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Understanding Antibiotic Resistance and Treatment Adherence in Urinary Tract Infections

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Abstract
Microorganisms associated with urinary tract infection (UTI) can generate microbial biofilm, and this community of microorganisms can be produced by several bacteria that indicate antimicrobial resistance. One hundred mid-stream urine specimens from patients attending Federal Medical Center, (FMC) Yenagoa were examined to determine the antimicrobial resistance and biofilm formation of bacteria. Specimens were studied by culture and biochemical tests. The multidrug resistance pattern was determined using the standard agar disc diffusion method and plasmid profiling. The production of biofilm was assessed utilizing phenotypic techniques, specifically the Congo red agar method. The relationship between biofilm formation was analyzed using Pearson correlation test, and the antibiogram of the isolates. Results from this study revealed the presence of *Citrobacter* spp. 8(21%), *Enterobacter* spp. 5(13.1%), *K. aerogenes* 10(26.3%), *P. mirabilis* 6(15.7%), *P. aeruginosa* 5(13.1%), and *S. aureus* 4(10.5%). *K. aerogenes* 10(26.3%) was the predominant isolate, while the least was *S. aureus* 4(10.5%). The highest incidence of UTI was seen within age brackets 20-25 years with 17(44.7%) and 25- 30 years with 13(34.2%). Statistically, there was a significant difference between the age distribution ($p< 0.05$). In comparison, females had the highest number of bacterial isolates with 27(71%) while males with 11(28.9%). There was a significant difference in the percentage of occurrence of bacterial isolates. ($p< 0.05$). Most of the gram-negative bacterial isolates showed resistance to the various antibiotics. *Citrobacter* spp. and *P. mirabilis* were both susceptible to levofloxacin, ampiclox, nalixidic acid and tarivid. *Enterobacter* spp. showed resistance to all the antibiotics. *K. aerogenes* and *P. aeruginosa* were both susceptible to levofloxacin and tarivid. *Staphylococcus aureus* was resistant to cefuroxime, imipenem, cefotaxime, cefexime, gentamycin, ciprofloxacin, and amoxicillin but susceptible to levofloxacin, azithromycin, erythromycin, and tarivid. Biofilm formation was observed with *Citrobacter* spp. and *P. mirabilis* while there was no biofilm formation by *Enterobacter* spp. and *S. aureus*. The plasmid curing before and after plasmid curing indicated that most resistant antibiotics before curing were resistant except for tetracycline. After curing ciprofloxacin, gentamicin, chloramphenicol, tetracycline became sensitive to *Citrobacter* spp., *Enterobacter* spp., and *P. mirabilis.* The degree of antibiotic resistance to certain microbes is mostly influenced by misuse of drugs, therefore sensitization to antibiotic resistance should be a priority by physicians.

Keywords: Antimicrobial Resistance, Plasmid analysis, Biofilm formation, Bacteria.

Introduction

Bacterial presence in urine is noteworthy regardless of the specific site of infection within the urinary tract and is regarded as a urinary tract infection (Michael & Adenike, 2016). The spectrum of UTI can span asymptomatic bacteriuria to severely symptomatic conditions, including urethritis, cystis, ureteritis, and pyelonephritis (Gupta & Trauter, 2018; Derese et al., 2016). In both the general population and healthcare settings, UTI stands out as a primary contributor to morbidity (Gabriel & Shehu, 2016; Vyas et al., 2015). Globally, UTIs affect approximately 150 million individuals annually, resulting in healthcare expenses exceeding $6 billion attributed to treatment and productivity loss. (Eticha, 2014). UTIs manifest across all age groups and genders, influenced by anatomical positioning, physiological changes, sexual activity, and contraceptive methods such as spermicide and diaphragm use. The absence of prostatic fluid, which acts as a natural antibacterial agent, contributes to this vulnerability. Notably, nearly 50% of women experience at least one UTI episode during their lifetime (Michael & Adenike, 2016; Wei & Piotr, 2016). Though UTIs occur less frequently in men compared to women, when the infection occurs, it tends to pose a more

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serious clinical challenge (Olson et al., 2015). Urinary tract infections (UTIs) have been linked to severe complications including frequent recurrences, bacteremia, renal failure, and preterm delivery (Flores et al., 2015). Gram-negative bacteria, particularly *Escherichia coli* (comprising 75–90% of cases), *Klebsiella* spp., and *Proteus* spp., alongside Gram-positive bacteria such as coagulase-negative staphylococcus (CoNS) and *Staphylococcus aureus*, have been implicated as the primary causative agents of UTIs (Gebremariam et al., 2019). Recent studies indicate a concerning trend of escalating antibiotic resistance among uropathogenic isolates globally, posing a significant challenge to treatment efficacy (Ravishankar & Thayanidhi, 2021).

