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Research Article

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Fecal Carriage of ESBL and Carbapenemase-Producing Enterobacteriaceae Among HIV Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia 2022Gc

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Abstract

Background: *Colonization in HIV-infected populations with extended-spectrum b-lactamase-producing Enterobacteriaceae is particularly problematic in low-income settings. Also, using antibiotics to treat infections in humans usually results in selecting pathogenic bacteria that become resistant to these drugs. The present study is intended to determine the magnitude of ESBL-PE and CPE carriage among HIV-infected patients attending Tikur Anbesa Referral Hospital in Addis Ababa, Ethiopia.*

Methods. A cross-sectional study was conducted in Tikur Anbesa Referral Hospital, Addis Ababa, Ethiopia, from April 01, 2021, to July 20, 2022, among 400 HIV-infected patients. Socio-demographic data and associated factors were collected using a pretested structured questionnaire. Stool specimens were collected using sterile stool cups and inoculated onto MacConkey agar. Extended-spectrum b-lactamase-producing Enterobacteriaceae was screened for antimicrobial susceptibility testing, and the more specific and sensitive techniques of extended-spectrum b-lactamase-producing Enterobacteriaceae detection techniques (disk diffusion test) in combination with double-disk synergy test was used for the detection and confirmation of the resistant Enterobacteriaceae. Biochemical identification and antimicrobial susceptibility testing, including lactamase production, were carried out. Ultimately, SPSS software (version 20) was used to evaluate the data, and a P-value of less than 0.05 was deemed statistically significant.

Results: *In the current study, 324 Enterobacteriaceae were isolated, of which E. coli accounted for the highest proportion, 32.1%, followed by K. pneumonia, 23.2%. The overall ESBL production rate was 74.1%, and the overall fecal carriage of carbapenemase-producing Enterobacteriaceae among HIV patients was 2.8 %. The predominant ESBL-producing Enterobacteriaceae was E. coli (26.9%), followed by K. pneumonia (19.6%). The predominant isolated Enterobacteriaceae is E.coli showed the highest resistance to ampicillin (97.6%), followed by Cefoxitin (95.9%), gentamicin (82.1%), and cotrimoxazole (79.7%). The isolated Enterobacteriaceae's overall multi-drug resistance (MDR) rate was 71.9%.*

Conclusion and Recommendation: *The magnitude of ESBL-PE and CPE was high in the study area. Therefore, strict infection control measure is needed for HIV patients to limit the infection and dissemination of these pathogens. Meropenem and imipenem were active against ESBL-PE.*

Keywords: *Fecal carriage, ESBL, Carbapenemase, Enterobacteriaceae, HIV patients.*

1. Introduction

1.1. Background

Extended-spectrum beta-lactamase (ESBLs) is an enzyme produced by certain bacteria hydrolyze extended-spectrum cephalosporin (1). ESBL and carbapenemase enzymes are the two most common enzymes located on plasmids and transposons and facilitate the transmission of resistance within or b/n species. Antibiotic resistance is the increasing infection control risk within healthcare settings, which is transferred to different bacteria by mobile genetic elements encoded by a plasmid [2].

So, rapid detection and isolation of the pathogen are essential for recognizing antimicrobial-resistant organisms, which is a fundamental problem in both hospital and community settings [3]. Production of ESBLs is a significant resistance mechanism

that affects the antimicrobial treatment of infections caused by *Enterobacteriaceae* and is a severe threat to the currently available antibiotics [4]. The widespread use of antibiotics has resulted in a global problem of antimicrobial resistance (AMR) [1,5].

Acute immunodeficiency syndrome (AIDS) is caused by severe bacterial infections, which can occur in people infected with the human immunodeficiency virus (HIV) [6]. This retrovirus particularly attacks and kills CD4 T cells, resulting in the dysfunction and deregulation of the immune system and, ultimately, AIDS [7]. Because of the weakened immune system, HIV-infected individuals can no longer ward off opportunistic infections [8].

Enterobacteriaceae are a large family of bacteria that commonly cause infections in healthcare settings and communities [9]. Several studies showed that *Enterobacteriaceae* infections are widely increased in HIV-positive patients [10]. *Enterobacteriaceae* infections in immune-compromised individuals are associated with considerable morbidity, mortality, and healthcare costs. In HIV patients, the effect of extended beta-lactamase spectrum *Enterobacteriaceae* infection is more diverse and ranges from asymptomatic colonization to gastroenteritis, meningitis wound infections, urinary tract infections, septicemia, and urinary tract infections [9]. This situation may increase the incidence and the progression of HIV infection to AIDS, an advanced stage of infection [11].

Enterobacteriaceae are recognized as one of the most basic public health problems globally due to the unexpected resistance character of these strains, even to the last classes of antibiotics [12]. Most of the ESBLs break down antibiotics such as penicillin and cephalosporin and alter their activity, which causes infections caused by ESBL-PE and carbapenemase-producing *Enterobacteriaceae* (CPE) are more challenging to manage (2, 13).Carriage has become more common over the previous ten years. of ESBL and infection with multidrug-resistant organisms (MDROs) such as ESBL-PE and CPE (14, 15). ESBL-PE and CPE pose a serious antibiotic management problem, as these genes are easily transferred from one organism to the other via plasmids. Many ESBL-PE and CPE are also resistant to the most common antimicrobial, which is the primary complex in surgeries, cancer treatment, and organ transplantation, which would increase morbidity and mortality in affected patients [16]. This problem is more pronounced in areas with no adequate infection control program, periodic surveillance, and multidrug-resistant bacteria detection laboratory facilities [17].

AMR affects both individuals as well as the entire healthcare system and has harsh implications for patients, not only in terms of their health outcomes but also in potentially devastating financial, social, and psychological effects [18,19]. Patients who are infected with pathogens that are resistant to most common standard treatments typically require more complicated treatment, take longer to recover, are a potential source of resistant bacteria, and are more likely to suffer treatment failure and death [18]. Furthermore, AMR also imposes enormous financial burdens on society as a whole; patients with AMR-related illnesses may miss many things, like working for more extended periods, which results in a loss of productivity and income [15,20].

Individuals living with HIV infection are at particular risk of acquiring MDRs due to the use of antibiotic prophylaxis and a high incidence of bacterial infections necessitating frequent antibiotic use [21]. Colonization in HIV-infected populations with ESBL-PE is particularly more significant and problematic in low-income settings [22]. *Enterobacteriaceae* infections in the immune-compromised individual are linked to significant rates of illness, death, and medical expenses [23].

1.2. Statement of the Problem

AMR, especially caused by ESBL-PE and CPE, has become a global public health concern [24], and infections caused by resistant organisms pose an essential challenge for treating both common and life-threatening diseases in low-income countries [21]. ESBL-PE and CPE infections are occasionally mostly reported from travelers returning from the tropics in sub-Saharan Africa [1] and have a high risk of ESBL-PE colonization, which suggests a high prevalence of ESBL-PE in Africa [25]. Even if the clonal distribution of different types of ESBL-PE and CPE genes were intensively studied in industrialized countries, the data about the burden of ESBL-PE and CPE are limited to sub-Saharan Africa [9].

These days, infections resulting from CPE and ESBL-PE represent an increasing burden for hospitals and community settings, affecting people of all ages and demographics. Furthermore, infections caused by bacteria that include the enzymes lactamase and carbapenemase are still linked to a high rate of morbidity and death around the globe. Addressing this issue is still critical because of the establishment of stable, quickly proliferating ESBL-producing clones with continuous horizontal gene transfer between genera and the selective pressure created by the widespread use of empiric broad-spectrum β-lactam antibiotics [26].

In hospitalized patients admitted to Ethiopia, the rate of gastrointestinal colonization and the patterns of antibiotic resistance of ESBL-PE and CPE grew significantly from year to year [27]. The rapid spread in Ethiopia is associated with a need for regular surveillance and antibiotic stewardship programs. Isolates of ESBL-PE and CPE from different regions of Ethiopia were searched exhaustively [24], even if published data regarding the pooled estimate of ESBL-PE and CPE are limited at the national level in Ethiopia.

A cross-sectional study conducted in 2018 in Ethiopia indicated that a total of 238 fermentative Gram-negative pathogens were isolated, of which; E.coli was the predominant isolate, followed by K. pneumonia and Ampicillin exhibited the highest rate of antibiotic resistance (100%), with trimethoprim/sulfamethoxazole coming in second. (81.9%) and 94.5% were MDR and of which, 8.8% and 0.8% were extensively and pan drug-resistant, respectively [28]. ESBL-PE and CPE have become the most commonly identified problem in hospitalized patients. The MDR rate for ESBL-PE and CPE is increased occasionally, which is the primary diagnostic difficulty for most doctors [29].

