Global Journal of
Health Science
(GJHS)

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IPRJB
INTERNATIONAL PEER REVIEWED JOURNAL AND BOOK PUBLISHING

Global Journal of Health Sciences
ISSN 2519-0210 (Online)
Vol 10, Issue 1, No.2, pp 14 - 37, 2025

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Prevalence of Uropathogenic *E. Coli*, Antimicrobial Susceptibility Profiles and Carriage of Extended-Spectrum Beta-Lactamases Genes at Mama Lucy Hospital, Kenya

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Article History

Received 6th November 2024

Received in Revised Form 11th December 2024

Accepted 13th January 2025

How to cite in APA format:

Chelangat, S., Ngugi, C., Song'oro, E., & Mwaniki, J. (2025). Prevalence of Uropathogenic E. Coli, Antimicrobial Susceptibility Profiles and Carriage of Extended-Spectrum Beta-Lactamases Genes at Mama Lucy Hospital, Kenya. *Global Journal of Health Sciences*, *10*(1), 14–37. https://doi.org/10.47604/gjhs.3153

Abstract

Purpose: Urinary tract infection (UTI) is a common bacterial infection affecting millions worldwide. *Escherichia coli* (*E. coli*) is the most prevalent causative agent of UTIs. This study aimed to determine the prevalence, antimicrobial susceptibility profiles and carriage of ESβL resistance genes among uropathogenic *E. coli* recovered from adults at Mama Lucy Hospital.

Methodology: A cross-sectional study was conducted. A purposive method was used to obtain 347 urine samples from patients who presented with symptoms suggestive of UTI and were cultured for *E. coli* using cysteine lactose electrolyte-deficient agar and eosin methylene blue agar. The collected urine samples were also subjected to dipstick analysis and microscopy. Questionnaires were used to collect sociodemographic data and possible risk factors for urinary tract infections. The recovered isolates were identified using conventional biochemical tests. Antimicrobial susceptibility profiles were determined using the Kirby diffusion disc method. The occurrence of ESβLs, including TEM, OXA, SHV, and CTX-M, was determined by polymerase chain reaction (PCR).

Findings: The overall prevalence rate of UTIs was 23.7%, whereas the *E. coli* prevalence rate was 13.3%. The isolates presented high levels of resistance to trimethoprim-sulfamethoxazole (81.3%), amoxicillin-clavulanic acid (66.7%), ciprofloxacin (62.5%), tetracycline (60.4%), ceftriaxone (54.2%), and cefoxitin (54.2%), whereas they were more susceptible to meropenem (14.6%), chloramphenicol (12.5%), and nitrofurantoin (8.3%). A total of 25 of the 46 *E. coli* isolates were screened for ESβL genes. TEM was the most common gene21/25 (84%), SHV12/25 (48%), OXA 7/25 (28%), and CTX-M *18/25* (66.1%), which indicates a high frequency of β-lactamase gene production among UTIs causing *E. coli*.

Unique Contribution to Theory, Practice and Policy: Based on these findings, current treatment guidelines should be revised to prevent increasing antimicrobial resistance through continuous surveillance, screening for extended-spectrum beta-lactamase genes, routine culture and antibiotic sensitivity testing. Additionally, the prevalence of UTIs should be continuously monitored to monitor trends that form a basis for preventive and treatment guidelines, such as policy development and prudent use of antibiotics, to reduce the increasing UTI burden in the population.

Keywords: *Urinary Tract Infections, Uropathogenic Escherichia Coli, Antibiotic Resistance, Extended Spectrum Beta-Lactamases*

INTRODUCTION

Urinary tract infections are infection of the urinary tract system, which includes the kidneys, ureters, bladders, and urethra. UTIs are caused by a wide range of microbes colonising the urinary tract, which include gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis.* Gram-positive bacteria include *Enterococcus faecalis, Streptococcus species Enterobacter species, Citrobacter* species and *Staphylococcus saprophyticu*s (Flores-Mireles et al., 2015). *E. coli* is the leading causative agent of urinary tract infections (UTIs), the most common bacterial infection encountered in clinical practice, and is responsible for significant morbidity and high medical costs (Alhashash, 2015). It accounts for 80–90% of pathogens causing urinary tract infections (UTIs), at least 80% of community-acquired infections and 65% of hospital-acquired infections. UPEC is responsible for approximately 75% of uncomplicated UTIs and up to 65% of complicated UTIs (Medina & Castillo-Pino, 2019). Urinary tract infections associated with UPEC occurs through biofilm formation, urothelial cell invasion and virulence factors such as fimbrial adhesins, invasins, iron-acquisition systems and toxins, which enhance the establishment of prolonged and recurrent infections (Zagaglia et al., 2022)

Urinary Tract Infections are the second most common bacterial infection affecting the urinary system, with a high prevalence globally, estimated at 150 million annually, and are a major public concern regarding mobility and financial cost (Flores-Mireles et al., 2015). Globally, the prevalence of UTIs ranges from 6% to 37% in developing countries (Uwaezuoke, 2016). At least 50% of women worldwide will experience a UTI at least once in their lifetime (Majumder et al., 2022). Women are significantly more likely to experience UTIs than men (Foxman, 2002). UTI is a common condition that affects people of all ages and genders. Women have a greater incidence of UTIs than men do because of anatomical factors such as a shorter urethral distance and proximity to the anus, urothelial mucosa adherence to the mucopolysaccharide lining, or other host factors (Haque et al., 2015). Other factors affecting women include sexual activity, contraceptive use, a history of UTIs and genetic factors (Scholes et al., 2000).

Statement of the Problem

Antibiotic resistance is a global health challenge developed in most antibiotic classes, rendering treatment challenging. Traditionally, broad-spectrum antibiotics have been the drugs of choice for treating community- and hospital-associated UTIs. Antibiotic resistance increases the cost of treatment, severe infection, complications and mortality (Llor & Bjerrum, 2014). Antibiotic resistance has increased due to gaps in the diagnosis of UTI infections, which have largely been attributed to the use of empirical treatment without primary urine culture; hence, the few cultures performed in a hospital setting may not be representative of the prevalence of antibiotic resistance in the community (Tony Mazzulli, 2012).

Additionally, self-medication is common in all parts of the world. Over-the-counter drugs have contributed to antimicrobial resistance due to the misuse of antibiotics. Overprescribing antibiotics is associated with an increased risk of adverse effects, more frequent reinfection and increased medicalization of self-limiting conditions (Llor & Bjerrum, 2014). Urine culture is currently considered the gold standard for UTI diagnosis, but reliance on dipstick tests and microscopy and gram stain which provide prompt results have led to under diagnosis in most Kenyan facilities (Onyango et al., 2018). Urine culture is the gold standard method for quantitative diagnosis of UTI. For proper diagnosis about 18 hours are required for bacterial

growth on culture media by standard laboratory techniques. Diagnosis is then delayed for about 24 - 48 hours for results leading to delayed treatment. Urine culture is also an expensive procedure which needs a well-equipped microbiology laboratory with highly experienced technicians for proper and accurate diagnosis.

Dipstick diagnosis parameters overestimate the prevalence of UTIs, which could result in unnecessary prescription and over prescription of antimicrobials. Additionally, urine dipstick parameters for UTI diagnosis, such as nitrite, have led to UTI underestimation and under prescription (Maina et al., 2023). These findings strongly imply the need to perform urine cultures for accurate UTI diagnosis and further research on emerging rapid UTI culture methods. Accurate diagnosis and appropriate use of antimicrobials for the treatment and prevention of urinary tract infections are necessary to reduce the burden and long-term consequences.

LITERATURE REVIEW

Urinary Tract Infections

Urinary tract infection (UTI), is a wide term for bacterial infections of the urethra, bladder, and kidneys. Other infectious agents that invade the urinary system include bacteria, viruses, and fungi (Foxman & Brown, 2003). UTIs are traditionally classified as uncomplicated or complicated UTIs, or by the site of infection. Infections might present themselves as symptomatic or asymptomatic. Urethritis and cystitis are examples of lower UTIs, while pyelonephritis and renal abscesses are examples of upper UTIs. Acute infections are usually caused by a single pathogen, whereas chronic infections are frequently caused by many pathogens.

UTI usually affects various parts of the urinary tract, but the bladder is the most affected organ. Statistics show UTIs are the most common outpatient infections, with more than 50-60% in adult women (Al-Badr & Al-Shaikh, 2013). Studies have shown that the prevalence of UTIs increases with age and even doubles in women aged above 65 years. However, several risk factors increase the likelihood of pathogens, sexual activity, and healthcare-associated UTIs.

