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PREVALENCE OF ANTIBIOTIC RESISTANCE UROPATHOGENIC *Escherichia coli* PRODUCING EXTENDED SPECTRUM BETA-LACTAMASE IN UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA

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ABSTRACT

The global prevalence and general spread of Uropathogens producing extended spectrum beta-lactamase (ESBL) has remained a critical health concern globally. The rise in the prevalence of antimicrobial-resistant uropathogenic bacteria globally has also become a major health concern, due to the increasing mortality rate. However, one of the pathogens which has been classified as a priority pathogen by World Health Organisation is ESBL-producing *Escherichia coli*. This study was carried out to assess antibiotic resistance and ESBL production among *E. coli* recovered from the archive of University College Hospital, Ibadan, Nigeria. From a total of 330 isolates retrieved from the archive of the hospital, 129 isolates were presumptively identified as *E. coli* by standard phenotypic methods and confirmed by the use of Oxoid 12E Microbact™ 2009 Gram-negative identification system. Majority of these isolates (67.4%) were Congo red positive and considered pathogenic. All the isolates were resistant to ampicillin, and tetracycline while significant percentage (95.5%) was resistant against gentamicin. 17.1% of the isolates demonstrated multidrug resistant phenotype with multiple antibiotic re-

INTRODUCTION

Escherichia coli (*E. coli*) was first described in 1885 by Theodor Escherich as *Bacterium coli commune* after being isolated from the faeces of infants. It was initially seen as a commensal in the large intestine of warm blooded animal hosts, synthesizing vitamin K2 and preventing the attachment of pathogenic

bacteria (Bentley and Meganathan, 1982, Hudault *et al*., 2001, Reid *et al*., 2001). *E. coli* belongs to the family Enterobacteriaceae which are anaerobic, facultative and Gram-negative rods. It possesses an extra-cytoplasmic outer membrane that consists of a lipid bilayer, lipoproteins, and capsule of lipopolysaccharide (LPS) that interfaces with the host environment (Johnson and Russo, 2005).

Based on genetic and clinical criteria, *E. coli* has been classified into three major groups including commensal *E. coli*, intestinal pathogenic (diarrheagenic) *E. coli*, and extra-intestinal pathogenic *E. coli* (ExPEC). Extra -intestinal pathogenic *E. coli* are capable of causing diseases outside the gastrointestinal tract. It has been shown that ExPEC can invade urinary tract, cerebrospinal fluid and blood stream causing urinary tract infections (UTIs), neonatal meningitis, and septicemia congruently (Govindarajan *et al*., 2024, Belmont-Monroy *et al*., 2025, Grome *et al*., 2026). ExPEC has been implicated in nosocomial pneumonia, osteomyelitis, and wound infections (Eisenstein and Jones, 1988, Russo and Johnson, 2000, Johnson and Russo, 2002). A previous investigation has shown that failure to diagnose UTIs early leading to a delay in the implementation of the right treatment, has been noted to cause renal scarring, hypertension and kidney failure, especially among infants (Omoregie *et al*., 2012).

Antimicrobial resistance (AMR) has become one of the most significant health challenges worldwide, positioning itself among the leading ten threats to human health (Olaitan *et al*., 2025). Recent statistics have shown that mortality rate of approximately 8.9 million was associated with bacterial infections in 2019, out of which 1.27 million were linked to AMR with an additional estimate of 4.95 million death attributable to its impact on a global scale (Naghavi *et al*., 2024, Olaitan *et al*., 2025). It has become increasingly worrisome as the incidence of AMR bacteria producing extended-spectrum beta-lactamases (ESBLs) has continued to rise, which in recent years has experienced an upsurge in both hospitals and communities (Ebrima *et al*., 2025). *Escherichia coli* and *Klebsiella pneumoniae* have been reported as the two major groups of bacteria connected with ESBL production (Wilson and Torok, 2018). The misuse of antibiotics in both human healthcare and animal farming, especially in Sub-Saharan Africa, has been described as the primary contributor to AMR (Moyo *et al*., 2023). Literature suggest that by 2030 in the absence of intervention, infections arising from AMR will constitute a significant danger to the world economy, especially among the low- and middle- income countries with sub-Saharan Africa countries having to deal with unequal burden (Sammaro *et al*., 2023).