The rates of gastrointestinal tract colonization caused by *Enterobacteriaceae* vary considerably in different geographical areas and change over time [2], and the mortality rates were high, ranging from 42% to 100% [3]. On the other hand, the spread of carbapenems-resistant Enterobacteriaceae (CRE) in healthcare settings is another crucial medical problem [4]. ESBLs also add to the burden on healthcare systems, causing

prolonged hospital stays. ESBL-PE often displays MDR phenotypic characteristics, further limiting the therapeutic options. A literature survey indicated little is known about the gastrointestinal carriage of ESBLs, carbapenemase enzyme producers, and antibiotic susceptibility patterns of *Enterobacteriaceae* in Ethiopia [4]. Therefore, this study was conducted to determine fecal carriage of ESBL and CPE by using the more specific and sensitive techniques of ESBL and CPE detection techniques (disk diffusion test) in combination with a double-disk synergy test, unlike the previous study among HIV patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

1.3. Significance of the Study

The global emergence, spread, and AMR of ESBL-PE and CPE have threatened the ability to treat infection and become an international public health concern [22,24]. Their rapid spread in Ethiopia is associated with a need for regular surveillance and antibiotic stewardship programs (30). Although ESBL-PE and CPE pause significant and devastating public health problems in both developed and developing countries, particularly in most African countries like Ethiopia, little is known about the prevalence of ESBL and carbapenemase among Enterobacteriaceae isolate. Furthermore, in microbiology laboratories, even in the capital city of Addis Ababa, extended-spectrum beta-lactam-resistant screening and phenotypic confirmatory tests availability are very rare and it is not well known [27,28]. So, this issue initiates the researcher to identify and show the proportion of ELBL-PE and CPE among HIV patients. Data generated from the current study will be used in the control and containment of resistant pathogens, to recommend the implementation of ESBL screening and confirmatory tests, and to guide microbiologists and infectious disease specialists in controlling and preventing resistant pathogens. Also, the current study's findings will be an input to recommend the implementation of ESBLs and CPE screening and confirmatory tests because early detection of these bacteria is important to control nosocomial infections and outbreaks. Knowing all the above objectives of the current study will provide substantial inputs of information and fill some of the significant limitations in the previous studies conducted in Ethiopia, as stated in the literature and statement of the problem parts. So, the current study was conducted to determine and compare the rates of and risk factors for intestinal carriage and acquisition of ESBL-PE and CPE among patients living with HIV infection in TASH, Addis Ababa, Ethiopia.

2. Literature Review

A worldwide increase in gastrointestinal colonization by ESBL-PE has been observed [31,32]. As global reports indicated, more than 50% of medicines are prescribed each year, dispensed, or sold inappropriately (18), and this causes an increase in AMR bacteria [33]. According to a 2021 report by the CDC, the estimated number of deaths worldwide due to drug-resistant infections is already approximately 700,000 each year, and this figure is likely to reach as high as 10 million per year by 2050 if AMR remains unchecked [34]. In Europe, a similar picture is developing, with an estimated 25,000 deaths attributable to antibiotic-resistant infections and as of 2014, nearly 25% of hospital-acquired infections in the United States were caused by antibiotic-resistant bacteria (including Carbapenem-resistant and Extended-spectrum β-lactam resistance *Enterobacteriaceae*) estimated by the US CDC as public health threats [35].

A case-control cross-sectional study conducted in Nepal by taking rectal specimens from 357 healthy volunteers (213 female and 144 male) and 119 HIV-positive individuals (82 female and 37 male) with a median age of 30 years indicated that ESBL colonization was found in 37.82% (CI 29.09, 47.16) among HIV positive individuals and 68.91% (CI 63.93, 73.49) among healthy HIV negative participants. The finding reported that HIV-positive individuals had a lower ESBL carriage rate (odds ratio 0.274 [CI 0.178, 0.423]) compared to healthy HIV-negative subjects ($p<0.01$) [36]. Another cross-sectional study conducted by collected rectal swabs from 215 ICU patients in Northern Thailand reported that a high prevalence of ESBL-PE carriage (62.3%) at ICU admission was observed, with Escherichia coli representing the predominant organism (67.5%) followed by Klebsiella pneumonia (19.4%) [37].

A report of the different previous studies indicated that Sub-Saharan Africa is the epicenter of the HIV epidemic, hosting an estimated 70% (approximately 25 million) of the world's HIV-infected population in 2014 [38]. TFrom a total of 595 newly diagnosed HIV-positive adults with a median age of 35 years, the study conducted in Tanzania found that 32.6% (194/595; 95% CI: 28.9–36.6]) were carriers of ESBL-PE. Participants with low CD4 count (<350 cells/μL) had a significantly higher prevalence of ESBL-PE carriage compared to those with CD4 count ≥350 cells/μL (26/58, 44.8%, vs. 168/537, 31.3%, p = 0.04). Additionally, the study's findings showed that one HIV-positive individual with a CD4 count of 132 cells/μL had phenotypic carbapenemase-producing *Enterobacteriaceae* [39].

Another hospital-based study conducted in Burkina Faso to assess the prevalence of digestive carriage in the hospital by taking 214 fecal samples, 101 from healthy volunteers and 113 from hospitalized patients in 2014, indicated that the prevalence of fecal ESBL-PE was 32%, of which 22% among healthy volunteers and 42% among HIV patients and *E. coli* (78%) mainly was isolated pathogen [40]. Also, a report of a prospective cross-sectional study conducted between April and June 2017 in South Africa among 200 children in a tertiary academic hospital who were screened by rectal swab for EBSL-PE and CRE indicated that overall, 48% of the children were colonized with at least one ESBL-PE, 8.3% (8/96) of these with 2 ESBL-PE and one other child was colonized with a CRE (0.5% (1/200)). Also, as the report of the study, the common colonizing ESBL-PE were *Klebsiella pneumoniae* (62.5%) and *Escherichia coli* (34.6%) [41].

The report of the cross-sectional study conducted at Gabon indicated that the overall colonization rate of ESBL-PE was 45% and increased from 33.6% at admission to 94.1% during hospitalization. The researchers also reported that the risk factors for ESBL-PE carriage were age <5 years, hospitalization for ≥5 days, and a hospital stay during the past year, and most of the isolated pathogens showed resistance to most antibiotics [25]. A study conducted by taking stool samples from 204 HIV-negative and 104 HIV-positive patients attending the Regional Hospital of Bafoussam-Cameroon from September 2016 to June 2017 indicated that the prevalence of *Enterobacteriaceae* infection among HIV-positive patients was 46.15% versus 27.47% among HIV-negative ones (P=0.0014) and the rate of *Enterobacteriaceae* infection according to CD4 count range was 20.83%, 37.5%, 35.42%, and 6.25% respectively for patients with CD4 T lymphocyte range up to 500, 300-500, 100-300 and

< 100 cells/mm^3. Also, as per the report of the study, the overall resistance rates were 27.72 and 34.66%, respectively, among isolates from HIV-negative and positive patients. The prevalence of ESBL-PE and CPE infection was 16.34, 12.5, and 4.81%, respectively, among HIV-infected patients versus 8.62, 8.65, and 0% among HIV uninfected patients (p = 0.0055) (42).

A cross-sectional study conducted from January 01 to May 30, 2017, in Ethiopia, indicated that the most frequent *Enterobacteriaceae* isolated among HIV patients were *E. coli* (53.5%) with a magnitude of ESBLs-E was 57.7% (246/426). The highest resistance level was seen to sulfamethoxazole-trimethoprim (77.0%), amoxicillin with clavulanic acid (71.6%), cefotaxime (62.2%), cefepime (60.3%) and ceftazidime (60.8%) with an overall magnitude of multi-drug resistance (MDR) level was 68.3%. Of ESBLs-E, 96.3% of them were MDR [22].

