoxicillin-clavulanic acid, which remained resistant to *P. aeruginosa.*

In Table 3, Cefotaxime exhibited the highest sensitivity of 25mm and 23mm zones of inhibition for *S. aureus* and *P. aeruginosa* respectively.

Table 2a: Zone of Inhibition (mm) of Antibiotic Susceptibility Test before Plasmid Curing

Bacterial Isolate	Inhibition by Antibiotics (mm)							
	CN	AZM	C	CPD	AUG	CTX	CIP	TE
Staphylococcus aureus	11	13	9	14	17	12	10	12
Pseudomonas aeruginosa	11	0	10	12	10	13	15	16

Key: CN=Gentamicin, AZM=Azithromycin, C=Chloramphenicol, CPD=Cefpodoxime, AUG =Amoxicillin clavlanic acid, CTX=Cefotaxime, CIP= Ciprofloxacin, TE=Tetracycline.

Table 2b: Zone of Inhibition of Antibiotic Susceptibility Test after Plasmid Curing

Bacterial Isolate	Inhibition by Antibiotics (mm)							
	CN	AZM	C	CPD	AUG	CTX	CIP	TE
Staphylococcus aureus	16	19	18	23	22	25	24	20
Pseudomonas aeruginosa	15	0	17	14	12	23	22	16

Key: CN=Gentamicin, AZM=Azithromycin, C=Chloramphenicol, CPD=Cefpodoxime, AUG =Amoxicillin clavlanic acid, CTX=Cefotaxime, CIP= Ciprofloxacin, TE=Tetracycline.

Table 3: Interpretation of Antibiotic Susceptibility Pattern of Bacterial Isolates Before and After Plasmid Curing

Antibiotics	Susceptibility test before curing						Susceptibility test after curing					
	S. aureus			*P. aeruginosa*			*S. aureus*			*P. aeruginosa*		
	S	I	R	S	I	R	S	I	R	S	I	R
Gentamicin	-	-	11	-	-	11	16	-	-	15	-	-
Azithromycin	-	-	13	-	-	0	19	-	-	-	-	0
Chloramphenicol	-	-	9	-	-	10	18	-	-	17	-	-
Cefpodoxime	-	-	14	-	-	12	23	-	-	-	-	14
Amoxicillin-clavulanic acid	-	-	17	-	-	10	22	-	-	-	-	13
Cefotaxime	-	-	12	-	-	13	25	-	-	23	-	-
Ciprofloxacin	-	-	10	-	-	15	24	-	-	22	-	-
Tetracycline	-	-	12	16	-	-	20	-	-	16	-	-

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

Figure 5 illustrates the multiple bands and the molecular weight of plasmid DNA discovered in multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* on an agarose gel stained with ethidium bromide. Lane M exhibits the marker DNA, a 23130bp DNA ladder, whereas lanes 1, 2, and 3, demonstrate the absence of plasmid bands. In lanes 4 and 5, multiple plasmid DNA bands with a size of 9466kbp were visible, signifying the presence of multidrug-resistant *S. aureus* and *P. aeruginosa*.

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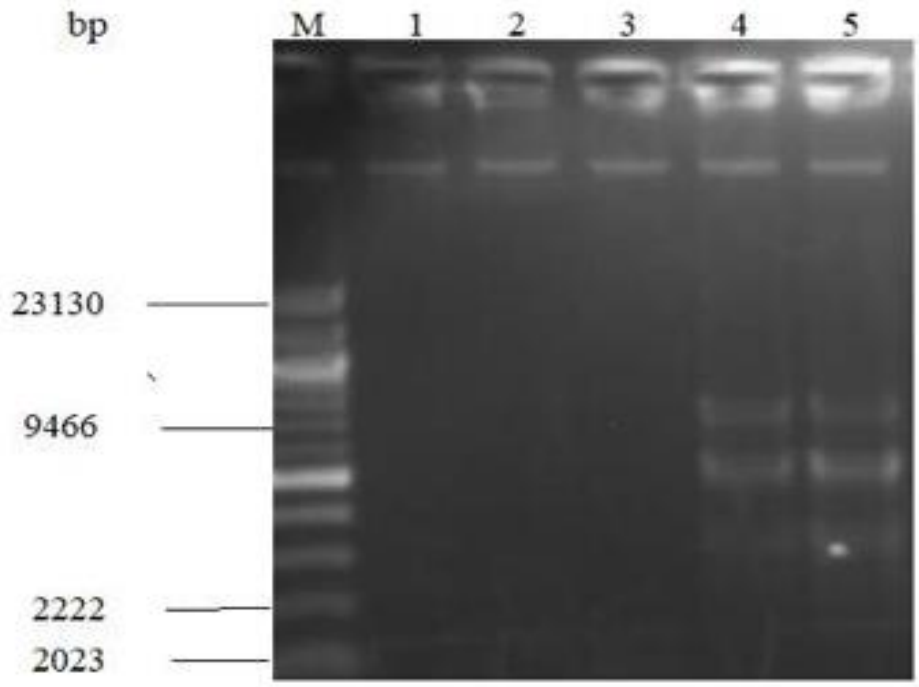

Figure 5: Molecular Weight of Plasmid DNA on an Agarose Gel stained with Ethidium Bromide
[Lane (M) marker DNA - 23130bp DNA ladder, lanes 1, 2, and 3: no plasmid band, lanes 4 and 5: 9466kbp multiple plasmid DNA bands of multidrug drug-resistant *S. aureus* and *P. aeruginosa* respectively].

