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Extended Spectrum Beta-Lactamase Production and Multi-Drug Resistant *Enterobacteriaceae* Among Urinary Tract Infection Suspected Patients Referred to Wudassie Advanced Medical Laboratory, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Tamirat Tamiru entitled: Extended Spectrum Beta-lactamase Production and Multi-Drug Resistant *Enterobacteriaceae* Among Urinary Tract Infection Suspected Patients Referred to Wudassie Advanced Medical Laboratory, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology specialty track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviation

AR	Antibiotic resistance
ATCC	American Type Culture Collection
CLSI	Clinical Laboratory Standards Institute
CDT	Combined disc Test
ESBL	Extended spectrum beta -lactamases
ESBL-E	Extended spectrum β-lactamases producing *Enterobacteriaceae*
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight
MDR	Multidrug-resistant
NF	Non-fermenter
SOP	Standard operating procedure
Spp	Species
SPSS	Statistical Package for social sciences statistical software
UTIs	Urinary Tract Infections
WAML	Wudassie advanced medical laboratory
WDC	Wudassie diagnostic center

Abstract

Background: Urinary tract infections (UTIs) are one of the commonly encountered infectious diseases globally, affecting approximately 150 million individuals each year. The rise of multidrug resistant (MDR) and extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae* has made the management of urinary tract infections more difficult which is becoming common public health concern worldwide

Objective: To assess the prevalence, antimicrobial susceptibility, and extended-spectrum beta-lactamase producing *Enterobacteriaceae* among UTI suspected patients referred to Wudassie advanced medical laboratory, Addis Ababa, Ethiopia.

Methods: A laboratory based cross-sectional study was conducted between December 2024 to March 2025. A total of 502 UTI suspected patients were included using convenient sampling technique. The identification of *Enterobacteriaceae* was done using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (EXS3000, Zybio Inc., China). Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion technique. Combination disk test (CDT) was used for confirmation of ESBL producing *Enterobacteriaceae*. Data ware entered in Epidata version 4.6 and exported to Statistical Package for the Social Sciences (SPSS) version 27 for analysis.

Result: Among 502 patients presenting with clinical symptoms of urinary tract infection (UTI), 29.4% (148/502) has positive culture for *Enterobacteriaceae*. The predominant species isolated were *Escherichia coli* 103(69.6%) and *Klebsiella pneumoniae* 25 (16.8%). The prevalence of extended spectrum β-lactamases producing *Enterobacteriaceae* (ESBLs-E) was 65.5% (97/148). The highest proportion of ESBL-producing isolate was *Klebsiella pneumoniae* (92.0%)..Among 148 *Enterobacteriaceae* isolated, 82.2% of them were multi-drug resistant (MDR).

Conclusion: The findings highlight a high prevalence of MDR and ESBL-producing *Enterobacteriaceae*. This finding emphasizes a critical need to implement comprehensive surveillance systems in hospital settings to enable the detection and tracking of ESBL-producing *Enterobacteriaceae*. Continuous monitoring of their dissemination is highly recommended for implementation of antibiotic stewardship programs.

Key words: Urinary tract infection, Multi drug resistance, Extended spectrum beta lactamase

1. Introduction

1.1 Background

Urinary tract infections (UTIs) can be caused by bacteria growing and existing anywhere in the kidney, ureter, bladder, and urethra [1]. Depending on the patient's clinical symptoms, a UTI may be upper (pyelonephritis) or lower (cystitis) [2]. UTI is the most frequent bacterial infection that affects both sexes. However, the occurrence is more frequently observed in women due to shorter urethra, the proximity of urethra to anus and colonization of vagina by intestinal flora [3,4].

Urinary tract infections (UTIs) are one of the commonly encountered infectious diseases globally, affecting approximately 150 million people annually. Many classes of antibiotics are used to treat UTIs, among them, the beta lactam antibiotics are among the most commonly used medications in clinical practice, because of their better safety profiles and broad-spectrum activities [5]. Numerous studies have indicated that the emergence of resistance is increasing due to the practice of self-medication and uncontrolled use of antibiotics prophylaxis and, thus creates the rise of resistant mutant by placing pressure on the bacterial populations [6].

UTI caused by antibiotic resistant *Enterobacteriaceae* makes empirical treatment of these infections difficult. These groups of bacteria employ multiple resistance mechanisms, including the production of enzymes such as extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases. Additionally, they utilize non-enzymatic strategies such as the activation of efflux pumps and reduced membrane permeability resulting from porin loss. ESBL, AmpC mediated beta lactamases and metallo-beta lactamase (MBL) are some of the enzymes not only produced by *Enterobacteriaceae* but also by other non-lactose fermenters causing UTIs [7].

Extended-spectrum beta-lactamases (ESBLs) hydrolyze and inactivate beta-lactam antibiotics, rendering them ineffective against infections caused by ESBL-producing bacteria. ESBL producing *Enterobacteriaceae* are resistant to penicillins, third-generation cephalosporins, and monobactams (aztreonam), but not to cephamycins (cefoxitin and cefotetan) and carbapenems [8]. Additionally, they are susceptible to β-lactamase inhibitors including clavulanic acid, sulbactam, and tazobactam [9,10].

Among *Enterobacteriaceae*, ESBLs have been commonly observed in *Klebsiella* and E. *coli spp*, though it has also been reported in other families of *Enterobacteriaceae* such as *Proteus spps, Enterobacter spps, Citrobacter spps, Serratia spps, Morganella spps, Providencia spps* and *Salmonella* [11]. The global rise in ESBL-producing *Enterobacteriaceae* (ESBL-E) has contributed significantly to multidrug resistance (MDR) in both community and hospital settings. ESBL- producing *Klebsiella pneumoniae and Escherichia coli*, have become a major global threat public health threat [12].

Genes responsible for ESBL production often located on large plasmids that carry antimicrobial resistance (AMR) genes which confer resistance to a broad range of antibiotics [13]. Thus, many ESBL-producing bacteria frequently exhibit resistance to multiple non-β-lactam antibiotics classes; such as, quinolones, tetracyclines, sulfonamides, aminoglycosides, trimethoprim sulfamethoxazole, and chloramphenicol, which are commonly used in treating severe bacterial infections [14-16].Although ESBL-E resists to many classes of antibiotics used for treatment of urinary tract infection, carbapenems remain last resort and effective treatment option for serious infection caused by *Enterobacteriaceae*[17].

Evaluating the presence of ESBL-producing *Enterobacteriaceae* at the local context is essential for understanding the resistance trends and impact of these infections, Unfortunately, there is limited information regarding the extent of ESBL-producing *Enterobacteriaceae* among UTI specially patients visiting private health facilities in Addis Ababa, Ethiopia. Additionally, it appears that nearly all clinical bacteriology laboratories in the country do not conduct ESBL testing. Therefore, this study aims to determine the prevalence of ESBL-producing and multidrug-resistant (MDR) *Enterobacteriaceae* among UTI suspected patients in Addis Ababa, Ethiopia.

1.2 Statement of Problem

Urinary tract infections are among the most frequently observed bacterial infections globally, impacting millions annually and placing a significant burden on community and hospital setting. It accounts for 35% of nosocomial infections [18] and approximately one million hospitalizations worldwide [19]. UTIs are the second most prevalent infection in community practice. In their lifetime, about 40% of women and 12% of men will experience at least one symptomatic infection. The increasing prevalence of MDR and ESBL-producing *Enterobacteriaceae* poses a significant threat to the effective management of UTIs [20].

MDR *Enterobacteriaceae*, including common UTI pathogens like *Klebsiella pneumoniae* and *Escherichia coli*, exhibit resistance to multiple classes of antibiotics, significantly limiting treatment options. ESBL-producing bacteria spread and pose bigger burden to developing countries. A recent finding revealed that that overall prevalence of healthcare-associated infections in developing countries (15.5%) is approximately double the average rate reported in Europe (7.1%). Some factors may contribute to this disparity are overcrowded healthcare facilities, frequent use of antibiotics without prescriptions, poorer hygiene in developed countries especially in hospitals [20]. This rise in MDR and ESBL-producing *Enterobacteriaceae* among UTI cases raises treatment failures, increased healthcare cost and spread of resistance which potentially creating a scenario where common infections become untreatable [13].

Addressing the growing threat of MDR and ESBL-producing *Enterobacteriaceae* in UTIs is crucial for guiding treatment decisions, implementing effective infection control measures, promoting the judicious use of antibiotics which is essential to minimize the emergence and spread of resistance and also the development of new antibiotics and alternative treatment strategies to combat MDR infections [10].

Although the phenomenon of antimicrobial resistance among ESBL–E isolates of various samples has been reported in Ethiopia [21,22], the issue has not been well described among ESBL–E isolates in urinary tract infection specially from patients visiting private health facilities such as Wudassie Advanced Medical Laboratory Hence this study will try to describe the bacterial profiles, their antimicrobial susceptibility, and the prevalence of MDR and ESBL producing *Enterobacteriaceae* among urine samples referred to a stand-alone diagnostic laboratory from different health facilities of Addis Ababa, Ethiopia

1.3 Significance of The Study

This study might help the policy maker and other stakeholders involved in infection control and antibiotic resistance prevention initiatives in establishing and promoting effective preventative strategies. The findings of this study hold substantial significance for private healthcare facilities, where diagnostic capacities and antimicrobial stewardship practices may vary widely. The finding of the study will also give insight to health program stakeholders so as to develop workable intervention for controlling the multi drug resistant and ESBL producing *Enterobacteriaceae*. Furthermore, it would assist physician in the selection of more effective, potentially reducing both treatment failures and unnecessary healthcare costs. moreover, it provides base line information for other researchers to conduct further research about MDR and ESBL producing *Enterobacteriaceae* and used as source of data.

2. Literature review

A comprehensive systemic review and meta-analysis was conducted on the global prevalence of co-existing ESBL-producing *K. pneumoniae* and *E. coli* in humans published from 1990 to 2022 in 101 studies, of which the pooled prevalence estimate (PPE) was 33.0% and 32.7% for *E.coli* and *K. pneumoniae* respectively. At the continental scale, Africa exhibited the highest pooled prevalence estimates (PPE), with 44.3% for ESBL-producing *Escherichia coli* and 32.8% for *Klebsiella pneumoniae*. On a country level, Thailand recorded the highest PPE for ESBL-producing *E. coli* at 52.1%, while the United States reported the highest PPE for *K. pneumoniae*, reaching 55.7% [23].

A review and meta-analysis conducted by Onduru OG *et al*., across Eastern, Central, and Southern Africa (ECSA) on 20,225 ESBL-producing isolates from various samples, of which 81% of the studies isolated both *K. pneumoniae* and *E. coli*. Of the studies, 63% reported predominance of E. coli among isolates, Klebsiella pneumoniae was dominant in 33%, and 5% of the studies reported that Proteus spp. was dominant A random pooled prevalence of ESBL-producing *Enterobacteriaceae* in human from 11 ECSA countries with available data was 38%. The pooled prevalence of ESBL-E in hospital settings was 41% and 34% in community settings [24].