Considering the central role that *E. coli* plays as a human pathogen and the various non-clinical reservoirs connected to it, through which persons can contract it, this study aimed to decipher the prevalence of antibiotic resistance profile of ESBL-production among pathogenic *E. coli* implicated in UTIs from University College Hospital (UCH) Ibadan, Oyo State, Nigeria.

MATERIALS AND METHODS

Ethical approval

Ethical approval for the collection of the clinical samples was duly obtained from the Oyo State Ministry of Health Research Ethics Committee (NHREC/OYOSHRIEC/10/11/22). This research was carried out in line with the World Medi-

cal Association (WMA) declaration of Helsinki on the principles for medical research involving human subjects and identifiable human material or data (WMA, 2024).

Study area

Pretested isolates of suspected *E. coli* from UTIs were collected from the Medical Microbiology and Parasitology Laboratory of UCH between May 28, 2022 and June 30, 2023. The tertiary hospital is located on latitude 7.40279° or 7° 24' 10" north and longitude 3.90361° or 3° 54' 13" east with open location of 6FV5CW33+4C and Open-Street -Map ID way of 82467201 (Figure 1). Currently, the hospital houses 1,000 bed spaces and 200 examination couches with occupancy rates ranging from 65% to 70%. (Retrieved 10 July 2025, at 22:57, UTC).

Figure 1: Locations of University College Hospital in Ibadan Metropolis, Oyo State, Nigeria

Determination of sample size

The sample size was determined using the single proportion method and prevalence of 12.3% as reported by Iseghohi et al. (2020).

$$n = \frac{Z^2P(1-P)}{D^2} \quad (1)$$

Where: n = sample size; Z = 1.96 for Confidence level at 95%; P = Prevalence rate at 24.6%; D = 0.05 for Marginal error at 5%. The sample size was calculated as 330.

Purity checks and identification of isolates

A total of 330 pre-tested clinical isolates of *E. coli* from UTIs were collected within the period of this study. The isolates were subculture using Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. Different tests described by Cowan (1974), Harrigan and McCance (1976), were used to

presumptively identify the isolates and then confirmed using Microbact™ Gram-Negative Bacilli 12E (Oxoid, UK). The stock cultures were maintained at 4°C and sub-cultured fortnightly to ensure viability of isolates.

Pathogenicity test of isolates

To discriminate between pathogenic and non-pathogenic isolates, *in-vitro* pathogenicity testing was performed by using Congo red dye binding activity test described by Zahid et al. (2016). Tryptic Soy agar supplemented with 0.03% Congo red dye and 0.15% bile salts was used. Each distinct colony of isolate was cultured on a separate plate, incubated at 37°C for 24 hours and cultures were left at room temperature for 48 hours to enable annotation of results. Red colonies were documented as Congo red positive (CR^+) and colonies that remained white or grey were considered Congo red negative (CR^-).

Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) of the identified Uropathogenic isolates were performed using Kirby–Bauer disc diffusion method against 13 antibiotics belonging to 8 classes of antibiotics according to Ajuga *et al.* (2021). Upon standardization of the bacterial isolates to a turbidity level equivalent to 0.5 McFarland standard, Mueller–Hinton agar plates were inoculated with the organisms using sterile swab sticks. After 5-min of pre-incubation, the test antibiotic discs were placed at equidistant on the petri dish. The plates were inverted and incubated at 37°C for 24 hours. Antibiogram was determined by comparing the zones of inhibition against standard breakpoints (CLSI, 2020).