Also, findings of research conducted on hospitalized patients admitted at Ethiopia's largest tertiary hospital of Addis Ababa by taking fecal samples/swabs from 267 patients indicated that the overall gastrointestinal colonization rate of ESBL-PE was 52% (95% CI), of which E. coil and *K.pneumoniae* accounted for 68% and 32% respectively. The finding also indicated that the fecal ESBL-PE carriage rate in neonates, children, and adults was 74%, 59%, and 46%, respectively, and the gastrointestinal colonization rate of ESBL-PE *E.coli* in neonates, children, and adults was 11%, 42%, and 42% respectively. The finding also showed that the overall carrier rate of ESBL-producing isolates resistant to carbapenem was 2% (5/267) [27].

Another hospital-based study conducted in 2019 in Ethiopia showed that from a total of 103 bacterial isolates, the proportions of ESBL-PE were significantly higher (82%, 90%, and 57% in NICU, orthopedic ward, and waste disposal area, respectively) compared to flies collected outside of the hospital compound (2% in the butchery) ($p \leq 0.001$). Also from 40 ESBL-genes detected, 85% were CTX-M-like, 83% TEM-like, 23% SHV-like & 2% CTX-M-2-like, and ESBL-producing bacteria showed higher rates of resistance against ciprofloxacin (66% vs. 5%), gentamicin (68% vs. 3%), piperacillin-tazobactam (78% vs. 5%), and trimethoprim-sulfamethoxazole (88% vs. 16%), compared to non-ESBL-producing bacteria [43]. The multidrug-resistant (MDR) ESBL-PE and CPE isolates have become a global threat to human health.

3. Objectives of The Study

3.1. General objective

- To determine fecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and carbapenemase-producing *Enterobacteriaceae* among HIV patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2022.

1.1. Specific objectives

- To determine the prevalence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* among HIV-infected patients attending Tikur Anbesa referral Hospital in Addis Ababa, Ethiopia; 2022.
- To determine carbapenemase-producing *Enterobacteriaceae* among HIV-infected patients attending Tikur Anbesa referral Hospital in Addis Ababa, Ethiopia; 2022.
- To determine the antimicrobial resistance pattern of *Enterobacteriaceae* among HIV-infected patients attending Tikur Anbesa referral Hospital in Addis Ababa, Ethiopia, 2022.
- To assess possible associated factors of antimicrobial resistance among HIV-infected patients attending Tikur Anbesa referral Hospital in Addis Ababa, Ethiopia, 2022.

4. Method and Material

4.1. Study setting (area)

The current study was conducted in Tikur Anbesa Specialized Referral Hospital (TASH), Addis Ababa, Ethiopia. The hospital is one of the specialized hospitals in Ethiopia, located in the capital city of Ethiopia, Addis Ababa. In addition to its health care activity, the hospital has an educational institute called the College of Health Sciences (CHS), which is Addis Ababa University (AAU). It is a professional health sciences college established in 2009/10. TASH is the college's teaching hospital. TASH is the largest specialized hospital in Ethiopia, with over 700 beds. It serves as a training center for undergraduate and postgraduate medical students, dentists, nurses, midwives, pharmacists, medical laboratory technologists, radiology technologists, and others who shoulder the community's and the country's health problems.

4.2. Study design

This hospital-based cross-sectional study was conducted from April 1, 2021, to July 20, 2022, to determine the fecal carriage of ESBL-PE and CPE among HIV patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopi2.

4.3. Population

4.3.1. Source population

In the current study, all HIV-infected individuals suspected of ESBL-PE and CPE infection at Tikur Anbesa specialized hospital during the study period were taken as a source population.

4.3.2. Study population

All newly HIV-infected individuals who were suspected of ESBL-PE & CPE infection and visited TASH during the study period were the study populations.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

This study can include all HIV-infected individuals who have not taken any antibiotics within one week and can provide stool samples.

4.4.2. Exclusion criteria

Individuals who have taken antibiotics within one week or cannot give a stool sample are critically ill, and taking HAART was excluded from the current study.

4.5. Sampling Method and Sample Size Calculation

4.5.1. Sampling Method

A systematic random sampling technique was implemented to select the study participants from the source population.

4.5.2. Sample Size Calculation

The minimum sample size required to conduct the current study was calculated using a single population proportion calculation formula, referring to a meta-analysis study finding that the overall proportional estimate of ESBL-producing *Enterobacteriacea*e was 61.8% (95% CI) (23), with consideration of the 95% confidence level and a 5% margin of error.

(P) =0.618, Confidence level ($Z_{a/2}$) =95%, Margin of error (d_2) =0.05

so that n= $\frac{(Z_{a/2})^2 xp\left(\frac{1-p)}{d2}\right)}{}$ So that n= $(1.96)^2$ (0.618) (0.382)/ $(0.05)^2$ = 362.8 = 363

Then, with consideration of a 10% non-responsive rate, the minimum sample size required for this study was 383.

4.6. Study Variable

4.6.1. Dependent variable

- ✓ The magnitude of fecal carriage of ESBL-PE
- ✓ The magnitude of fecal carriage of CPE
- ✓ Antimicrobial resistance pattern of *Enterobacteriacea*e from fecal specimen

4.6.2. Independent Variables

- ✓ Soci demographic factors like age, gender, clinical diagnosis
- ✓ Type of isolated bacteria
- ✓ Type of antimicrobial drug
- ✓ Antimicrobial resistance pattern

4.7. Data Collection Procedure

4.7.1. Socio-demographic data

Structured questionnaires were used to collect data related to socio-demographic and clinical data following obtaining informed consent from the study participants aged≥ 18 years and assent from participants aged ≤18 years old from their parents or guardians by data collectors using written informed consent. Clinical data of the patients, such as the history of hospitalization in the past 12 months, historyof antibiotic use in the past two or three months, and other clinical data, were collected from the medical record book of the participants. For participants who cannot read and write, the information sheet was read to them, and a witness signed it before data collection. All this information was collected by the principal investigator in collaboration with data collectors and concerned bodies.

4.7.2. Stool sample/rectal swab sample collection

A stool sample was collected from each HIV patient using a sterile stool cup. Then, the stool sample was transported to the Microbiology laboratory for analysis of stool culture. For participants who cannot give stool (neonates and patients),rectal swabs were collected by an experienced nurse after obtaining informed consent from the participants and their parents or guardians. Then, the swab was put in Cary-Blair transport media containing a test tube until it reached the Microbiology laboratory for bacteriological analysis.

4.7.3. Stool culture and identification

The stool sample/rectal swab sample collected from each participant was inoculated onto MacConkey agar and incubated aerobically at 37°C for 18 to 24 hrs. After the incubation period had been achieved, all of the culture plates were examined for the growth of Enterobacteriaceae. *Then, for those plates that showed bacterial growth, MacConkey agar was used to differentiate lactose fermenter pathogens from non-lactose fermenters. Then, lactose fermenter colonies were selected and inoculated on Xylose-Lysine Deoxycholate agar to observe further characteristics, and also pure colonies were taken for identification. As necessary, re-subculture onto MacConkey agar was performed to get pure colonies. Finally, the colony or all of the isolated* Enterobacteriaceae *were further characterized and identified by Gram staining, colony characteristics, and conventional biochemical tests, namely H2S production, lysine decarboxylase,lactose fermentation, indole, citrate utilization, urea hydrolysis, gas production, and mannitol fermentation as outlined in Annex II. Then, the pure colony was used for subsequent antibiotic susceptibility tests.*

4.7.4. Antibiotic Susceptibility Testing

The Kirby-Bauer disk diffusion method was used to perform antimicrobial susceptibility testing, and the results were expressed as susceptible, intermediate, or resistant according to the CLSI guidelines (CLSI, 2019). The inoculum was prepared using the 0.5 McFarland turbidity standard.

So to test AST; Mueller Hinton agar (MHA) plates were inoculated and antimicrobial disks including amikacin (30 µg), amoxicillin-clavulanic acid (AMC: 20/10 µg), ampicillin (AM:10µg), aztreonam (ATM: 30 µg), cefepime (FEP:30µg), cefotaxime (CTX: 30 µg), Cefoxitin (30µg), ceftazidime (CAZ: 30 µg), ceftriaxone (CRO: 30 µg), chloramphenicol (CL:30µg), ciprofloxacin (CIP: 5µg), cotrimoxazole (COT:25µg, gentamicin (CN: 10 µg), imipenem (IMP: 10µg), kanamycin (K: 30 µg), meropenem (MEM: 10 µg), norfloxacin (NOR: 10µg), tetracycline (TE:30µg), trimethoprim-sulfamethoxazole (SXT: 1.25/23.75µg), were applied to the plate for susceptibility test (51). All the used or selected antibiotic diskswere Oxoid, United Kingdom brands.