In addition, studies have shown that the nature of pathogens varies between hospital and community settings. UTIs create a significant personal and societal burden with a high number of hospital visits yearly related to UTIs. In addition, recurrent infections are often associated with increased physician visits with mostly women suffering from recurrent UTIs severely impacting their quality of life. According to studies, *Escherichia coli* is the common cause of diseases outside the gastrointestinal tract. Various independently evolved strains of *E. coli* are the primary cause of bloodstream and urinary tract infections. Epidemiological and in vitro evidence shows that multiple toxins common to many strains of *E. coli* may be involved in the immune evasion and colonization ability of *E. coli*.

A previous study reviewed the virulence factors of the UPEC and how they interact with the host. UTIs are among the most common infectious diseases, and *E. coli* is the most frequently isolated pathogen from uncomplicated cases of *E. coli*. However, *E. coli* must overcome several host defence mechanisms, including invading neutrophils, urine flow, and endogenous antimicrobial factors (Katongole et al., 2020). Therefore, the UPEC must harbour several virulence factors to enable the pathogen to resist various defence mechanisms. UTIs do not only localize in the urinary tract in young children and often resemble other febrile illnesses.

Global Journal of Health Sciences
ISSN 2519-0210 (Online)
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Healthcare-associated urinary tract infections also called nosocomial UTIs are the fifth most common infection in hospital settings. About 75% of hospital-acquired UTIs are associated with urinary catheters. During hospitalization about 12-16% of inpatients have indwelling catheters hence increased risk of having catheter-associated urinary tract infection(CAUSTI). Longer duration of catheterization is a major risk factor of CAUSTI and can lead to serious complications such as bacteremia, endocarditis and meningitis.

Uropathogenic *Escherichia Coli*

Escherichia coli belongs to the vast group of Gram-negative rods known as Enterobacteriaceae, and they cause primary and opportunistic infections in humans. *E. coli* is the most common cause of UTIs about 60-90%. *E. coli* is lactose-fermenting bacteria, often referred to as coliforms, aerobes and facultative anaerobes, non-sporing and motile. Certain *E. coli* serotypes are more common in urine infection, which is likely due to their frequent presence in the colon rather than their inherent pathogenicity for the urinary tract. *E. coli* in urine are invasive and pathogenic since they are intestinal flora that has virulence factors such as 1-fimbriae, P fimbriae, S fimbriae, and alpha-hemolysis aid the invasion, colonization, and evasion of the host (Tarek et al., 2024)

Extended Beta-lactamases

Extended-spectrum beta-lactamases are enzymes produced by Enterobacteriaceae which can break down active ingredients in commonly used antibiotics rendering them inactive. ESBLs are class A β-lactamases that hydrolyze penicillin, oxyimino-cephalosporins, and monobactams but not cephamycins or carbapenems and are inhibited *in vitro* by clavulanate (Bradford, 2001). Beta-lactam antibiotics such as cephalosporins, monobactams, cephamycins and carbapenems, constitute the main classes of UTI treatment. Resistance to β-lactams is due primarily to bacterially produced β-lactamase enzymes that hydrolyze the β-lactam ring, thereby inactivating the drug (Bush & Bradford, 2016). The production of extended-spectrum β-lactamases (ESBLs) is a significant resistance mechanism that impedes the antimicrobial treatment of infections caused by *Enterobacteriaceae* and is a serious threat to currently available antibiotic treatment (Shaikh et al., 2015)

ESBLs are most common in *Klebsiella pneumoniae* and *Escherichia coli* (Mehrgan & Rahbar, 2008). Plasmids are small circular DNA in bacterial cells that carry essential genes for survival under any adverse conditions. The most common ESBL genotypes are the SHV, TEM, and CTX-M.SHV genes are encoded by self-transmissible plasmids that carry resistance genes to other classes of antibiotics therefore becoming widespread and clinically significant. CTX-Ms hydrolyses cefotaxime rather than ceftazidime. TEM hydrolyses penicillins and narrow-spectrum cephalosporins. OXA hydrolyzes oxacillin and cloxacillin. Other clinically important types include; PER, BEL-1, BES-1, SFO-1, TLA, and IBC (Dhillon & Clark, 2012). ESBLs are often plasmid-mediated, contain genes encoding ESBLs conferring resistance to other classes of antimicrobial agents and are readily transmissible between strains and different species of the Enterobacteriaceae family. The production of ESBLS is a major global health concern, causing treatment failure due to antibiotic resistance. Over time, bacterial strains exposed to a wide variety of β-lactam antibiotics have produced β-lactamases that mutate dynamically and continuously, increasing the activity of these bacteria even against recently developed β-lactam antibiotics (Shaikh et al., 2015).

The emergence of extended-spectrum beta-lactamase-producing gram-negative bacteria has caused increasing problems worldwide through antimicrobial resistance. The prevalence of

community- and hospital-acquired infections caused by *E. coli* produced by ESβL is increasing, posing a threat to public health (Rahal, 2009). Early identification of ESβL-producing bacterial isolates causing clinical illness is highly important for appropriate treatment as well as for effective infection control in hospitals. The correct management of infection is important for reducing new antibiotic resistance patterns in the future (Picozzi et al., 2014). The variation in ESBL-producing strains among studies might be attributable to local antibiotic prescribing practices, the extensive use of broad-spectrum antibiotics, especially third-generation cephalosporins, and the prevalence of drug-resistant pathogens in the locality. The detection of UTI-causing pathogens and their resistance to commonly prescribed antibiotics is essential for improvements in the treatment and management of UTIs. The prevalence of extended-spectrum beta-lactamase isolates will guide continuous screening and surveillance in policy formulations and encourage the prudent use of antimicrobial agents.

Research Gaps

Mama Lucy hospital serves a population of 2.25 million people annually and is located in a densely populated area in Nairobi County mainly a residential area and has numerous informal settlements. High population density, poor sanitation and access to clean water contribute to rising prevalence of UTI infections. Limited research has been done on the prevalence of Uropathogenic *E. coli*, the predominant causative agent of UTI since several studies focuses on all uropathogens causing UTI. The association between sociodemographic, lifestyle and clinical factors with UTI remain unclear. This study adds new knowledge and information on risk factors associated with UTI and also prevalence of UPEC in Mama Lucy Hospital.

Urinary Tract Infection affects millions of people worldwide posing a threat to healthcare affecting quality of life and has also contributed to financial, social and clinical burdens. Antibacterial resistance has resulted in five million deaths globally and annual mortality is set to increase annually. First-line treatment drugs against uncomplicated UTIs are no longer effective and resistance has spread to several classes of antibiotics including drugs that treat complicated UTIs. Limited relevant data on current effective treatment regimens against UTI to prevent rising UTI cases. Antimicrobial resistance endangers health and pose a challenge to modern medicine therefore the findings in this study will guide in empirical treatment.

Limited research has been done on the association between multi-drug resistant uropathogenic *E. coli* causing UTI and ESBLs causing antimicrobial resistance. Research has not been done extensively on prevalence of ESBLs resistance genes in Kenya and its correlation to UPEC. The rise in antimicrobial resistance globally is attributed to beta-lactamases genes which breakdown active components of antibiotics rendering them inactive. This underscores the importance of the study to determine the rising prevalence, recurrent and persistent UTI infection attributed to ESBLs. The data will guide in policy making, continuous screening and surveillance of ESBLS. This study is important in understanding the prevalence of UPEC and the trends of extended beta-lactamase production in Mama Lucy Hospital guiding treatment and prevention measures which reduce the overall UTI burden.

METHODOLOGY

Study Site, Design and Ethical Approval

This was a cross-sectional study carried out at Mama Lucy in Nairobi, Kenya, between November 2022 and January 2023. Mama Lucy Kibaki Hospital is a county referral level-5 hospital located in the Embakasi constituency, Nairobi County. The hospital plays a crucial role in providing all-around healthcare services to the residents of Nairobi city and surrounding

counties, with a bed capacity of 137. The services offered include emergency care, surgical services, diagnostic services, outpatient services, inpatient services, maternity services, mental health services, and pharmacy and community outreach services. The land elevation is 1579.5 m (5180.7 feet), and the coordinates are (1° 17' 58.56" S36° 48' 31.5684" E). The area consists mainly of residential and informal settlements. The study targeted both adult inpatients and outpatients at hospitals presenting with urinary tract infection symptoms. Ethical approval for this study was obtained from the Jomo Kenyatta University of Technology Institutional Ethics Review Committee (IERC), and a license was obtained from the National Commission for Science and Technology and Innovation (NACOSTI).