This systematic review and meta-analysis conducted by Khadka C et al., on the prevalence of ESBL among the members of the *Enterobacteriaceae* from studies published between 2011 and 2021 in Nepal. 65 articles were systematically reviewed. The overall pooled prevalence of ESBL-producers in *Enterobacteriaceae* was 29 % with high heterogeneity. Escherichia coli was the predominant ESBL-producing member of the *Enterobacteriaceae* family, followed by Citrobacter spp. and Klebsiella spp. The prevalence of ESBL-PE increased from 18.7 % in 2011 to 29.5 % in 2021. A strong positive correlation ($r = 0.98$) was observed between ESBL production and MDR in *Enterobacteriaceae*. ESBL-PE isolates showed high resistance to ampicillin, cephalosporins, and amoxicillin-clavulanic acid [25].

A systematic review and meta-analysis conducted on antimicrobial resistance and ESBL production in uropathogenic *Escherichia coli* (UPEC) from 2014 to 2023. A total, 15791 study participants were assessed for UTI, from this review the pooled prevalence of UPEC-associated UTI was 9.2%.34 studies reporting MDR profiles of 1022 UPEC isolates. The pooled MDR profile

of UPEC was calculated to be 66.28%. 11 studies that examined ESBL production among 342 cases of UPEC. The overall pooled ESBL production rate among UPEC was found to be 29.16% [26]

A cross-sectional study conducted by Eltai *et al*., Doha, Qatar from February to June of 2017.Total of 727 urine Samples were collected from children (0–15 years of age). Out of this 635 *Enterobacteriaceae* positive urine cultures, from this 201 (31.7%) were found to be ESBL producing bacteria. *E. coli* species were the most prominent with prevalence rate of 83% ($n = 166$), followed by *Klebsiella pneumoniae* 11% ($n = 22$) and the rest 6% included *Citrobacter koseri*, *Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter amalonaticus*. All ESBL-producing isolates showed 100% resistance to ampicillin, and to all cephalosporins including cephalothin, cefazolin, ceftriaxone and cefepime. Low resistance was recorded to carbapenems. Resistance ranged between 2.5% to meropenem, ertapenem and 10% to imipenem. Among the β-lactam/β-lactamase inhibitor combinations, 9% were resistant to piperacillin/tazobactam, whereas 99% were resistant to amoxicillin/clavulanic acid. Regarding aminoglycosides, all the isolates were susceptible to amikacin, and 24.4% of which were resistant to gentamicin. The resistance prevalence to other classes of antibiotics namely, cefoxitin, nitrofurantoin, trimethoprim/sulfamethoxazole and ciprofloxacin were 19.4%, 13%, 59.7% and 36%, respectively [27].

A cross-sectional study conducted by Raya GB *et al*, Siddhi Memorial Hospital (SMH) in Bhaktapur, Nepal from April 14, 2017 to April 13, 2018. 5545 non-repeated urine samples from the children with symptoms of UTI. A significant growth of uropathogens were observed in 203 samples (3.7%). *Escherichia coli* ($n = 158$, 77.8%) and *Klebsiella pneumoniae* ($n = 30$, 14.8%) were common among the uropathogens. Among them, 80.3% were resistant to amoxicillin and 51.2% were resistant to cotrimoxazole. Most of them were susceptible to amikacin, nitrofurantoin, and ofloxacin. MDR were detected in 34.5% ($n = 70/203$) and ESBL producers in 24.6% ($n = 50/203$) of them [28].

A prospective cross-sectional study conducted by Shakiya *et al.,* at Alka Hospital, Lalitpur to assess the prevalence of ESBL production among the Gram-negative rods isolated from urine samples. A total of 2209 non-repetitive mid-stream urine (MSU) samples were collected during the study period (March to September 2014). A total of 451 samples showed significant bacteriuria

with 365 (80.9%) *E. coli*, 17 (3.8%) *Klebsiella pneumoniae* and 3 (0.7%) *Klebsiella oxytoca*. *E. coli* were highly resistant to amoxicillin 269 (73.69%) and least resistant to imipenem 7(1.9%). Likewise, most *Klebsiella* spp. were highly resistant to amoxicillin 18 (90%) and least resistant to gentamycin 0 (0%) and imipenem 0(0%). Of 451 isolates, 236 (52.3%) were found MDR strains. By combined disk test, 33 (91.7%) *E. coli* and 3 (8.3%) *Klebsiella* spp. were found ESBL producers [29].

A cross-sectional study conducted by Mogald EH *et al*., from February to July 2018. A total of 144 isolated Gram-negative bacteria from different clinical sample sources were collected from two main tertiary care referral hospitals in the Khartoum state (Omdurman and Bahri Teaching Hospitals). The result showed that out of 144 isolates, *Escherichia coli* (47.9%) were predominant isolate followed by, *Klebsiella pneumoniae* (25%) . Most of the isolated *Enterobacteriaceae* were from urine sample. *E. coli* with the frequency of 51 (35.4%) and *K. pneumonia* 22 (15.37%) . The prevalence of ESBL were higher in *K.pneumonia* (38.9%) than *E. coli* (34.8%). All isolated *E. coli* were sensitive to nitrofurantoin and tigecycline. There was a high prevalence of MDR *Enterobacteriaceae* [30].

A retrospective cross-sectional study conducted by Djim-Adjim-Ngana K *et al*., in Garoua, the capital of the North Cameroon region in 2017. 144 strains of *Enterobacteriaceae* from suspected UTIs cases were identified and isolated. These included Escherichia coli, Klebsiella pneumoniae, Enterobacter cloaceae Enterobacter aerogenes, Citrobacter koseri, Proteus mirabilis, and Morganella morganii. E. coli (72.9%; 105/144) and Klebsiella pneumoniae (20.1%; 29/144) were the most commonly isolated strains, accounting for 93% of all isolates. Out of the 144 *Enterobacteriaceae* isolates, 59 (41%) were multi drug resistant, with E. coli (76%) being the most abundant MDR strain, followed by K. pneumoniae (21%). The double synergy test (DDST) revealed that ESBL producers were of 24.3% (35/144). E. coli were the most common ESBL producer (77.1%;27/35), followed by K. pneumoniae (20% ;7/35) and E. aerogenes (2.9%) of the 35 detected ESBL-producing isolates [31].

A prospective cross-sectional study conducted by Mohammed ES *et al*., in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Minia University, Egypt from June 2018 to December 2018. A total of 440 *Enterobacteriaceae* strains were isolated from urine specimens of 440 patients suffering from UTI. Antimicrobial susceptibility and phenotypic tests

identified 311 (70.6%) isolates as ESBL producers. The frequency of ESBL production were 211/311 (69.6%) in *E. coli*, 53/71 (74.6%) in *K. pneumoniae,* 40/40 (100%) in *Citrobacter* spp. and 7/15 (46.6%) in *Proteus* spp. isolates. Out of 311 ESBL positive isolates, 308 (99%) isolates were positive for ESBL genes indicating high sensitivity of the phenotypic tests [32].

A study conducted by Fares R, et al., at referral hospitals in Tebessa province, Northeast Algeria, from 2018 to 2020.400 UTI-causing strains were found and categorized as *Enterobacteriaceae* throughout this time. 68.5% (274/400) of the *Enterobacteriaceae* isolates were found to be multi-drug resistant (MDR) strains based on the findings of antimicrobial susceptibility tests. The prevalence of ESBL producing *Enterobacteriaceae* was (59.5%; 238/400) and E. coli (85/238) was the highest ESBL producer. The ESBL producing *Enterobacteriaceae* were resistant to cephalosporins and penicillins. However, those ESBL producing *Enterobacteriaceae* were sensitive to nitrofurantoin (87.8%) and imipenem (80.2%) [33].

A hospital-based cross-sectional study conducted by Abayneh M et al., in Jimma University Specialized hospital, Southwest Ethiopia, 2016. 74 (21.6%) of urine samples were confirmed as positive urine culture of which 63 (85.1%) were *E. coli* and 11 (14.9%) were *K. pneumoniae.* ESBL-producing phenotypes were detected in 23% (n = 17) of urinary isolates, of which *Escherichia coli* accounts for 76.5% (n = 13) and *K. pneumoniae* for 23.5% (n = 4). ESBL-producing phenotypes showed high resistance to cefotaxime (100%), ceftriaxone (100%), and ceftazidime (70.6%), while both ESBL-producing and non-ESBL-producing isolates showed low resistance to amikacin (9.5%), and no resistance were seen with imipenem [34].

A cross-sectional study conducted by Agegnew A et al., in Hawassa Comprehensive Specialized Hospital, southern Ethiopia. from February 1, 2018, to July 30, 2018, among children presenting to the outpatient department of Hawill besa Comprehensive Specialized Hospital, from 284 urine specimens, growth was detected in 90 specimens, and a total of 96 (33.8%) bacterial species were identified. Among these, 75% (n = 72/96) were *Enterobacteriaceae* with *E. coli* (44.4%, n = 32/72), *K. pneumonia* (27.8%, n = 20/72), *Klebsiella oxytoca* (8.33%, n = 6/72), *Providencia* spp. (5.6%, n = 4/72), *Citrobacter diversus* (4.16%, n = 3/72), *Enterobacter cloacae* (2.8%, n = 2/72), *Proteus mirabilis* (2.8%, n = 2/72), and *Klebsiella ozaenae* (4.16%, n = 3/72) being most common isolates. Majority of isolates were resistant to ampicillin (95.8%), amoxicillin/clavulanic

acid (94.4%), trimethoprim-sulfamethoxazole (86.1%), and gentamycin (86.1%), while better susceptibility were observed for ciprofloxacin (47.2%), norfloxacin (45.8%), meropenem (40.3%), and nitrofurantoin (26.4%). 62 (86.1%) were non susceptible to three or more drugs belonging to different antibiotics classes ESBL producing *Enterobacteriaceae* accounted for 58.3% (42/72) of the total isolates, of which 71.4% (30/42) were confirmed as ESBL producers. ESBL-producing *Enterobacteriaceae* were resistant to amoxicillin/clavulanic acid (96.7%), ampicillin (96.7%), trimethoprim-sulfamethoxazole (96.7%), gentamycin (96.7%), cefotaxime (96.7%), ceftriaxone (90%), and tetracycline (86.7%) as compared to ESBL non-producers [35].

3. Objectives

3.1 General objectives

- To assess Extended Spectrum Beta-Lactamase Production and Multi-Drug Resistant *Enterobacteriaceae* among Urinary Tract Infection Suspected Patients Referred to Wudassie Advanced Medical Laboratory, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine the prevalence of *Enterobacteriaceae* among urine samples referred to WAML
- To determine the antimicrobial susceptibility pattern of *Enterobacteriaceae* from urine samples referred to WAML
- To determine prevalence the ESBL producing *Enterobacteriaceae* from urine samples referred to WAML

4. Hypothesis

H0 = The prevalence of ESBL producing *Enterobacteriaceae* is similar to study conducted in Tikur Anbessa Specialized hospital 61.7% of ESBL-E [36].

5. Material and methods

5.1 Study area

The study was conducted on patients referred to Wudassie Advanced Medical laboratory (WAML) from different health facilities (hospitals, health centers, clinics, institution) found in Addis Ababa. WAML is a private diagnostic laboratory found in Arada Sub city, Addis Ababa, Ethiopia, with 5 branches located different part of the city. It is founded in 2020 and known by core laboratory testing, including chemistry, hematology, full microbiological capabilities. It is accredited by Ethiopian Accreditation Service (EAS) with specific scopes since 2024. The laboratory runs an average of 400 different microbiological tests per a month.

5.2 Study design and period

A cross-sectional study was conducted from December 2024 to March 2025.

