

ANTIBIOTIC SUSCEPTIBILITY AND PLASMID SCREENING OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* AMONG PATIENTS WITH URINARY TRACT INFECTION

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Abstract

An infection attacking any part of the urinary system is termed Urinary Tract Infection (UTI) which has continued to be a serious healthcare challenge globally. This research work, therefore, was aimed at determining the prevalence of antibiotic susceptibility and plasmid profile of *Staphylococcus aureus* and *Escherichia coli* among patients with urinary tract infections. A total of 50 urine specimens were collected and analyzed by culture, Gram staining and biochemical tests. Antibacterial susceptibility test and plasmid profile analysis were carried out on the isolates. *E. coli* had a high prevalence of 68.57% compared to *S. aureus*, which had a prevalence of 31.43%. This study showed the age and sex distribution of students with bacterial isolates from urine specimens with $P \geq 0.05$, which implies that there was no significant difference between the age and sex distribution of students with pathogenic bacteria from urine. For the percentage distribution of bacterial isolates by sex, the prevalence of the bacterial isolates was seen to be higher in females with 62.50% for *Escherichia coli* and 54.55 for *Staphylococcus aureus* while males had 37.50% for *Escherichia coli* and 45.45% for *Staphylococcus aureus*. *E. coli* Antibiotic susceptibility profile $P \geq 0.05$ which implies that there is no significant difference in the antibiotic susceptibility profile of Gram-negative bacteria. This high rate of resistance could be associated with self-prescription, incomplete dosage, or indiscriminate use of antibiotics. Bacteria strains resistance to multiple antibiotics exists among patients suffering from UTIs. However, *E. coli* was highly susceptible to streptomycin (95.83%) and gentamycin (100%) while *S. aureus* Antibiotic susceptibility profile $P \leq 0.05$, which implies that there is significant difference in the antibiotic susceptibility profile of *Staphylococcus aureus*. It was susceptible to all the antibiotics used with Ciprofloxacin (100%) and Aminopenicillin (90.91%) having the highest susceptibility rate. Plasmid profiling using Gel electrophoresis, shows no plasmid in amplification bands of *E. coli*,

while the presence of plasmid in amplification band of *Staphylococcus aureus* at 9466bp was seen.

Keywords: Urinary Tract Infection (UTI), Antibiotic susceptibility, Plasmid profile, *Escherichia coli*, and *Staphylococcus aureus*.

1. Introduction

Urinary tract infections (UTIs) are common bacterial infections that affect different components of the urinary tract, such as the kidneys, bladder, urethra, and prostate. (Selim et al., 2022). This condition has both direct and indirect impacts on individuals' lives and is a significant global concern. Additionally, these diseases are increasingly becoming a prominent contributor to morbidity. It is estimated that urinary tract infections (UTIs) will affect approximately 150 million individuals worldwide annually. (Flores-Mireles, Walker, Caparon, & Hultgren, 2015). Healthcare expenses in the United States are estimated to amount to \$6 billion. (Shrank, Rogstad, & Parekh, 2019). Urinary tract infections (UTIs) rank as the second most common cause of illness in humans. In the United States, urinary tract infections (UTIs) lead to approximately one million visits to the emergency room and 100,000 hospitalizations annually. Urinary tract infections (UTIs) can be categorized as either community-acquired (CAUTI) or nosocomially acquired (N-UTI). Community-acquired UTIs occur within the community, typically within 48 hours of hospital admission, while nosocomially acquired UTIs manifest 48 hours after hospital admission or three days after discharge. (Despotovic et al., 2020). The prevalence of urinary tract infections (UTIs) is influenced by various factors including age, gender, catheterization, inpatient therapy, and prolonged antibiotic use. The majority of urinary tract infections (UTIs) are caused by Gram-negative bacteria (90%), while they account for only 10% of bacterial infections overall. (Seifu&Gebissa, 2018). *Escherichia coli* is the primary causative agent in the majority of urinary tract infections (UTIs), accounting for approximately 65-90% of infections. (Lee, Lee, & Choe, 2018). In addition to *Escherichia coli*, other uropathogens contribute to urinary tract infections (UTIs). These include *Enterococcus* species, *Klebsiella pneumoniae*, *Citrobacter* species, *Pseudomonas aeruginosa*, and coagulase-negative staphylococci (CoNS). Typically, urinary tract infections attributed to *Staphylococcus aureus* are uncommon. Nevertheless, in specific