Mama Lucy Hospital

Figure 1: Location of Mama Lucy Hospital in Nairobi County, adapted from Google 2024.

Sampling Strategy and Sample Size

A purposive sampling technique was used to recruit eligible participants until a sample size of 346 was achieved. The purpose of the study was adequately explained to the participants, and consent was obtained before enrollment in the study. Structured questionnaires were used to obtain information on sociodemographic factors and clinical and lifestyle factors.

The sample size was determined using the *Fisher* formulae. The estimated prevalence of UTI is 27.6% in Kenya (Wanja et al., 2021)

Sample Collection

All 346 clean-catch midstream urine samples were collected into 20 ml sterile screw-capped universal bottles. Adult participants were instructed on how to collect clean catch midstream urine to test for urinary tract infections. The samples were assigned unique identification codes linked to the participant's questionnaire. The urine samples were transported to the microbiology laboratory within 4 hours in a cooler at 4°C. The samples were then processed in the laboratory within two hours for maximum recovery of the organisms.

Urine Sample Analysis

Macroscopic and Dipstick Urinalysis

The physical appearance of the urine samples was macroscopically analyzed based on various parameters, such as colour, clarity, presence of blood, or sediments. Normal urine was pale to dark yellow or amber and clear. Turbidity may be caused by excessive cellular material or protein in the urine

Urine dipsticks were used to rapidly detect various parameters in urine samples (Maina et al., 2023). Approximately 10 ml of midstream urine sample was collected in a sterile container, and dipstick analysis was performed within 10 minutes at the study site. To avoid contamination, a portion of the urine sample was transferred to a separate sterile container for later use in culture before dipstick analysis was performed. Combostik 10 dipsticks were dipped into urine samples immediately removed and allowed to sit for 1–2 minutes (according to the manufacturer's instructions). Leucocyte esterase and red blood cells were recorded as negative, trace, 1+ (small), 2+ (moderate), or 3+ (large), whereas nitrites were recorded as negative or positive.

Urine Culture

Urine aliquots of 10 µl were plated directly on CLED and EMBA media (OXOID, UK) and then incubated aerobically at 37°C for 18–24 hours (Rahman et al., 2018)(Serrano & Penuliar, n.d.). Bacteria identified based on colony morphology, colour, and colony count yielding bacterial growth over ≥10 5 CFU/mL were interpreted as significant UTI cases and met the threshold of UTI positivity; lower values ≤10 5 CFU/mL or mixed cultures were considered no significant growth, no growth or contaminants. Colonies that appeared yellow on CLED agar and metallic green sheen colonies on EMB agar were identified as *E. coli. E. coli* ATCC-25922 was used as a positive quality control strain, and *S. aureus* ATTC 25923 was used as a negative control.

Gram Staining

Gram staining was performed on all the isolates subcultured on Mueller–Hinton agar, which isolates *E. coli* to obtain pure cultures. The Gram-staining method was used to confirm that the gram-negative bacteria were red (Tripathi & Sapra, 2020).

Biochemical Tests

Biochemical tests were performed using conventional biochemical test protocols to identify *Escherichia coli*. Briefly, each potential was tested via the following biochemical tests: the triple sugar iron test, citrate utilization test, methyl red-Voges-Proskauer test, lysine indole motility test, and urea utilization test. *E. coli* ATCC 25922 was used as a positive control, and *S. aureus* ATCC 25923 was used as a negative control to compare the validity of the results.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed according to the Kirby–Bauer disc diffusion method (Bauer, 1996) on Mueller–Hinton agar (OXOID, UK) based on criteria recommended by the Clinical Laboratory Standards Institute (CLSI 2021). *E. coli* ATCC 25922 was used as a positive quality control strain to determine the potency of the antibiotic discs and the quality of the media. Briefly, a bacterial suspension was prepared, and the turbidity of the suspension was compared with 0.5 McFarland tubes to standardize the inoculum size. The following panels of antibiotics were used: ceftriaxone (CR0, 30 µg), cefuroxime (CXM, 30 µg), ceftazidime

(CAZ, 30 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TCY, 30 µg), meropenem (MEM, 10 µg), chloramphenicol (CHL, 50 µg), trimethoprim-sulfamethoxazole (SXT, 25 µg), kanamycin (KAN, 30 µg), cefoxitin (FOX, 30 µg), cephalexin (CPN, 30 µg), cefotaxime (CTX, 30 µg), cefepime (FEP, 30 µg), nitrofurantoin (NIT, 300 µg), amoxicillin-clavulanic (AMC, 10 µg) and ampicillin (AMP, 30 µg).

Antimicrobial susceptibility tests were performed using plates A and B. Plate A was used to screen for potential extended-spectrum beta-lactamase (ESBL) production. The antibiotics used were penicillin (AMP), 3rd generation cephalosporins (CTX, CXM, CAZ, CRO), cefoxitin (FOX), fourth-generation cephalosporins (FEP) and beta-lactamase inhibitors in the middle (AMCs). Observations of the synergy zones that form when cephalosporins are combined with beta-lactam inhibitors have been made. Plate B antibiotics include CIP (targeting quinolones and fluoroquinolone resistance), kanamycin (aminoglycoside resistance), CHL, TCY, NIT, SXT, MEM, and CPN, which are used as treatments for the management of UTIs. All the plates were incubated at 37°C for 18–24 hours. The resistance profiles were reported as sensitive, intermediate, and resistant by measuring the diameter of the zone of inhibition based on the CLSI standard 2021. Isolates that were resistant to three or more antibiotics were considered multidrug resistant (Magiorakos et al., 2012).

Detection of Extended-Spectrum B-Lactamases

Extended-spectrum β-lactamase production among the isolates was determined using the double disk diffusion synergy method following the CLSI 2021 guidelines. Isolates that showed synergy between amoxicillin-clavulanic, ceftazidime, and ceftriaxone were identified as probable ESBL producerswhich increased in size toward amoxicillin clavulanate(Rawat & Nair, 2010). Furthermore, if the zones of inhibition were ≤25 mm for ceftriaxone, ≤22 mm for ceftazidime, and ≤27 mm for cefotaxime, the isolate was considered a potential ESBL and further tested by the double disk synergy method (Pandit et al., 2020)

DNA Extraction

The DNA used as a template for PCR was extracted from pure isolates using the thermal lysis method (Dashti et al., 2009) at 50°C for 15 minutes. Briefly, a sterile swab was used to obtain a pea-sized inoculum from the culture plate, which was subsequently transferred to a corresponding tube containing 500 µl of PCR water. The tubes were placed on a heating block for 12 minutes. After cooling, the tubes were centrifuged at 1400 rpm for 5 minutes. The supernatant (DNA) was transferred to a sterile tube and stored at -20°C. The concentration and purity were checked using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific-US).

Detection of ESBL Genes

PCR amplification of selected ESβL genes (*bla* $_{TEM}$, *bla* $_{OXA}$, *bla* $_{SHV}$, and *bla* $_{CTX-M)}$ was carried out by conventional PC. In a final volume of 25 µl, 1 µl of DNA template, 0.5 µl of both forward and reverse primers as indicated in **Table 1**, 18 µl of PCR water and 5 µl of Taq 5X master mix (New England Biolabs) were used. The PCR mixture was used as a negative control, and appropriate positive controls were used depending on the gene. Amplification was conducted in a programmable thermal cycler (Biometrics Trio) where the PCR conditions were as follows: initial denaturation at 95°C for 30 seconds, final denaturation at 55°C for 30 seconds, annealing temperatures of (*bla* $_{TEM}$, *bla* $_{SHV)}$ at 50°C and for (*bla* $_{OXA}$ *bla* $_{CTX-M)}$ at 60°C for 1 minute, initial extension at 35°C for 1 minute and a final extension at 680°C for 5 minutes for 30 cycles. After amplification, 7 µl of each reaction mixture was separated on 1.5% agarose gels stained with gel red at 100 v for 35 minutes in TBE and visualized under UV light, and

gel images were recorded with the aid of a gel documentation system (Azure Biosystems). The sequences of primers used were as follows:

Table 1: PCR Amplification Primers

Genes	ESBL Primer Sequence	Annealing temp	Expected size(bp)
TEM	F-5'ATGAGTATTCAACATTTCCGTG 3' R-5'TTACCAATGCTTAATCAGTGAAG 3'	50	865(*Zhang et al.,2017)*
SHV	F-5'TTATCTCCCTGTTAGCCACC-3' R-5'GATTTGCTGATTTCGCTCGG 3'	50	795(*Zhang et al.,2017)*
CTX-M	F-5'ATGTGCAGYACCAGTAARGTKATGGC 3' R-5'TGGGTRAARTARGTSACCAGAAYCAGCGG-3'	60	*593 (Moubarecket al.,2005)*
OXA	F-5'ATGAAAAACACAATACATATCAACTTCGC3' R-5'GTGTGTTTAGAATGGTGATCGCATT 3'	60	820(*Juma et al.,2017)*

Data Analysis

The prevalence of *E. coli* was derived by dividing confirmed positive cases by the total number of urine cultures performed. By dividing the total number of urine cultures performed by the number of UTI-positive patients, the study's UTI prevalence rate was calculated and reported as a percentage. Antimicrobial susceptibility profiles were analysed using WHONET software, and the data were generated as bar graphs. The data were entered into a Microsoft Excel spreadsheet. The data were analysed via the Statistical Package for Social Sciences (SPSS) version 28, 2023. The associations between sociodemographic factors and risk factors for UTIs were investigated using logistic regression analysis to generate adjusted odds ratios with 95% confidence intervals. An alpha of less than 0.05 ($P < 0.05$) was considered significant. The labelled gel images were used to represent the ESBL genes.

RESULTS

Study Population

The study involved adult patients seeking treatment at Mama Lucy Kibaki Hospital. A total of 346 patients were recruited during the study between November 2022 and January 2022: 329 (95.09%) outpatients and 17 (5.16%) inpatients. There were 312 (90.2%) women and 34 (9.8%) men. The age group with the highest UPEC cases was 18--34 years (22), and those >64 years had the
Least cases of *E. coli* infection in UTIs.

Global Journal of Health Sciences
ISSN 2519-0210 (Online)
Vol 10, Issue 1, No.2, pp 14 - 37, 2025

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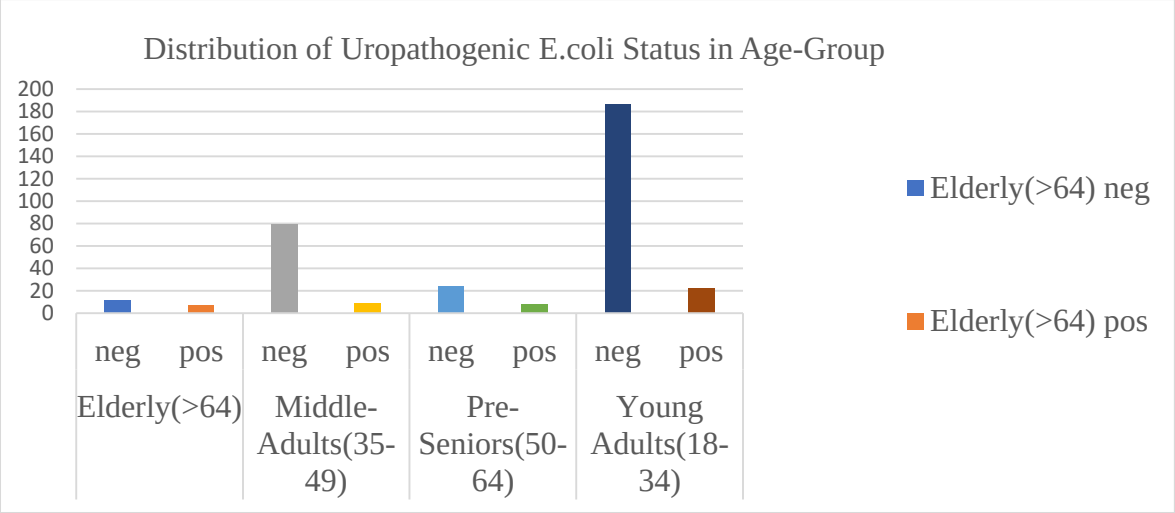

Figure 2: Distribution of E. coli among Different Age Groups

Prevalence of UTI and *E. coli* Isolates

The overall prevalence of UTIs was 82/346 (23.70%), and the prevalence of *E. coli* isolates in the study population was 13.3%. The most predominant isolates were gram-negative (75.61%), while gram-positive isolates accounted for 24.39% of the total. Among all the recovered isolates, *E. coli* was the most common uropathogenic 46 (56.1%), followed by *Klebsiella pneumoniae 12 (14.63%), Pseudomonas aeruginosa 6 (7.32%), Enterococcus faecalis 4(4.87%), coagulase-negative Staphylococcus aureus saprophyticus 9(10.98%)*

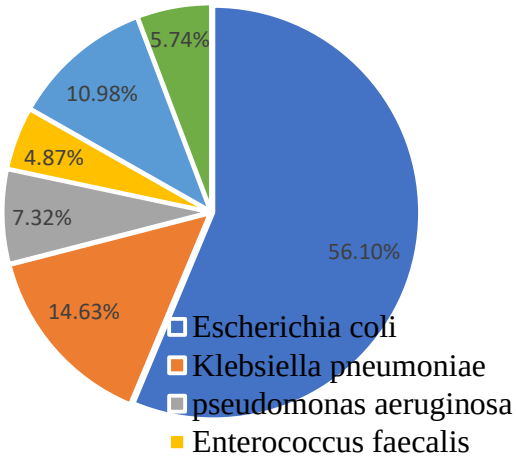

Figure 3: Distribution of Uropathogens among UTI Isolates

Sociodemographic Factors Associated with UTI Infection

There was a significant association between UTIs and education level. Both secondary (P=0.006) and tertiary-level education (P=0.001) were statistically significant. The level of education and sex were statistically significant (P=0.028). The age group of 35--49 years (middle-aged adults) was statistically significant (p=0.027) and hence was associated with UTIs. All the other factors, except education, age group (35--49 years) and sex, were not statistically significant (p>0.05). The majority of cases were female (90.2%), which suggests

that UTIs are more prevalent among women than men. Patient type, marital status, age group and occupation are not associated with the likelihood of having a UTI but may be contributing factors to UTI infection.

Table 2: Sociodemographic Factors Associated with UTI Infection

Variable		(N=Number of Cases) N=346	%	OR	95% Confidence interval		P. Value
					Lower bound	Upper Bound	
Gender	Female Male	312 34	90.2 9.8	- 0.196	- -3.850	- -0.163	- 0.028
Patient type	Inpatient Outpatient	17 329	4.9 95.1	- 0.271	- -2.586	- 0.045	- 0.058
Age group	18-34 35-49 50-64 >68	208 88 32 18	60.1 25.4 9.2 5.2	0.255 0.201 0.854 -	-2.761 -3.048 -1.595 -	0.067 -0.177 1.320 -	 0.061 0.027 0.831 -
Marital-status	Married Not married Widowed	246 85 15	71.1 24..6 4.3	3.191 5.295 -	-0.644 -0.245 -	3.575 4.157 -	 0.222 0.091 -
Occupation	Self/employed Not employed	210 136	60.7 39.3	- 0.706	- -1.091	- 0.383	- 0.350
Education	Primary Secondary Tertiary	63 184 99	18.2 53.2 28.6	- 0.317 0.167	- -1.985 -2.994	- -0.328 -0.682	- 0.006 0.001

Lifestyle and Clinical Factors Associated with UTI Infection

There were no statistically significant differences in lifestyle factors or clinical risk factors. All variables were not statistically significant, with a P value>0.05.