5.3 Population

5.3.1 Source population

All urinary tract infection suspected patients referred to Wudassie Advanced Medical Laboratory during the study period.

5.3.2 Study population

All urinary tract infection suspected patients who referred to Wudassie Advanced Medical Laboratory during the study period and who fulfill the eligibility criteria.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

- Patients who had clinically suspected for UTI and referred to WAML.
- Patients of all age group

5.4.2 Exclusion criteria

- Patients who received antibiotics within 7 days before presentation.

5.5. Study variables

5.5.1. Dependent variables:

- Profiles of *Enterobacteriaceae*
- Antimicrobial susceptibility pattern of *Enterobacteriaceae*

- Prevalence of ESBL producing *Enterobacteriaceae*

5.5.2. Independent variables:

- Age
- Sex
- Health facility type
- Inpatient/outpatients

5.6. Sample size calculation and Sampling method

5.6.1 Sample size calculation

The sample size was calculated using a single population proportion formula by taking the prevalence (p) of ESBL Producing *Enterobacteriaceae* Isolates from Patients with Urinary Tract Infections at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia, (P= 61.7% (0.617)) [36]. Considering 95% confidence interval (Zα/2), 5% margin of error (d) the sample size was calculated using the following standard formula.

$$n = \frac{(z_{\alpha/2})^2 \cdot pq}{d^2}$$

Where,

n=minimum sample size needed

p=proportion (28%)

q= 1-p

Z=significance level at confidence interval of 95%

d=margin of error (0.05)

Z α/2=value of standard normal distribution corresponding to significant level of alpha (α) 0.05 which is 1. 96

$$n= \underline{(1.96)^2*0.617(1-0.617)} = 363$$

$(0.05)^2$

n=363, by considering 10% non-response rate,

The total sample size(n) was **400**

The final sample size was increased to **502** to improve the representativeness of the data and generalizability of the findings.

5.6.2. Sampling Method

Convenient sampling method was used.

5.7. Measurement and Data collection

5.7.1 Data collection procedure

Trained laboratory personnel's, who took training from the principal investigator employed a pre-structured laboratory data collection form to gather socio-demographic data from study participants.

5.7.2. Laboratory analysis

5.7.2.1 Urine specimen collection, handling and transport

A clean catch morning mid-stream urine specimen (20ml) was collected from patients suspected UTI using a sterile screw-capped, wide-mouth container and labeled with the unique sample number, date and time of collection. After collection the specimens were transported immediately to the microbiology laboratory at Wudassie Advanced Medical Laboratory, using an icebox, and stored at 2-80c if testing is delayed beyond 2 hours.

5.7.2.2 Culture and Bacterial identification

A small volume of urine specimen (0.001 mL) was inoculated directly onto MacConkey Agar (Oxoid, Basingstoke, UK) and 5% sheep Blood agar (Oxoid, Basingstoke, UK) using a sterile calibrated inoculating loop and the plates were incubated at 37°C for 24 hours under standard atmospheric conditions [37]. Bacterial growths of $\geq 10^5$ CFU/mL was considered significant bacteriuria. All isolate was identified by Matrix-Assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (MALDI-TOF MS) (EXS3000, Zybio Inc., China).

5.7.2.3 Principle of MALDI-TOF

The sample for analysis by MALDI-TOF MS is prepared by mixing or coating with solution of an energy-absorbent, organic compound called matrix. When the matrix crystallizes on drying, the sample entrapped within the matrix also co-crystallizes. The sample within the matrix is ionized in an automated mode with a laser beam. Desorption and ionization with the laser beam generate singly protonated ions from analytes in the sample. The protonated ions are then accelerated at a fixed potential, where these separate from each other on the basis of their mass-to-charge ratio (m/z). The charged analytes are then detected and measured using time of flight (TOF) analyzers During MALDI-TOF MS (EXS3000, Zybio Inc., China) analysis, the m/z ratio of an ion is measured by determining the time required for it to travel the length of the flight tube. Based on the TOF information, a characteristic spectrum called peptide mass fingerprint (PMF) is generated or analytes in the sample [38].

5.7.2.4 Antibiotic susceptibility testing

Antimicrobial susceptibility was done by using the Kirby-Bauer disc diffusion method and categorized as susceptible, intermediate or resistant according to CLSI 2024 guideline. Bacterial suspension adjusted to 0.5 McFarland turbidity inoculums was prepared and inoculated on Muller-Hinton Agar (MHA) plates (Oxoid LTD, Basingstoke, Hampshire, United England) and antimicrobial discs were applied to the plate. The antibiotic discs used in this study were ampicillin (AM: 10 µg), amoxicillin-clavulanic acid (AMC: 20/10 µg), ceftazidime (CAZ: 30 µg), cefotaxime (CTX: 30 µg), cefepime (FEP: 30 µg), gentamicin (GEN: 10 µg), amikacin (30 µg), nitrofurantoin (NI: 300 µg), and sulfamethoxazole-trimethoprim (SXT: 3.75/1.25 µg), ciprofloxacin (CIP: 5 µg), meropenem (MER: 10 µg).

5.7.2.5 Detection and Screening of ESBL production

ESBL production of *Enterobacteriaceae* was screened considering the zone of inhibition diameters produced by cefotaxime (30 µg) or ceftazidime (30 µg) in the disk diffusion technique. *Enterobacteriaceae* isolates with zone of inhibition diameters of ≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime were considered as likely ESBL producers according to the CLSI 2024[40].

5.7.2.6 Storing and Isolate Holding

Enterobacteriaceae isolates that tested positive during ESBL screening were sub-cultured onto 5% sheep blood agar to obtain fresh bacterial colonies for phenotypic confirmation of ESBL production. The purified isolates were then preserved in tryptic soy broth supplemented with 20% glycerol and stored at –80°C.

5.7.2.7 Confirmation of ESBL production

ESBL production among potential ESBL-producing isolates were confirmed phenotypically using combined disc method (CDT). Comparison of the zone of inhibition was made for the cefotaxime (30µg) and ceftazidime (30µg) discs alone versus that of the cefotaxime-clavulanic acid (30 µg/10 µg) and ceftazidime (30 µg) -clavulanic acid (30 µg/10 µg) placed at appropriate distance on MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight (18–24 h) at 37 °C. An increase in the inhibition zone diameter of ≥ 5 mm for a combination disc versus ceftazidime or cefotaxime disc alone was confirmed as ESBLs production as per CLSI 2024 guideline [40].

5.8. Data quality assurance

To ensure data quality, the data collection form was pre-tested and the data that that has been collected was thoroughly checked on spot and daily for their completeness, accuracy, and clarity. All of the pre-analytical, analytical and post analytical steps were carried out in strict adherence to the Standard Operating Procedures (SOPs) of the microbiology laboratory at WAML to assure the quality of laboratory results.

5.8.1. Pre analytical phase

A Socio-demographic characteristic of patients were collected using pre-structured laboratory data collection sheets. All urine culture specimens were collected by well-trained laboratory personnel by following standard operating procedure. When specimens reach the laboratory, it was checked to ensure that the correct specimen had been sent and the name on the specimen will be the same as that on specimen collection form. To avoid sample contamination leak proof and sterile sample container was used. Expiry date of medias, antibiotic disk and reagents, sterility and performance of medias and antibiotics was checked by known standard strains of *E. coli ATCC 25922*.

5.8.2 Analytical Phase

Proper aseptic techniques were used when collecting and handling urine samples. Sterility of the culture media was checked with indicator tape and by incubating 3–5% of the batch at 37 °C for

24 hours. Media performance was further evaluated by inoculating with control strains. American Type Culture Collection (ATCC) reference strains were used for ESBL detection, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively [40].

5.8.3. Post Analytical Phase

During the post-analytical phase, test results were documented using each patient's unique identification number. Reporting was adhered to a standardized format and terminology to ensure consistency and clarity. All laboratory reports were double cheeked for accuracy before being released. For long-term preservation, purified bacterial cultures were maintained tryptic soy broth supplemented with 20% glycerol at -80°C and were sub-cultured monthly to maintain viability.

5.9 Data analysis and interpretation

Data was entered using Epidata version 4.6 and analyzed using SPSS software (version 27). Descriptive statistics were employed to analyze both dependent and independent variables using frequencies and cross-tabulations. The final results were presented in narrative form, as well as through tables and graphs.

5.10 Operational and definition of terms

Multi Drug Resistance: MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories [39].

Urinary Tract Infection: is an infection that affects a part of the urinary tract [40].

5.11. Ethical considerations

Ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, and the Department of Laboratory Sciences before starting the thesis work; protocol number DRERC/783/24/MLS and permission was obtained Wudassie Advanced Medical Laboratory. To ensure confidentiality, data was linked to a study code number. Confidentiality was ensured, and unauthorized persons had no access to the data. Only the principal investigator and advisors of the principal investigator could access the data.

5.12. Dissemination of the result

The results of this study will be reported in a formal document and presented to the Department of Medical Laboratory sciences, Addis Ababa University. Besides this, the finding of the research will be presented and communicated to the international community through conferences and publication in a peer reviewed journal.

6. Results

6.1. Demographic characteristics of patients

A total of 502 patients were recruited, 55.8% (280/502) were females and the mean age of the patients was 46 years (±20.9 SD). The majority of the patients 26.7% (134/502) were in age group of 46-60 years. Most of the patients were from outpatient (56.4% ,283/502) and 81.5% (409/502) private hospitals.

Table 1: Socio-demographic characteristics, health facility type and patient type in WAML, Addis Ababa, Ethiopia from Dec 2024 to Mar 2025.

Variables		**Frequency**	**Percentage**
Sex	Male	222	44.2%
	Female	280	55.8%
Age (Years)	≤15	46	9.2%
	16-30	80	15.9%
	31-45	120	23.9%
	46-60	134	26.7%
	≥61	122	24.3%
Health facility type	Private	409	81.5%
	Public	93	18.5%
Patient tatus	Outpatient	283	56.4%
	Inpatient	219	43.6%

6.2. Frequency of *Enterobacteriaceae* isolates

Out of 502 patients who developed clinical signs of UTI, microbial growth was detected in 183 samples. Of these, 179 (97.8%) were positive for bacterial growth, while the remaining 4 (2.2%) were attributed to fungal species. Among the bacterial isolates, 158 (88.3%) were identified as gram-negative bacteria and 21 (11.7%) as gram-positive bacteria. Among total urines processed,29.4% (148/502) had a positive culture for *Enterobacteriaceae*. The majority of *Enterobacteriaceae* isolates were from 64.8% (96/148) female and 68.2% (101/148) outpatients. Among the 148 *Enterobacteriaceae* isolates, *E. coli* with 69.6% (103/148) was the most frequent isolates followed by *K. pneumoniae* with 16.8% (25/148). From the 103 *E. coli* isolates, 71.8% (74/103) were isolated from female and 72.8% (75/103) were isolated outpatient.