individuals, *S. aureus* can result in ascending colonization and infection in the urinary tract, frequently originating from staphylococcal bacteremia in other sites like endocarditis. The presence of indwelling catheters and other instruments in the urinary tract increases the risk of *S. aureus* transmission. *Staphylococcus aureus* has flourished in the human population due to its adaptability and capacity to develop antibiotic resistance, establishing itself as a significant pathogen in various circumstances (Sakr, Brégeon, Mège, Rolain, & Blin, 2018). Methicillin-resistant *Staphylococcus aureus* (MRSA) accounts for over 50% of *Staphylococcus aureus* infections acquired in hospital settings. (MRSA). (Fukunaga et al., 2016). The emergence of antibiotic resistance in *Staphylococcus aureus* has been linked to the production of β -lactamases and other related mechanisms mediated by plasmids.

Drug resistance in microorganisms emerges through various mechanisms, such as the incorporation of foreign DNA into bacterial chromosomes, horizontal gene transfer, and genetic alterations. The patterns of resistance exhibited by microorganisms can vary across different regions, countries, states, hospitals of varying sizes, and even between hospitals and the community. In Dohuk city, Iraq, antibiotic resistance is worsening as a result of antibiotic overuse and misuse (Nauc  r et al., 2021). A comprehensive national surveillance system for antibiotic resistance is lacking, and the available data is insufficient to accurately quantify the extent of the problem. (Gharbi et al., 2019). In clinical settings, it is crucial and advantageous to identify the bacteria responsible for urinary tract infections (UTIs) and determine their resistance to commonly prescribed antibiotics. This information greatly improves the effectiveness of empirical treatment. The objective of the current study was to emphasize the bacterial causes of UTIs and assess the resistance patterns of *E. coli* and *S. aureus* isolates.

2. Urinary Tract Infection (UTI) Causing Bacteria

Urinary Tract Infection (UTI) is an infection involving any part of the urinary system, including the kidneys, bladder, ureters, and urethra. UTIs remain among the most frequently reported bacterial infections globally and contribute significantly to hospital visits and antibiotic consumption (Flores-Mireles et al., 2020).

Recent global surveillance reports indicate that UTIs account for a substantial proportion of both community-acquired and hospital-acquired infections, especially among women, catheterized patients, and immunocompromised individuals (World Health Organization [WHO], 2023).

Etiology of UTI

The majority of UTIs are caused by Gram-negative bacteria, particularly *Escherichia coli*, which accounts for 65–90% of cases (Bunduki et al., 2021). Other important uropathogens include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus* spp., and *Staphylococcus aureus* (Chenoweth et al., 2020). The increasing involvement of Gram-positive organisms such as *S. aureus* is often linked to catheter-associated infections and hospital settings (Muder et al., 2020).

Pathogenesis of UTI

UTI development begins with bacterial colonization of the periurethral region followed by ascension into the urinary tract. Bacterial virulence factors such as fimbriae, adhesins, and toxins facilitate attachment and invasion of urinary epithelium (Kostakioti et al., 2020).

Biofilm formation enhances bacterial survival, protects against host immune responses, and reduces antibiotic effectiveness, contributing to persistent and recurrent infections (Chenoweth et al., 2020).

***Escherichia coli* in UTI**

Uropathogenic *Escherichia coli* (UPEC) remains the leading cause of UTI worldwide. Its pathogenicity is associated with virulence factors such as P-fimbriae, type 1 fimbriae, and iron acquisition systems (Stapleton et al., 2020).

However, antimicrobial resistance among *E. coli* isolates has increased significantly, limiting treatment options and complicating empirical therapy (Larramendy et al., 2020).

***Staphylococcus aureus* in UTI**

Although less common, *Staphylococcus aureus* is an important opportunistic uropathogen, particularly in catheterized or hospitalized patients (Muder et al., 2020).

Methicillin-resistant *S. aureus* (MRSA) strains present a major clinical challenge due to multidrug resistance and limited treatment options (De Oliveira et al., 2020). Its ability to form biofilms further enhances persistence and treatment failure.

Antibiotic Resistance in UTI Pathogens

Antibiotic resistance is a growing global health threat affecting UTI management. Misuse of antibiotics, self-medication, and incomplete dosage significantly contribute to resistance development (Van Hoek et al., 2020).

Extended-spectrum beta-lactamase (ESBL)-producing organisms are increasingly reported among *E. coli* isolates worldwide, making standard antibiotics less effective (Larramendy et al., 2020).