Global Journal of Health Sciences
ISSN 2519-0210 (Online)
Vol 10, Issue 1, No.2, pp 14 - 37, 2025

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Table 3: Lifestyle Factors and Clinical Factors Associated with UTI Infections

Variable		Odd ratio	95% Confidence Interval Lower	Upper	P. value
Antibiotic history	No	-	-	-	-
	Yes	1.893	-0.798	1.975	0.369
UTI knowledge	Yes	2.001	-0.166	1.660	0.117
	No	-	-	-	-
Completion of dosage	Yes	4.317	-0.768	6.352	0.239
	No	-	-	-	-
Doctor's prescription	Yes	0.389	-1.883	0.051	0.062
	No	-	-	-	-
Hospital visit	Chronic issues	0.189	-4.362	1.201	0.234
	Urinary issues	0.292	-3.733	1.600	0.359
	Other issues	0.233	-3.894	1.334	0.274
Toilet	Flash &pit	2.656	- 1.437	2.914	0.375
	Pit	1.062	-0.647	0.762	0.867
	Flash	-	-	-	-
Genital hygiene	Front-back	0.344	-2.394	0.462	0.158
	Back -front	-	-	-	-
Handwashing	Yes	0.422	-3.498	4.104	0.621
	No	-	-	-	-
Undergarment change(daily)	Twice	0.617	-1.294	0.262	0.209
	Thrice	0.526	-3.156	1.151	0.520
	Once	-			-
Undergarment drying	Sun drying	1.520	-0.273	1.143	0.278
	Not-sun drying	-	-	-	-

Forest Plot Summarizing Sociodemographic, Lifestyle and Clinical Factors

The logistic regression model shows 77.85% accuracy in distinguishing *E. coli*-positive and -negative UTIs. Variables such as sex, level of education and age group (35--49 years) were statistically significant, and all other factors were not statistically significant, as shown in **Table 1** and **Figure 4**. The colour of the points indicates statistically significant variables: red (significant) and blue (not significant). Coefficients on the x-axis indicate the association between the variable and the outcome. The confidence intervals are represented by horizontal lines extending from each point.

Global Journal of Health Sciences
ISSN 2519-0210 (Online)
Vol 10, Issue 1, No.2, pp 14 - 37, 2025

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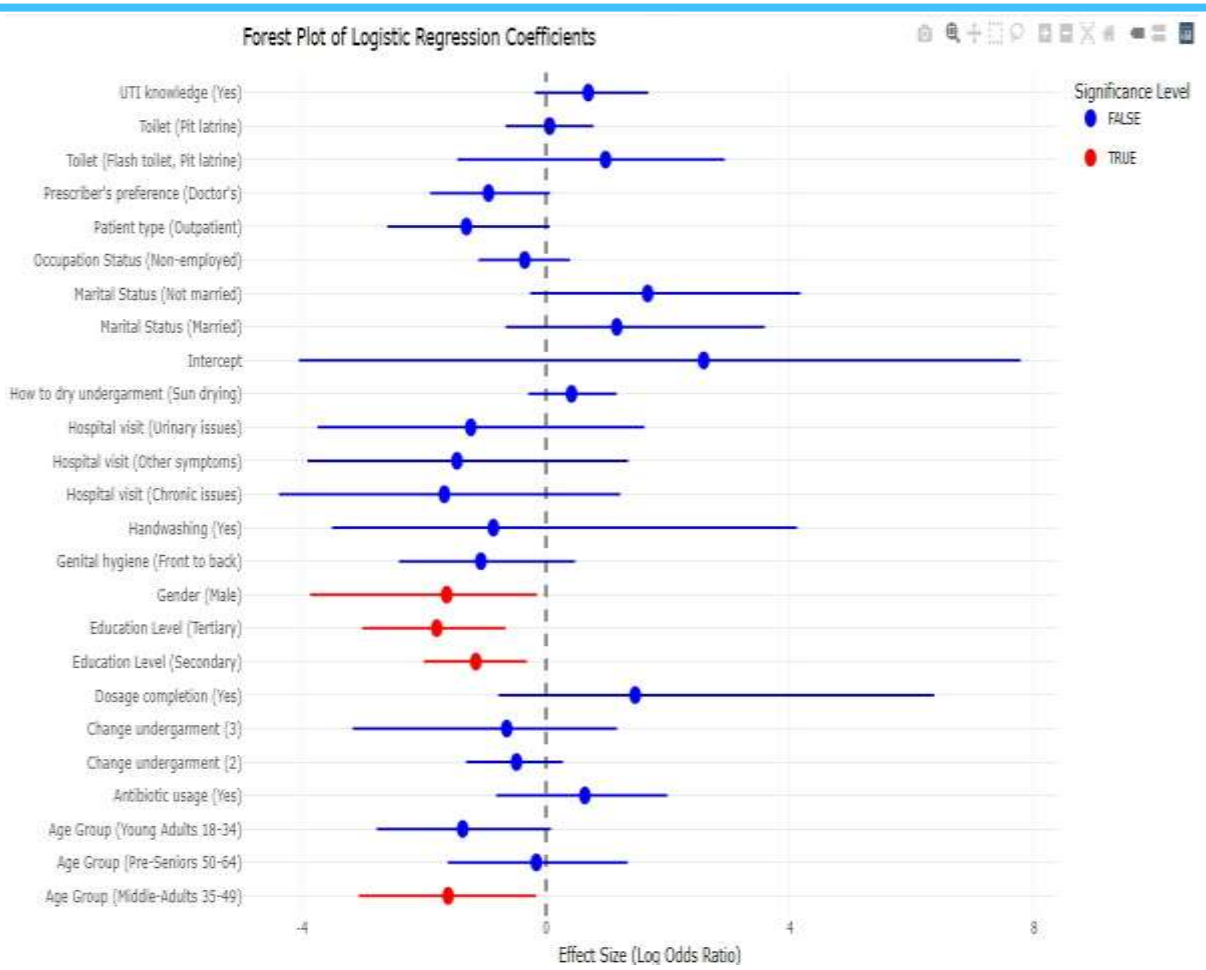

plotly: Link to the attached forest plot.

Figure 4: Forest Plot Showing the Summary of Sociodemographic, Clinical and Lifestyle Factors

Antimicrobial Susceptibility Profiles

E. coli isolates were subjected to antimicrobial susceptibility testing. There were 46 *E. coli* isolates. Overall, the percentage of resistance to commonly used antibiotics in UTI treatment was as follows, as shown in **Figure 4**: trimethoprim-sulfamethoxazole (81.25%), amoxicillin/clavulanic acid (66.67%), ciprofloxacin (62.50%), tetracycline (60.42%), ceftriaxone (54.17%) and cefoxitin (54.17%) had the highest resistance rates. Meropenem (14.58%), chloramphenicol (12.50%), and nitrofurantoin (8.33%) had the lowest resistance rates and were hence effective in UTI treatment. Multidrug resistance was detected in 45 isolates, 98% of which were MDR-resistant.

Global Journal of Health Sciences
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Antimicrobial Susceptibility Profiles

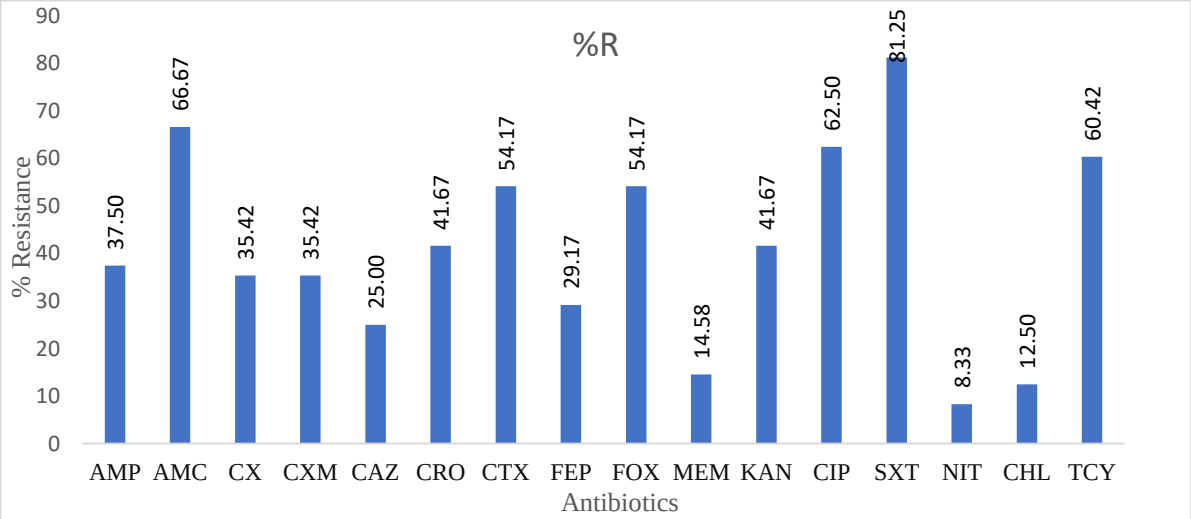

Figure 5: Antimicrobial Susceptibility Profiles among E. Coli Isolates

Keywords: *AMP- ampicillin, AMC-amoxicillin-clavulanic, CX-cefalexin, CXM-cefuroxime, CAZ-ceftazidime, CRO-ceftriaxone, CTX-cefotaxime, FEP-cefepime, FOX-cefoxitin, MEM-meropenem, KAN-kanamycin, CIP-ciprofloxacin, SXT-trimethoprim sulfamethoxazole, NIT-nitrofurantoin, CHL-chloramphenicol, TET-tetracycline.*

ESBL Genes

Among the 46 *E. coli* isolates, 24 (52.17) % were confirmed by molecular analysis as ESβL gene producers. Among the ESβL genotypes, *bla TEM* 21/25 (84%) was the most prevalent, followed by *bla SHV12/25* (72%), *bla CTX-M 18/25* (66.1%), and the OXA gene 7/25 (28%). Over half (54.8%) of the ESBL-producing *E. coli* isolates had the *TEM, CTX-M, and SHV genes.* In our study, *bla* TEM was detected at a predominant rate compared with other ESBL genotypes, as previously reported.In addition, ESBL genotypes and $bla_{CTX\text{-}M}$ were also detected in our study. The beta-lactamase gene bla_{SHV} was detected in more than 72% of the ESBL-producing *E. coli* isolates. The beta-lactamase gene bla OXA, which is the least detected gene, was detected in only 7/25 (28%) isolates.