4.8. Phenotypic Detection Of Extended-Spectrum Beta-Lactamase

4.8.1. Screening for ESBLs producing isolate

Enterobacteriaceae that showed an inhibition zone size of less than or equal to 22 mm (≤ 22 mm) with antibiotics ceftazidime (30µg), less than or equal to 25 mm (≤25 mm) with ceftriaxone (30 µg), and or less than or equal to 27 mm (≤ 27mm) with cefotaxime (30 µg) were considered as ESBL producersand were recruited for further phenotypic confirmation of ESBL production by using combination disc diffusion test & Double Disk Synergy Test (5). Additionally, all *Enterobacteriaceae* that showed a zone of inhibition less than or equal to 19 mm (≤ 19 mm) for imipenem or meropenem were suspected as carbapenemase producers (44, 45).

4.8.2. ESBLs Phenotypic Confirmation with combination Disc test (CDT)

Ceftazidime (30 µg) & cefotaxime (30 µg), and cefepime alone, as well as their combination with Clavulanic acid (30 µg g/10 µg) acid (ceftazidime + clavulanic acid (30 µg/10 µg) and cefotaxime (30 µg) + clavulanic acid (30 µg/10 µg) and cefepime + clavulanic acid) were placed at a distance of 25 mm, center to center,on the MHA plate that was inoculated with a bacterial suspension of 0.5 McFarland turbidity standard and then was incubated overnight (18 – 24 hrs) 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 ESBL producers. In all other cases, the results were considered harmful for ESBL producers (6).

4.8.3. Phenotypic confirmation of carbapenemase production

Isolates that showed a zone of inhibition ≤ 19mm for imipenem or meropenem were confirmed by a rapid Carba NP test kit for the presence of carbapenemase (46). It is a hydrolyzed-based assay for the rapid detection of carbapenemase-producing Enterobacteriaceae. Enterobacteriaceae isolates that showed red color on the control well (well d) and yellow, light orange, or dark orange on the test well (well e) or orange on the control well (well d)and yellow on the test well (well e) were confirmed as positive for carbapenemase production. On the other hand, Enterobacteriaceae isolates that showed red color on thecontrol well (well d) and red color on the test well (well e) or orange color on the controlwell (well d) and orange on the test well were confirmed (well e) as unfavorable forcarbapenemase production as the manufacturer instruction.

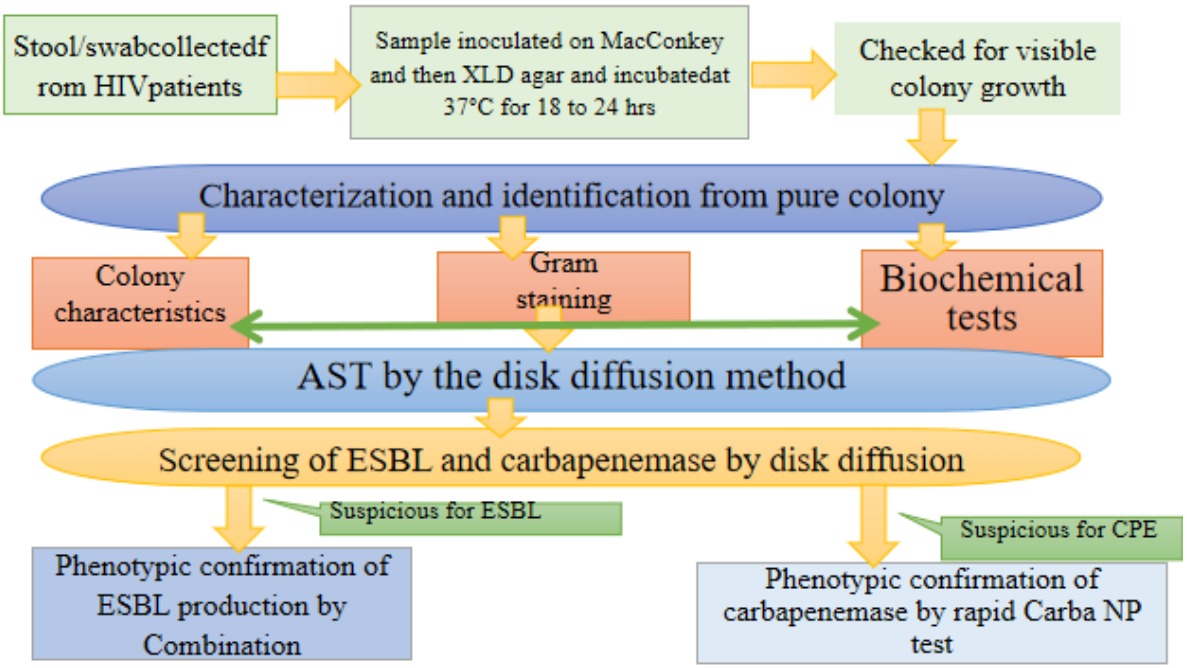

Figure 1: Flow chart for identification, susceptibility testing, ESBL, and carbapenemase screening and phenotypic confirmation of Enterobacteriaceae among HIV patients at TASH, Addis Ababa, Ethiopia, 2022.

4.9. Quality Control

To achieve the required result quality, standard operating procedures (SOP) were strictly followed, and the stools/ rectal swab specimen was processed and transported as soon after receipt as possible. If there was an uncontrolled delay in processing the specimen, the recommended preservation method, like a refrigerator, was used. Before testing, the culture media was sterilized and visually evaluated for cracks, the presence of a freezing substance, bubbles, and contaminants.

The quality of the new batch culture media was checked using ATCC 25922 *E.coli* standard strain. Before applying glycerol (15%) with TSB (Trypticase Soy Broth) for storage, QC was tested for *Escherichia coli* ATCC 25922 standard strain. For the ESBLs confirmatory test, ESBLs positive *K. pneumoniae* ATCC 700603 and ESBL harmful *E. coli* ATCC 25922 control strains were used. *K. pneumoniae* ATCC BAA-1705was used as a positive control, and *K. pneumonia* ATCC BAA-1706 was used as a negative control for carbapenemase detection. Finally, the isolated *Enterobacteriaceae* wasstored at -20^{0}C using 15% TSBS (29, 33).

4.10. Data Entry and Analysis

After double-checking and cleaning the data gained from the data collection form, data was entered into Epi Info Version 3.5.4. Then, the data was exported to Statistical Package for Social Sciences (SPSS) version 25 to calculate descriptive statistics (median, percentages, or frequency) and to analyze the bi-variant logistic regression, which was used to observe the relationship between the dependent variable and independent variables. The independent variables that showed P-value ≤ 0.25 bi-variant logistic regression analysis were further selected for multivariable logistic regression analysis. Then, an independent variable that showed a p-value ≤ 0.05 in the multivariate logistic regression analysis was considered significantly associated with the dependent variable. Finally, the results were presented in words, graphs, and tables.

4.11. Operational Definitions

- ✓ **Multi-drug Resistance (MDR)** occurs when a bacterium is simultaneously resistant to two or more antimicrobials belonging to different chemical classes (47).
- ✓ **ESBL-producing *Enterobacteriaceae* (ESBL-PE)**: an enzyme produced by *Enterobacteriaceae* that hydrolyze penicillins, first, second, and third cephalosporins, and monobactam, except for cephamycins, clavulanate, and carbapenems (48).
- ✓ **Carbapenemase-producing *Enterobacteriaceae* (CPE)**: an enzyme produced by *Enterobacteriaceae* that hydrolyzes all the beta-lactams, including carbapenems (49).

4.12. Ethical Considerations

The study started with obtaining an approval letter from the Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory, College of Health Science, Addis Ababa University. Also, a written permission letter was obtained from the Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia. Also, following a clear explanation about the objective, purpose, and procedures of the study to the study participants and parents or guardians, written consent or assent form was gained from the study participants with an age greater than or equal to 18 years and with an age of fewer than 18 years old respectively. For participants who could not read and write, the information sheet was read to them, and a witness signed the document stating that the process had been conducted appropriately. The confidentiality of all study participants was maintained.

4.13. Dissemination of Result

The findings of the current study have been submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, and also disseminated to the respective microbiology laboratories of TASH, Addis Ababa City Administration Health Bureau, Federal Ministry of Health Bureau, other Policymakers, and other concerned bodies related to this public health issue programs according to the university and other Ethical Regulations. Finally, the entire manuscript will

be published in an International or national peer-reviewed journal.