Table 2: Distribution of Enterobacteriaceae isolates among UTI suspected patients against demographic characteristics, health facility type and patient types in WAML, Addis Ababa, Ethiopia from Dec 2024 to May 2025

Variables	***Enterobacteriaceae***								
	E. coli	***K.pneumoniae***	***E. cloacae***	***C.freundii***	***K. oxytoca***	***P. mirabilis***	***M.morganii***	***S.marcascens***	**Total**
Age in years									
<15	7(70%)	2(20%)	0(0%)	0(0%)	1(10%)	0(0%)	0(0%)	0(0%)	10(6.7%)
16-30	14(82.3%)	2(11.7%)	0(0%)	0(0%)	1(5.8%)	0(0%)	0(0%)	0(0%)	17(11.5%)

31-45	21(70%)	4(13.0%)	3(10%)	1(3.3%)	0(0%)	0(0%)	0(0%)	1(3.3%)	30(16.8%)
46-60	22(61.1%)	10(27.7%)	0(0%)	3(8.3%)	0(0%)	1(2.7%)	0(0%)	0(0%)	36(24.3%)
>61	39(70.9%)	7(12.7%)	3(5.4%)	0(0%)	2(3.6%)	3(5.4%)	1(1.8%)	0(0%)	55(37,1%)
Gender									
Female	2(2%)	3(3.1%)	74(77%)	1(1%)	12(12.5%)	0(0%)	3(3.1%)	1(1%)	96(64.8%)
Male	2(3.8%)	3(5.7%)	29(55.7%)	3(5.7%)	13(25%)	1(1.9%)	1(1.9%)	0(0%)	52(35.2%)
Health facility type									
Private	96(70%)	22(16%)	5(3.6	4(2.9%)	4(2.9%)	4(2.9%)	1(0.7%)	1(0.7%)	137(92.5%)
Public	7(63.6%)	3(27.2%)	1(9%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	11(7.5%)
Patient status									
Outpatient	75(74.2%)	12(11.8%)	2(19%)	4(3.9%)	4(3.9%)	3(2.9%)	1(0.9%)	0(0%)	101(68.2%)

Inpatient	28(59.5%)	13(27.6%)	4(8.5%)	0(0%)	0(0%)	1(2.1%)	0(0%)	1(2.1%)	47(31.8%)

6.3. Antibiotics resistance pattern of *Enterobacteriaceae*

The antibiotics resistance pattern of *Enterobacteriaceae* isolated from urine specimens against 11 antibiotics were presented in Table 3**.** Among the antibiotics tested, ampicillin (98.6%) showed higher resistance rate, followed by amoxicillin-clavulanic acid (92.5%), trimethoprim-sulfamethoxazole (86.4%), ciprofloxacin (77.0%), cefotaxime (71.6%), ceftazidime (65.5%), and cefepime (58.1%). Gentamicin also showed a notable resistance rate of 43.4%. In contrast, meropenem (12.8%), amikacin (25%), and nitrofurantoin (36.4%) had lower resistance rates. Out of 148 Enterobacteriaceae isolates, 122(82.2%) were multidrug-resistant, and 8.1% (12) showed resistance to all tested antibiotics.

Among the tested antibiotics, *Klebsiella pneumoniae* exhibited the highest resistance to cefotaxime (92.0%), cefepime (88.0%), and ceftazidime (92.0%). High resistance was also noted against amoxicillin-clavulanic acid (100%) and gentamicin (80.0%), although it showed relatively lower resistance rates to meropenem (56.0%) and amikacin (56.0%). In contrast, *Escherichia coli* showed resistance, to sulfamethoxazole-trimethoprim (83.4%), followed by amoxicillin-clavulanic acid (89.3%) and ciprofloxacin (72.8%). Resistance to third and fourth-generation cephalosporins was also substantial—70.8% to cefotaxime, 53.3% to cefepime, and 63.1% to ceftazidime. The most effective antibiotics against *E. coli* were meropenem and amikacin, with resistance of 2.9% and 17.4%, respectively.

Table 3: Distribution of antibiotics resistance level of Enterobacteriaceae isolated from UTI patients at WAML, Addis Ababa, Ethiopia from Dec2024 to Mar 2025.

Isolates (number)	**Distribution of antibiotics resistance among *Enterobacteriaceae* isolates (n (%))**										
	AMP	**AMC**	**CAZ**	**CTX**	**FEP**	**NI**	**AK**	**GEN**	**CIP**	**SXT**	**MER**
***E. coli* (*n* = 103)**	101 (98.0 %)	92 (89.3 %)	65 (66.0 %)	73 (60.8 %)	55 (53.3 %)	26 (25.2 %)	18 (17.4 %)	32 (31.0 %)	75 (72.8 %)	86 (83.4 %)	3 (2.9%)
***K. pneumoniae* (*n* = 25)**	25 (100 %)	25 (100 %)	23 (92.0 %)	23 (92.0 %)	22 (88.0 %)	18 (81.8 %)	14 (56.0 %)	20 (80.0 %)	23 (92.0 %)	23 (92.0 %)	14 (56.4 %)
***E. cloacae* (n = 6)**	6 (100 %)	6 (100 %)	2 (33.3 %)	2 (33.3 %)	2 (33.3 %)	2 (33.3 %)	2 (33.3 %)	2 (33.3 %)	4 (66.6 %)	5 (83.3 %)	2 (33.3 %)
***C. freundii* (*n* = 4)**	4 (100 %)	4 (100 %)	1 (25.0 %)	1 (25.0 %)	2 (50.0 %)	1 (25.0 %)	0 (0.0%)	0 (0.0%)	4 (100 %)	0 (0.0%)	0 (0.0%)
***K. oxytoca* (*n* = 4)**	4 (100 %)	4 (100 %)	3 (75.0 %)	4 (100 %)	3 (75.0 %)	3 (75.0 %)	2 (50.0 %)	3 (75.0 %)	3 (75.0 %)	4 (100 %)	0 (0.0%)
***P. mirabilis* (*n* = 4)**	4 (100 %)	4 (100 %)	2 (50.0 %)	2 (50.0 %)	1 (25.0 %)	4 (100 %)	1 (25.0 %)	2 (50.0 %)	3 (75.0 %)	4 (100 %)	0 (0.0%)
***M. morganii* (n = 1)**	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	0 (0.0%)	1 (100 %)	1 (100 %)	1 (100 %)	0 (0.0%)

***S.marcasecens* (*n* = 1)**	1 (100 %)	1 (100 %)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100 %)	0 (0.0%)	1 (100 %)	1 (100 %)	1 (100 %)	0 (0.0%)
Total Resistance (N =148)	146 (98.6 %)	137 (92.5 %)	97 (65.5 %)	106 (71.6 %)	86 (58.1 %)	54 (36.4 %)	37 (25.0 %)	61 (41.2 %)	114 (77.0 %)	128 (86.4 %)	19 (12.8 %)

Abbreviations: AMC: amoxicillin-clavulanic acid; AMP: ampicillin; CIP: ciprofloxacin; GEN: gentamicin; STX: trimethoprim-sulfamethoxazole; NI: nitrofurantoin; CAZ: ceftazidime; CEP: cefepime; AK: amikacin; CXT: cefotaxime; MEM: meropenem.

6.4. Multi-drug resistant *Enterobacteriaceae*

Overall, 82.2% (122/148) of the *Enterobacteriaceae* isolates were MDR. Among this E. *coli* and *K. pneumoniae* contributed to 54.7% (81/148) and 16.2% (24/148) of the observed MDR, respectively. The highest MDR level were observed among in *K. oxytoca*, *C. freundii*, and *M. morganii*, with all isolates (100%), followed by *K. pneumoniae* (96.0%, 24/25), *E. coli* (78.6%, 81/103), *P. mirabilis* (75%, 3/4) and *E. cloacae* (50.0%, 2/4). *S. marcascens* was found to be non-MDR. Only 1.4% (2/148) of the *Enterobacteriaceae* were susceptible for all antibiotics tested (Table 3). From all MDR *Enterobacteriaceae,* the predominant were *E. coli* (54.7%; 81/148) and *K. pneumoniae* (16.2%, 24/148).

Table 4: Multidrug resistance level of Enterobacteriaceae among UTI patients to different classes of antibiotics, at WAML, Addis Ababa, Ethiopia from Dec 2024 to Mar 2025.

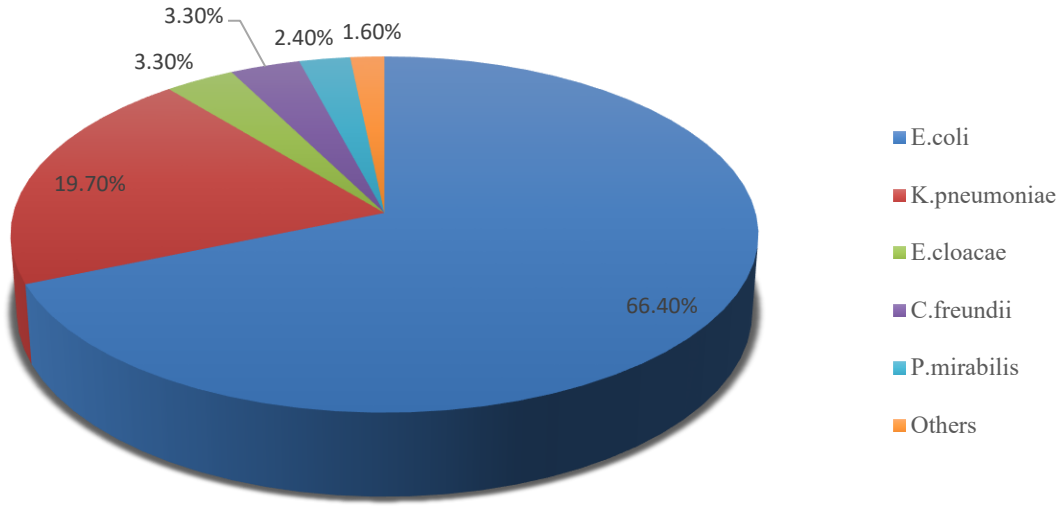
isolates (number)	Level of antibiotics resistance ((number (%))								Total MDR-E (>R3)
	R0	R1	R2	R3	R4	R5	R6	R7	
***E. coli* (103)**	2(1.9%)	11(10.7%)	9(8.7%)	14(13.5%)	41(39.8%)	18(17.4%)	7(6.7%)	1(0.9%)	81(78.6%)
***K. pneumoniae* (25)**	0(0.0%)	1(4.0%)	0(0.0%)	2(8.0%)	2(8.0%)	4(16.0%)	7(28.0%)	9(36.0%)	24(96.0%)
***E.cloacae* (4)**	0(0.0%)	1(25.0%)	1(25.0%)	2(50.0%)	0(0.0%0	0(0.0%)	0(0.0%)	2(50.0%)	2(50.0%)
***C.freundii* (4)**	0(0.0%)	0(0.0%)	0(0.0%)	2(50.0%)	2(50.0%)	0(0.0%)	0(0.0%)	2(50.0%)	4(100.0%)
***K.oxytoca* (4)**	0(0.0%)	0(0.0%)	0(0.0%)	1(25.0%)	0(0.0%)	2(50.0%)	1(25.0%)	0(0.0%)	4(100.0%)
P. mirabilis (4)	0(0.0%)	0(0.0%)	1(25.0%)	0(0.0%)	1(25.0%)	1(25.0%)	1(25.0%)	0(0.0%)	3(75.0%)
***M. morganii* (1)**	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(100.0%)	0(0.0%)	0(0.0%)	1(100.0%)
***S. marcascens* (1)**	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Total (N = 148)	2(1.3%)	13(8.7%)	11(7.4%)	21(14.1%)	47(31.7%)	26(17.5%)	16(10.8%)	12(8.1%)	122(82.4%)

R0-Absence of resistance to any antibiotic category, R1- resistance to one antibiotic category, R2- resistance to two antibiotic categories, R3- resistance to three antibiotic categories, R4- resistance

to four antibiotics category, R5- resistance to five antibiotic categories, R6 -resistance to six antibiotic category and R7- is resistance to seven antibiotic categories used for treatment

Out of a total of 122 Multidrug-resistant (MDR) *Enterobacteriaceae* isolates, the most frequently identified species was *Escherichia coli*, accounting for 66.4% (81/122) followed by *Klebsiella pneumoniae* 19.7% (24/122), *Citrobacter freundii* 3.3% (4/122), *Enterobacter cloacae* 3.3% (4/122), *Klebsiella oxytoca* 3.3% (4/122), and *Proteus mirabilis* 2.4% (3/122). The remaining *Enterobacteriaceae* isolates compromising 1.6% (2/142).