Plasmid-Mediated Resistance

Plasmids are mobile genetic elements responsible for horizontal gene transfer of resistance genes among bacteria. They play a major role in the rapid spread of multidrug resistance in clinical isolates (Tenover, 2021). Plasmid-borne genes often encode beta-lactamases and other resistance determinants, contributing significantly to treatment failure in UTIs.

Antibiotic Susceptibility Testing

The Kirby-Bauer disk diffusion method remains the standard technique for antimicrobial susceptibility testing. Interpretation is guided by Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute [CLSI], 2023).

Recent updates emphasize standardized breakpoints to improve global comparability of antimicrobial resistance data (Humphries et al., 2023).

3. Methods

Study Location

This study is primarily hospital based. It was carried out at Federal University Otuoke, Bayelsa State. It is located within Latitude 4°47'51.3" N. Longitude 6°19'41.7" E and latitude 4°48'.04"

N. Longitude 6°19'27.0" E.

Ethical Clearance

The ethical clearance was obtained from the ethical committee of Federal Medical Centre Otuoke.

Specimen Collection

The study is tagged on both male and female outpatients and inpatients in Federal Medical Centre Otuoke, Bayelsa State, presenting with symptoms and signs of UTI which include dysuria, polyuria, fever, nausea, and flank pain will be sampled for in this study. Baseline demographic data includes age, sex, and risk factors such as catheterization, history of UTI, also out and in patients will be collected.

Isolation and Identification of Microorganisms

The urine specimens from all the patients will be cultured on CLED agar (Oxoid LTD, UK) and identified to determine the microorganism involved. Inoculation of urine specimen is done using sterile calibrated wire loop inoculating 0.001ml of urine specimen onto CLED agar (Oxoid LTD, UK). The cultured media will then be incubated at 37°C for 24 hours. For the media which had no growth after 24hrs incubation will be further incubated up to 48 hours before declaring absence of bacterial growth/negative. The significant growth will be identified further using Gram staining and biochemical reactions (Kolawale et al., 2009).

Characteristics of the Isolates

Each urine isolates will be identified based on their colonial morphology, cultural characteristics which include color, surface, appearance, crystal

violet stain, Lugol's iodine stain and safranin stain. All isolates obtained is preserved in the incubator and stored at 37o C. the stored isolates will be used to carry out biochemical tests.

Gram staining

Oxidase

Principle: The Oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless. The preserved isolates were touched with a swab stick and transferred on the surface of a filter paper, with a drop of oxidase reagent on the isolates on the filter paper, if it turns purple color within 5-20 seconds it is positive, anything outside from that is negative (Manges et al.,2017).

Catalase

Principle: The essence of catalase is to check if the organism has enzyme called catalase that it can use to breakdown hydrogen peroxide, water and oxygen. The presence of the enzyme in bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and rapid transferred on the surface of a filter paper, with a drop of hydrogen peroxide on the isolates on the filter paper, if there is elaboration of oxygen bubbles it is catalase positive, but if no rapid bubbles it is negative (Mushtaq et al., 2019).

Citrate

Principle: The basic principle of this test is to detect the ability of an organism which can utilize citrate as a sole source of carbon for their metabolism wit resulting alkalinity. The citrate enzyme hydrolyses the citrate to form oxaloacetic acid (Ferry, 2014). An already prepared citrate reagent with a blue color was transferred into a test and slanted, after the citrate has solidified the preserved isolates were inoculated and streaked pm the citrate reagent in the test tube with a wire loop and incubated for 24hours, if the color changes from blue to green it is citrate positive, but if no color change it is citrate negative (Lloyd et al., 2016).

Antibiotics Susceptibility Testing

Multi-disk Diffusion Technique

Swab sticks are used to pick the various isolate that are in the incubator. The surface of the

Nutrient agar will be streaked using swab stick containing the inoculums. The plates are streaked in four directions to ensure even distribution. The dried labeled circles to be used as sensitivity disc will be placed on the inoculated plates using a sterile forceps. The plates are then incubated for 24 hours at 37°C to check the zone of inhibition (Mezger et al., 2015).

Plasmid profiling

Extraction of plasmid from bacteria isolates was carried out using TENS-miniprep method (Zhou et al., 1999). An overnight bacterial culture on agar plates was harvested using a sterile inoculating loop and transferred into 100 µl of nutrient broth medium. Each Eppendorf tubes (5) were vortexed using a vortex machine (SciFinetech vortex mixer microfield) at high speed to resuspend the cells completely. 300 µl 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. 150 µl 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 900 µl of 100% ethanol (absolute) which has been pre-cooled to -20°C. 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 500 µl 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 at a safe and sterile atmosphere. The plasmids extracted were packed in a Ziploc bag and was 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 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°C.