5. Result

5.1. Socio-demographic characteristics of study participants

A total of 383 HIV-infected patients were included in this study, of which 56.9% (218/383) were female participants, 88.0% (338/383) were adults (aged between 15 to 85 years with a mean age of 39.7 years old),11% (42/383) children (age between 2 months to 13 years old with a mean age of 7.8 years) and neonates 1%(4/383) (age between 4 to 22 days with a mean age of 12 days) were included. The majority of the study participants were urban residents (97.9%, 375/383), were married once (54.6%, 209/383), were diploma and above holders (35.8%, 137/383), and were governmental employers (32.6%, 125/383) (Table 1).

Table 1: Socio-demographic characteristics of study participants at TASH, Addis Ababa, Ethiopia, from April 01, 2021, to July 20, 2022.

Variables and Category		Frequency	Percent
Sex of Participants	Male	165	43.1
	Female	218	56.9
	Total	383	100.0
The age group of participants	Neonate	4	1.0
	Children	42	11.0
	Adult	337	88.0
	Total	383	100.0
A resident of the study participants	Urban	375	97.9
	Rural	8	2.1
	Total	383	100.0
Marital status of the participants	Single	84	21.9
	Married	209	54.6
	Divorced	90	23.5
	Total	383	100.0
Educational status	Illiterate	38	9.9
	Primary	87	22.7
	Secondary	121	31.6
	Diploma and above	137	35.8
	Total	383	100.0
Occupational status	Unemployed	53	13.8
	Governmental	125	32.6
	Nongovernmental	79	20.6
	Daily Labour	85	22.2
	Housewife	41	10.7
	Total	383	100.0

5.2. Clinical profile of the study participants

Of a total of 383 study participants, 13.1% (50/383) had a history of antibiotic usage in the past two months, 14.9% (57/383) had a history of chronic diseases, 18.3 % (70/383) had a history of diarrhea, and 17.2% (66/383) had a history of hospitalization in the past twelve months. Also, from 383 HI patients who participated in the current study, 18.8% (72/383) had not started HAART, 24.0% (92/383) had started HAART within less than six months, 27.4% (105/383) had stayed for 6-12 months with HAART, and the rest 29.8% (114/383) were had taken HAART for more than 12 months. Also, of the total participants, 29.8% (114/383) were in stage 3 and stage 4 levels of HIV as recommended by WHO (50), 32.4% had a viral load greater than 1000 copies/ml, and 77.8 % had a CD4 count less than or equal to 350 cells/ml (Table 2).

Table 2: Table 1 Clinical and HIV-Related Findings of Study Participants at Tash, Addis Ababa, Ethiopia, From April 01, 2021, To July 20, 2022.

Clinical Variables		Frequency	Percent
Antibiotic use last two months	Yes	50	13.1
	No	333	86.9
History of chronic diseases	Yes	57	14.9
	No	326	85.1
History of diarrhea	Yes	70	18.3
	No	313	81.7
History of hospitalization in the past 12 months	Yes	66	17.2
	No	317	82.8
Previous ICU stay	Yes	4	1.0
	No	379	99.0
HAART experience	Not started	72	18.8
	Less than six months	92	24.0
	6-12 months	105	27.4
	> 12 months	114	29.8
HIV stage	Stage 1	84	21.9
	Stage 2	114	29.8
	Stage 3	114	29.8
	Stage 4	71	18.5
Current viral load (copies ml)	Not detected	88	23.0
	≤ 1000 copies /ml	171	44.6
	> 1000 copies /ml	124	32.4
CD4 counts (cells/mL)	=/<350	298	77.8
	>350	85	22.2

5.3. Prevalence of Enterobacteriaceae

A total of 324 *Enterobacteriaceae* were isolated among 383 HIV patients who participated and who brought stool specimens. The most predominant isolates were *E. coli (32.1%*/123/324), followed by K. pneumonia (23.2%/89/324), *salmonella* 8.6%/33/324), and *K.oxytoca* (6.3%/24/324) (Figure 2).

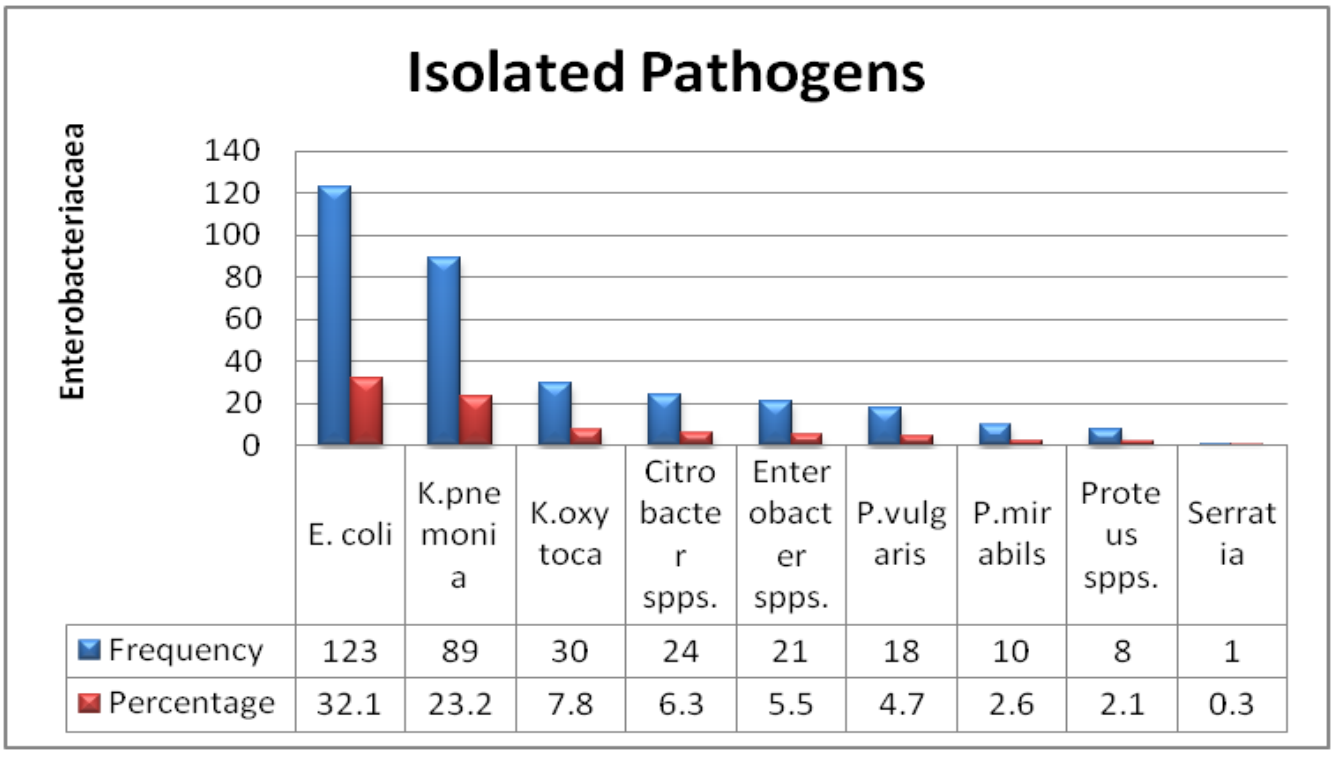

Figure 2: The Frequency of Enterobacteriaceae Isolated from Stool Culture Was Collected from HIV Patients at Tash, Addis Ababa, Ethiopia, From April 01, 2021, To July 20, 2022.

5.4. The magnitude of ESBL Producing Enterobacteriaceae

From 324 isolated *Enterobacteriaceae* in the current study, the overall ESBL production was 74.1% (240/324), confirmed by a combination disk test. Of these, 26.9% (87/324) were *E. coli*, 19.6% (64/324) were *K.pneumonia*, and 8.6% (28/324) were *K.oxytica*. The distribution of ESBL-producing *Enterobacteriaceae* varied among isolated species, and the highest ESBL-PE was observed in *proteus spps.* And *Serratia spps.* 100% (8/8), followed by *K. oxytoca 93.3%* (28/30), *Citrobacter* (75.%, 18/24), P.vulgaris (72.2%, 13/18), *K. pneumonia* 71.9% (64/89) and *enterobacter* (71.4%, 15/21). No ESBL production was observed in *Shigella* and *Salmonella spps.* (Figure 3).

