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Prevalence and Antibiotics Susceptibility 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. This research aimed to determine the prevalence and antibiotic susceptibility 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°C for 24 hours. All the isolates were subjected to biochemical tests. The modified Kirby Bauer disc diffusion method was used for susceptibility testing and results were interpreted according to Clinical Laboratory Standard Institute. The results show that out of the 117 study subjects, 17 were identified as *E. coli* which accounts for (14.5%). It was found that the females category (8.5%) were more susceptible to UTI than males (6.0%), and the participants 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 outcome of antibiotic susceptibility test on *E. coli* isolates were extremely 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 data obtained from this research suggests that antibiotic prescriptions should be managed by recommendations. Antibiotic use should be monitored both in clinical settings and in the general community to reduce infection rates.

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

INTRODUCTION

The family Enterobacteriaceae is enormous and diversified. Members of this family are peritrichous, Gram-negative, facultative anaerobic bacilli that range in size from 0.3 to 1.0 x 1.0 to 6.0 μm. 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, diarrhea, 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-resistant 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 extensive 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 upsurge 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 β -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 the North-west geopolitical zone of Nigeria with latitude 12.3667 and longitude 4.6333oE. 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 891 km². The inhabitants are predominantly Hausa people their tribe. Trading and agriculture is the main occupations of the people especially in the rural areas. The local

government has a market that is traditionally held 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 of the Kebbi State Ministry of Health before administering the work. Informed consent both written and oral was obtained from all participants.

Sample Population

The study population includes all the patients referred to the laboratory department, Patients from all sex and age groups, and categorically 117 samples were 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 samples were collected in a sterile universal container in the process of collecting patients were advised to wash their hands with soap and water in other to avoid contamination of urine samples. The samples were placed in a cool box, then the samples were immediately transported to the 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. A standard aseptic method was used to prepare the media according to the manufacturer's instructions.

Isolation and identification of Escherichia coli

Cultural examination

The 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 a light microscope according to Ali et al., (2017).

Determination of Significant Bacteriuria

The Significant bacteriuria was determined by a bacterial count greater than 1 x 10⁵/ml, while a bacterial count less than 1 x 10⁵/ ml was regarded as 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 smeared on a clean, free grease glass slide with a sterile wire loop, and 3 drops of hydrogen peroxide were added, where a favorable outcome was indicated by the presence of gaseous bubbles.

B- Indole Test

A single culture colony of bacteria was inoculated into peptone water broth and cultured there for 24 hours at 37°C in an incubator. Two to three drops of Kovac's reagent were applied and subsequently incubated. 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 incubated 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 indicative of positive results.

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 charts, and bar charts were used for the descriptive and inferential analysis using the Excel spreadsheet.

Antimicrobial Susceptibility Test (AST)

In microbiology laboratory of faculty of life science, Kebbi State University of science and technology aleiro, by 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 disc was subsequently pressed down to ensure absolute and comprehensive contact with the agar surface. The discs were distributed evenly so that they were no closer than 24 mm from each site. The plates were inverted and placed in an incubator at a temperature of 37°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 patients attending General Hospital Jega, Kebbi State were analyzed using the following microbiological culture screening method, where 17 were confirmed to be positive and 100 were negative. The positive culture was also subjected to a series of biochemical tests for classification: such as microscopic examination, catalase, citrate, indole production test as well as gas and hydrogen sulphide production tests using Kligler iron agar (KIA), MR-VP test, also carried out to confirm their characteristics using the 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, and 51- above. The result of the study revealed that patients between the ages 11-20yrs are the most frequently encountered age group that has the highest UTI incidence with 5 positive cases (4.27%) while patients within the age group of 41-50 have the least UTI incidence with 1 positive case (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 females are more prone to Urinary Tract Infection 10(8.5%) as compared to males 7(6.0%), The outcome of this study demonstrated that the occurrence of UTI was significantly greater in female patients compared to male ones. This outcome aligns with earlier research carried out in North-East and South-West Nigeria. The high frequency of infection in females is associated with several factors, such as a wider and shorter urethra in comparison to that of males, the lack of antimicrobial properties in prostatic fluid, hormonal fluctuations that affect bacterial attachment to mucosal

surfaces, and urethral trauma during sexual activity, as described in Figure 2 below.