Complex aggregates of bacteria immersed in a self-produced matrix and affixed to a surface, one another, or both are known as bacterial biofilms. Substances such as proteins (e.g., fibrin), polysaccharides (e.g., alginate), and extracellular DNA (eDNA) make up the biofilm matrix, provide protection and facilitate bacterial survival. Biofilm-dwelling bacteria employ various diverse tactics to circumvent the body's immune response, including being latent, which shields them from immune detection, thereby inducing local tissue damage and subsequently triggering acute infections. Within the biofilm structure, bacteria adapt to environmental challenges such as oxygen deprivation and nutrient scarcity by modulating their metabolism, gene expression, and protein synthesis, resulting in diminished metabolic activity and reduced cell proliferation (Donlan, 2002; Stoodley & Hall-Stoodley, 2009). Furthermore, these adaptations confer heightened resistance to antimicrobial therapies through mechanisms such as target inactivation or reduced dependence on cellular functions targeted by antimicrobials. Biofilm infections often trigger concurrent activation of both innate and acquired host immune responses, fail to effectively eliminate the biofilm pathogen, and instead worsen tissue damage (Moser et al., 2017).

UTI-associated microorganisms possess the capability to form biofilms, which may comprise one or multiple bacterial species exhibiting resistance to antimicrobial tolerance. Predisposing host factors such as age, diabetes, prolonged hospital stays, and catheterization contribute to this phenomenon (Ahmed & Avasara, 2009). According to the National Institutes of Health (NIH), biofilm formation in the urothelium, prostate stones, and implanted foreign bodies is a major concern in urology, where it can pose a serious threat (Tenke et al., 2006). Biofilms represent the population of bacteria thriving on biotic and abiotic surfaces, encapsulated within a self-produced extracellular matrix predominantly composed of exopolysaccharides (EPS), proteins, and various micromolecules including DNA. This matrix constitutes approximately 90% of the biofilm biomass (Flemming & Wingender, 2010). The presence of this extracellular matrix shields bacteria from host immune responses and hampers the effective penetration of antibiotics (Stewart, 2002). Notably, biofilm-forming uropathogens exhibit heightened antimicrobial resistance compared to planktonic (free-floating) bacteria.

Materials and Methods

Ethical Approval: Before commencing the study, ethical approval was acquired from the Directorate of Research and Quality Assurance at Federal University Otuoke. Subsequently, the approval was submitted to the Ethical Board of Federal Medical Centre, Yenagoa, Bayelsa State. Bacterial Culturing and Identification: A cross-sectional study design was employed to collect 100 mid-stream urine specimens from patients attending the Federal Medical Centre, Yenagoa. The urine specimens obtained were inoculated into MacConkey agar (Chaitanya agro biotech, India), Cysteine Lactose Electrolyte Deficient (CLED) agar (Hi flown biotech, Berkshire, UK), Cetrimide agar (Hi Media, India) for 24hrs at 37^{o}C. Observed microbial colonies were subcultured on nutrient agar plates. Identification and characterization of bacterial isolates were through colonial morphology, Gram stain, and biochemical tests such as catalase, coagulase, urease, citrate, motility, indole, and sugar fermentation (Cheesbrough, 2006).

Antibacterial susceptibility test: Suspension of each of the test organisms was made by collecting a loop-full of colonies from each plate and overnight incubating at a temperature of 37°C in sterile physiological saline. The overnight broth cultures of organisms were quantified by the guidelines set by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). Antibiotic sensitivity testing was carried out using the Kirby Bauer disk diffusion method, and the antibiotic's sensitivity, intermediate, and resistance to bacteria were determined using Clinical and Laboratory Standards Institute (CLSI) guidelines. A bacterial suspension matching the turbidity standard of 1.5 McFarland was prepared for inoculation. A sterile swab stick was utilized to dip into the prepared bacterial suspension and then inoculated into the Mueller-Hinton agar plate by swabbing the suspension evenly across the surface of the plate. Sterile forceps were then used to place antibiotic disks onto the inoculated agar plate. The discs (Celtech Diagnostic) contained the following antibiotics for positive: amoxicillin clavulanate - AUG (30µg), cefotaxime - CTX (25µg), ceftriaxone sulbactam - CRO (45µg), cefexime - ZEM (5µg), levofloxacin - LBC (5µg),