Figure 1



Others -*S. marcascens and M. morganni*

Figure 1: Distribution of major MDR isolate among the total MDR Enterobacteriaceae among UTI patients at WAML, Addis Ababa, Ethiopia from Dec 2024 to Mar 2025

6.5. Magnitude of ESBL producing *Enterobacteriaceae*

Among *Enterobacteriaceae* isolates, 72.3% (107/148) were positive for the screening test of ESBL production. Of the 107 screening positive isolates, 97 (90.6%) were confirmed ESBL producers by the combination disk test, resulting in an overall ESBLs positivity of 65.5% (97/148) (Table 5).

Table 5: Distribution of major ESBL-producing Enterobacteriaceae and MDR among UTI patients in type of patient at WAML, Addis Ababa, Ethiopia from Dec 2024 to March 2025.

Isolate collection Site	**ESBL-producing *Enterobacteriaceae* (n (%))**	**Major ESBL-producing *Enterobacteriaceae***		**MDR *Enterobacteriaceae* (n (%))**
		***E. coli* (n (%))**	***K.pneumoniae* (n (%))**	
Outpatient	64.3% (65/101)	61.3% (46/75)	91.6% (11/12)	77.2% (78/101)
Inpatient	68.0% (32/47)	64.2% (18/28)	92.3% (12/13)	93.6% (44/47)
Total	65.5% (97/148)	62.1% (64/103)	92.0% (23/25)	82.4% (122/148)

ESBL production showed notable variation across *Enterobacteriaceae* species. *Klebsiella oxytoca (4/4)* and *Morganella morganii* (1/1) exhibited the highest intra-species ESBL production rates, with both showing 100% positivity. This was followed closely by *Klebsiella pneumoniae*, where 92.0% (23/25) of isolates were ESBL producers. Moderate levels of ESBL production were seen in *Escherichia coli* and *Proteus mirabilis*, with 62.1% (64/103) and 50% (2/4), respectively. On the other hand, lower frequencies were observed among *Enterobacter cloacae* and *Citrobacter* species, with 33.3% (2/6) and 25% (1/4) ESBL-positive isolates, respectively (Figure 2). A significantly higher proportion of MDR was found among ESBL-producing Enterobacteriaceae (95.8%; 93/97) than in non-ESBL-producing isolates (54.9%; 28/51).

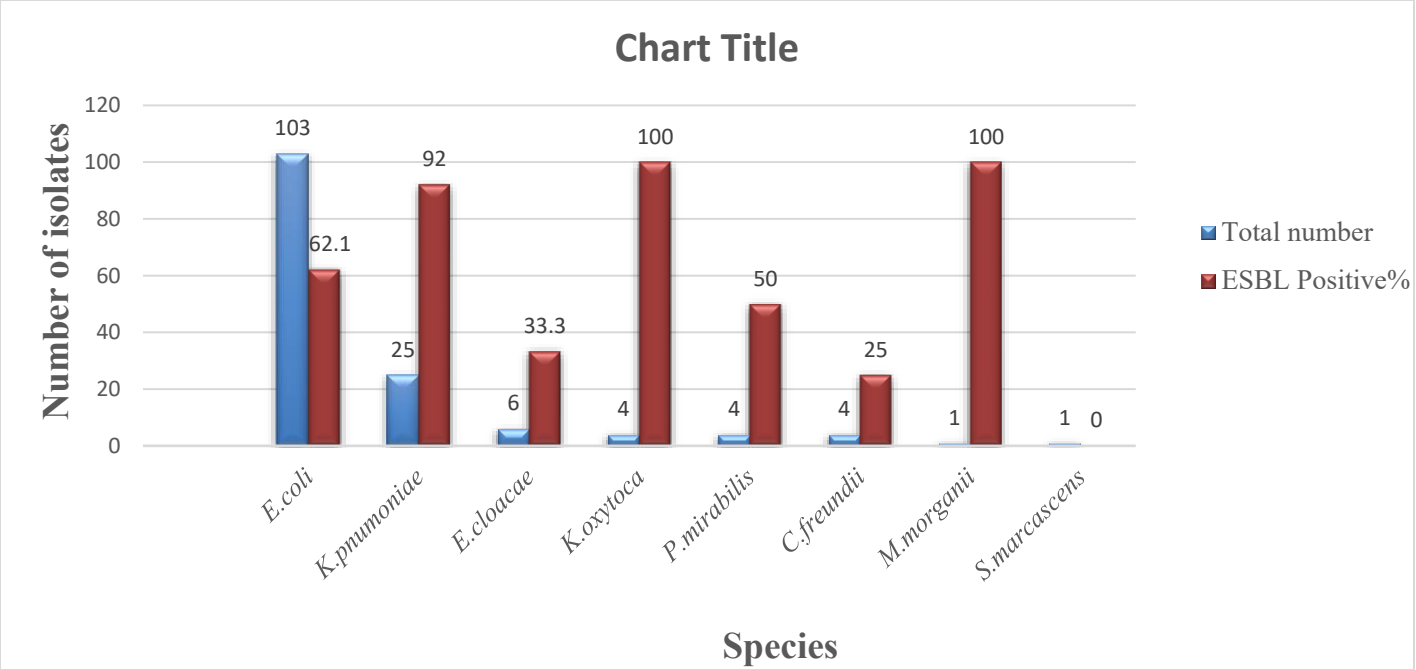
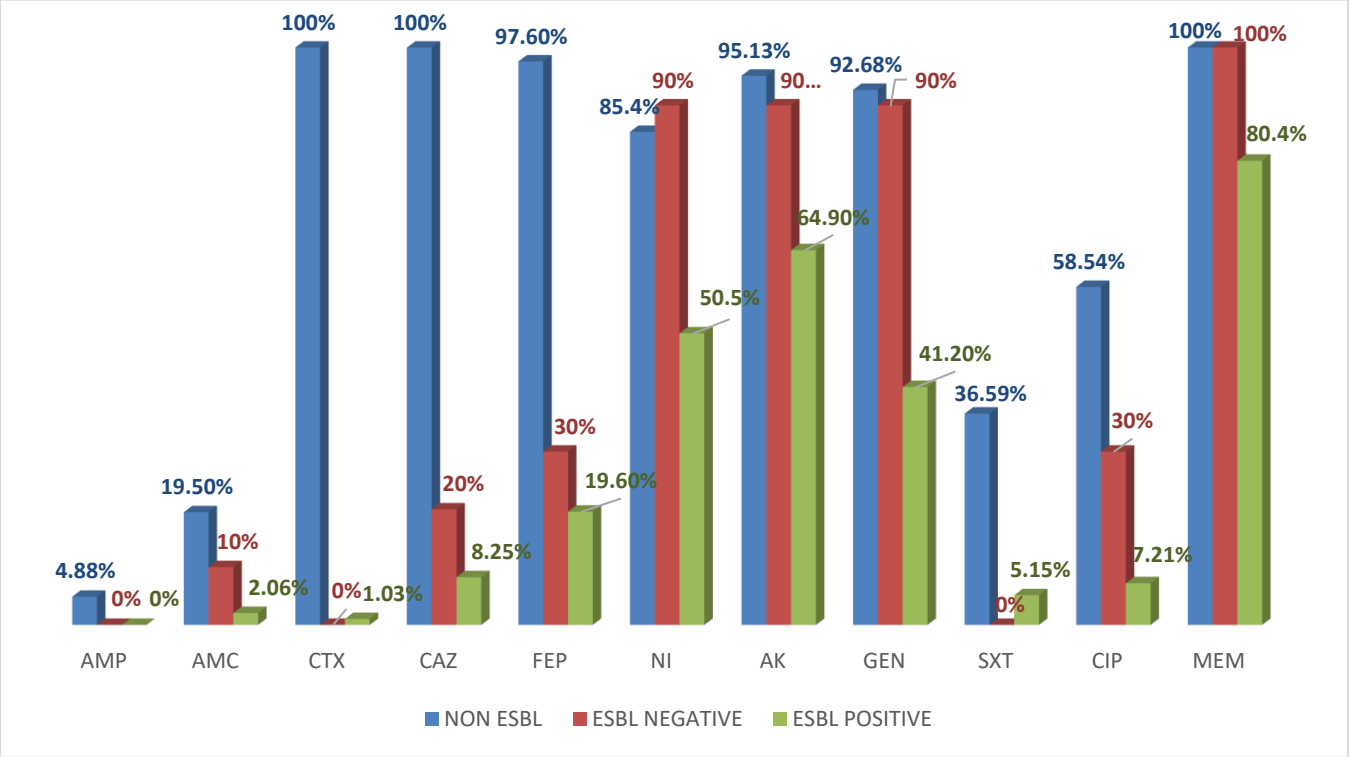


Figure 2: Frequency of ESBL producing *Enterobacteriaceae* among UTI patients at WAML from Dec 2024 to Mar 2025

6.6. Antibiotics susceptibility pattern of ESBLs-E

Meropenem (80.4%), amikacin (64.9%), and nitrofurantoin (50.5%) were the most effective antibiotics against ESBL-producing *Enterobacteriaceae.*Gentamicin, ciprofloxacin, and cotrimoxazole showed lower susceptibility, with only 41.2%, 7.2%, and 5.2% respectively. In contrast, non-ESBL-suspicious isolates (those ESBL screening negative showed higher sensitivity to meropenem (100%), amikacin (95.1%) and gentamicin (92.7%). Additionally, nitrofurantoin and ciprofloxacin were effective against non-ESBL isolates with sensitivity of 85.4% and 58.6% respectively (Figure 3).

Figure 3



Abbreviations: AMC: amoxicillin/clavulanate; AMP: ampicillin; CIP: ciprofloxacin; GEN: gentamicin; STX: trimethoprim-sulfamethoxazole; NI: nitrofurantoin; CIP: ciprofloxacin; CTX: cefotaxime; CAZ*: ceftazidime;* AK: *amikacin;* FEP: cefepime; MEM: meropenem

Figure 3: Antibiotics susceptibility pattern of ESBLs-positive, ESBLs-negative and Non-ESBLs *Enterobacteriaceae* among UTI patients at WAML between Dec 2024 to Mar2025.

7. Discussion

ESBL-producing Enterobacteriaceae has become a critical global concern. The spread of these enzymes significantly reduces the effectiveness of broad-spectrum antibiotics, leading to serious treatment challenges and influencing patient outcomes.