Multiple Antibiotic Resistance (MAR) index and multidrug-resistant status of isolates were also determined from the results of the antibiotic susceptibility testing. The MAR index was calculated using the formula a/b, where "a" is the total number of antibiotics to which the test organisms were resistant and "b" is the total number of antibiotics against which the test organisms were tested. The multidrug-resistance was considered on the basis of resistance to three or more classes of antibiotics.

Phenotypic detection of ESBL

The presence of ESBL was determined phenotypically by the double-disc synergy test. A standard suspension of the isolate was spread evenly on the surface of Mueller–Hinton agar plates. Discs of cefuroxime, ceftazidime and cefoxitin, 30µg each were placed at an equidistant of 20 mm from a centrally placed amoxicillin/clavulanate disc containing 20/10µg. The plates were incubated at 37°C for 24 hours and patterns of zones of inhibition were noted. Isolates that showed a champagne cork (keyhole appearance) with potentiation toward amoxicillin/clavulanate disc were considered ESBL-producers (CLSI, 2020) and for each test isolate the procedure was repeated in duplicate.

Statistical analysis

Data obtained from the antibiotic susceptibility test were analyzed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, DC, USA). The prevalence and occurrence from this study were expressed in percentage (%). Statistical significance was determined and compared at $p \leq 0.05$.

From the total of 330 suspected isolates of *E. coli* collected, 129 (39.1%) isolates were presumptively identified as *E. coli* (Figure 2). Of these 129 isolates of *E. coli* tested for

pathogenicity using Congo red dye, 87 (67.4%) isolates demonstrated Congo red binding activity and were considered CR^+ (Figure 3).

An analysis of the Antibiogram showed that majority of the pathogenic isolates were significantly resistant to ampicillin, tetracycline 87 (100%) and gentamicin 83 (95.5%) – Figure 4. Mid-level resistance was recorded against kanamycin 20 (22.7%), cefuroxime 24 (27.3%) and amoxicillin-clavulanic acid 38 (43.7%). Least resistance was observed against ceftriaxone 8 (9.1%), meropenem 15 (17.5%), and cefoxitin 18 (20.8%) – Figure 4. An assessment of the susceptibility rate showed that all (100%) the isolates were sensitive to nitrofurantoin and ofloxacin whereas, ciprofloxacin, ceftazidime, and meropenem demonstrated significant scores of 79 (90.9%), 71 (81.8%), and 64 (73.1%), congruently (Figure 4).

An evaluation of the resistance pattern revealed a total of 6 resistance patterns among the isolates (Table 1). The isolates in this study exhibited similar levels of distribution among the different MAR indices with the MAR index ranging from 0.46 to 0.62 (Figure 5). Considering resistance to more than three antibiotic classes, a total of 22 (17.1%) of the isolates demonstrated multi-drug resistant (MDR) phenotype, exhibiting resistance to three or more antibiotic classes (Table 1).

Based on the phenotypic assessment of ESBL-production, a mid-level occurrence of 42 (48.1%) isolates demonstrated a keyhole appearance among the 87 uropathogenic *E. coli* isolates. All the non-pathogenic strains (51.9%) were ESBL-negative (Figures 6).