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 horizontal gel casting tray of which two (2) combs had been inserted prior to the pouring and the gel was left to set at room temperature. The combs were carefully removed and $4\mu\text{l}$ each of the plasmid samples was mixed with $1\mu\text{l}$ of the loading dye giving $5\mu\text{l}$ 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 nineteen (19) samples ran 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 sub-cultured by streaking on a nutrient agar and incubated at 37°C for 24 hours. The overnight bacteria culture were harvested in 1ml of lysogeny broth each, labelled and incubated at 37°C for 24hours. 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 aluminium foil because acridine orange is light sensitive. The tubes were incubated at 37°C for 24 hours in a rotary incubator.

Application of Antibiotics Sensitivity Disc

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 by using Kirby-Bauer disc diffusion method according to the recommended standard of Clinical Laboratory Standard Institute (CLSI) the discs (Maxicare Medical Lab) contained the following antibiotics for positive: Rocephin R (25µg), Ciprofloxacin CPX (10µg), Streptomycin S (30µg), Septrin SXT (30µg), Pefloxacin PEF (10µg), Gentamicin CN (10µg), Ampiclox APX (30µg), Zinnacef Z (20µg), Amoxicillin AM (30µg). The discs used for Gram-negative (Rapids Labs International) contained the following: Ceftazidime CAZ (30µg), Cefuroxime CRX ((30µg), Gentamicin GEN (10µg), Ciprofloxacin CPR (5µg), Ofloxacin OFL (5µg), Amoxycillin/Clavulanate AUG (30µg), Nitrofurantoin NIT (300µg), Ampicillin AMP (10µg). The results were recorded after 24 hours of incubation and measurement was taken using the diameter of the inhibition zone around each disc. The antibiotics' sensitivity and resistance to the organisms were determined using the Clinical Laboratory Standards Institute guideline (Clinical and Laboratory Standards Institute [CLSI], 2020).

Statistical Analysis

Analysis of data will be collected and analyzed. Patients' names will not be used, numbers and letters will be used to label the samples. The raw data were entered into excel spreadsheet and later imported to statistical packages for social sciences (SPSS version 15) for analysis. The antibacterial activity is reported in terms of diameter of the zones of inhibition (mm). Comparison of means of zones of inhibition will be done using student t-test since there is more than one variable in consideration and values of ($p < 0.05$) will be considered statistically significant.

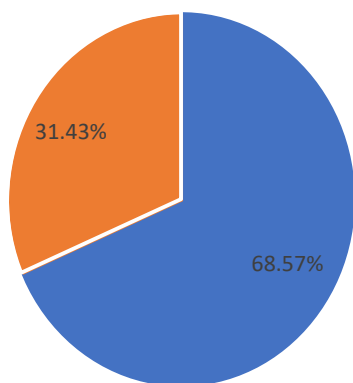
Analysis of Results

Out of 50 urine samples, a total number of 35 samples were obtained. The isolates are *Escherichia coli* and *Staphylococcus aureus*.

TABLE 3.1: Percentage Frequency Distribution of Bacterial Isolates from urine.

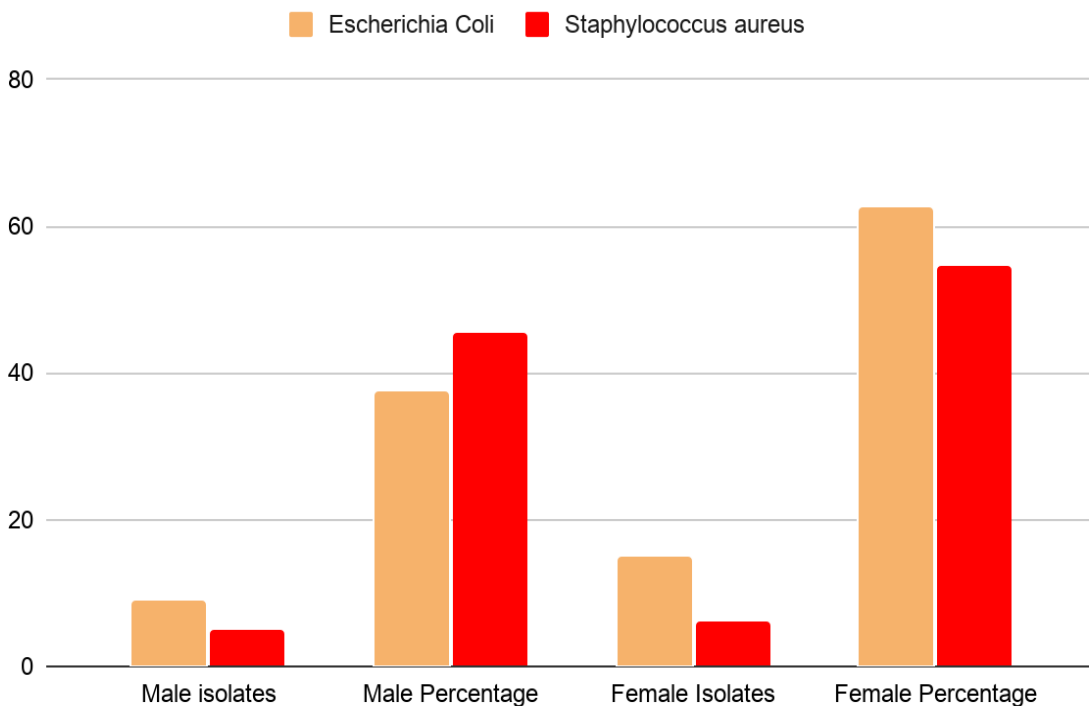
Isolates	Number of isolates	Percentage (%)
<i>Escherichia coli</i>	24	68.57
<i>Staphylococcus aureus</i>	11	31.43
Total	35	100

Table 3.1 Above shows the frequency distribution of bacterial isolates from urine of students in Federal University Otuoke. *Escherichia coli* recorded the highest isolate 24(68.57%) while the lowest was *Staphylococcus aureus* with 11(31.43%). There is no significant difference ($P \geq 0.05$) between the number of isolates and percentage occurrence of both *Escherichia coli* and *Staphylococcus aureus*.



■ *Escherichia coli* ■ *Staphylococcus aureus*

FIGURE 3.1: Presentation of Percentage distribution of bacterial isolates from urine specimens.



	<i>Escherichia Coli</i>	<i>Staphylococcus aureus</i>
Male isolates	9	5
Male Percentage	37.5	45.45
Female Isolates	15	6
Female Percentage	62.5	54.55

Figure 3.2: Above shows the percentage distribution of bacterial isolates by sex. The prevalence of the bacterial isolate was seen to be higher in females.

Table 3.2: Antibiotics susceptibility profile of Gram-negative bacteria isolated (*Escherichia coli*) from the urine samples using Gram negative antibiotics.

Antibiotics	<i>Escherichia coli</i>		
	n=24		
	%		
	NS	NI	NR
OFX	21(87.50)	3(12.50)	0(0.00)
PEF	19(79.17)	5(20.83)	0(0.00)
CPX	0(0.00)	24(100)	0(0.00)
AU	0(0.00)	0(0.00)	24(100)
CN	24(100)	0(0.00)	0(0.00)
S	23(95.83)	1(4.17)	0(0.00)
CEP	0(0.00)	0(0.00)	24(100)
NA	0(0.00)	0(0.00)	24(100)
PN	0(0.00)	0(0.00)	24(100)
SXT	0(0.00)	0(0.00)	24(100)

There is no significant difference ($P \geq 0.05$) in the antibiotic susceptibility profile of Gram-negative bacteria *Escherichia coli* isolated from urine specimens of students at Federal University Otuoke showing a high resistant rate.

KEY:CN(gentamycin-10mcg),AU(augmentin-30mcg),CPX(ciprofloxacin-10mcg),SXT(septrin-30mcg),PN(ampicilin-30mcg),S(streptomycin-30mcg),CEP(ceporex-10mcg),OFX(tarivid-10mcg), NA(nalidixic acid-30mcg), PEF(reflacine-10mcg), NS-number of sensitive, NI-number of intermediate, NR-number of resistant,%-percentage.

Table 3.3: Antibiotics susceptibility profile of Gram-positive bacteria (*Staphylococcus aureus*)

isolated from the urine samples.

Antibiotics		<i>Staphylococcus aureus</i>	
		n=11	
		%	
		NS	NR
		NI	
Ampicillin	9(81.82)	1(9.09)	1(9.09)
Penicillin	7(63.64)	4(36.36)	1(11.11)
Amikacin	10(90.91)	1(9.09)	0(0.00)
Vancomycin	9(81.82)	2(18.18)	0(0.00)
Aminopenicillin	10(90.91)	1(9.09)	0(0.00)
Ciprofloxacin	11(100)	0(0.00)	0(0.00)

Keys: N = Number, S = Susceptibility/Sensitivity, R = Resistance

There is a significant difference ($P \leq 0.05$) in the antibiotic susceptibility profile of Gram-positive bacteria *Staphylococcus aureus* isolated from urine specimens of students at Federal University Otuoke showing a high susceptible rate.

Table 3.4: Susceptibility test before curing

Bacteria Isolates	CN	AZM	C	CPD	AUG	CTX	CIP	TE
<i>Escherichia coli</i>	1.2	1.2	0.7	-	1.1	1.1	-	0.9
<i>Staphylococcus spp</i>	1.1	1.3	0.9	-	-	1.15	1.0	1.2

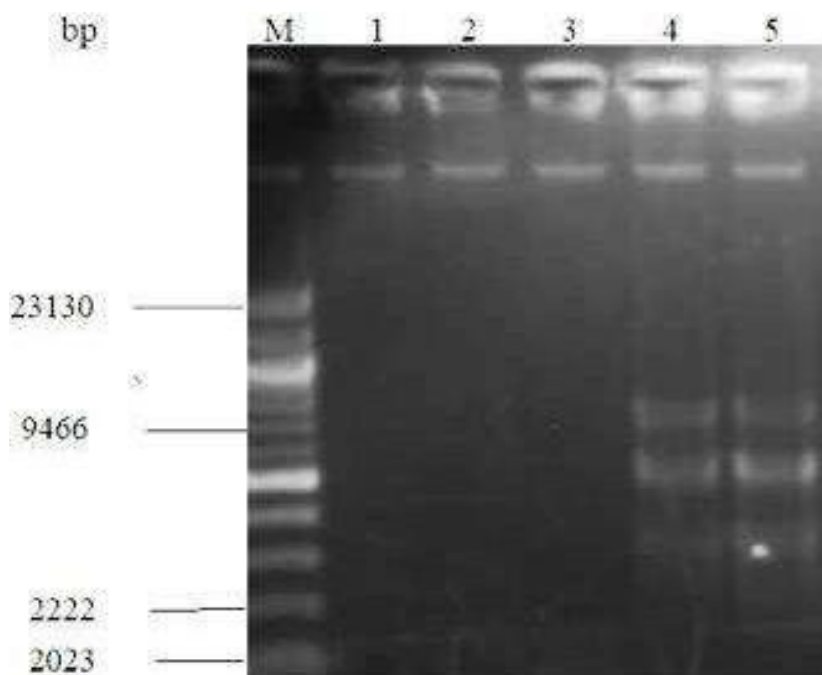
All measurement were taken in centimetre (CM). TE, Tetracycline, AZM, Azithromycin, C, Chloramphenicol, CIP, Ciprofloxacin, CN, Gentamicin, CTX, Cefotaxime, AUG,

Amoxicillin-clavulanic acid, CPD, Cefpodoxime.

Table 3.5: Susceptibility test after curing

Bacteria Isolates	CN	AZM	C	CPD	AUG	CTX	CIP	TE
<i>Escherichia coli</i>								
<i>Staphylococcus spp</i>	1.1	1.0	0.9	-	-	1.15	1.0	0.5

All measurement were taken in centimeter (CM). TE, Tetracycline, AZM, Azithromycin,



C, Chloramphenicol, CIP, Ciprofloxacin, CN, Gentamicin, CTX, Cefotaxime, AUG, Amoxicillin-clavulanic acid, CPD, Cefpodoxime.

Figure 3.3: Gel electrophoresis.

Lane M, 1Kb DNA ladder, Lanes 1-4 shows no plasmid in amplification bands of *E. coli*, while lane 5 shows the presence of plasmid in amplification band of *Staphylococcus aureus* at 9466bp, in gel electrophoresis.

4. CONCLUSION

The main cause of the UTIs is Gram-negative bacteria. Streptomycin and Gentamycin may be drugs of choice for Gram-negative bacteria whereas Aminopenicillin and Ciprofloxacin may be drugs of choice for Gram-positive bacteria. The main cause of the UTIs was found as Gram-negative bacteria hence, prescription of antibiotics based on susceptibility tests would help in reduction of antibiotic resistance. The high prevalence of E. coli in the female gender could be due to the proximity of the anus to the vagina.

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