In this study, the prevalence of ESBL producing *Enterobacteriaceae* was 65.5%, which aligns closely with findings from Ethiopia(67.0%)[43], Tanzania(64%)[44], Cameroon(64.7%)[45] and Uganda (64.9%)[46], Compared to a previous study, the prevalence of ESBL production found in the current study is notably lower than from a study in Bahir dar, Ethiopia (85.8%) [47].The ESBL prevalence was also lower than those reported in Egypt(70%)[48], this high prevalence might be attributed to the fact that the study was conducted on clinical samples ,which are more likely to yield resistant strains..

On the contrary, this study reported a higher prevalence of ESBL-producing isolates compared to other findings from Addis Ababa (57.7%) [49], Adama (25%) [50], Chad (47.7%) [51], South Africa (59.0%) [52], and Sudan (45.2%) [53], suggesting a rising trend in the spread of ESBL-producing Enterobacteriaceae across Africa, which may be attributed to the frequent empirical use of third-generation cephalosporins, However, lower prevalence rates have been reported in developed regions like USA, Europe [54], Australia [55], and several Asian countries [56, 57].This may be attributed to variations in infection prevention protocols, duration of hospitalization and enhanced nursing practices that effectively reduce the transmission and acquisition of ESBL-producing strains. .

Similar to previous finding, *E. coli* remained the predominant uropathogen, with *K. pneumoniae* being the second most common [43]. Although *E. coli* was the most commonly isolated organism (69.5%), *K. pneumoniae* exhibited the highest rate of ESBL production (92.0%), with *E. coli* at 62.1%. A similar trend was observed in studies conducted in Ethiopia [58,59]. However, other studies from Ethiopia reported *E. coli* as the predominant ESBL producer compared to *K. pneumoniae*—with rates of 58% for *E. coli* and 23% for *K. pneumoniae* [60]. Comparable findings were also reported in Cameroon (*E. coli* 82%, *K. pneumoniae* 74.3%) [61] and Iraq (*E. coli* 71.7%, *K. pneumoniae* 26.7%) [62].

The prevalence of ESBL-E was notably higher in healthcare-associated isolates compared to those from the community, consistent with findings from studies in Ghana and Rwanda [63, 64]. This may be attributed to well-established risk factors commonly present in hospital settings, such as prolonged hospital stays, underlying comorbidities, prior antibiotic exposure, and the use of urinary catheters. The detection of ESBL-producing isolates in community-acquired urinary infections is concerning due to the limited therapeutic options, as these strains are often multidrug-resistant.

In this study, the highest level of resistance was observed against ampicillin (98.6%), followed by amoxicillin-clavulanic acid (92.5%), sulfamethoxazole-trimethoprim (86.4%), ciprofloxacin (77.0%), cefotaxime (71.6%), ceftazidime (65.5%), and cefepime (58.1%). These findings are consistent with previous reports from Addis Ababa, Ethiopia [46]. High levels of resistance were observed in *Escherichia coli* against sulfamethoxazole-trimethoprim (77.6%), amoxicillin-clavulanic acid (70.0%), and ciprofloxacin (64.0%) and also resistance to third- and fourth-generation cephalosporins was notable, with rates of 54.8% for cefotaxime, 53.5% for cefepime, and 53.1% for ceftazidime. Similar findings have been reported in earlier research carried out in Addis Ababa, Ethiopia [22], Nigeria [65] and Tanzania [66].

In this study, multidrug resistance (MDR) was detected in 93 (95.8%) of the ESBL-producing isolates, compared to only 28 (54.9%) of the non-ESBL producers, which aligns closely with findings from Addis Ababa, Ethiopia [11]. This indicates that ESBL-producing strains exhibit significantly higher resistance levels, highlighting a greater burden of MDR among these isolates.

The current study found that ESBL-producing *Enterobacteriaceae* isolates were most susceptible to meropenem (80.4%), followed by amikacin (64.9%) and nitrofurantoin (50.5%). These results align with previous findings from Addis Ababa, Ethiopia [22] and Nigeria [65]. This suggests meropenem, amikacin, and nitrofurantoin appear to remain effective choices for treating infections due to ESBL-producing *Enterobacteriaceae*.

8. Strengths of the study

- ✓ A major strength of this study lies in the use of MALDI-TOF for bacterial identification. This advanced diagnostic method enabled accurate and rapid identification of *Enterobacteriaceae* isolates at the species level, enhancing the reliability of the microbiological findings.
- ✓ The magnitude of ESBLs and MDR in the city was done in a relatively larger number of urine specimens and isolates than in earlier studies

9. Limitations of the study

- ✓ This study lacked detailed demographic and clinical data of the participants, which limited the ability to assess risk factors and clinical outcomes associated with multidrug-resistant and ESBL-producing *Enterobacteriaceae.*

10. Conclusion

The study found a significant prevalence of ESBL-producing and multidrug-resistant *Enterobacteriaceae* among patients suspected of having UTIs, especially in hospitalized patients and those attending private hospitals. The most common ESBL-producing bacteria were *Klebsiella pneumoniae* and *Escherichia coli*. These ESBL producers demonstrated much higher resistance rates to several antibiotics, including trimethoprim-sulfamethoxazole, ciprofloxacin, cefepime, and gentamicin, compared to non-ESBL-producing strains. Meropenem, amikacin, and nitrofurantoin showed better activity against these resistant bacteria.

11. Recommendation

The increasing prevalence of multidrug-resistant (MDR) organisms and extended-spectrum beta-lactamases (ESBLs) highlights the urgent need to advance clinical bacteriology research and enhance laboratory diagnostic capacities for accurate detection and surveillance of antimicrobial resistance. Therefore, routine screening of ESBL-producing *Enterobacteriaceae*, along with strong infection prevention and control measures. In addition, raising awareness about antimicrobial resistance through comprehensive education programs, enforcing rigorous antimicrobial stewardship practices, and promoting the judicious use of antibiotics by healthcare providers are essential. Moreover, researchers should focus on identifying the dominant ESBL genes circulating in Ethiopia to gain deeper insights into local resistance patterns and mechanisms.

References

1. Tesfa T, Baye Y, Sisay M, Amare F, Gashaw T. Bacterial uropathogens and susceptibility testing among patients diagnosed with urinary tract infections at Hiwot Fana Specialized University Hospital, Eastern Ethiopia. SAGE open medicine. 2021; 9:20503121211001162.
2. Grabe M, Bartoletti R, Bjerklund Johansen TE, Cai T, Çek M, Köves B*, et al*., Guidelines on urological infections. European Association of Urology; 2015. Подготовила Мария Арефьева по материалам сайта: http://www. infectweb. com/only/lec_mem_19/naber. html. 2015.
3. Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. International journal of general medicine. 2011 19:333-43.
4. Nielubowicz GR, Mobley HL. Host–pathogen interactions in urinary tract infection. Nature Reviews Urology. 2010 ;7(8):430-41.
5. Tekele SG, Teklu DS, Tullu KD, Birru SK, Legese MH. Extended-spectrum Beta-lactamase and AmpC beta-lactamases producing gram negative bacilli isolated from clinical specimens at International Clinical Laboratories, Addis Ababa, Ethiopia. PLoS One. 2020;15(11): e0241984.
6. Benyagoub E, Alkhudhairy MK, Benchaib SM, Zaalan A, Mekhfi Y, Teyebi N, *et al*., Isolation frequency of uropathogenic strains and search for ESBL producing Enterobacteriaceae isolated from patients with UTI in Bechar (Algeria). Anti-Infective Agents. 2021;19(3):303-16.
7. Fares R, Debabza M, Mechai A. Detection and prevalence of extended spectrum β-lactamases production among Enterobacteriaceae isolated from urinary tract infections. Biosystems Diversity. 2023 ;31(2):163-9.
8. Kunishima H, Ishibashi N, Wada K, Oka K, Takahashi M, Yamasaki Y, Aoyagi T, Takemura H, Kitagawa M, Kaku M. The effect of gut microbiota and probiotic organisms on the properties of extended spectrum beta-lactamase producing and carbapenem resistant Enterobacteriaceae including growth, beta-lactamase activity and gene transmissibility. Journal of Infection and Chemotherapy. 2019 Nov 1;25(11):894-900.

9. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrobial agents and chemotherapy. 1995 ;39(6):1211-33.
10. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrobial agents and chemotherapy. 1995 ;39(6):1211-33.
11. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrobial Resistance & Infection Control. 2019; 8:1-2.
12. Khadka C, Shyaula M, Syangtan G, Bista S, Tuladhar R, Singh A, *et al*., Extended-spectrum β-lactamases producing Enterobacteriaceae (ESBL-PE) prevalence in Nepal: A systematic review and meta-analysis. Science of the Total Environment. 2023 :166164.
13. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. American journal of infection control. 2006 ;34(5): S20-8.
14. Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. Clinical microbiology reviews. 2018 ;31(2):10-128.
15. Shyaula M, Khadka C, Dawadi P, Banjara MR. Systematic review and Meta-analysis on extended-spectrum β-lactamases producing Klebsiella pneumoniae in Nepal. Microbiology insights. 2023; 16:11786361221145179.
16. Thenmozhi S, Moorthy K, Sureshkumar BT, Suresh M. INTERNATIONAL JOURNAL OF PURE &APPLIED BIOSCIENCE. Int. J. Pure App. Biosci. 2014;2(3):207-26.
17. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, Van Duin D, Clancy CJ. Infectious Diseases Society of America guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with difficult-to-treat resistance (DTR-P. aeruginosa). Clinical Infectious Diseases. 2021;72(7): e169-83.
18. Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade KA, Kolawole CF. Prevalence of urinary tract infections (UTI) among patients attending

Dalhatu Araf Specialist Hospital, Lafia, Nasarawa state, Nigeria. International journal of medicine and medical sciences. 2009;1(5):163-7.

19. Öztürk R, Murt A. Epidemiology of urological infections: a global burden. World journal of urology. 2020; 38:2669-79.
20. Abayneh, M., Tesfaw, G. and Abdissa, A., 2018. Isolation of Extended-Spectrum β-lactamase-(ESBL-) Producing Escherichia coli and Klebsiella pneumoniae from Patients with Community-Onset Urinary Tract Infections in Jimma University Specialized Hospital, Southwest Ethiopia. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2018*(1), p.4846159.
21. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrobial Resistance & Infection Control. 2019; 8:1-2.
22. Bitew A, Tsige E. High Prevalence of Multidrug-Resistant and Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: A Cross-Sectional Study at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia. Journal of tropical medicine. 2020;2020(1):6167234.
23. Ramatla, T., Mafokwane, T., Lekota, K., Monyama, M., Khasapane, G., Serage, N., *et al*., 2023. "One Health" perspective on prevalence of co-existing extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae: a comprehensive systematic review and meta-analysis. *Annals of Clinical Microbiology and Antimicrobials*, *22*(1), p.88.
24. Onduru OG, Mkakosya RS, Aboud S, Rumisha SF. Genetic determinants of resistance among ESBL-producing enterobacteriaceae in community and hospital settings in east, central, and Southern Africa: A systematic review and meta-analysis of prevalence. Canadian Journal of Infectious Diseases and Medical Microbiology. 2021;2021(1):5153237
25. Khadka C, Shyaula M, Syangtan G, Bista S, Tuladhar R, Singh A*, et al*., Extended-spectrum β-lactamases producing Enterobacteriaceae (ESBL-PE) prevalence in Nepal: A systematic review and meta-analysis. Science of the Total Environment. 2023:166164.