Discussion

Urinary tract infection remains a menace in public health and the major cause of death despite the different available therapeutic options for its treatment. In this study conducted among female students with urinary tract infections at Federal University Otuoke, Bayelsa State Nigeria, a total of 28(56%) isolates was obtained. The prevalence of *P. aeruginosa* was 28.57% while that of *S. aureus* was 71.43%. Earlier studies in Bushenyi (Uganda) 2015, Mulago (Uganda) 2011, and Awka (Nigeria) 2016 reported high rates of *S. aureus* of 45/103 (43.7%), 9/40 (22.5%), and 60/215 (28%), respectively (Ekwealor *et al*., 2016; Odoki *et al*., 2015; Mwaka *et al*., 2011). Our study indicated *S. aureus* as the prevalent organism. The high prevalence of *S. aureus* in this study differs from these previous studies; and it could be attributed to its increasing involvement in UTI, improper hand washing, endogeneous infection, and poor hygiene practices among the females or the specific focus on *S. aureus* and *P. aeruginosa*.

This finding is in agreement with the study conducted by Yaser and Haveen, (2023) which also identified *S. aureus* as the prevalent isolate in UTI. Notwithstanding, *E. coli* has been implicated as the common uropathogen in urinary tract infections (Mlugu *et al*., 2023).

Demographic data pertaining to positive cases of bacterial infection were analyzed across different age groups, revealing that there is no statistically significant difference (P>0.05). The uropathogens obtained in this study demonstrated a significant rate of susceptibility to gentamicin, ciprofloxacin, streptomycin, and levofloxacin, particularly in the case of *S. aureus*, and tarivid, ciprofloxacin, and septrin in the case of *P. aeruginosa.* This observation may be attributed to responsible antibiotic usage among individuals or the absence of antibiotic-resistant bacterial strains and the development of resistance among the research population. A total of 18(64.29%) isolates exhibited resistance to more than two antibiotics, classifying them as multidrug-resistant (MDR).

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This finding of the study aligns with the research conducted by Alabi and Lawal, (2018) and Wolters, (2020). The high percentage of resistance observed may be due to poor water sources because the study location is among the high-risk flood areas, overuse of antibiotics, and overcrowded conditions in the various female hostels leading to the spread of resistance strains. Additionally, the isolates may carry plasmids conferring antibiotic resistance, as reported by Ngwai *et al.* (2014). Multidrug-resistant *S. aureus* and *P. aeruginosa* isolates were shown to possess plasmids with a molecular weight of 9466base pair respectively as seen with the plasmid analysis. This indicates that their resistance may not be chromosomal inherited but acquired as also suggested by Van Hal *et al*. (2009) and Ebele *et al*. (2022); or by horizontal gene transfer which is a key player in the development of multidrug organisms and spread of antibiotic resistance as reported by Roos and Klemm (2006). The acquisition of resistance may have been influenced by environmental factors where plasmids between strains and species of organisms may have spread. It was also observed that multiple antibiotics-resistant *S. aureus* and *P. aeruginosa* which were resistant before plasmid curing became sensitive to the antibiotics after curing with ethidium bromide.

However, *P. aeruginosa* remained resistant to azithromycin, cefpodoxime, and amoxicillin-clavulanic acid, after curing. Additionally, *S. aureus* and *P. aeruginosa* displayed the highest sensitivity of 25mm and 23mm zones of inhibition respectively to cefotaxime. This finding is consistent with the research conducted by other scientists who reported that plasmids encode genes that confer resistance to antibiotics that are found in nature in particular environmental niches (Kroll *et al*., 2010). The findings of this study also align with the study conducted by Ojo *et al*. (2014) which showed that resistance displayed by some bacterial isolates, including *S. aureus*, *P. aeruginosa*, and *P. mirabilis*, is primarily plasmid-mediated. Hence, our study demonstrates the relationship between plasmids and various types of drug resistance, pointing out that plasmids contribute to the spread of drug resistance among bacteria.

In conclusion, *S. aureus* and *P. aeruginosa* are among the bacteria associated with urinary tract infections in female students of Federal University Otuoke Bayelsa State, Nigeria.

Fearsomely, high infection rates are brought on by these antibiotic-resistant bacteria. The findings from this study revealed a high susceptibility rate of *S. aureus* to *g*entamicin and streptomycin, while *P. aeruginosa* to tarivid. Conversely, there was a significant level of resistance in *S. aureus* to ampiclox and *P. aeruginosa* to ampicillin. To address this issue, the development of alternative tools for treatment options is crucial. Furthermore, it is essential to emphasize the importance of maintaining proper personal hygiene practices, and avoid the use of contaminated water and overcrowding conditions to reduce the risk of urinary tract infections.

We acknowledge some limitations of this study. The study presents data from a setting where information on AMR is highly limited, small sample size is used due to the number of female students willing to give their consent and be included for the study. Molecular characterization of isolates and detection of resistance genes were also not undertaken.

Acknowledgment

We would like to acknowledge Yaba College of Technology's College Central Laboratory for their unwavering support.

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