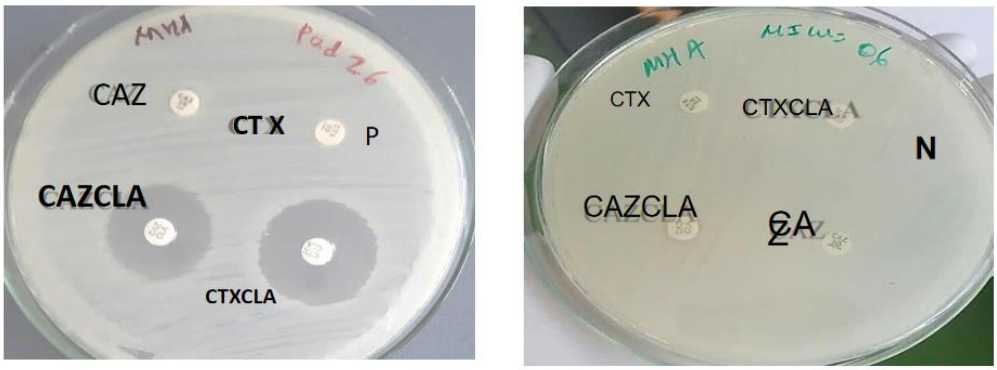

Figure 3: ESBL Positive (P) And ESBL Negative (N) Enterobacteriaceae Using Combination Disk Method from Stool Culture Collected from HIV Patients at Tash, Addis Ababa, Ethiopia From April 01, 2021, To July 20, 2022.

5.5. The magnitude of carbapenemase-producing Enterobacteriaceae

In our study, the overall magnitude of carbapenemase-producing *Enterobacteriaceae* was 2.8 % (9/347). Of the nine carbapenemase-producing *Enterobacteriaceae* isolated in the current study, thehighest percentage of carbapenemase producers was *K.pneumoniae*, whichwas 55.6% (5/9), followed by *K.oxytica*, 33.3% (3/9) and *E.coli*, 11.1% (1/9) (Figure 4).

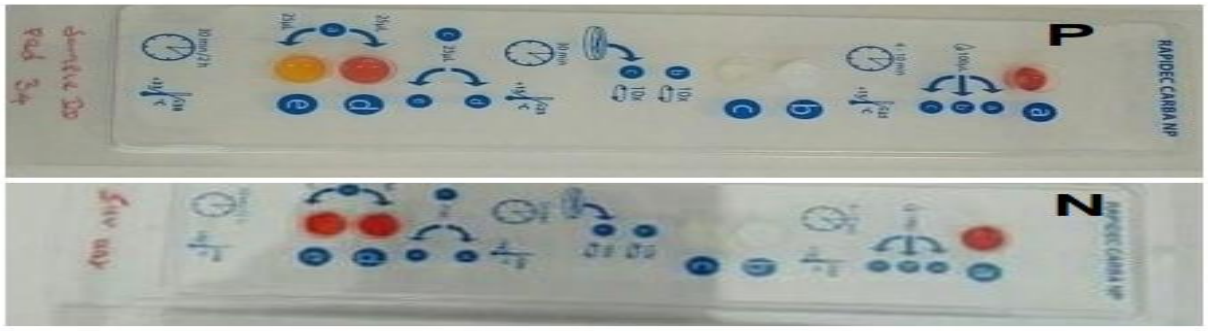

Figure 4: Carbapenemase Positive (P) And Carbapenemase Negative (N) Using Carba Np Test from The Fecal Sample Culture Collected from HIV Patients at Tash, Addis Ababa, Ethiopia From April 01, 2021, To July 20, 2022.

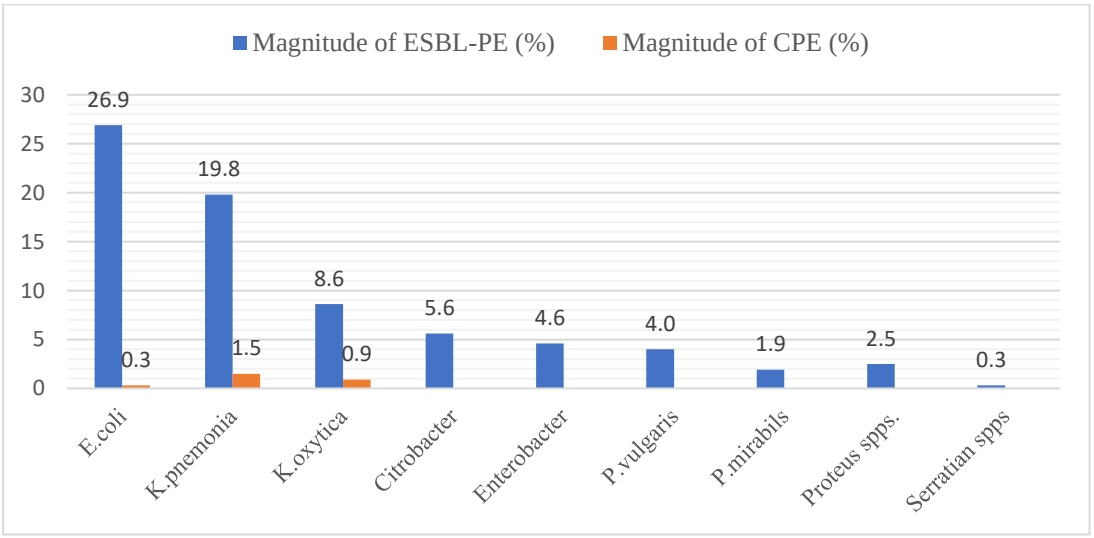

Figure 5: Frequency of ESBL-Producing Enterobacteriaceae And Carbapenemase-Producing Enterobacteriaceae From Stool Culture Collected from HIV Patients at Tash, Addis Ababa, Ethiopia From April 01, 2021, To July 20, 2022.

5.6. Antimicrobial resistance pattern of Enterobacteriaceae

The current study tested antimicrobial susceptibility for all the isolated *Enterobacteriaceae* against fourteen selected antibiotics. The highest level of resistance was observed to ampicillin (97.8%), followed by ceftriaxone (92.0%), gentamicin (85.8%), cotrimoxazole(78.7%), amoxicillin-clavulanic acid (59.3%), ceftriaxone (57.7%), and cefotaxime (52.5%) respectively. A low level of resistance was recorded against imipenem (7.1%) and meropenem (7.1%), ciprofloxacin (18.5%), and ceftazidime (11.1%) (Table 3). E.*coli* isolates showed the highest resistance to ampicillin (97.6%), followed by Cefoxitin (95.9%), gentamicin (82.1%), cotrimoxazole (79.7%), tetracycline (64.2%) and amoxicillin-clavulanic acid (52.0%). Also, *K. pneumoniae* isolates showed high resistance to ampicillin (100%), Cefoxitin (89.9%), gentamicin (89.9%), cotrimoxazole (85.4%), andamoxicillin-clavulanic acid (74.2%) and somewhat sensitive for tetracycline (7.9%), imipenem (6.7%), and meropenem (6.7%) also as the current finding showed 100% resistance (Table 3).

Table 2: Antimicrobial resistance pattern of Enterobacteriaceae isolated at TASH, Addis Ababa, Ethiopia from April 01, 2021, to July 20, 2022.