Table 4: Proportions of ESBLs in UPEC that Cause UTIs

Gene	**n=25**
Bla CTX-M	21(85%)
Bla SHV	12(72%)
Bla TEM	18 (66.1)
Bla OXA	7(28%)
Total DNA Isolates	58

Figures 6-9 below present the electrophoresis gel results for Bla TEM, Bla OXA, Bla CTX-M, and Bla SHV detected in adults with UPEC-causing UTIs.

Global Journal of Health Sciences
ISSN 2519-0210 (Online)
Vol 10, Issue 1, No.2, pp 14 - 37, 2025

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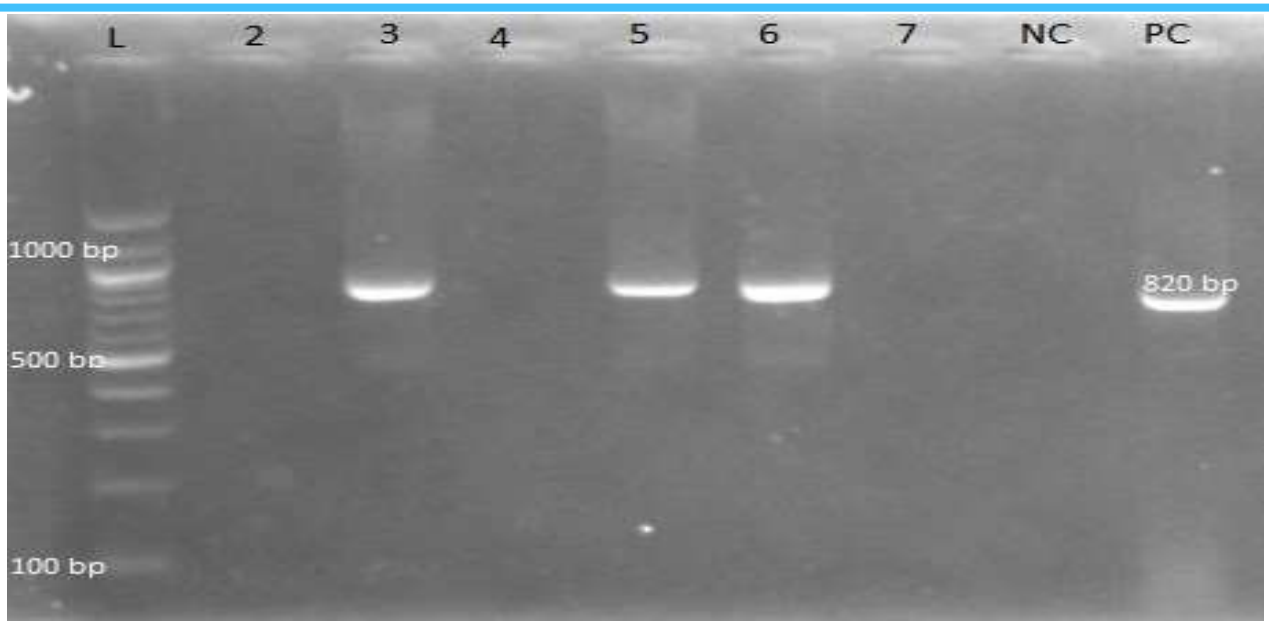

*Figure 6: Electrophoresis gel results for **OXA (820 Bp)** **L**-Molecular size ladder (1000Bp), the **NC**-negative control sterile PCR water, **the** PC-positive control (E. coli ATCC 29522), and the numbers 2--7 target gene genes.*

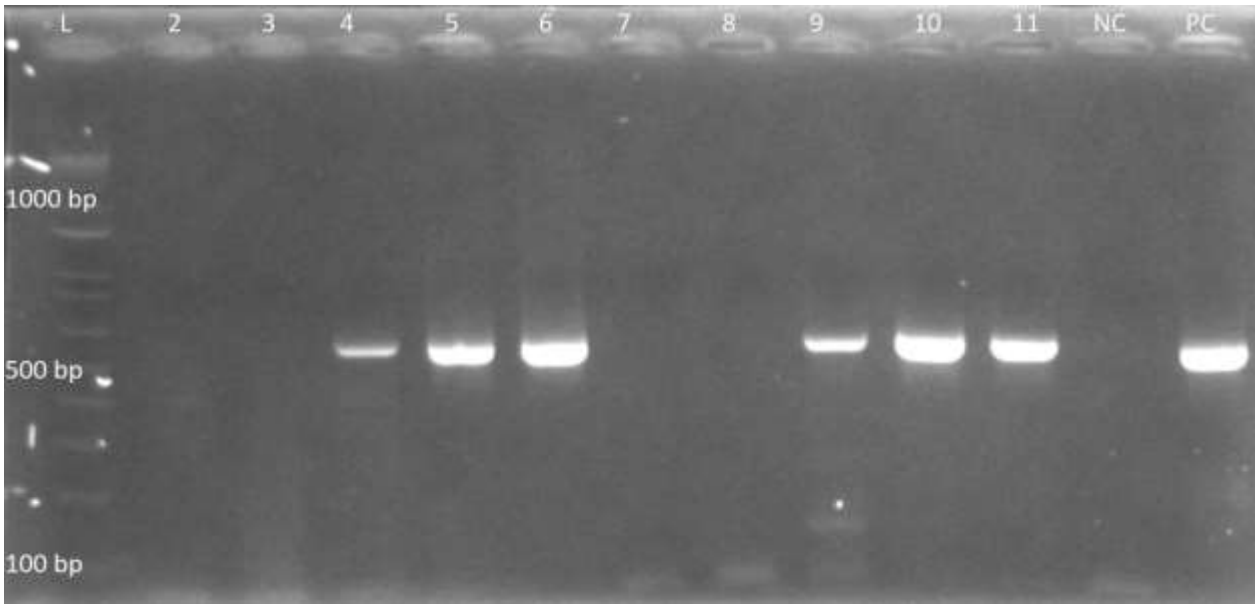

*Figure 7: Electrophoresis gel results for the **CTX-M (593 bp)** **L**-Molecular size ladder (1000Bp), the **NC**-negative control sterile PCR water, **the** PC-positive control (E. coli ATCC 29522), and the numbers 2--11 target genes.*