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ciprofloxacin - CIP (5µg), Imipenem/cilastatin - IMP (10/10µg), cefuroxime - CXM (30µg), ofloxacin - OFX (5µg), erythromycin - ERY (15µg), gentamycin - GN (10µg), and azithromycin - AZN (15µg). The discs used for Gram-negative (Celtech Diagnostic) contained the following: amoxicillin clavulanate - AUG (30µg), cefotaxime - CTX (25µg), imipenem/cilastatin – IMP (10/10µg), ofloxacin - OFX (5µg), gentamycin - GN (10µg), nalidixic acid - NA (30µg), nitrofurantoin NF (300µg), cefuroxime – CXM (30µg), ceftriaxone sulbactam – CRO (45µg), ampiclox – ACX (10µg), cefexime – ZEM (5µg), and levofloxacin - LBC (5µg). The discs were gently pressed down to ensure contact with the agar. The plates were then incubated at 37°C for 18-24 hours to allow for the growth of the bacteria. After incubation, the plates were observed. The clear zones (zones of inhibition) around each antibiotic disk indicating the effectiveness of the antibiotics against the bacteria, were measured using calipers or a ruler and recorded in millimeters. The zone diameters were then compared to a standard reference chart or interpretive criteria provided by the Clinical and Laboratory Standards Institute (CLSI) and the results were categorized as susceptible, intermediate, or resistant based on the zone sizes and the specific antibiotic (CLSI, 2015; CLSI 2020).

Plasmid Extraction: Extraction of plasmid from bacteria isolates was carried out using the TENS-miniprep method (Zhou et al., 1990). An overnight bacterial culture on agar plates was harvested using a sterile inoculating loop and transferred into 100µl of nutrient broth medium. Each Eppendorf tube was 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 contained the isolates harvested cells, all the tubes were vortexed to mix the cells and the reagents completely. After vortexing, the tubes were spun using a microcentrifuge (Beckman Coulter Microfuge) for 5 minutes at the highest revolution per minute (14,000 rpm) to pellet the cell debris and chromosomal DNA. After spinning, the supernatant was transferred into a fresh labelled sterile Eppendorf tube, where it was mixed with 900µl of 100% ethanol (absolute)which was 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 3 hours in a safe and sterile atmosphere. The plasmids extracted were kept inside the freezer for further use.

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 100 ml of 1 X 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 500C. Two (2) 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 (2) combs were inserted before the pouring and the gel was allowed to solidify 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 80 volts for 45 minutes. Plasmids were visualized on ultraviolet (UV-Transilluminator) and the bands were photographed using gel documentation.

Antibiotics Sensitivity test before curing: A 24-hour multidrug-resistant bacterial isolate from the previous susceptibility testing was used before the curing. The bacterial isolates were swabbed with a sterile swab stick on Mueller-Hinton agar plates. Antibiotic susceptibility testing was performed by using the Kirby-Bauer disc diffusion method according to the recommended standard of the Clinical Laboratory Standard Institute (CLSI) the discs (Rapids Labs International) contained the following antibiotics; gentamycin GEN (10µg), ciprofloxacin CIP (5µg), amoxycillin/clavulanate AUG (30µg), chloramphenicol C (30µg), cefotaxime CTX (30µg), azithromycin AZM (15 µg), tetracycline TE (30µg), and cefepime FEP (30µg). The results were recorded after 24 hours of incubation and measurement was taken using the diameter of the inhibition zone around each disc and interpreted as sensitive, intermediate, and resistant using CLSI guidelines (CLSI, 2020).

Plasmid Curing with Acridine Orange: After gel documentation, the two (2) bacterial isolates that produced bands were subjected to curing by treatment with acridine orange. The preserved bacterial isolates were subcultured by streaking on nutrient agar plates and incubated at 370C for 24 hours. The overnight bacteria cultures were harvested

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in 1ml of lysogeny broth, each labelled and incubated at 370C 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 (colour 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 aluminium foil because acridine orange is light sensitive. The tubes were incubated at 37^{o}C for 24 hours in a rotary incubator.

