Antibiotics Resistant Pattern of Uropathogenic *Escherichia coli* Isolated From Patient Attending General Hospital Jega, Kebbi State, Nigeria.

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Abstract

Urinary tract infections (UTIs) continue to be the most prevalent infections diagnosed among patients, as well as the primary cause of hospitalization. Uropathogenic *Escherichia coli* is the most predominant isolate in both the community and hospital at large, and the frequency of Antibiotics-resistant *E. coli* is rising, globally. The aim of this research was to determine antibiotic resistance pattern of Uropathogenic *E. coli* among patients attending General Hospital Jega. Kebbi State Nigeria. A total of 117 urine samples were collected from patients *each sample was streaked using a sterilized platinum wire loop onto the surface of freshly prepared MacConkey agar plates which was then incubated at 37^{o}C for 24 hours. All the isolates were subjected to* biochemical t*est.* Modified Kirby Bauer disc diffusion method was used for susceptibility testing and results were interpreted according to Clinical Laboratory Standard Institute. *The results showed that out of the 117* study *subject,* 17 were identified as *E. coli* which account for (14.5%). It was found that the females category (8.5%) were more susceptible to UTI than males (6.0%), and the participant between the age range of 31-40 were the most commonly infected (5.9%), and 11-20 years were the least (1.0%) respectively, The result of antibiotic susceptibility test on *E. coli* isolates were highly resistance to Cephalexin with 100%, Amoxicillin/clavulanic-acid, 47.1% Cefpodoxime and Tetracycline 41.2%, Meropenem 23.5%, Cefotaxime 17.6% and Ceftazidime and Cefoxitin 11.8% respectively. The isolates were further identified using polymerase chain reaction (PCR) methods. The data obtain from this research suggests that antibiotic prescriptions should be managed in accordance with recommendations. Antibiotic use should be monitored both in clinical settings and in the general community in order to reduce infection rate.

Keyword: Antibiotics resistance, *E. coli*, Prevalence, Urinary Tract Infection.

INTRODUCTION

The family Enterobacteriaceae are enormous and diversified. Members of this family are peritrichous, Gram-negative, facultative anaerobic bacilli that ranges in size from 0.3 to 1.0 x 1.0 to 6.0 m. The majority of the bacteria in this family are chemoorganotrophic, which means they have both a respiratory and a fermentative metabolism. They grow well and are most metabolically active between 25 and 35°C (Ali *et al*., 2017).

Gram-negative *Escherichia coli* is a rod-shaped, facultatively anaerobic, non-sporulating, motile pathogen with a high rate of global dissemination. It is feasible to isolate it from veterinary, environmental, and clinical sources. Certain strains of *E. coli* are responsible for the majority of clinical and environmental induced illnesses. The human gastrointestinal system is home to numerous *Escherichia coli* types of bacteria. Despite being commensals, they have the potential to cause a variety of diseases, such as meningitis, diarrhoea, septicemia, and urinary tract infections (UTIs) (Musa *et al*., 2023).

The anatomy of the infection determines whether a urinary tract infection (UTI) is upper or lower: the lower urinary system includes the bladder and urethra, while the upper urinary tract includes the ureters and kidneys (Forbes *et al*., 2007). Women experience UTIs more frequently than men do (Boye *et al*., 2012). The development of UTIs is particularly influenced by the structure of the female urethra. The female urethra is smaller than the male urethra in comparison, and it is also located adjacent to the warm, moist, and microbially rich perirectal region. Because of the shorter urethra, bacteria can reach the bladder more easily in the female host. According to Eugene *et al*., (2007), the urinary system is second only to the respiratory tract in terms of developing microbial infections, particularly in females.

Urinary Tract Infection brought on by *E. coli* is treated with a variety of antibiotic classes, such as cephalosporins, carbapenem, aminoglycosides, and fluoroquinolones. There are fewer therapeutic alternatives available, notably for UTIs, for which considerable antibiotic use has been observed in both community and hospital settings due to the worrisome increase in the rate at which these strains acquire antibiotic resistance genes (Musa *et al*., 2023). Their harmless strains can continue to coexist with other organisms as commensals as long as they do not pick up genetic components that express hazardous proteins. Variables related to virulence may eventually result in these illnesses (Onanuga *et al*., 2019). Multidrug resistance (MDR) development is a natural process, but poor antibiotic use, unhygienic conditions, poor food handling, and inadequate superbug infection prevention and control techniques have all aided in its development and promoted its spread. The ability to treat these conditions is

threatened by the emergence of multidrug-resistant bacteria. These microorganisms are particularly resistant to the common antimicrobials (Fagan *et al*., 2015). Multidrug resistance is defined as the inability to respond to at least one agent in three or more antimicrobial categories, whereas extensively drug resistance is defined as the inability to respond to at least one agent in all but two or fewer antimicrobial categories.

One of the global health problems, UTI accounts for over 8.1 million annual visits to healthcare providers and affects almost 150 million individuals each year (Bitew *et al*., 2017). Major repercussions from untreated UTIs frequently lead to higher treatment expenses and mortality (Fahim, 2021). Due to the use of antimicrobial drugs, resistance has emerged among several bacterial strains (Tanwar *et al*., 2014). UTIs are the most common bacterial infections contracted in hospitals and the general population. According to Ekwealor *et al*., (2016), *E. coli* is the most common uropathogen because most of them originate from the host's gut microflora and enter the bladder through the urethra (Ugwu *et al*., 2020). Treatment for urinary tract infections (UTIs) has led to an increase in bacteria's resistance to widely used antibiotics, placing a heavy financial and health cost on society (Tille *et al*., 2015). *Escherichia coli* could develop antibiotic resistance by efflux pumps, antibiotic-inactivating enzymes, alterations to permeability or targets, acquired plasmids encoding -lactamases, or through enzymes that modify aminoglycosides (Abejew *et al*., 2014).

MATERIALS AND METHODS

Study Area

Jega is a local government situated in, Kebbi State, The local government is located in North-west geopolitical zone of Nigeria located with latitude 12.3667 and longtitude 4.6333^{o}E. The local government is bounded by Sokoto State to the north and east, Niger state to the south. It has a total land area of 891km2. The in-habitant are predominantly Hausa people by their tribe. Trading and agriculture is the main occupation of the people especially in the rural areas. The local government has a market that is traditionally holds on Friday, while the business activities continue even after the market. The local government has a total population of 193,352 people as projected from the (NPC, 2006) (Figure 1).

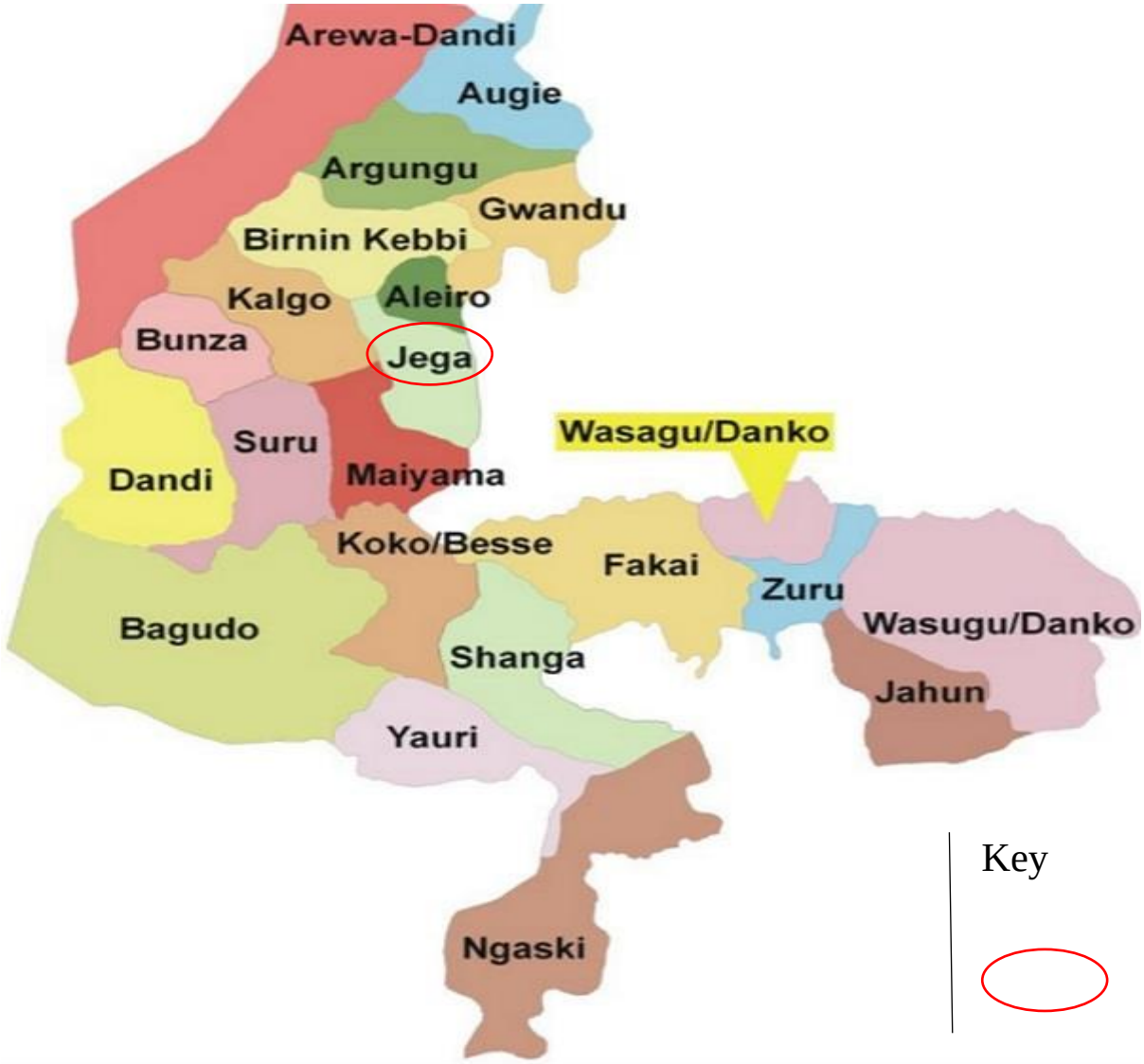


Fig 1: Map of Kebbi State showing the study area (Jega). Copyright ©2023 Nigeria Zip Codes. All Rights Reserved**.**

Ethical Clearance

Ethical approval for this study was obtained from the ethical review Committee from Kebbi State Ministry of Health before administering the work. Informed consents both written and oral were obtained from all participants.

Sample Population

The study population include all the patient referred to laboratory department, Patient from all sex and age groups, categorically 117 samples was obtained from General hospital Jega, Kebbi State.

Study design

This was a Cross-sectional and hospital-based study.

Sample Collection

A minimum of 117 clean catch midstream urine sample was collected in a sterile universal container in the process of collecting patients were advised to wash hand with soap and water in other to avoid contamination of urine sample. The sample were placed in ice cool box, then the sample were immediately transported to Microbiology Department Laboratory Kebbi State University of Science and Technology Aliero for further analysis (Kalgo *et al*., 2022).

Sample Processing

A loopful (0.01 ml) of urine sample was inoculated on MacConkey agar plate using a sterilized wire loop, and it was then incubated aerobically at 37°C for 24 hours for bacterial growth.

Preparations of Culture Media Used

In this study Mueller Hinton Agar (MHA), Nutrient Agar, and MacConkey Agar (MAC) were the media used. Standard aseptic method was used to prepare the media in accordance with the manufacturer's instructions.

Isolation and identification of *Escherichia coli*

Cultural examination

Morphological characteristics of colonies were studied on MacConkey agar. The colonies were recorded after 24hrs of incubation at 37˚C.

Microscopic examination

A single colony of each isolate was fixed on a clean grease free slide to study gram stain, under light microscope according to Ali *et al.*, (2017).

Determination of Significant Bcteriuria

The Significant bacteriuria were determined by a bacterial count greater than 1 x 10^5/ml, while a bacterial count less than 1 x 10^5/ ml were regarded insignificant bacteriuria

Biochemical tests

The suspected isolates were subjected to a series of biochemical tests to confirm the organisms as mentioned by Ali *et al*., (2017) and as follows.

A- Catalase Test

A single colony of each bacteria isolate was picked, which was then smear on a clean, free grease glass slide with a sterile wire loop, and 3 drop of hydrogen peroxide was added, where a favourable outcome indicated by the presence of gaseous bubbles.

B- Indole Test

A colony was inoculated into peptone water broth and incubate for 24 hours at 37°C. Two to three drops of Kovac's reagent were added after incubation. Positive results are shown by the presence of a pink ring at the top.

C- Citrate utilization test (Simmon's Citrate slant)

An overnight colony of subculture isolate was streaked into a simmon citrate agar slant and incubate for 24 to 48 hours at 37°C. Positive results are indicated by a shift from the medium colour to blue.

D- Methyl-red test

A single colony of bacteria isolate was inoculated in MR-VP broth and allowed to grow for 24 hours in an incubator at 37°C. Three to four drops of the methyl red reagent were added after incubation. Red media colour conversion is an indicative of positive result.

E- Vogas-Proskauer test

A single culture colony of bacteria was inoculated into MRVP broth and cultured there for 24 hours at 37°C in an incubator. Afterward, four drops of VP2 and two drops of VP1 were added. After 15 minutes, the appearance of red colour indicates a positive result.

Statistical analysis

Statistical tools such as diagrams, Pie chart and bar chart were used for the descriptive and inferential analysis using the excel spread sheet.

Antimicrobial Susceptibility Test (AST)

In Microbiology laboratory of Facualty of Life Science, Kebbi State University of Science and Technology Aleiro, the disc diffusion method, which involve a modified version of the Kirby Bauer technique, which has been accurately standardized by the Clinical and Laboratory Standards Institute (CLSI), was employed to ascertain the antimicrobial susceptibility pattern of all the isolates (Abdu *et al*., 2018). In brief the colonies were first suspended in a saline solution, after which the inoculums were modified to achieve a turbidity level that corresponds to a 0.5 McFarland standard. Subsequently, a sterile cotton swab was immersed into the modified suspension. The swab was subsequently rotated numerous times and applied with significant pressure onto the interior wall of the tube, positioned above the level of the fluid. By performing this action, any excess inoculum adhering to the swab was successfully reduced. The dehydrated exterior of a Mueller-Hinton agar plate was then subjected to inoculation through the process of swabbing the swab across the entire pure surface of the agar. Using sterile forceps, antimicrobial discs were dispensed onto the surface of the inoculated agar plate.

Each individual disc was subsequently pressed down to ensure absolute and comprehensive contact with the agar surface. The discs were distributed evenly so that they are no closer than 24 mm from each site. The plates were inverted and placed in an incubator at a temperature of 37^{o}C for 24 hours. The diameters of the inhibition, as determined by visual observation without the aid of any instruments, were measured, along with the diameter of the disc itself. The measurement of the zones was conducted to the nearest whole millimeter using a ruler, which was positioned on the back of the inverted Petri-plate. The organisms were categorized as either susceptible, intermediate, or resistant to the agents that were subjected to testing (Kalgo *et al*., 2022).

Results and Discussion

Isolation and identification of *Escherichia coli*

A total of one hundred and seventeen (117) midstream urine samples collected from patient attending General hospital Jega, Kebbi State were analyzed using the following microbiological culture screening method, where 17 were confirmed to be positive and 100 are negative. The positive culture were also subjected to series of biochemical test for classification: such as microscopic examination, catalase, citrate, indole production test as well as gas and hydrogen sulphide production test using Kligler iron agar (KIA), MR-VP test, were also carried out to confirm their characteristics using microbiological standard procedure of identifying isolates **Table 1**.

The study participants were categorized among the following age groups 1-10yrs, 11-20yrs, 21-30yrs, 31-40yrs, 41-50yrs, 51- above. The result of the study revealed that patient within the age 11-20yrs are the most frequently encounter age group that have the highest UTI incidence with 5 positive cases (4.27%) while patient within the age group of 41-50 have the least UTI incidence with 1 positive cases (1.0%), as described in **Figure 1** below: The reason of occurrence may be due to frequent sexual intercourse, use of contraceptive spermicidal agents, diaphragms and menopause for women and enlargement of the prostate gland for men. This type of finding has earlier been reported in Bangladesh and Borno North East Nigeria, and in contrast; the sex category revealed that female are more prone to Urinary Tract Infection 10(8.5%) as compared in male 7(6.0%), This result indicated that the female patients had higher prevalence of UTI than in males. This result is consistent with other studies performed in North-East and South-West Nigeria. A numbers of factors are associated with high prevalence of infection in females such as shorter and wider urethra in females than in males, lack of

antimicrobial properties of prostatic fluid as in males, hormonal changes which affect the mucosal adherence of bacteria and trauma of urethra during sexual intercourse, as described in **Figure 2** below.

Features	***E. coli***
Colony on MacConkey agar	Red colonies, circular, low convex, smooth, translucent, Lactose fermenters colony
Gram staining	Gram negative, rod shaped, pink color
Indole	Positive
Catalase	Positive
Motility	Positive
H2S production	Negative
Gas production	Positive
Citrate utilization	Negative
MR-VP	Positive/Negative

Table 1: Cultural, microscopic and biochemical properties of *E. coli*

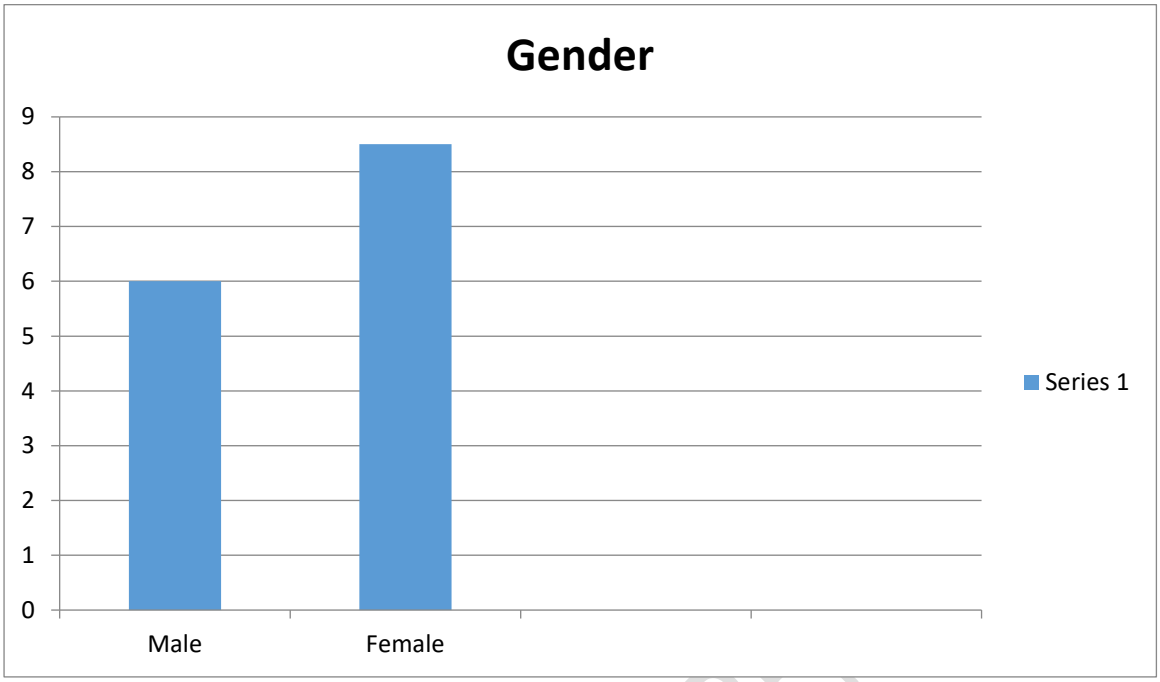


Figure 1: Prevalence of *E. coli* according to sex

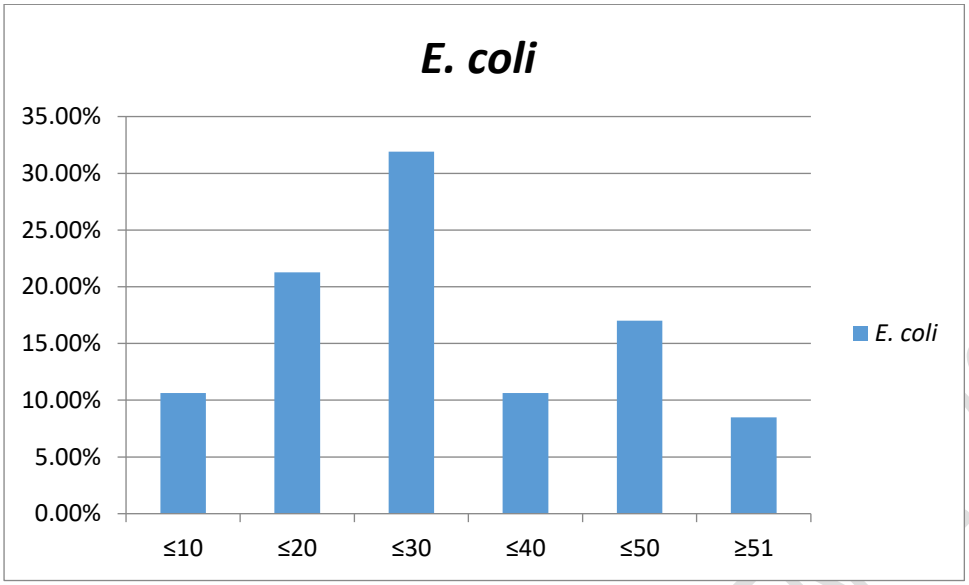
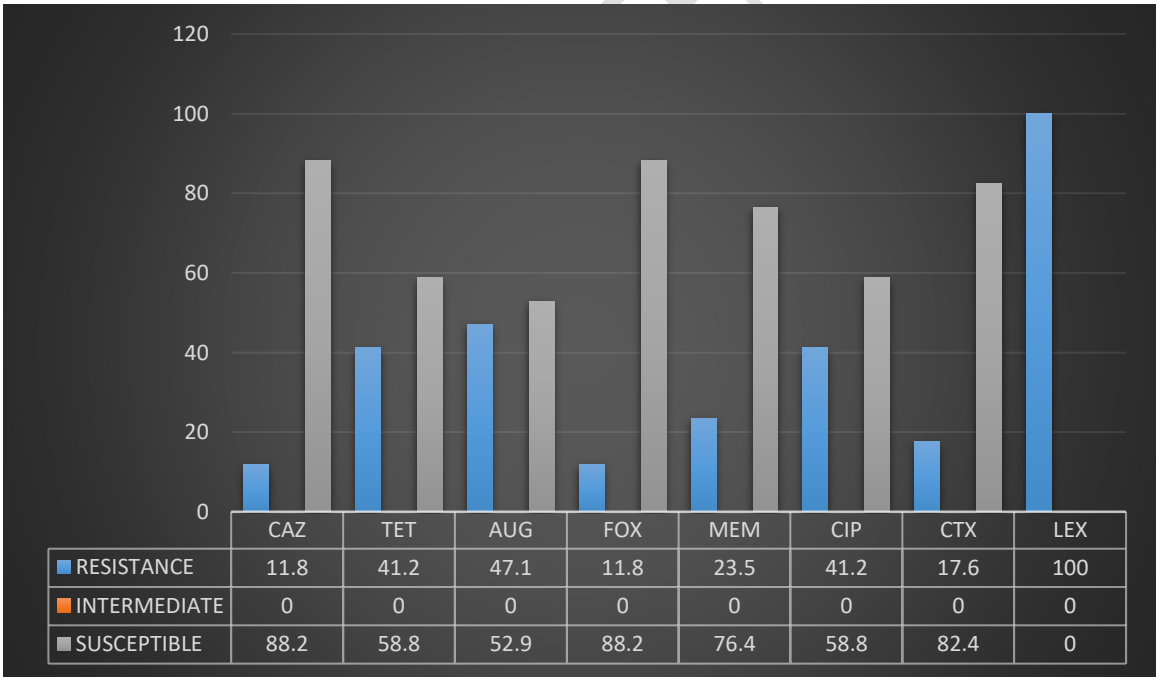


Figure 2: Prevalence of *E. coli* according to different age group

Antimicrobial Resistance of *E. coli* isolates

In this study, the selected isolates were examined for their susceptibility to the following antibiotics by disc diffusion method, eight (8) different antibiotics which also belong to different classes and categories. From the test conducted 17(51.9%) of all confirmed *Escherichia coli* isolates were tested with eight different antibiotics. It was found that *E. coli* were resistant to Ceftazidime,(CAZ10µg/disk), 2(11.8%), Cefpodoxime,(CPD30µg/disk), 7(41.2%), Augumentin,(AMX/CA30µg/disk), 8(47.1%), Tetracycline,(TET30µg/disk), 7(41.2%), Meropenem,(MEM30µg/disk), 4(23.5%), Cefotaxime,(CTX30µg/disk), 3(17.6%), Cephalexin,(LEX30µg/disk) 17(100%) and Cefoxitin,(FOX30µg/disk), 2(11.8%) as presented in **figure 3** below: However this result is in consistent and similar with other finding by previous studies conducted from federal medical center Birnin Kebbi by Musa *et al*., (2023) resistance to Cefotaxime with 81.5%, also according to Haque *et al*., (2023). Tetracycline 46.66% and Amoxicillin 100% are resistance to *E. coli* According to Kalgo *et al*., (2023) report the resistance of Ceftazidime and Cefotaxime with 100%.



	CAZ	TET	AUG	FOX	MEM	CIP	CTX	LEX
RESISTANCE	11.8	41.2	47.1	11.8	23.5	41.2	17.6	100
INTERMEDIATE	0	0	0	0	0	0	0	0
SUSCEPTIBLE	88.2	58.8	52.9	88.2	76.4	58.8	82.4	0

Conclusion

Our result suggests that the incidence of UTI was higher in females than males. The *Escherichia coli* resistance profile reveals that Carbapenems: Cephalexin is highly resistance while Cefoxitin and Ceftazidime were least resistance against the isolated *E. coli* It is

recommended that for appropriate treatment and prevention of bacterial resistance, the clinicians should prescribe antibiotic after having the culture sensitivity results.

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