26. Asmare Z, Erkihun M, Abebe W, Tamrat E. Antimicrobial resistance and ESBL production in uropathogenic Escherichia coli: a systematic review and meta-analysis in Ethiopia. JAC-Antimicrobial Resistance. 2024;6(3): dlae068.
27. Eltai, N.O., Al Thani, A.A., Al-Ansari, K. *et al.* Molecular characterization of extended spectrum β -lactamases enterobacteriaceae causing lower urinary tract infection among pediatric population. *Antimicrob Resist Infect Control* **7**, 90 (2018). https://doi.org/10.1186/s13756-018-0381-6\
28. Raya GB, Dhoubhadel BG, Shrestha D, Raya S, Laghu U, Shah A,. Multidrug-resistant and extended-spectrum *et al.,* beta-lactamase-producing uropathogens in children in Bhaktapur, Nepal. Trop Med Health. 2020; 48:65. doi: 10.1186/s41182-020-00251-6. PMID: 32774128; PMCID: PMC7397599.
29. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL production among E. coli and Klebsiella spp. causing urinary tract infection: a hospital-based study. The open microbiology journals. 2017; 11:23.
30. Moglad EH. Antibiotics Profile, Prevalence of Extended-Spectrum Beta-Lactamase (ESBL), and Multidrug-Resistant Enterobacteriaceae from Different Clinical Samples in Khartoum State, Sudan. International Journal of Microbiology. 2020;2020(1):8898430.
31. Djim-Adjim-Ngana K, Mbiakop BW, Oumar LA, Munshili Njifon HL, Tchinda Fossi C, Enyegue EL, *et al.*, Phenotypic characterization and epidemiology of extended-spectrum β-lactamase-producing Enterobacteriaceae strains from urinary tract infections in Garoua, Cameroon. Frontiers in Public Health. 2023; 11:11870934.
32. Mohamed ES, Khairy RM, Abdelrahim SS. Prevalence and molecular characteristics of ESBL and AmpC β-lactamase producing Enterobacteriaceae strains isolated from UTIs in Egypt. Antimicrobial Resistance & Infection Control. 2020; 9:1-9.
33. Fares R, Debabza M, Mechai A. Detection and prevalence of extended spectrum β-lactamases production among Enterobacteriaceae isolated from urinary tract infections. Biosystems Diversity. 2023;31(2):163-9.
34. Abayneh M, Tesfaw G, Abdissa A. Isolation of Extended-Spectrum β-lactamase-(ESBL-) Producing Escherichia coli and Klebsiella pneumoniae from Patients with Community-Onset Urinary Tract Infections in Jimma University Specialized Hospital, Southwest

Ethiopia. Canadian Journal of Infectious Diseases and Medical Microbiology. 2018;2018(1):4846159.

35. Agegnehu A, Worku M, Nigussie D, Lulu B, Tadesse BT. Pediatric febrile urinary tract infection caused by ESBL producing Enterobacteriaceae species. BioMed research international. 2020;2020(1):6679029.
36. Seman A, Sebre S, Awoke T, Yeshitela B, Asseffa A, Asrat D, *et al*., The magnitude of carbapenemase and ESBL producing Enterobacteriaceae isolates from patients with urinary tract infections at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia. InAdvances in Microbiology, Infectious Diseases and Public Health: Volume 16 2021 (pp. 117-128). Cham: Springer International Publishing.
37. Karah N, Rafei R, Elamin W, Ghazy A, Abbara A, Hamze M, *et al*., Guideline for urine culture and biochemical identification of bacterial urinary pathogens in low-resource settings. Diagnostics. 2020 ;10(10):832.
38. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol. 2015; 6:791. doi: 10.3389/fmicb.2015.00791. PMID: 26300860; PMCID: PMC4525378
39. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al*., Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012;18(3):268-81.
40. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA, 2024.
41. Michael T Sweeney, Brian V Lubbers, Stefan Schwarz, Jeffrey L Watts, Applying definitions for multidrug resistance, extensive drug resistance and pan drug resistance to clinically significant livestock and companion animal bacterial pathogens, Journal of Antimicrobial Chemotherapy, Volume 73, Issue 6, 2018, Pages 1460–1463,
42. WHO. Antimicrobial Resistance. Fact Sheet-2017. [Cited 2018 Nov 21]; Available from: http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en
43. Beyene D, Bitew A, Fantew S, Mihret A, Evans M. Multidrug-resistant profile and prevalence of extended spectrum β-lactamase and carbapenemase production in

fermentative Gram-negative bacilli recovered from patients and specimens referred to National Reference Laboratory, Addis Ababa, Ethiopia. PloS one. 2019 25;14(9):e0222911.

44. Moremi N, Claus H, Rutta L, Frosch M, Vogel U, Mshana SE. High carriage rate of extended-spectrum beta-lactamase-producing Enterobacteriaceae among patients admitted for surgery in Tanzanian hospitals with a low rate of endogenous surgical site infections. Journal of Hospital Infection. 2018 1;100(1):47-53.
45. Bayaba S, Founou RC, Tchouangueu FT, Dimani BD, Mafo LD, Nkengkana OA, Founou LL, Noubom M. High prevalence of multidrug resistant and extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections in the West region, Cameroon. BMC Infectious Diseases. 2025 24;25(1):115.
46. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. BMC pharmacology and Toxicology. 2015; 16:1-6.
47. Moges F, Eshetie S, Abebe W, Mekonnen F, Dagnew M, Endale A, Amare A, Feleke T, Gizachew M, Tiruneh M. High prevalence of extended-spectrum beta-lactamase-producing Gram-negative pathogens from patients attending Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Amhara region. PloS one. 2019 15;14(4): e0215177.
48. Mohamed ES, Khairy RM, Abdelrahim SS. Prevalence and molecular characteristics of ESBL and AmpC β-lactamase producing Enterobacteriaceae strains isolated from UTIs in Egypt. Antimicrobial Resistance & Infection Control. 2020; 9:1-9.
49. Tekele SG, Teklu DS, Tullu KD, Birru SK, Legese MH. Extended-spectrum Beta-lactamase and AmpC beta-lactamases producing gram negative bacilli isolated from clinical specimens at International Clinical Laboratories, Addis Ababa, Ethiopia. PLoS One. 2020 12;15(11): e0241984.
50. Mulisa G, Selassie L, Jarso G, Shiferew T, Zewdu A, Abebe W, Belachew F, Sewunet T. Prevalence of extended Spectrum Beta-lactamase producing Enterobacteriaceae: a cross sectional study at Adama hospital, Adama, Ethiopia. J Emerg Infect Dis. 2016;1(1):1-6.

51. Ouchar Mahamat O, Lounnas M, Hide M, Dumont Y, Tidjani A, Kamougam K, Abderrahmane M, Benavides J, Solassol J, Bañuls AL, Jean-Pierre H. High prevalence and characterization of extended-spectrum ß-lactamase producing Enterobacteriaceae in Chadian hospitals. BMC infectious diseases. 2019; 19:1-7.
52. Peirano G, van Greune CH, Pitout JD. Characteristics of infections caused by extended-spectrum β-lactamase–producing Escherichia coli from community hospitals in South Africa. Diagnostic microbiology and infectious disease. 2011 ;69(4):449-53.
53. Dirar M, Bilal N, Ibrahim ME, Hamid M, Hamid ME. Resistance patterns and phenotypic detection of β-lactamase enzymes among Enterobacteriaceae isolates from referral hospitals in Khartoum State, Sudan. Cureus. 2020 ;12(3).
54. Hoban DJ, Lascols C, Nicolle LE, Badal R, Bouchillon S, Hackel M, Hawser S. Antimicrobial susceptibility of Enterobacteriaceae, including molecular characterization of extended-spectrum beta-lactamase–producing species, in urinary tract isolates from hospitalized patients in North America and Europe: results from the SMART study 2009–2010. Diagnostic microbiology and infectious disease. 2012 ;74(1):62-7.
55. Chua KY, Stewardson AJ. Individual and community predictors of urinary ceftriaxone-resistant Escherichia coli isolates, Victoria, Australia. Antimicrobial Resistance & Infection Control. 2019; 8:1-1.
56. Azargun R, Sadeghi MR, Hossein M, Barhaghi S, Kafil HS, Yeganeh F, Oskouee MA, Ghotaslou R. The prevalence of plasmid-mediated quinolone resistance and ESBL-production in Enterobacteriaceae isolated from urinary tract infections. Infection and drug resistance. 2018 :1007-14.
57. Eltai NO, Al Thani AA, Al-Ansari K, Deshmukh AS, Wehedy E, Al-Hadidi SH, Yassine HM. Molecular characterization of extended spectrum β-lactamases enterobacteriaceae causing lower urinary tract infection among pediatric population. Antimicrobial Resistance & Infection Control. 2018; 7:1-9.
58. Siraj SM, Ali S, Wondafrash B. Extended-spectrum β-lactamase production in Klebsiella pneumoniae and Escherichia coli at Jimma University specialized hospital, south-west, Ethiopia. Molecular Microbiology Research. 2015 23;5.
59. Desta K, Woldeamanuel Y, Azazh A, Mohammod H, Desalegn D, Shimelis D, Gulilat D, Lamisso B, Makonnen E, Worku A, Mannerqvist K. High gastrointestinal colonization

rate with extended-spectrum β-lactamase-producing Enterobacteriaceae in hospitalized patients: emergence of carbapenemase-producing K. pneumoniae in Ethiopia. PloS one. 2016 ;11(8): e0161685.

60. Seman A, Sebre S, Awoke T, Yeshitela B, Asseffa A, Asrat D, Abebe T, Mihret A. The magnitude of carbapenemase and ESBL producing Enterobacteriaceae isolates from patients with urinary tract infections at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia. InAdvances in Microbiology, Infectious Diseases and Public Health: Volume 16 2021:17 (pp. 117-128). Cham: Springer International Publishing.
61. Verma S, Kalyan RK, Gupta P, Khan MD, Venkatesh V. Molecular characterization of extended spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae isolates and their antibiotic resistance profile in health care-associated urinary tract infections in North India. Journal of Laboratory Physicians. 2023 ;15(02):194-201.
62. Abubaker KT, Anwar KA. Antimicrobial susceptibility and integrons detection among extended-spectrum β-lactamase producing Enterobacteriaceae isolates in patients with urinary tract infection. PeerJ. 2023 ;11: e15429.
63. Obeng-Nkrumah N, Twum-Danso K, Krogfelt KA, Newman MJ. High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. The American journal of tropical medicine and hygiene. 2013;89(5):960.
64. Muvunyi CM, Masaisa F, Bayingana C, Mutesa L, Musemakweri A, Muhirwa G, Claeys GW. Decreased susceptibility to commonly used antimicrobial agents in bacterial pathogens isolated from urinary tract infections in Rwanda: need for new antimicrobial guidelines. The American journal of tropical medicine and hygiene. 2011 ;84(6):923.
65. Umar, U. Y., Giwa, F. J., Ibrahim, A., & Gachi, F. S. (2023). Antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing uropathogens in aminu Kano teaching hospital, Northwestern Nigeria. *Annals of African medicine*, *22*(4), 508–514. https://doi.org/10.4103/aam.aam_155_22
66. Moyo SJ, Aboud S, Kasubi M, Maselle SY. Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at Muhimbili National Hospital in Tanzania. Tanzania Journal of Health Research. 2010 Sep 15;12(4):233-6.