Antibiotic susceptibility testing of isolates after curing**:** Following curing, the three (3) bacterial isolates in acridine orange broth incubated in a shaking/rotary incubator were brought out and each was swabbed with a sterile swab stick on Mueller-Hinton agar plates. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to the recommended standard of the Clinical Laboratory Standard Institute (CLSI, 2020). The discs (Rapids Labs International) contained the following antibiotics: gentamycin GEN (10µg), ciprofloxacin CIP (5µg), amoxicillin/clavulanate AUG (30µg), chloramphenicol C (30µg), cefotaxime CTX (30µg), azithromycin AZM (15 µg), tetracycline TE (30µg), and cefepime FEP (30µg). The results were recorded after 24 hours of incubation and measurement was taken using the diameter of the inhibition zone (mm) around each disc and interpreted as sensitive, intermediate, and resistant using CLSI guidelines (CLSI, 2020; Hudzicki, 2009).

Detection of biofilm production via phenotypic methods (Congo Red agar method): The method for the identification of biofilm formation was described by Freeman et al. (1989). The method of Congo Red Agar (CRA) consisting of Brain heart infusion broth (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L, Himedia). Positive results were indicated by the formation of black colonies with a dry crystalline texture, signifying biofilm producers, while negative results were indicated by pink-coloured colonies for non-biofilm producers.

Statistical analysis: The data obtained from the results were subjected to the Analysis of Variance (ANOVA) test to determine the significant difference at a 95% confidence limit. The association between the biofilm formation capability and the antimicrobial susceptibility was determined using the Pearson correlation test. All tests were considered significant at $p<0.005$.

Results

Out of the 100 urine specimens examined, 38 isolates were obtained. The organisms identified were *Citrobacter* spp., *Enterobacter* spp., *K. aerogenes*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*. Among the bacteria isolates identified, Gram-negative organisms were more prevalent. *K. aerogenes* 10(26.3%) was the highest isolate followed by *Citrobacter* spp. 8(21%), while the least was *S. aureus* 4(10.5%). The highest incidence of UTI was seen within age brackets 20-25 years with 17(44.7%) and 25- 30 years with 13(34.2%) as shown in Table 1**.** Statistically, a notable variance was observed in the distribution of ages at ($p< 0.05$).

Table 1: Age distribution of patients with UTI attending the FMC

Age Interval (Year)	Sex Male(%)	Female(%)	Total number of specimen	Number of isolates (%)
10-15	6	7	13	2 (5.3)
15-20	10	12	22	6 (15.8)
20-25	18	21	39	17 (44.7)
25-30	12	14	26	13 (34.2)
Total	46	54	100	38 (100)

Key: % - Percentage

Table 2 indicates the percentage distribution of the bacterial isolates by sex. The distribution of bacterial isolates is as follows; *Citrobacter* spp. 8(21%), *Enterobacter* spp. 5(13.1%), *K. aerogenes* 10(26.3%), *P. mirabilis* 6(15.7%), *P. aeruginosa* 5(13.1%), and *S. aureus* 4(10.5%). *K. aerogenes* 10(26.3%) was seen as the highest isolate while the least was *S. aureus* 4(10.5%). *Citrobacter* spp. and *K. aerogenes* were the predominant organisms among the females. In comparison, females had the highest number of bacterial isolates with 27(71%) while males with 11(28.9%). *K. aerogenes* was the highest isolate seen in females with 8(21%), followed by *Citrobacter* spp., 7(18.4%) while the least

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isolate was *P. aeruginosa* with 2(5.2). For the males, the highest isolates were *P. mirabilis* and *P. aeruginosa* with 3(7.8%) respectively, while the least were *Citrobacter* spp., *Enterobacter* spp., and *S. aureus* with 1(2.6%) respectively. There was a marked difference in the percentage of occurrence of bacterial isolates at ($p< 0.05$).

Table 2: Percentage occurrence distribution of bacterial isolates by sex.