Antibiotics	**List of bacterial isolates**									**Total resistance (324)**
	E. coli (123)	***K.pnemonia (89)***	***K.oxytoca (30)***	***Citrobacter spps. (24)***	***Enterobacter spps. (21)***	***P.vulgaris (18)***	***P.mirabilis (10)***	***Proteus spps. (8)***	***Serratia (1)***	
AMC	64 (52.0%)	66 (74.2%)	15(50.0%)	13(54.2%)	14(66.7%)	10(55.6%)	5(50.0%)	4(50.0%)	1(100%)	192 (59.3%)
AMP	120 (97.6%)	89(100%)	30(100%)	24 (100%)	18 (85.7%)	18 (100%)	9 (90.0%)	8 (100%)	1 (100%)	317 (97.8%)
CHL	33 (26.8%)	17 (19.1%)	4 (13.3%)	2 (8.3%)	3 (16.7%)	0	1 (10.0%)	0	0	60 (18.5%)
CAZ	21 (17.1%)	11 (12.4%)	0	0	2 (9.5%)	1 (5.6%)	0	1(12.5%)	0	36 (11.1%)
CIP	6 (4.9%)	43 (48.3%)	5 (16.7%)	2 (8.3%)	1 (4.8%)	3 (16.7%)	0	0	0	60 (18.5%)
COT	98 (79.7%)	76 (85.4%)	25 (83.3%)	18 (75.0%)	15 (71.4%)	11 (61.1%)	6 (60.0%)	5 (62.5%)	1 (100%)	255 (78.7%)
CRO	64 (52.0%)	56 (62.9%)	18 (60.0%)	13 (54.2%)	15 (71.4%)	11 (61.1%)	5 (50.0%)	4 (50.0%)	1 (100%)	187 (57.7%)
CTX	56 (45.5%)	43 (48.3%)	22 (73.3%)	10 (41.7%)	17 (81.0%)	9 (50.0%)	5 (50.0%)	7 (87.5%)	1 (100%)	170 (52.5%)
FEP	6 (4.9%)	3 (3.4%)	5 (16.7%)	2 (8.3%)	1 (4.8%)	3 (16.7%)	0	0	0	20 (6.2%)
FOX	118 (95.9%)	80 (89.9%)	27 (90.0%)	24 (100%)	14 (77.8%)	18 (100%)	9 (90.0%)	7 (87.5%)	1 (100%)	298 (92.0%)
GM	101 (82.1%)	80 (89.9%)	23 (76.7%)	24 (100%)	17 (81.0%)	16 (88.9%)	9 (90.0%)	7 (87.5%)	1 (100%)	278 (85.8%)
IMP	10 (8.1%)	6 (6.7%)	3 (10.0%)	0	2 (9.5%)	2 (11.1%)	0	0	0	23 (7.1%)
MER	10 (8.1%)	6 (6.7%)	3 (10.0%)	0	2 (9.5%)	2 (11.1%)	0	0	0	23 (7.1%)
TE	79 (64.2%)	7 (7.9%)	6 (20.0%)	1 (4.7%)	5 (23.8%)	18 (100%)	1 (10.0%)	1 (12.5%)	0	118 (36.4%)

Note: AMP: ampicillin, FOX: Cefoxitin, GM: gentamicin, CIP: ciprofloxacin, COT: cotrimoxazole: IMP: imipenem, MER: meropenem, AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, CRO: ceftriaxone: TE: tetracycline, FEP: cefepime: CHL: chloramphenicol

5.7. Multi-drug resistance pattern of Enterobacteriaceae

Of 324 *Enterobacteriaceae* isolated in the current study, 71.9% (233/324) showed multi-drug resistance (resistance to greater than or equal to 3 antibiotics in a different class). Also, from a total of 123 *E.coli* isolated in the current study, 72.4% (89/123) showed multi-drug resistance (resistance to greater than or equal to 3 antibiotics in a different class). K. Pneumonia is this study's second most prevalent isolated *Enterobacteriaceae*, showing 66.3% (59/123) of multi-drug resistance (Table 4).

Table 4: Multi-Drug Resistance Patterns of Enterobacteriaceae Isolates at Tash, Addis Ababa, Ethiopia From April 01, 2021, To July 20, 2022.

Antibiotic resistance level (n (%)							
Isolates(number)	**R0**	**R1**	**R2**	**R3**	**R4**	**≥R5**	**Total MDR Isolates (R≥3)**
E. coli (123)	1(0.8)	13(10.6)	20(16.3)	33 (26.8)	21(17.1)	35(28.5)	89 (72.4)
K.pnemonia (89)	0	19(21.3)	11 (12.4)	22(24.7)	21(23.6)	16(18.0)	59(66.3)
K.oxytoca (30)	1(3.3)	2(6.7)	6(20.0)	7(23.3)	8(26.7)	6(20.0)	21(70)
Citrobacter spps. (24)	0	3(12.5)	2(8.3)	8(33.3)	5(20.8)	6(25.0)	19(79.2)
Enterobacter spps. (21)	0	2(9.5)	4(19.0)	4(19.0)	5(23.8)	6(28.6)	15(71.4)
P.vulgaris (18)	0	1(5.6)	1(5.6)	7(38.9)	5(27.8)	4(22.2)	16(88.9)
P.mirabils (10)	0	2(20.0)	1(10.0)	2(20.0)	3(30.0)	2(20.0)	7(70.0)
Proteus spps. (8)	0	0	2 (25.0)	3(37.5)	1(12.5)	2(25.0)	6(75.0)
Serratia (1)	0	1(100.0)	0	0	0	0	1(100.0)
Total (n=324)	2(0.6)	43 (13.3)	47(14.5)	86(26.5)	69(21.3)	77 (23.8)	233(71.9**)**
Note: R0: resistance to no antibiotics; R1-7: resistance to 1, 2, 3, 4, and 5 antibiotics; ≥R3:resistance to 3 or more antibiotics from different classes.							

5.8. Relationship between independent factors and ESBL-PE fecal carriage

An analysis was conducted using bivariant and multivariate logistic regressions to ascertain the relationship between the independent and dependent variables. In the bi-variable analysis, variables like marital status, age, residence, intake of antibiotics, chronic diseases, presence of diarrhea, stage of HIV, viral load, and CD4 count were not eligible for multivariable analysis (P-value> 0.25). On the opposite, in bi-variable analysis, independent variables like sex, educational status, occupational status, history of hospitalization, experience with HAART, and previous history of ICU were eligible for multivariate analysis (P-value< 0.25), and all these variables were recruited for multivariate analysis. However, in the multivariate analysis, all variables did not significantly correlate with the isolation of ESBL-producing *Enterobacteriaceae* in the fecal sample (Table 5).

Table 3: Factors associated with fecal carriage of ESBL-producing Enterobacteriaceae isolates at TASH, Addis Ababa, Ethiopia, from April 01, 2021, to July 20, 2022.

Variable	**Category**	**Bacterial growth**		**Bi-variant analysis**	**Multivariate analysis**	
		Yes	**No**	**AOR (95%, CI)**	**AOR (95%, CI)r**	**P-value**
Sex	Male	135	30	1.448(.830-2.526)	1.346 (.735-2.466)	.336
	Female	189	29	Ref**	Ref**	
Educational status	Illiterate	31	7	Ref**	Ref**	
	Primary	71	16	1.837 (.689-4.893)	2.219 (.807-6.101)	.122
	Secondary	100	21	1.833 (.855-3.930)	1.840 (.838-4.042)	.129
	Diploma and above	122	15	1.708 (.837-3.486)	1.739 (.836-3.615)	.139
Occupational status	Unemployed	41	12	2.707 (.803-9.131)	2.384 (.648-8.768)	.191
	Governmental	107	18	1.556 (.495-4.895)	1.384 (.414-4.626)	.598
	Nongovernmental	64	15	2.168 (.670-7.019)	1.917 (.568-6.476)	.294
	Daily Labour	75	10	1.233 (.362-4.197)	.968 (.265-3.535)	.961
	Housewife	37	4	Ref**	Ref**	
Hospitalization	Yes	52	14	1.627 (.833-3.178)	1.771 (.882-3.554)	.108
	No	272	45	Ref**	Ref**	

HAART experience	Not started	62	10	1.253 (.518-3.030)	1.232 (.499-3.043)	.651
	< 6 months	73	19	2.022 (.939-4.354)	2.095 (.950-4.619)	.067
	6-12 months	88	17	1.501 (.690-3.263)	1.403 (.628-3.133)	.409
	> 12 months	101	13	Ref**	Ref**	
Previous history of ICU	Yes	2	2	5.649 (.78- 40.919)	4.698 (.584-37.79)	.146
	No	322	57	Ref**	Ref**	

Discussion

As global reports indicated, the gastrointestinal carriage of ESBL-PE and carbapenemase-producing Enterobacteriaceae has become a major challenge for hospitalized patients worldwide. Infections caused by ESBLPE and carbapenemase-producing Enterobacteriaceae, usually multi-drug resistance, make the treatment option challenging (51). In the current study, a total of 324 Enterobacteriaceae were isolated, of which E. coli accounted for the highest proportion 32.1% (123/324), followed by K. pneumonia 23.2% (89/324) and K.oxytica 7.8% (30/324). The current finding was comparable with the previous study conducted in Tanzania, which reported that a total of 244 isolates of Enterobacteriaceae were isolated from 194 participants, and E. coli was the predominant microbe, 209/244 (85.7%), followed by Klebsiella pneumonia, 33/244 (13.5%) (39), a study conducted in Nigeria (a total of 240 Enterobacteriaceae were isolated from stool collected from 100 HIV patients; of which 41.6% E.coli, 13.8 K. pneumonia) (52) and a study done in University of Gondar Comprehensive Specialized Hospital Gondar, Ethiopia 186 Enterobacteriaceae isolated from a total of 161 HIV participants with E. coli 60% and K. pneumonia 16.13% predominance (53). This finding was somewhat higher compared with a previous study conducted in Nepal (37.82% of Enterobacteriaceae isolated) (54), Zimbabwe (16.0% Enterobacteriaceae isolated) (38), and the survey conducted in Western Cameroon, which reports the overall prevalence of Enterobacteriaceae among HIV patient was 46.15% (42).