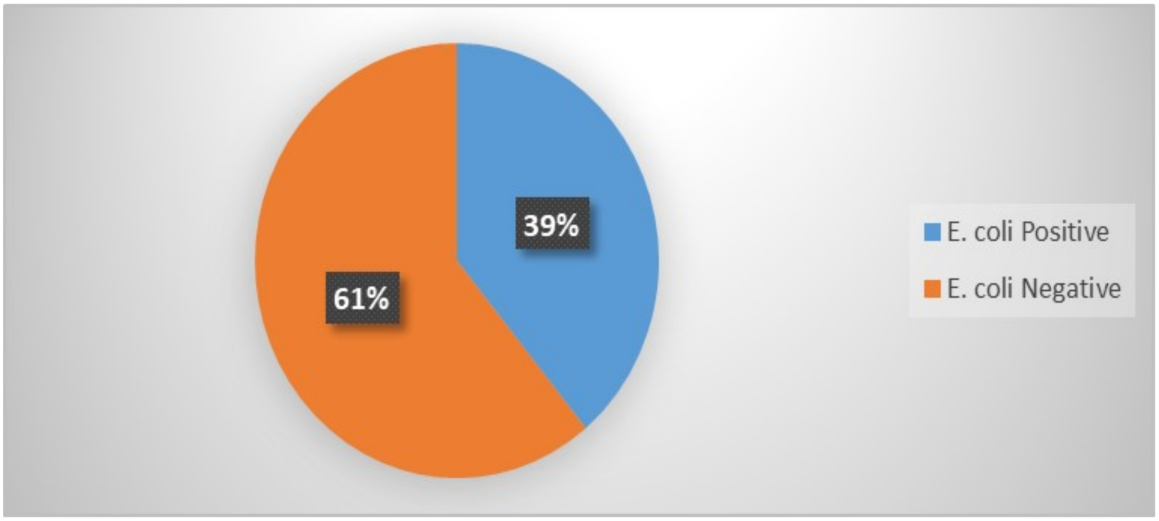


Figure 2: Percentage prevalence of *E. coil* in UTI isolates collected from the archive of UCH, Ibadan

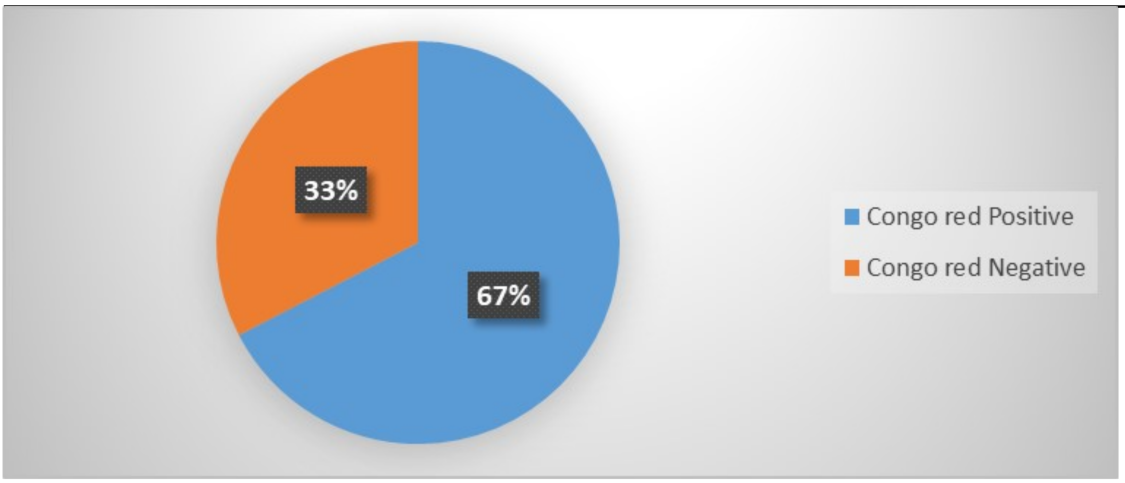


Figure 3: Percentage distribution of pathogenic *E. coli* in UTI isolates collected from the archive of UCH, Ibadan