Annexes

Annex I: SOP for urine sample collection

1. Label the container with the date, the name and number of the patient, and the time of collection

2. Give the patient suitable container.

3. Instruct the patient before the collection, preferably with illustration.

4. Tell the patient not to touch the inside or rim of the container

Male

- If not circumcised, draw back the foreskin
- Begin to urinate, but pass the first portion into the toilet.
- Collect the mid-portion of urine into the container, and pass the excess into the toilet.

- Female

- Squat over the toilet and separate the labia with one hand.
- Avoid the first portion of urine into the toilet.
- Collect the mid-portion of urine into the container and pass the excess into the toilet.

Annex II: SOP for media preparation

A. SOP for preparation of MacConkey Agar (MAC)

MacConkey agar is selective and differential medium to distinguish gram negative *Enterobacteriaceae* and lactose fermenting bacteria from non-lactose fermenters. Crystal violet is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Procedure for preparation:

Prepare as instructed by the manufacturer.

1. Suspend 51.1g of powder in 1 liter of distilled or deionized water.
2. Heat and boil until completely dissolved with frequent agitation.
3. Sterilize in autoclave at 1210C for 15 minutes
4. Cool to 45-50 0C
5. Mix well and dispense by dispenser (15-20 ml) aseptically into sterile petri dishes.
6. Leave standing for thirty minutes to solidify.
7. Perform sterility testing as described before.
8. Label the bottom of each plate with date of preparation and batch number.

Store the culture media plates upside down at 2-80C sealed in plastic bags to reduce chances of contamination. Test Samples for performance, using stable, typical control culture

B. SOP for preparation of Blood agar plate (BAP)

Blood agar plate is a non-selective medium for the isolation and cultivation of many pathogenic microorganisms. Organisms can be differentiated by their hemolysis on this agar.

Blood Agar Base formulation has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can be used as a general-purpose growth media without adding blood.

Procedure

Measure 500ml of distilled water using a measuring cylinder.

1. Transfer the distilled water into a 1litre capacity conical flask.
2. Weigh 20g of Blood Agar Base powder using a weighing balance.
3. And then add into the 500ml of distilled water and mix thoroughly.
4. Boil until completely dissolved
5. Autoclave at 121°C for 15 minutes.
6. Allow to cool to 45-50°C in a water bath.
7. Once the medium has been melted and cooled to 45-50 ºC
8. Add 5-10% of defibrinated sterile sheep blood, in this case you can recuperate
9. Rotate the flask or bottle slowly to create a homogeneous solution.
10. Aseptically add 25 ml of sterile defibrinated sheep blood with constant shaking.
11. Label on the bottom top of the blood agar plates the batch number & date prepared.
12. Store the culture media plates upside down at 2-80C sealed in plastic bags to reduce chances of contamination. Shelf life: up to sixteen weeks provided there is no change in texture

C. SOP for Preparation of Mueller Hinton Agar (MHA)

It used for antibiotic susceptibility testing of non-fastidious organisms.

Procedure for preparation

1. Weigh, dissolve by heat the medium as instructed by the manufacturer.

2. Adjust the pH of the medium to 7.2–7.4.

3. Autoclave at 121°C at 15 psi for 20 minutes. Do not over heat.

4. Pour into 150 mm or 100 mm diameter sterile petri dishes to a depth of 4 mm (about 25 ml per plate).

- It should be dispensed into plates on a level surface so that the depth of the medium is uniform because too thin medium may give falsely large inhibition zones and too thick medium may give falsely small zones.

Annex III: SOP for Antimicrobial susceptibly tests (Disc diffusion)

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

Test Inoculums Preparation

- 3 to 5 pure colonies of the same morphological type will be selected from blood agar plate. The colonies are transferred into a tube containing 4 to 5 ml of Tryptone soy broth.
- The turbidity of the broth culture will be adjusted with 0.5 McFarland standards.
- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.
- Left for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Application of Disks to Inoculated Agar Plates

- The predetermined series of antimicrobial disks is dispensed onto the surface of the inoculated agar plate.
- The plates are inverted and placed in an incubator at 37 degrees centigrade

Interpreting Results

After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition are measured, including the diameter of the disk. Zones are measured to the nearest whole millimeter, using sliding calipers which is held on the back of the inverted plate.

Annex IV: SOP for MALDI-TOF (EXS3000 Series)

The automatic microbial mass spectrometry detection system is based on the matrix assisted laser desorption ionization (MALDI) method to ionize sample molecules into ions, and then detect samples with different m/z through the Time of Flight (TOF) The time when the ion arrives at the detector is converted into a peptide fingerprint, which is matched and searched with the pre-stored database to complete the identification of microorganisms. This product uses matrix-assisted laser analysis and ionization time-of- flight (MALDI-TOF) mass spectrometry technology to identify bacteria and fungi.

Detection Principle

The matrix and sample on the sample target are transformed from molecules to ions under the trigger of laser energy. At the same time, a high voltage is applied to the sample target to form a high-voltage electric field in the ion source. The sample ions and the matrix ions accelerate under the action of the electric field. When the ions fly out of the ion source, they fly in the flight tube at a constant speed., Until iHt reaches the detector, the time for ions with different mass-to-charge ratios to reach the detector is different. The ions finally generate electronic signals on the detector, and then undergo processing such as digital converters to obtain digital signals on the computer, and finally through the computer Processing and analysis can obtain the mass spectrum of the corresponding sample. Search the sample's mass spectrum and microbial protein fingerprint library to realize the search and identification of the sample.

Equipment And Reagents

1. Microbe Sample Pretreatment Kit
2. Fresh bacteria sample
3. Centrifuge tube（1.5ml）
4. Pipette and tips (1ul, 200ul, 1000μL)
5. Ultra-pure water or sterile water
6. Absolute alcohol
7. Vortex mixer
8. Centrifuge machine

Procedure

1. Transfer the appropriate amount of bacteria isolates (without transferring any medium) into a 1.5 ml centrifuge tube containing 300 μL ultrapure water, fully suspend;

2. Add 900 μL ethanol and mix well;

3. Centrifuge at 12, 000 rpm for 2-3 min, remove and discard the supernatant. Centrifuge at the same speed for another 1 min and remove the residual supernatant. Let the sediment dry at room temperature for 5 min;

4. Add 20 μL microorganism lysate I to the pellet and mix well with pipette (for gram-positive bacteria and some fungi, let the solution stand at room temperature for 5 min);

5. Add 20 μL microorganism lysate II, mix well, and centrifuge at 12, 000 rpm for 2 min;

6. Add 1 μL supernatant to the target plate and allow it to dry. Cover with 1 μL matrix solution and allow it to dry.

7. Put the target plate into the Mass Spectrometry System for analysis

Normal working condition

- Ambient temperature: 10 °℃~30℃;
- Relative humidity: ≤85%;
- Power condition: 100-240 V, 50/60 Hz, 500 VA;
- There is no strong mechanical vibration and electromagnetic interference source around;
- Avoid direct exposure to strong light;
- There should be a good grounding environment;
- The ground is level and the inclination is less than 1/200;
- For indoor use, the room should be clean and dust-free, with good ventilation.

Description of report of Data

The software has the dual functions of spectrum collection and microbial identification. After the mass spectrum is collected, the obtained mass spectrum is matched and searched with the standard

library to complete the microbial identification of the sample. The software can directly display the identification results of the strains, and display the confidence level of the identification results of each sample site in color. The software uses a three-point system for scoring rules. When the score value is ≥2.0, the identification result is displayed as green, which represents the possible species level identification. The higher the score value, the higher the confidence of the species level; when the score value is ≥1.7 and <2.0, the identification result is displayed as yellow, which represents the possibility for genus level identification, the higher the score, the higher the confidence of the genus level; when the score value is less than 1.7, the identification result is displayed in red, indicating that the identification result is unbelievable, that is, an unreliable identification.

Limitations

Limitations of the application of automatic microbial mass spectrometry detection system:

(1) Analyze patient samples directly;

(2) Analyze mixed cultures;

(3) Identify the microorganisms in the liquid culture medium;

(4) Identify highly pathogenic microorganisms;

(5) Identify microorganisms of the types of bacteria not included in clinical trials;

(7) Identify the serotype.

Annex V: Data collection format

I. Patient identification

Characteristics of patients	
1.Sample ID	
2.Age	_____
3.Sex	A. Male B. Female
4.Facility type	
5.Inpatient/Out patient	

II. Laboratory Data

1. Date of specimen collection | | | |

2. Bacteria isolated by MALDI-TOF_____

3.Score given by MALDI-TOF _____

3.Antibiotics susceptibility result for *Enterobacteriaceae* isolates

	Antibiotics		AM	AMC	CAZ	CTX	NI	MER	SXT	GEN	AK	FEP	CIP
	Drug Susceptibility Test	S											
		I											
		R											

Annex VI: Ethical clearance letter

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Addis Ababa UNIVERSITY
Collage of Health Sciences

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Date: 27/12/24

Ref.No. MLS/ 339/24 ነጋ

Departmental Research and Ethics Review Committee (DRERC) decision

Meeting No: 028/2024

Protocol number: DRERC/783/24/ MLS/

Protocol title: Extended Spectrum Betalactamase Production and Multidrug Resistant Enterobacteriaceae Among Urinary Tract Infection Suspected Patients Referred to Wudassie Advanced Medical Laboratory, Addis Ababa, Ethiopia

Principal investigators: Tamirat Tamiru

Institute: **AAU-CHS CLS**

Elements reviewed [√] Attached [ ] Not attached

Review of revised application [ ] Yes [√] No

Date of previous review: _____

Decision of the meeting: [√] **Approved** [ ] Approved with recommendation

[ ] Approved on Condition (Major revision) [ ] Disapproved

Obligation of the PI:

1. Should comply with the standard international and national scientific and ethical guidelines
2. All the amendments and changes made in protocol and consent form needs DRERC approval
3. The PI should report any updates to DRERC within 10 days of the event.
4. End of the study, including manuscripts and thesis works should be reported to the DRERC/ Department

Departmental Research and Ethics Review Committee (DRERC) Approval period: Jan 1, 2025 to November 1 2025.

Follow up report expected in

2 months_____√_____ 6 months_____ 9 months_____ one year_____

Chairperson, DRERC: Mikias Negash, Chair of the day **Chair Person for DMLS:** Melatwork Tibebeu

Signature: _____ **Sigpature:** _____

Date: 27/12/2024 **Date:** 27/12/2024

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School of Medical Laboratory Technology

ADDIS ABABA UNIVERSITY

Annex VII: Institutional Consent form

Wudassie and Family Business PLC

Wudassie Diagnostic Center

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OUR REF. NO. WDC/K10/1265/17 DATE Dec 5/2024

To Whom It May Concern,

Subject: Institutional Consent for Academic Research

This letter is to confirm that Wudassie Advanced Medical Laboratory has granted permission to Mr. Tamirat Tamiru, a postgraduate student from Addis Ababa University, to conduct research titled: Extended Spectrum Beta-Lactamase Production and Multi-Drug Resistant *Enterobacteriaceae* Among Urinary Tract Infection Suspected Patients Referred to Wudassie Advanced Medical Laboratory, Addis Ababa, Ethiopia.

Bethelhem Enkuberhan Fisseha
Imaging & Advanced Laboratories
Admin Manager

Wudassie Diagnostic Center

Declaration

The undersigned declares that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: Tamirat Tamiru (B.Sc.) Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Dr. Kassu Desta (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Regasa Diriba (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia