Fecal carriage of Extended-spectrum Beta-lactamase and Carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, North Shoa, Amhara Regional State, Ethiopia.

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A Research thesis submitted to Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology, and Parasitology in partial fulfilment of the requirements for the degree of Master of Science in Medical Microbiology

June 2021

Addis Ababa, Ethiopia

Acknowledgments

First and foremost, I would like to express my gratitude to the Almighty God for giving me the strength and wisdom throughout my career. Secondly, I would like to express my deepest gratitude to my advisors Dr. Yimtubezinash Woldeamanuel, and Prof. Daniel Asrat, for their intellectual counsel, follow-up, and direction during the study process. Mr. Melese Hailu has my sincerest gratitude for his unwavering support during the preparation of this study. My heart full gratitude would also go to Mr.Birhanu Yitayew, Mr.Assaye Mitku, Mr. Kassu Desta, and Ms. Saba G/Michael for their support of reagents to conduct this research. My deepest gratitude also goes to Mr. Getabalew Engidaye for his technical assistance during data analysis.

I would also like to convey my gratitude to the Addis Ababa University Department of Microbiology, Immunology, and Parasitology for giving me this opportunity to conduct this research. Special thanks go to Debre Berhan Comprehensive Specialized Hospital in particular Getinet Mamo for the primary permission to conduct this research in the Microbiology laboratory utilizing the necessary tools and supplies.

My sincere gratitude will also extend to Debre Berhan Comprehensive Specialized Hospital laboratory staffs, for their technical and coordination support of the entire laboratory work. My unforgettable acknowledgment goes to Debre Berhan University to give me this opportunity particularly the Department of Medical laboratory science. Last but not least, I would like to acknowledge the study participants from which the sample was collected.

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List of Abbreviations and Acronyms

AMR	Antimicrobial Resistance
ARB	Antibiotic Resistance Bacteria
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Cell Culture
CDC	Centre of Disease Control and prevention
CLSI	Clinical Laboratory Standard Institute
CPE	Carbapenemase Producing- *Enterobacteriaceae*
CRE	Carbapenem Resistance- *Enterobacteriaceae*
ESBL	Extended-Spectrum Beta- Lactamase
ESBL-PE	Extended-Spectrum Beta-Lactamase Producing *Enterobacteriaceae*
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
MDR	Multi-Drug Resistance
MHA	Muller Hinton Agar
NICU	Neonatal Intensive Care unit
QC	Quality Control
SOP	Standard Operating Procedure
TSB	Trypticase Soya Broth
WHO	World Health Organization
XLD	Xylose lysine deoxycholate

Abstract

Background: The rapid spread of extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing *Enterobacteriaceae* (CPE) has become a major public health issue. Gastrointestinal carriage of ESBL and CPE are the significant risk factors for infection and dissemination of resistance clones in the hospital. Therefore, conducting research on the carriage rate of ESBL and carbapenemase-producing *Enterobacteriaceae* is very important to prevent nosocomial infection and the spread of antimicrobial-resistant bacteria in the hospital setting.

Objective: To determine the fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, North Shoa, Amhara Regional State, Ethiopia.

Methods: A hospital-based cross-sectional study was conducted among 383 hospitalized patients from November 2020 to March 2021. Stool sample or rectal swab was aseptically collected and cultured on MacConkey agar and Xylose lysine deoxycholate agar. The isolated *Enterobacteriaceae* were further identified by conventional biochemical tests. Screening of ESBL production was done by using cefotaxime and ceftazidime. All *Enterobacteriaceae* were screened for carbapenemase production using meropenem and imipenem. Confirmation of ESBL production was done using the combination disk method. Carbapenemase production was confirmed by a rapid Carba NP test. Data was entered using Epi data version 3.1 then analyzed using Statistical Package for Social Sciences software version 25. P-value ≤ 0.05 was considered as statistically significant.

Results: A total of 347 *Enterobacteriaceae* were isolated. The overall magnitude of ESBL-PE and CPE was 47.3% (n=164/347) and 5.5% (n=19/347) respectively. The predominant extended-spectrum beta-lactamase-producing *Enterobacteriacea*e (ESBL-PE) were *E. coli* 25.9% (n=90/347) and *K. pneumoniae* 15.9% (n=55/347). *K. pneumoniae* 3.5% (n= 12/347) was the highest carbapenemase producer. The overall multidrug resistance rate was 87.8% (n=305/347). The highest resistance was observed to ampicillin (98.3%), followed by gentamicin (80.7%), and tetracycline (73.3%) respectively. History of antibiotic use in the past 3 months (p≤0.000), admission in a neonatal intensive care unit (p=0.023), and presence of chronic disease (p≤0.000) were independently associated with fecal carriage of ESBL-PE. History of hospitalization in the past 12 months showed statistical significance with fecal carriage of CPE (p= 0.02).

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

Keywords: Fecal carriage, ESBL, carbapenemase, *Enterobacteriaceae,* Debre Berhan, Ethiopia, hospitalized patients

1. Introduction

1.1. Background

The *Enterobacteriaceae* family is a large and diverse collection of Gram-negative rods that reside in the gut of humans and animals. They are the most common sources of both community and hospital-acquired infections. These bacteria also cause a variety of infections, including urinary tract infections (UTIs), bloodstream infections, pneumonia, peritonitis, meningitis, and device-associated infections (Nordmann *et al.*, 2012a, Diriba *et al.*, 2020).

Antibacterial agents of the beta-lactam group are the commonly prescribed antibiotics for the treatment of infections caused by multi-drug resistant (MDR) *Enterobacteriaceae*. However, the emergence of resistance to beta-lactam antibiotics has become a major challenge for the treatment of severe nosocomial infection (Andrew *et al.*, 2017).

The production of beta-lactamases is the main mechanism of resistance to beta-lactam antibiotics in *Enterobacteriaceae*. Among the beta-lactamases, the production of ESBLs and carbapenemase are the most common. ESBL enzymes can break or hydrolyze many beta-lactam antibiotics including penicillins, cephalosporins, and monobactam except for cephamycins, clavulanate, and carbapenems (Mohamudha *et al.*, 2010).

The emergence phenomenon of extended-spectrum beta-lactamase in *Enterobacteriacea*e has consequently increased the consumption of carbapenems. These antibacterial agents are the crucial treatment option for life-threatening nosocomial or hospital-acquired infections. The rise of carbapenem resistance may imperil or halt the advancement of current medical treatments. It is clear that very few novel antibiotics will be discovered in the near future, making the issue of carbapenem-resistant *Enterobacteriaceae* of primary importance worldwide (Nordmann et al., 2012a).

Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) has spread worldwide and has become endemic in several countries since their first description in 1983 (Ghafourian *et al.*, 2015). Their spread is primarily due to ESBL-encoding genes, which are frequently carried by mobile genetic elements, such as plasmids, that facilitate their dissemination (Mahamat *et al.*, 2019).

Fecal carriage of ESBL-PE and CPE are the significant risk factors for infection with antibiotic-resistant bacteria for hospitalized patients since the bacteria can spread from

colonized persons to others by hand carriage as well as contaminated food and water (Mulki *et al.*, 2017, Ruh *et al.*, 2019, Mahamat et al., 2019). Therefore, local epidemiological data on the carriage of ESBL-PE and CPE is very important to prevent nosocomial infection and the spread of antimicrobial resistance in hospitals.

1.2. Statement of the problem

Antimicrobial resistance is an emerging global health issue. Over the past decade, there has been an increasing prevalence of carriage and infection with multidrug-resistant organisms (MDROs) such as ESBL and carbapenemase-producing *Enterobacteriacea*e (CPE) (Woerther *et al.*, 2013).

Infections caused by MDR organisms have increased mortality compared to those caused by non-MDR bacteria and they cause a huge economic burden, estimated at over 20 billion dollars per year in the US only (DiazGranados *et al.,* 2005). The Centres for Disease Control and Prevention estimated that at least 23,000 people die annually in the USA as a result of infections caused by an antibiotic-resistant organism (CDC, 2013). Similarly, according to a recent report, antibiotic resistance is estimated to cause around 300 million premature deaths by 2050, with a loss of up to $100 trillion to the global economy. This worsening situation results in leaving clinicians without antibiotics to treat infected patients (Munita and Arias, 2016).

ESBL-producing *Enterobacteriaceae* accounts for a major risk worldwide in hospital settings. The problem is worrying because ESBL enzymes can hydrolyze almost all beta-lactams except carbapenems and cephamycins. In addition, these enzymes are usually encoded by genes found on highly mobile plasmids, providing the ability of clonal and horizontal transfer. These plasmids can also confer resistance genes to other classes of antibiotics including aminoglycosides, trimethoprim, sulphonamides, tetracyclines, and chloramphenicol (Cantón, 2006). ESBL and carbapenemase are easily spread within and between *Enterobacteriaceae*, because they are plasmid and transposon (mobile genetic element) mediated (Wilmore *et al.*, 2017).

The gastrointestinal tract is the principal reservoir for *Enterobacteriaceae* infections, whether they are acquired in the hospital or the community. Additionally, the gastrointestinal tract is the place where the exchange of resistance genes between bacteria happens and antibiotic treatment selects the over-growth of resistant bacteria. Consequently colonization by ESBL and carbapenemase-producing *Enterobacteriaceae* is one of the most important risk factors for antibiotic-resistant bacterium infection (ARB)(Rolain, 2013). These infections pose a great challenge which increases hospital stay and cost leading to the increased morbidity and mortality rates due to the limited therapeutic options (Lynch III *et al.,* 2013). It has been reported that infections caused by

ESBL-PE have a fatality rate ranges from 42% to 100%. On the other hand, the CRE associated infection mortality rate reaches up to 70% (Friedman *et al.*, 2017)

Carbapenems are the last choice of antibiotics for the treatment of multidrug-resistant *Enterobacteriaceae* (MDR-E) infections. However, carbapenem-resistant *Enterobacteriaceae* (CRE) is an ongoing public health problem globally (Nordmann *et al.*, 2011, Codjoe and Donkor, 2017). Resistant to carbapenems by the production of carbapenemase is becoming a challenge though limiting the treatment option leads to failure of beta-lactam therapy which may result in high economic loss and mortality (Thomson, 2010).

In 2017 WHO has published a list of antibiotic resistance priority pathogens; which presents a great concern for humans to which new antibiotics are urgently needed. According to this report carbapenem resistant *Enterobacteriaceae* (CRE) are grouped as critical that need new antibiotics to use against them urgently (WHO, 2017b).

Carriage of ESBL and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients are a threat to the future of antibiotic treatment. The high burden of ESBL and carbapenemase-producing *Enterobacteriaceae* among healthy asymptomatic individuals have a significant public health effect, for the treatments of both hospital and community-acquired infections. Since these commensals translocate to sterile sites and cause untreatable infection (Babu *et al.*, 2016).

Fecal carriage of ESBL and carbapenemase-producing *Enterobacteriaceae* has been increasingly reported worldwide over the last decade. The highest carriage prevalence has been described in Asia whereas prevalence rates are lower in Europe and North America (Islam *et al.*, 2017, Pilmis *et al.*, 2018). However, data on the fecal carriage of ESBL and carbapenemase-producing *Enterobacteriaceae* in Sub-Saharan Africa including Ethiopia is scarce (Mahamat et al., 2019).

Although infection caused by ESBL and carbapenemase-producing *Enterobacteriaceae* is a global threat; the burden is higher in low-income countries like Sub-Saharan Africa (SSA) where, widespread self-medication, overcrowding of hospitals, absence of antibiotic prescription guidelines, poor infection control practices, and poor hygiene and antibiotic misuses are common (Vialle-Valentin *et al.*, 2012).

Previously few research have been undertaken in the context of infection caused by ESBL-PE and carbapenem-resistant *Enterobacteriaceae* in Ethiopia (Abayneh and Worku, 2020).

However, little is known regarding the gastrointestinal carriage rate of ESBL-PE and CPE in hospitalized patients (Aklilu *et al.*, 2020, Desta *et al.*, 2016). To our knowledge there is no study conducted in Debre Berhan Comprehensive Specialized Hospital regarding fecal of carriage extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* Therefore, knowing the magnitude of fecal carriage of ESBL and carbapenemase-producing *Enterobacteriaceae* is needed for the prevention and control of nosocomial infection and dissemination of antimicrobial-resistant strains. Hence, this study aimed to determine the fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients in Debre Berhan Comprehensive Specialized Hospital (DBCSH), North Shoa, Amhara Regional State, Ethiopia.

1.3. Significance of the study

This study shows the antimicrobial resistance pattern of beta-lactamases and non- beta-lactamases producing *Enterobacteriaceae* from the fecal specimen of hospitalized patients at Debre Berhan Comprehensive Specialized Hospital which helps for the rational use of antibiotics and careful selection of empirical therapy. In addition, the detection of ESBL and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients gives local baseline data for infection control and epidemiological surveillance. This study provides information on the prevalence of fecal carriage of MDR *Enterobacteriaceae*. This will further help stakeholders to amend or develop a new infection control program in the hospital. This study will also be used as a reference for further research.