Global Journal of Health Sciences
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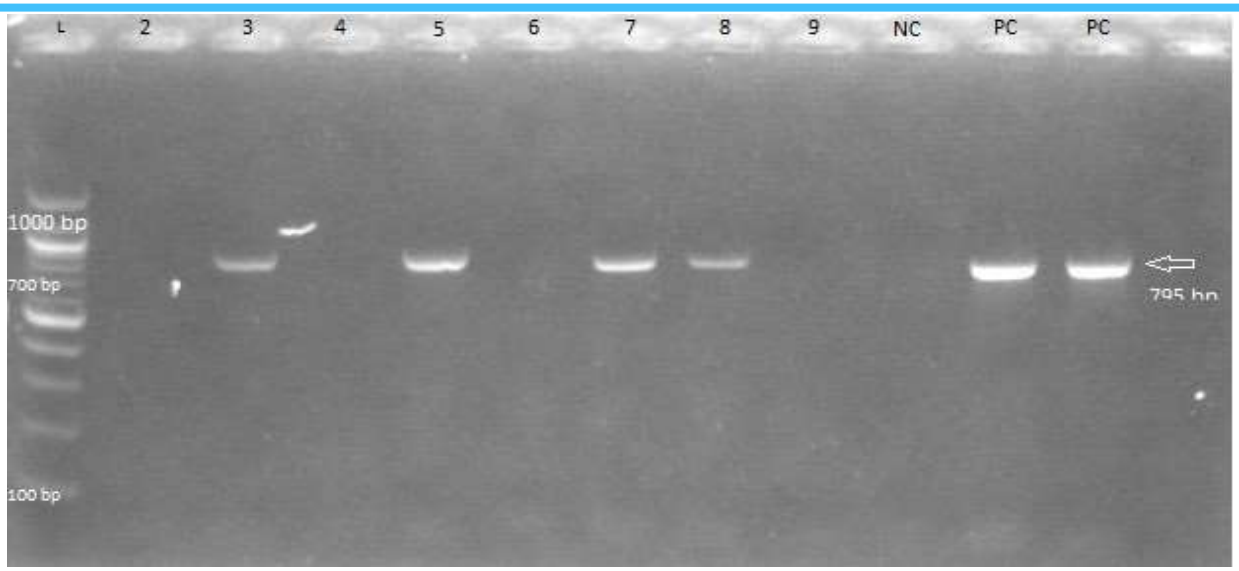

*Figure 8: Electrophoresis gel results for the **SHV (795 bp)** **L**-Molecular size ladder (1000Bp), the **NC**-negative control sterile PCR water, **the** PC-positive control (E. coli ATCC 29522), and the numbers 2--9 target genes.*

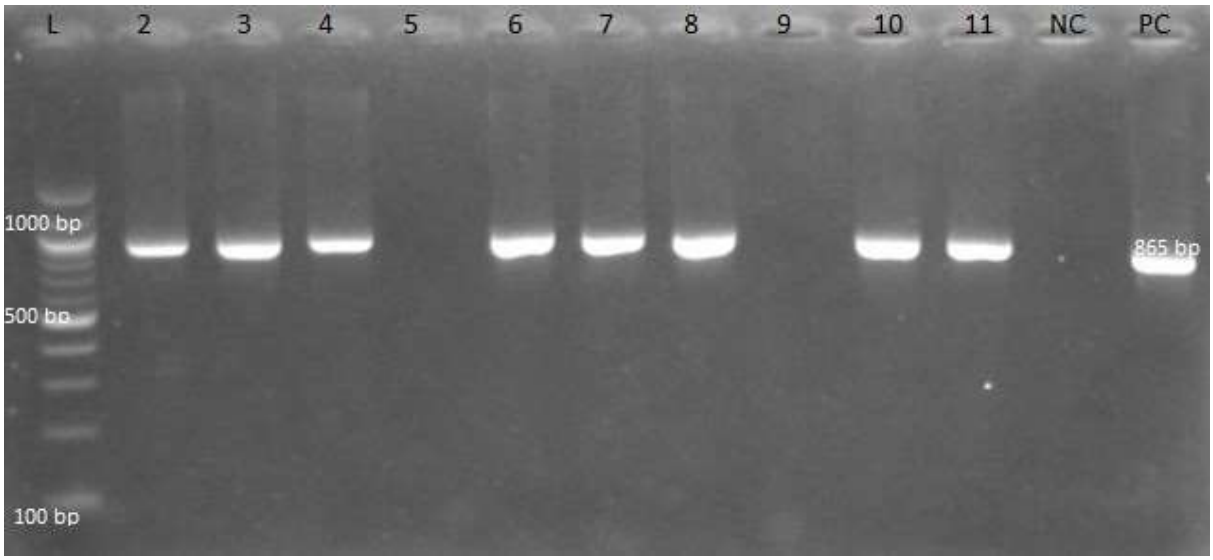

*Figure 9: Electrophoresis gel results for the **TEM (865 Bp)** **L**-Molecular size ladder (1000Bp), **NC**-negative control sterile PCR water, **PC**-positive control (E. coli ATCC 29522), and the numbers 2-11 target genes.*

Discussion

UTI infection, especially *E. coli* infection, is the most common bacterial infection in healthcare settings globally. This study was designed to determine the prevalence of urinary tract infections and the antibiotic resistance patterns of bacterial isolates and to screen for extended-spectrum beta-lactamases, particularly *E. coli,* among adult patients seeking treatment. The overall prevalence of UTIs associated with significant bacteriuria is 23.7%, which falls within the global range of 13%-33% indicating a higher burden on the population (Ikram et al., 2015). In Ethiopia, studies have reported prevalence rates of 23% and 27.35% comparable to the findings of this study (Abate et al., 2020). A similar study performed in Kiambu reported a

prevalence of 27.6% comparable to the findings of this study, and a study performed in Ruiru reported a higher prevalence of 32.7% (Wanja et al., 2021)(Maingi et al., 2023). In Uganda, a prevalence of 32.2% was reported to be higher than our findings but within the global range (Odoki et al., 2019). In Tanzania, the prevalence of UTIs was 27.4% comparable to our findings (Silago et al., 2022).

UTI prevalence variations occur due to geographical distributions, population variation and sample size. In terms of lifestyle, low standards of sanitation and hygiene are additional factors believed to contribute to the increasing prevalence of UTIs. In terms of healthcare, access to healthcare insurance is not as good as universal insurance but higher in Kenya lower in Tanzania and lowest in Uganda. Limited health funding has limited access to effective UTI treatment and a high level of self-medication has resulted in high community prevalence. Challenges in the healthcare systems such as long queues, short consultation time, recurrent infection and high treatment expenses are comparable in Kenya, Uganda and Tanzania.

E. coli was the predominant etiological agent, with a prevalence of 13.3% in the study population. This was comparable to a study performed at Mulago Hospital, Uganda, with a prevalence of 10% (Odongo et al., 2020). Similar to other studies, *E. coli* emerged as the most uropathogenic 46 (51.6%) causing UTIs, which is comparable to findings in Kenya of 57.1% and 44.5% respectively (Maingi et al., 2023) (Onyango et al., 2018). This finding aligns with other studies showing that *E. coli* is a primary causative agent of UTIs. The predominance of gram-negative isolates was 63 (76.83%) over that of gram-positive isolates was 19 (23.17%), in contrast to findings in Uganda (81.8%) (Makeri et al., 2023). These findings emphasise the importance of gram-negative bacteria in UTIs, indicating their critical role in causing UTIs, antimicrobial resistance, and being carriers of extended-spectrum beta-lactamases.

UTIs are common in both inpatients and outpatients, but in this study, outpatients accounted for 95.09%, whereas inpatients accounted for 5.16%. These findings reflect diverse representations of individuals presenting with UTI symptoms as outpatients, which is in agreement with the findings of a study performed in Uganda (Odoki et al., 2019). UTI affects women (90.2%) more than men (9.8%) because of anatomical factors, such as a shorter urethra and hence a shorter distance for bacteria to reach the bladder. *E. coli* adherence to the urinary tract, uses integral virulence factors and other factors such as sexual activity, pregnancy and menopause, contribute to UTIs in women (Odoki et al., 2019). All age groups are affected by UTIs, but 18–34-year-old participants had the highest number of cases since the highest peak number of uncomplicated UTIs occurred during the years of maximum sexual activity, which is in agreement with the findings of this study (Medina & Castillo-Pino, 2019). The 35–49-year-old (middle-aged adults) group was significantly different from the UTI group (P=0.027).

Knowledge of UTIs and treatment-seeking behaviour contributes immensely to the UTI burden in the community. This study indicates that the level of education was statistically significant. Participants with secondary (P=0.006) and tertiary education levels (p=0.001) were statistically significant, with $P<0.05$. Sex was also statistically significant (P=0.028), and females (90.2%) were more likely to have UTIs than males (9.8%) because of predisposing anatomical factors such as a shorter urethral distance and proximity to the anus. Sociodemographic factors other than level of education and sex and factors such as type of patient, marital status and occupation were not statistically significantly associated with UTIs ($P>0.05$). The method of prescription contributes to increasing the incidence of UTI infection. Over-the-counter drugs have resulted in the misuse of antibiotics, primarily due to treatment without proper diagnosis. Clinical factors such as completion of dosage and prior antibiotic history have led to an increase in

antimicrobial resistance since most participants admitted not to complete the dosage. Lifestyle factors and general hygiene practices may not be statistically significant but are risk factors for UTI infections.