Bacterial isolates	Sex		Total number of specimen	Total Number of isolate(%)
	Male(%)	Female (%)		
Citrobacter spp	1(2.6)	7(18.4)	19	8 (21.0)
Enterobacter spp	1(2.6)	4(10.5)	13	5 (13.1)
K. aerogenes	2(5.2)	8(21.0)	25	10 (26.3)
P. mirabilis	3(7.8)	3(7.8)	14	6(15.7)
P. aeruginosa	3(7.8)	2(5.2)	17	5 (13.1)
S. aureus	1(2.6)	3(7.8)	12	4(10.5)
Total	11(28.9)	27(71.0)	100	38(100)

Key:
% - Percentage

The antibiogram of Gram-negative bacterial isolates is shown in Figure 1. Most of the gram-negative bacterial isolates showed resistance to the various antibiotics. *Citrobacter* spp. and *P. mirabilis* were both susceptible to levofloxacin, ampiclox, nalixidic acid and tarivid, while resistant to ceftraxone sulbactam, imipenem, cefuroxime, cefexime, amoxicilin-clavulanate, cefotaxime and gentimicin. *Enterobacter* spp. showed resistance to all the antibiotics. *K. aerogenes* and *P. aeruginosa* were both susceptible to levofloxacin and tarivid while resistant to ampiclox, nalixidic acid ofloxacin, ceftraxone sulbactam, imipenem, cefuroxime, cefexime, amoxicilin-clavulanate, cefotaxime and gentimicin.

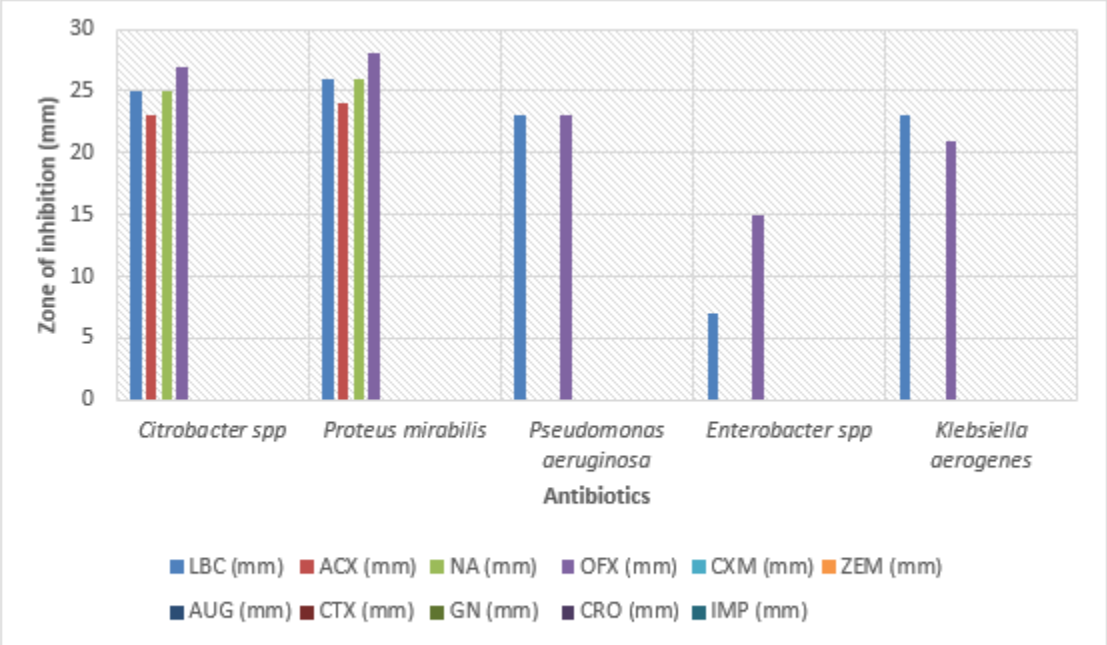

Fig 1: Antibiogram of Gram-negative bacterial isolates.

Key: LBC; levofloxacin-5mcg., CRO; ceftriaxone sulbactam-45mcg, ACX; ampiclox-10mcg, IMP; imipenem-10mcg, CXM; cefuroxime-30mcg, ZEM; cefixime-5mcg, AUG; amoxicillin clavulanate-30mcg, CTX; cefotaxime-25mcg, GN; gentamycin-10mcg, NA; nalidixic acid-30mcg, OFX; tarivid- 5mcg,

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Figure 2 shows the antibiogram of Gram-positive bacterial isolate. *S. aureus* was resistant to cefuroxime, imipenem, cefotaxime, cefixime, gentamicin, ciprofloxacin, and amoxicillin but susceptible to levofloxacin, azithromycin, erythromycin, and tarivid.