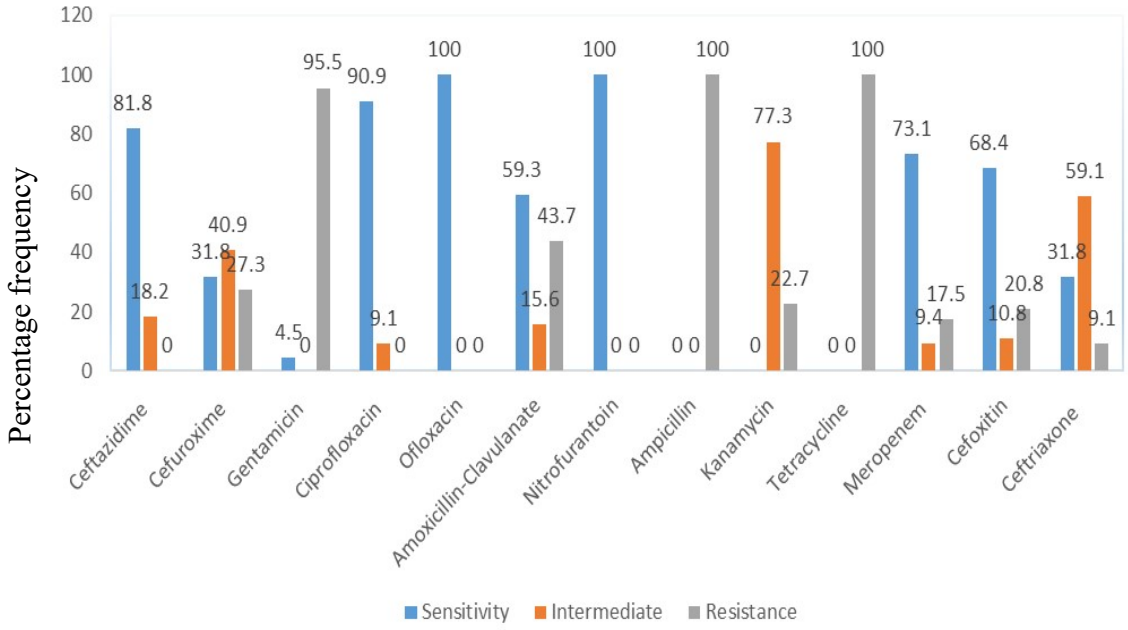


Figure 4: Percentage susceptibility pattern of pathogenic *E. coli* in UTI to antibiotics used in this study

Table 1: Antibiotic phenotypes of pathogenic *E. coli* in UTI used in this study

Resistance Phenotype	No. of Antibiotics	No. of Isolates
GEN, AMC, AMP, TET, MRO, FOX	6	11
GEN, AMC, AMP, TET, MRO, FOX, CTR	7	2
GEN, AMC, AMP, KAN, TET, MRO, FOX	7	3
CRX, GEN, AMC, AMP, TET, MRO, FOX	7	4
CRX, GEN, AMC, AMP, TET, MRO, FOX, CTR	8	1
CRX, GEN, AMC, AMP, KAN, TET, MRO, FOX	8	1

Keys: CAZ - ceftazidime (30µg), cefuroxime - CRX (30µg), OFL - ofloxacin (5µg), GEN - gentamicin (10µg), CIP - ciprofloxacin (5µg), AMC - amoxicillin-clavulanate (30µg), TET - tetracycline (30µg), NIT - nitrofurantoin (300µg), AMP - ampicillin (30µg), KAN - kanamycin (30µg), MRO - meropenem (10µg), FOX - cefoxitin (30µg) and CTR - ceftriaxone (30µg)

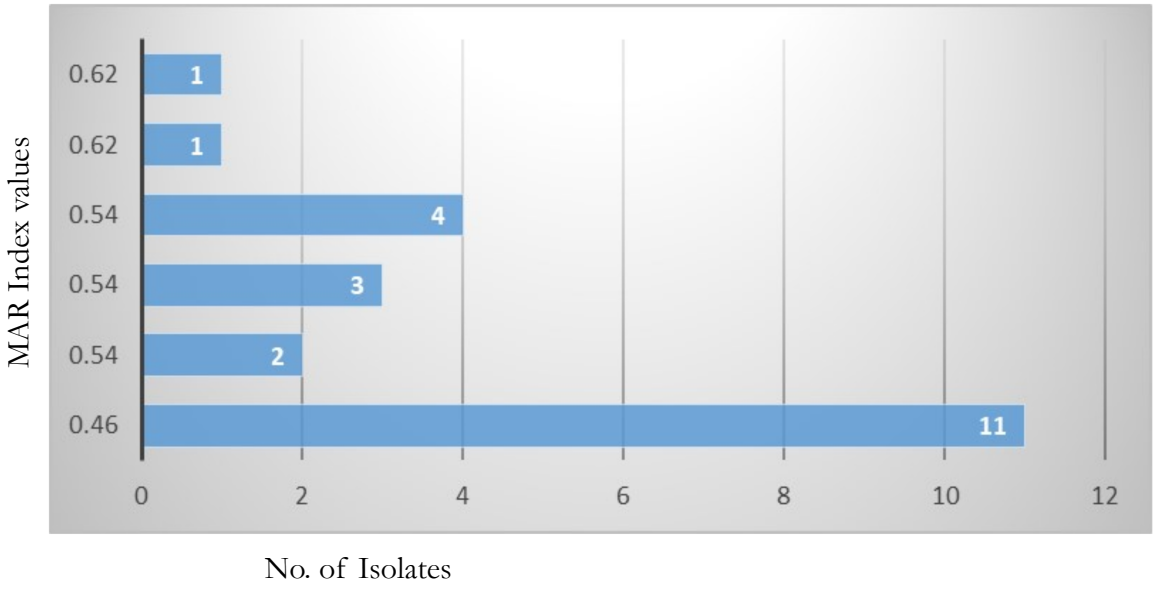


Figure 5: MAR index distribution of *E. coli* in the UTI isolates from the study

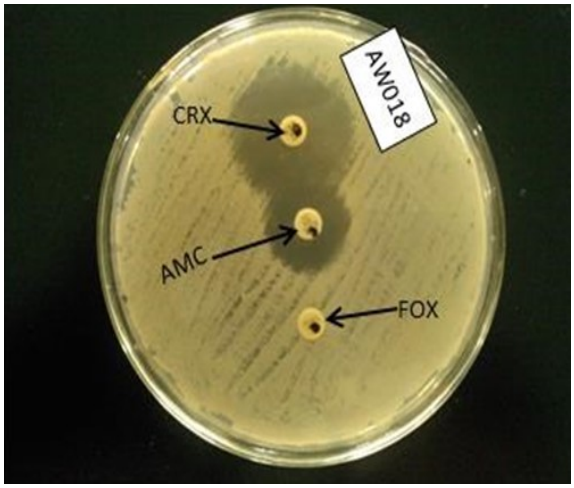
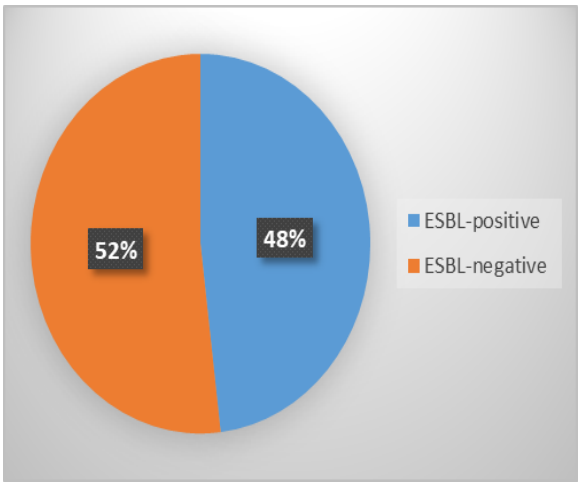


Figure 6: (A) Keyhole effect of ESBL-positive isolate *coli* among the UTI isolates. CRX - ceftriaxone (30µg),



(B) Percentage occurrence of ESBL-producing *E.* AMC - amoxicillin-clavulanate (30µg), FOX - cefoxitin (30µg)

DISCUSSION

The general dissemination and global prevalence of Uropathogenic *Escherichia coli* producing extended spectrum beta-lactamase (ESBL) remain a critical health concern globally. This is because of its propensity in conferring antibiotic resistance to beta-lactams, hence, prolonging therapeutic complexity. In many Nigerian hospitals, beta-lactams account for about 25% of antibiotics administered yearly (Oduyebo *et al.*, 2017, Umeokonkwo *et al.*, 2019, Fowotade *et al.*, 2020) and resistance to these antibiotics is of great concern. WHO has included third generation cephalosporin-resistant *E. coli* and carbapenem-resistant *E. coli* to their recent documentation of Bacterial Priority Pathogen List (BPPL) (Olaitan *et al.*, 2025). Consequently, this research was conducted with the aim of deciphering the antibiotic resistance of ESBL producing *E. coli* isolated from UTI in a tertiary health facility.

In this study, a prevalence of ESBL-producing *E. coli* was revealed to be 48.1% out of a pool of 129 identified *E. coli* of which 67.4% were considered pathogenic. The mid-level prevalence of ESBL production detected is consistent with majority of the previous reports across the globe (Nwafia *et al.*, 2019, Tufa *et al.*, 2020, Ajuga *et al.*, 2021, Ebrima *et al.*, 2025). As ESBL production rises among bacterial isolates in different countries of the world (Lob *et al.*, 2016, Alqasim *et al.*, 2018), previous reports have shown rates ranging from 33% to 91% (Monira *et al.*, 2017, Alqasim *et al.*, 2018, Hassuna *et al.*, 2020, Pandit *et al.*, 2020, Tufa *et al.*, 2020). In Nigeria, rates as low as 24% and as high as 100% have also been reported in the past in validation of the global rise in the trend of ESBL production among Enterobacteriaceae (Igwe *et al.*, 2016, Agbagwa and Aminofifori, 2017, Nwafia *et al.*, 2019, Onanuga *et al.*, 2019, Ugwu *et al.*, 2020).

Escherichia coli has been described as one of the antibiotic-resistant bacteria with significant concern in clinical settings (Olaitan *et al.*, 2025). However, clinical environments

have often been associated with higher levels of antibiotic exposure and have led to the selection of resistant strains (Ajuga *et al*., 2021). Resistance rates described in clinical settings are comparatively high, with rates exceeding 50% as reported against many antibiotics (Kibret and Abera, 2011, Igwe *et al*., 2016, Monira *et al*., 2017; Tuem *et al*., 2018; Pormohammad *et al*., 2019, Ajuga *et al*., 2021). This trend is consistent with current findings against the antibiotics used in this current study. All the isolates were resistant to ampicillin and tetracycline while significant percentage of the population 95.5% was resistant to gentamicin. However, mid-level resistance was observed against Amoxicillin-clavulanic acid. The different antibiotic resistance phenotypes expressed by isolates in this study further correlate with findings documented by Onifade *et al.* (2015) and Lohani *et al.* (2019). Most of the *E. coli* isolates reported by these authors expressed significant resistance to trimethoprim, ceftazidime, gentamicin, cefuroxime, chloramphenicol, cefixime, and augmentin.

However, unlike some previous reports from within and outside Nigeria which observed MDR rates range of 52% to 100% (Igwe *et al*., 2016, Monira *et al*., 2017, Makanjuola *et al*., 2018, Ramírez-Castillo *et al*., 2018, Onyeadi and Agbagwa 2019), the rate of MDR observed in this study was much lower, 17.1% while the MAR index range of 0.46 to 0.62 may be considered to be mildly high. This finding is similar to observation by Pormohammad *et al.* (2019) who reported 22% MDR of *E. coli* in their study.

CONCLUSIONS

There were 67.4% of pathogenic *E. coli* associated with UTI in this tertiary health facility. There is also a prevalence of 48.1% of ESBL-producing *E. coli* with 17.1% multidrug-resistance characteristics and 0.46 to 0.62 MAR index. The drug of choice for the treatment of infections caused by these isolates may be any of the following: nitrofurantoin, ofloxacin, ciprofloxacin, ceftazidime, and meropenem.

This study has established the significance of good antibiogram evaluation as a baseline for empirical diagnosis, epidemiological surveillance, antibiotic prescription, and disease management.

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