Antimicrobials commonly prescribed for UTI treatment worldwide include cephalosporins, semisynthetic penicillins with or without beta-lactamase inhibitors, trimethoprim-sulfamethoxazole and fluoroquinolones (Hryniewicz et al., 2001). Several studies indicate that commonly used antibiotics are no longer effective against *E. coli*. This poses a significant challenge to the treatment of UTIs and highlights the need for new and effective treatment strategies. Antibiotic resistance patterns revealed concerning data, whereby a study on UPEC revealed a variable degree of resistance. These results are comparable to those of a study conducted in Kenya (Wanja et al., 2021). *E. coli* isolates presented high resistance rates to trimethoprim-sulfamethoxazole (81.25%), amoxicillin/clavulanic acid (66.67%), ciprofloxacin (62.50%), tetracycline (60.42%), ceftriaxone (54.17%), and cefoxitin (54.17%). In European countries, the resistance of UPEC to antibiotics ranges from 14.6-60% (Kot, 2019)

Trimethoprim-sulfamethoxazole has increased resistance as one of the first-line treatments for uncomplicated UTIs, which is in agreement with findings in Kenya with 100% resistance (Omwenga et al., 2022). Fluoroquinolones such as ciprofloxacin are highly resistant in developing countries, ranging from 55.5–85.5% which is in agreement with our study(Kot, 2019). The increased resistance to fluoroquinolones is due to increased use in patient outpatient services. Amoxicillin-clavulanic therapy is also the first-line therapy for the treatment of complicated UTIs; hence, resistance varies regionally. This resistance poses a serious challenge in selecting appropriate empirical treatment options for UTIs caused by *E. coli*. The elevated resistance to these antibiotics signifies the need for a cautious approach to their utilization and highlights the urgency in developing alternative treatment strategies.

Some *E. coli* isolates are sensitive to antibiotics. Meropenem (14.58%), chloramphenicol (12.50%), and nitrofurantoin (8.33%) exhibited notably lower resistance, suggesting their potential efficacy in treating UTIs caused *by E. coli.* Nitrofurantoin is the first-line treatment for uncomplicated cystitis; hence, resistance is quite low. A study in Uganda reported that the sensitivity of nitrofurantoin was 70% which is comparable to that reported in a study in Kenya, where nitrofurantoin resulted in 14% resistance (Odongo et al., 2020) (Wanja et al., 2021). The identification of these antibiotics as having lower resistance rates is encouraging, emphasizing their importance as potential alternative treatments in scenarios where commonly used antibiotics fail due to resistance.

Factors such as geographical, social, economic, and lifestyle factors have contributed to increased antibiotic resistance in this study, which is comparable to findings in Afghanistan (Joya et al., 2022). From a geographical and social perspective, a significant number of UTI patients rely on over-the-counter medications, lack medical facilities, lack UTI knowledge and do not complete dosage; hence, this practice is a major contributor to the development of drug resistance. Economically, many individuals lack the resources to undergo urine culture tests and are therefore treated empirically, a practice that further exacerbates the rate of resistance. Lifestyle factors such as poor sanitation and contaminated water have strongly contributed to the increase in UTI cases (Joya et al., 2022).

Gram-negative bacteria are major carriers of extended-spectrum beta-lactamase genes. The CTX-M, TEM, OXA and SHV genes are significant forms of ESBL and are present in many important clinical pathogens around the world (Paterson & Bonomo, 2005). This study

revealed that 84% of the *E. coli* samples presented the TEM gene *in 21/25* (84%), the SHV gene in *12/25* (48%), the CTX-M gene in 18/25 (72%), and the OXA gene in 7/25 (28%) according to the results of the PCR screening. The frequency of β-lactamase genes in the *E. coli* population is indicated by these percentages, which also indicate the possibility of β-lactam resistance. These findings align with research showing the presence of Bla resistance genes among *E. coli* isolates (Mitsan et al., 2020). However, the most prevalent gene was Bla CTX (53.3%), followed by BLA TEM (33.3%), which slightly varied from our study. The epidemiology of ESBL genes rapidly varies due to other factors, such as gene expression, enzyme activity, resistance mechanisms, or phenotypic testing methods. The geographical difference in the distribution of ESBL genotypes contributes to variations in the prevalence of genes and the overuse of antibiotics (Flores-Mireles et al., 2015). Moreover, studies have indicated that the prevalence of ESBL producers is variable in different regions of the world, as detected by phenotypic detection tests (Abrar et al., 2018). This study provides evidence for the predominance of TEM-producing *E. coli* among UPEC isolates in Kenya, which is in agreement with many reports demonstrating the changing epidemiology of ESBL-producing *E. coli* worldwide. Understanding these findings is crucial in guiding effective treatment strategies and addressing antimicrobial resistance. There is a need for strengthening laboratory diagnostic systems for continuous surveillance and screening of ESBLs resistance genes which have continuously increased antimicrobial resistance and multi-drug resistance in current treatment regimens. Emphasis on the development of vaccines and development of drug-sensitive rapid diagnostic kits which will reduce the turnaround time for culture method and also proper diagnosis reducing inconsistent results. There is a need to review current treatment guidelines eliminating drugs that are no longer effective for UTI treatment hence reducing the prevalence and recurrent UTI infections.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study highlights the substantial burden of UTIs, particularly those caused by *E. coli*, and the pressing issue of antibiotic resistance. The creation of awareness of UTIs should be emphasized in the community, thus increasing the knowledge, prevention and management of UTIs and reducing their burden. The emergence of resistance to commonly prescribed antibiotics signifies a critical need for judicious antibiotic use, comprehensive surveillance, and the development of new therapeutic strategies to combat UTIs effectively. Furthermore, the identification of antibiotics with lower resistance rates provides hope, highlighting the importance of these options in the management of UTIs. Our study results suggest that the use of meropenem, chloramphenicol, and nitrofurantoin by clinicians can be used in the management of UTIs. Urine culture should be the fundamental diagnostic tool for UTIs, hence reducing the incidence of UTIs and forming a basis for proper treatment, therefore reducing antibiotic resistance. Continuous screening for extended-spectrum beta-lactamases through PCR and sequencing of the genes will guide the management of UTIs. Future studies should focus on the development of novel methods of treatment that focus on rapid tests and virulence marker antibiotics, which will be vital for better patient care and the global fight against antimicrobial resistance.

Recommendations

Based on the conclusion above, further research on all the uropathogens associated with UTI should be determined to ascertain the UTI burden in the community. Antimicrobial resistance

prevalence should be minimized by adequate patient education, proper diagnosis, regulation of the unauthorized sale of antibiotics and also misuse of antibiotics which ensures proper treatments. Continuous screening and surveillance of Extended beta-lactamase genes associated with UTIs should be performed to identify resistance genes which contribute to antibiotic resistance to current treatment regimens. This study recommends the need to combine phenotypic and molecular methods to understand various aspects of antibiotic resistance in developing countries.

Study Limitations

This study faced several limitations that can be addressed in future studies.

1. This study focused only on uropathogenic *E. coli* isolates; other uropathogens were not included in terms of antimicrobial susceptibility. Future studies should include all the uropathogens causing UTIs.
2. We only conducted the study at one study site; hence, it was not representative of the vast number of UTI infections in the community. Future studies should use several study sites for better comparison.
3. We were able to screen a few selected ESBL genotypes. This provides a basis for continuous screening of other ESBL genes causing resistance, and future studies should include all ESBL genotypes.

Acknowledgements

The authors wish to thank the study participants for taking part in this study. We also thank the **Mama Lucy Hospital** Administration for allowing us to conduct the study in the facility. Special thanks to the clinicians and laboratory technologists for their guidance, cooperation and assistance during sample collection. We also wish to thank **KEMRI/CMR** (molecular biology), particularly Susan Wambui Kiiru, Kenneth Malombe, Alex Mogita, and Maureen Miriti. Hussein Mwita assisted in the data analysis. We would like to thank the **AFRICA**-ai-**JAPAN** Project Innovation Research Fund for facilitating major parts of the study.

Funding

This research was funded by the **AFRICA**-*ai*-**JAPAN** Project Innovation Research Fund**.**

Ethics Approval

Study ethical approval was sought from the Jomo Kenyatta University of Technology Institutional Ethics Review Committee (IERC) ref: JKU/2/4/896B, and a license from the National Commission for Science and Technology and Innovation (NACOSTI) ref: 613454 was obtained.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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