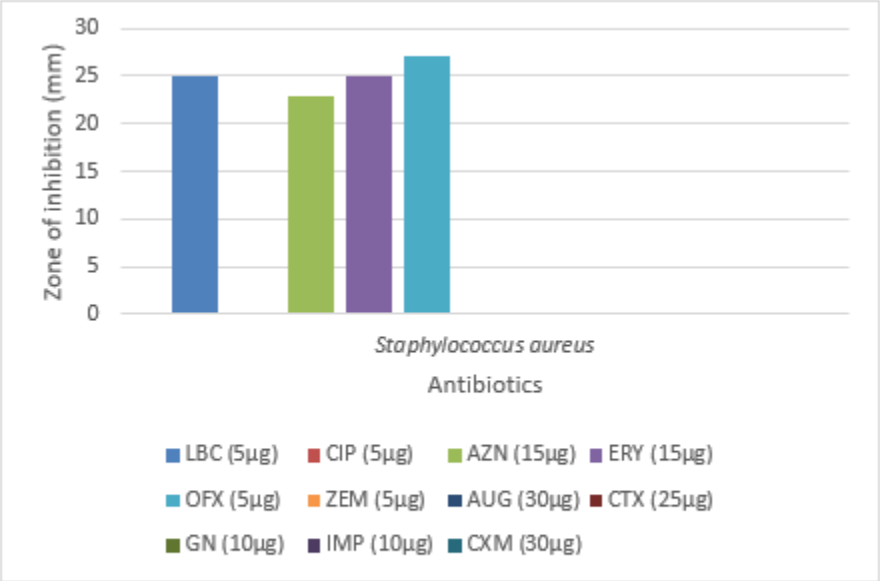

Fig 2: Antibiogram of Gram-positive bacterial isolate.

The antibiotic susceptibility test of bacterial isolates before plasmid curing is displayed in Table 3. All bacteria strains were resistant to the antibiotics except *Ctirobacter* spp which was sensitive to tetracycline with a zone of inhibition of 15mm before curing.

Table 3: Antibiotic susceptibility test for bacterial isolates before plasmid curing.

Bacterial isolates	AUG	CTX	C	TE	FEP	CIP	CN	AZM
P. mirabilis	0	13	8	0	0	13	6	2
Citrobacter spp	0	0	12	15	0	0	5	0
Enterobacter spp	0	0	6	4	0	11	10	0

Key: AUG- amoxicillin clavulanate 30µg, CTX- cefotaxime 30µg, C- chloramphenicol 30µg, TE- tetrecycline 30µg, FEP- cefepime 30µg, CIP- ciprofloxacin 5µg, GN- gentamicin 10µg, AZN- azithromycin 15µg.

The result as shown in Table 4 reveals the antibiotic susceptibility test of bacterial isolates after plasmid curing. Some of the bacterial isolates which were resistant before curing became susceptible to the antibiotics after curing. Before curing, *Enterobacter* spp. and *P. mirabilis* exhibited resistance to all the antibiotics. Upon curing, *Citrobacter* spp. became highly susceptible to ciprofloxacin and gentamicin with zones of inhibition of 30mm and 21mm respectively. *Enterobacter* spp was susceptible to chloramphenicol (24mm), tetracycline (21mm), ciprofloxacin (22mm), and gentamicin (25mm). *P. mirabilis* was susceptible to chloramphenicol (22mm), ciprofloxacin (23mm) and gentamicin (18mm).

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Table 4: Antibiotic susceptibility test for bacterial isolates after plasmid curing

Bacterial isolate	AUG	CTX	C	TE	FEP	CIP	CN	AZM
P. mirabilis	6	23	22	14	6	23	18	7
Citrobacter spp	6	7	11	7	8	30	21	8
Enterobacter spp	6	7	24	21	6	22	25	7

Key: AUG- amoxicillin clavulanate 30µg, CTX- cefotaxime 30µg, C- chloramphenicol 30µg, TE- tetrecycline 30µg, FEP- cefepime 30µg, CIP- ciprofloxacin 5µg, GN- gentamicin 10µg, AZN- azithromycin 15µg.

Table 5 shows the biofilm formation by the bacterial isolates using the phenotypic method *Citrobacter* spp. and *P. mirabilis* produced biofilm while there was no biofilm formation by *Enterobacter* spp. and *S. aureus* biofilm formation.

Table 5: Detection of biofilm production by phenotypic method via Congo red agar

Organism	Description	Remark
Citrobacter spp	The colour of the medium turns from red to black after 24 h incubation	Positive
*Enterobacter*spp	The colour of the medium remains the same after24hincubation	Negative
P. mirabilis	The colour of the medium turns from red to black after 24h incubation	Positive
S. *aureus*	The colour of the medium remains the same after 24h incubation	Negative

The plasmid DNA on an agarose gel stained with ethidium is shown in Figure 3. The result revealed the presence of plasmid bands of *Enterobacter* spp., *Citrobacter* spp., and *P. mirabilis*, and the absence of *S. aureus.*

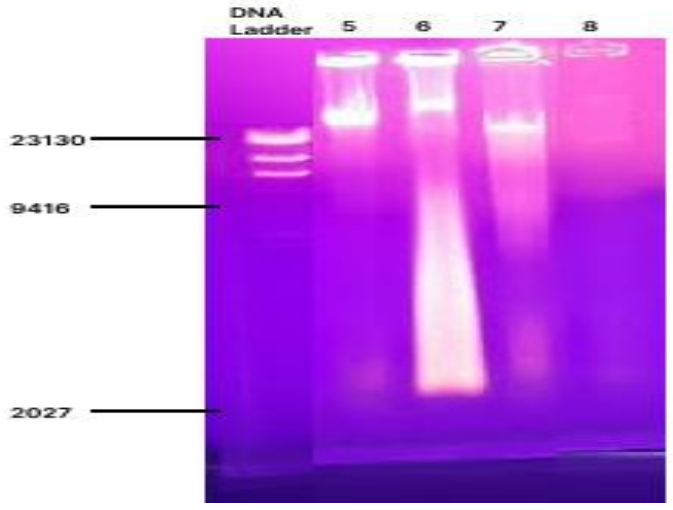

Fig 3: Molecular weight of plasmid DNA on an agarose gel stained with ethidium bromide.

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Lane (M) marker DNA - 23130bp DNA ladder, lanes 5, 6, and 7; plasmid DNA bands of multidrug drug-resistant *P.mirabilis*, *Citrobacter* spp, and *Enterobacter* spp at the molecular weight above 23130bp respectively. Lane 8; indicates no plasmid of *S. aureus.*

Discussion

Urinary tract infection is defined by the substantial existence of bacteria in urine, independent of the specific urinary tract site affected, and is prevalent across all age groups and genders (Gabriel & Shehu, 2016). Contributing factors encompass anatomical position, physiological alterations, sexual activity, utilization of contraceptive methods such as spermicide and diaphragms, and insufficiency of prostatic fluid, which serves as a natural antibacterial agent (Michael & Adenike, 2016; Vyas et al., 2015). Meanwhile, over time some antibiotics used for treating such infections have been found to become resistant, thereby creating a loophole that needs to be filled. The identified bacterial isolates in this study were *Citrobacter* spp., *Enterobacter* spp., *K. aerogenes*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus.* Among which the prevalence of the most identified isolates, *K. aerogenes* was 10(26.3%), followed by *Citrobacter* spp. 8(21%), while the least was *S. aureus* 4(10.5%). Gram-negative bacteria were the most predominant bacteria isolated in this study. This is similar to the report of Awoke et al. (2019) in which *Klebsiella* spp had 23.3% of 76.7% of gram-negative bacteria identified while *S. aureus* had 11.60% of 23.3% of gram-positive bacteria. The presence of these bacteria could be associated with their consistency in UTIs, seasonal changes (Simmering et al., 2021) level of education, and poor toilet hygiene.

Our findings show that UTIs, which have different routes of contraction, were common within the age group of 20-25 years with several isolates totalling 17/38 (44.7%). This result can be compared with the report by Odoki et al. (2019) that stated the prevalence of bacterial UTI was highest in the age group 20–29 with 28/86 (32.6%) Contrarily, another research conducted in Southwest Ethiopia and Kenya reported their findings from examined inpatients within the age group between 21 -30 years (Awoke et al., 2019; Mosonik & Kombich, 2023). In comparison, females had the highest number of bacterial isolates with 27/38 (71%), while the males had 11/38 (28.9%). This can be attributed to the nearness of the female urethral opening to the vagina and anus (Gould et al., 2010). This is similar to the report by Odoki et al. (2019) whose finding showed the prevalence of UTI was greater among females with 66 out of 176 (37.5%) in contrast to 20 out of 91 (22%) among males.