In the current study, the antimicrobial sensitivity patterns of fourteen selected commonly used antibiotics were checked for all of the isolated Enterobacteriaceae and the finding indicated that a high level of resistance was observed to ampicillin (97.8%) followed by Cefoxitin (92.0%), gentamicin (85.8%), cotrimoxazole (78.7%), and amoxicillin-clavulanic acid(59.3%) and the current finding was in comparison with the finding of the previous study conducted in University of Gondar Comprehensive Specialized Hospital Gondar, Ethiopia (51), Debre Berhan Comprehensive Specialized Hospital, Ethiopia (55), Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Amhara, Ethiopia (56), and a study conducted in Arba Minch General Hospital, Arba Minch, Ethiopia (57). On the other hand, a low level of resistance rate was recorded against imipenem (7.1%), meropenem (7.1%), and ceftazidime (7.1%). The current finding was comparable with the finding of the previous study conducted in Debre Berhan Comprehensive Specialized Hospital, Ethiopia (55), Addis Ababa, Ethiopia (58), and Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Amhara, Ethiopia (56).

From a total of 324 isolated *Enterobacteriaceae*, the predominant isolated *Enterobacteriaceae* is *E.coli* which accounts for 38.0% (123/324), and *E.coli* showed the highest resistance to ampicillin (97.6%), followed by Cefoxitin (95.9%), gentamicin (82.1%), and cotrimoxazole (79.7%). This was in agreement with the study conducted in Gondar, Ethiopia (51), Debre Berhan, Ethiopia (55), Bahir Dar, Ethiopia (56), and a survey conducted in Arba Minch General Hospital, Arba Minch, Ethiopia (57). The second most prevalent *Enterobacteriaceae K. pneumoniae* showed the highest rate of resistance against ampicillin (100%), gentamicin (89.9%), Cefoxitin (89.9%), cotrimoxazole (85.4%), and amoxicillin-clavulanic acid (74.2%) and lower resistance for cefepime (3.4%), imipenem (6.7%), meropenem (6.7%) and tetracycline (7.9%). This was nearly comparable with the report of the previous study conducted in Nepal (54), Zimbabwe (38), Western-Cameroon (42), Bahir Dar, Ethiopia (56), and a survey conducted in Arba Minch General Hospital, Arba Minch, Ethiopia (57). The possible reason for the high resistance pattern for most isolated pathogens in the commonly used antibiotics may be due to inappropriate prescription of antibiotics and self-medication practice. So, the increase in resistance patterns for those commonly available antibiotics is the primary health threat for most developing countries, which is closely related to the lack of resources in developing countries. In addition, if there is poor hand hygiene, these resistant bacteria can spread from one patientto another via healthcare workers' contaminated hands, predisposing the patients to infection by antibiotic-resistant bacteria.

The overall multi-drug resistance (MDR) rate of the isolated *Enterobacteriaceae* MDR in the current study was 71.9%. The current finding was relatively comparable with the findings of the previous studies done in Northwest Ethiopia (MDR 87.4%) (59) in Nepal (MDR 96.84 %) (60), in Debre Berhan, Ethiopia (55), and comparatively higher with the finding of the study conducted in Addis Ababa, Ethiopia (MDR 42.1%) (61). This inconsistency might be due to the indiscriminate use of antibiotics, poor hygienic practices in the study area, increased MDR strain through time due to selective pressure, and differences in the study population.

The overall ESBL production rate in the current study was 74.1% (240/324), and the predominant ESBL-producing *Enterobacteriaceae* was *E. coli (*26.9%), followed by *K. pneumonia* (19.6%). The current finding was in line with the finding of the previous studies conducted in Nepal (68.91%) (54), Dar es Salaam, Tanzania (59.7%)(62) and was relatively higher than the finding of the study conducted in Gondar, Ethiopia (19.9%) (53), Dar es Salaam, Tanzania (34.3%)(51), a community setting in Tanzania (32.6%) (63), Zimbabwe (13.7%)(38) and Debre Berhan, Ethiopia (47.3%)(55). This variation might be due to the difference in thestudy population, HIV status of the patients, drug intake habits of the participants, hygiene-related variations, variations in antibioticresistance prevention measures, and variations in the method of ESBL detection. In addition to the above reasons, the potential reason for the difference in magnitude of ESBL-producing *Enterobacteriaceae* among HIV patients may be due to several factors such as variation in type and frequency of isolates, sample size, study participants, and geographical location.

The current study found that the overall fecal carriage of carbapenemase-producing *Enterobacteriaceae* among HIV patients was 2.8 %. This finding was in agreement with the finding of a study conducted in Western Cameron (4.81%)(42)

and Arba Minch, Ethiopia (1.43%) (57), and higher compared with a study conducted in South Africa (no carbapenemase-producing *Enterobacteriaceae* is isolated)(64). This variation may be possible due to differences in carbapenemase detection method, methodological differences, sample size, and study participants.

7. Strengths and Limitations of The Study

7.1. Strength of the study

- Rapid Carba NP test, which is highly sensitive and specific, was used to detect carbapenemase-producing *Enterobacteriaceae*.
- This study has tried to investigate factors associated with fecal carriage of ESBL and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients,which had not been documented previously in Ethiopia.

7.2. Limitations of the study

- AmpC beta-lactamase was not phenotypically confirmed due to a lack of confirmatory kits.
- A molecular test for ESBL and carbapenemase gene characterization was not done from isolated *Enterobacteriaceae*.
- Healthy participants from the community were not included as controls.

Conclusion

The current study finding indicated that the overall incidence of extended-spectrum- beta-lactamase-producing *Enterobacteriaceae and* fecal carriage of multi-drug resistance of *Enterobacteriaceae were* high among HIV patients. The highest resistance was recorded against ampicillin (97.6%), Cefoxitin (95.9%), gentamicin (82.1%), and cotrimoxazole (79.7%). The predominant ESBL-producing *Enterobacteriaceae* were *E. coli (38.0%, 123/324)* and *K. pneumonia* (27.5%, 89/324). ESBL-producing *Enterobacteriaceae* showed high resistance to ampicillin, Cefoxitin, gentamicin, and cotrimoxazole.

The overall magnitude of carbapenemase-producing *Enterobacteriaceae* was 2.8 %. The predominant carbapenemase producer was *K.pneumoniae (*55.6%), followed by *K.oxytica* (33.3%) and *E.coli (*11.1%). Also, the AST finding of the current study indicated that carbapenemase-producing *Enterobacteriaceae* were resistant to most beta-lactam antibiotics. In this study, no independent variable is seen that has a significant association with the rate of carbapenemase-producing *Enterobacteriaceae.*

Recommendations

The high rate of carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae and carbapenemase-producing Enterobacteriaceae among HIV patients is the primary medical treatment. Therefore.

- Strict infection prevention measures should be implemented to limit thedissemination of ESBL-PE and CPE in the study area.
- Rational use of antibiotics should be applied to prevent the cross-transmissionand occurrence of resistant strains.
- Large-scale research that can assess a wide geographical area with a largepopulation needs to be done.
- Nationwide active surveillance of antibiotic resistance, including hospital-basedand community-based, should be employed.
- Early screening of ESBL-PE and CPE is recommended.

List of Abbreviations

AMR: Antimicrobial Resistance
AST: Antimicrobial susceptibility testing
ATCC: American Type Culture Collection
CDDT: Confirmatory disk Diffusion test
CLSI: Clinical and Laboratory Standards Institute
DDST: Double disk synergy test
DRERC: Department of research and ethics review committee
ESBL: Extended spectrum β-lactamases
MAC: MacConkey agar
MBL: Metallo beta-lactamases
MDR: Multidrug Resistant
MHA: Mueller Hinton agar
TSB: Tryptose Soya Broth
SHV: sulfhydryl variable
SMART: Study for Monitoring Antimicrobial Resistance Trends
WHO: World Health Organization

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