Table 1: microbiological features of E. coli

Features	<i>E. coli</i>
Colony on MacConkey agar	Red colonies, circular, low convex, smooth, translucent, Lactose fermenters colony
Gram staining	Rod shaped, Gram negative, pink color
Indole	Affirmative
Catalase	Affirmative
Motility	Affirmative
H ₂ S production	Adverse
Gas production	Affirmative
Citrate utilization	Adverse
MR-VP	Affirmative e/Adverse

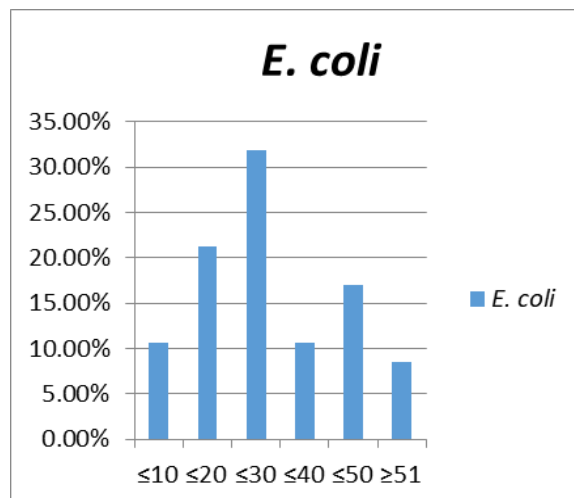


Figure 2: frequency of E. coli in relation to various age groups

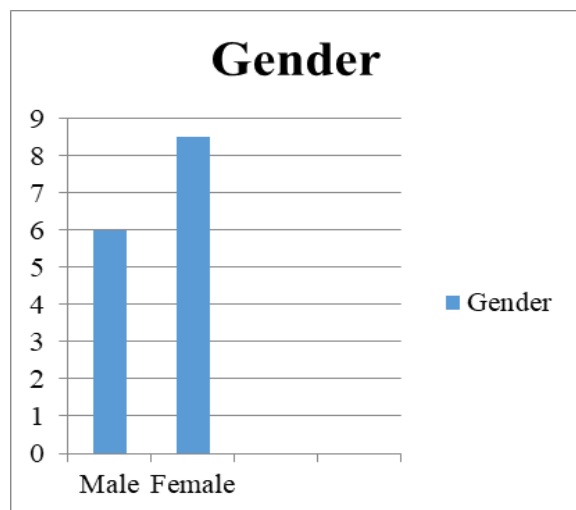


Figure 1: Prevalence of E. coli according to sex

Antibiotics Susceptibility of E. coli isolates

In this investigation, the particular isolates were scrutinized for their susceptibility to the following antibiotics by disc diffusion technique, ten (10) different antibiotics that also belong to different classes and categories. From the test conducted 17(51.9%) of all confirmed Escherichia coli isolates were tested with ten different antibiotics. It was found that E. coli were resistant to Cephalexin (LEX30µg/disk) 17(100%), Gentamicin (GEN, 30µg), 17(100%), Ampicillin (AMP, 10µg), 17(100%). Followed by Augumentin, (AMX/CA30µg/disk), 8(47.1%). However, the most sensitive antibiotics against the isolates was Ceftazidime, (CAZ10µg/disk), 15(12.8%), Cefoxitin, (FOX30µg/disk), 15(12.8%) Cefotaxime,(CTX30µg/disk), 14(12.0%), Meropenem,(MEM30µg/disk), 13(11.11%), Cefpodoxime,(CPD30µg/disk), 10(8.5%), and Tetracycline,(TET30µg/disk), 10(8.5%), as presented in figure 3 below: However, this result is inconsistent and similar with other finding conducted from federal medical centre 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%, Third generation cephalosporins, namely cefixime, cefotaxime, ceftazidime, and ceftriaxone, exhibit antimicrobial sensitivity to varying degrees: 80%, 75%, 70%, and 63%.

CONCLUSION

According to this research, UTIs were more frequently in women than in men The Escherichia coli resistance data indicate that Carbapenems: Cephalexin is highly resistance while Cefoxitin and Ceftazidime were least resistance against the isolated E. coli It is advised that after receiving the culture sensitivity data, practitioners should prescribe antibiotics for the proper course of therapy and to prevent the development of bacterial resistance.

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