The gram-negative isolates; *Citrobacter* spp., *Enterobacter* spp., *K. aerogenes*, *P. mirabilis*, and *P. aeruginosa* were mostly resistant to imipenem, amoxicillin, ceftraxone sulbactam, cefuroxime, cefexime, amoxicillin-clavulanate, cefotaxime, and gentimicin but susceptible to levofloxacin and tarivid. This is in agreement with the findings of Gould et al. (2010) but a slight divergence from the findings of Awoke et al. (2019) in which *Proteus mirabilis*, *Citrobacter* spp, and *Klebsiella aerogenes* were sensitive to amoxicillin and cefotaxime. This implies the usage of levofloxacin in treating the aforementioned bacterial isolates would yield a good result as the drugs remain effective having been reviewed by several researchers (Arnold et al., 2013). Furthermore, the gram-positive isolate; *S. aureus*, was susceptible to levofloxacin, erythromycin as reported by Nwankwo and Nasiru (2011) and azithromycin (Madeeha et al., 2021). *S. aureus* was also noted to be susceptible to tarivid in this study, but in contrast to the finding by Sagay et al. (2022) which reported resistance. Generally, *S. aureus* was resistant to cefuroxime, imipenem, cefotaxime, cefexime, gentamycin, ciprofloxacin, and amoxicillin as observed.

Biofilm production was observed with *Citrobater* spp and *P. mirabilis* while *Enterobacter* spp and *S. aureus* were found to be non-biofilm producers. The biofilm production can be linked to their pathogenicity, or inactivation of the target cells by the bacteria to evade the mechanism of action of antibiotics, as similarly reported by Moser et al. (2017). The result of the bacteria strain susceptibility to antibiotics before plasmid curing showed multidrug-resistant bacteria; *Enterobacter* spp. and *Proteus mirabilis* to the antibiotics. The high resistance was seen with augmentin and cefepime. However, *Citrobacter* spp. was susceptible to tetracycline. After curing, *Citrobacter* spp showed high susceptibility to ciprofloxacin and gentamicin, *Enterobacter* spp was susceptible to chloramphenicol, tetracycline, ciprofloxacin, and gentamicin, and *P. mirabilis* susceptible to chloramphenicol, ciprofloxacin, and gentamicin. This shows that bacteria plasmids are the key factor responsible for their ability to resist the effect of certain antibiotics. Thus, if the plasmid of a bacteria is successfully removed, it becomes less efficient against antibiotic mechanisms.

These findings are in line with the report of Veerachamy et al. (2014) in which the plasmid of some bacteria, *E. coli*, *S. aureus*, *Citrobacter* spp, and *Enterobacter* spp, were cured and further examined to know their reaction or susceptibility to antibiotics. In addition, the plasmid analysis revealed that multiple drug-resistant *P. mirabilis*, *Citrobacter* spp, and *Enterobacter* spp carry plasmids with molecular weight of above 23130bp

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respectively while *S. aureus* lack plasmids. This is in line with the findings of Akter et al. (2021), on antibiotic resistance, in which *E. coli* and *Bacillus subtilis* were analyzed for the presence of plasmid DNA, which further show that the isolates contain plasmid with different weights (approximately 1.5-15kb range). Likewise, Chowdhury et al. (2020) reported that the major mechanism that scientists evolve around for plasmid profiling and molecular characterization involves the outflow of antibiotics that is accountable for the existence of multi-component pumps which is mainly associated with gram-negative bacteria. The plasmid DNA observed to be higher than the DNA ladder does not signify that it is larger than the DNA ladder but could be attributed to high voltage during the gel electrophoresis, which can cause distortions in the migration patterns of DNA fragments, plasmid DNA, and DNA ladder bands. The faster migration of DNA through the gel could cause uneven heating of the gel, resulting in altered DNA bands thereby making it difficult to precisely determine the sizes of the DNA fragments (Green & Sambrook, 2012).

Conclusion

Globally, UTI accounts for about 40% of the different hospital-acquired infections with several bacterial pathogens. Antimicrobial resistance has been linked with biofilm formation, which is a complex community of microorganisms capable of producing extracellular polysaccharide matrix on damaged tissue, and this makes it difficult for certain infections to be thoroughly treated. It becomes necessary that routine studies and constant surveillance are necessary for healthcare institutions to enable the generation of a homegrown solution in the fight against the problem of multidrug-resistant gram-negative bacteria. However, infections caused by these gram-negative bacteria can be effectively treated considering the result of the antibiotic sensitivity test, of this study, which affirms the fact that levofloxacin, in combination with other antibiotics such as tarivid, is effective for curbing bacteria, both gram-negative and gram-positive, associated with urinary tract infection.

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