2.Literature review

2.1 Overview of antimicrobial resistance

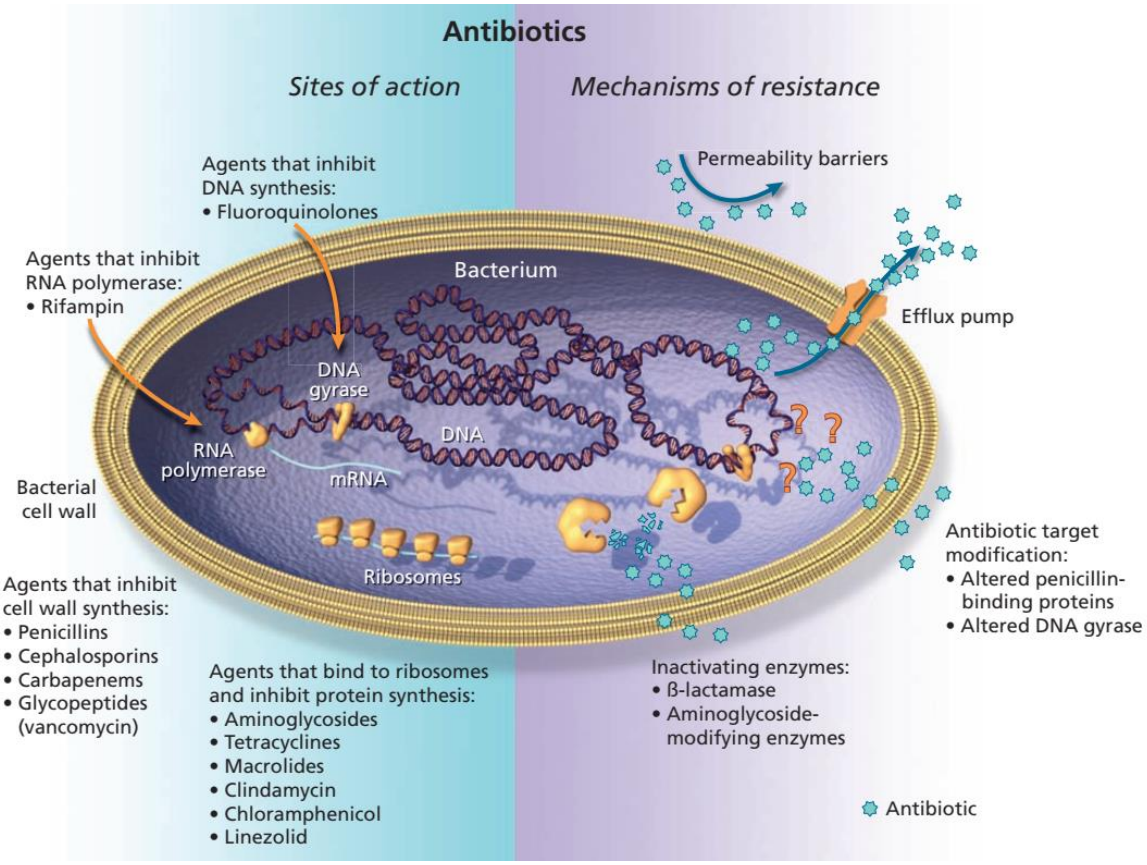
Antimicrobial resistance (AMR) is quickly spreading across the globe and is a significant threat to public health. The gastrointestinal tract plays a major role in the development of antibiotic resistance and harbouring the microorganisms as commensals. Asymptomatic fecal carriage of MDR bacteria had been reported from several countries and continents with wide differences in carriage prevalence rates between geographic areas (Sharma and Arjuna, 2017).

AMR is increasing in both healthcare settings and the community around the globe. The dissemination of MDR *Enterobacteriaceae* particularly extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* (CPE), is alarming. Severe infections due to MDR *Enterobacteriaceae* are associated with the worst outcomes and increased mortality, especially when adequate antibiotic therapy is delayed. Low- and middle-income countries are mostly affected but precise data from their hospitals are often deficient (Sangare *et al.*, 2017).

In Africa, the incidence of ESBL-producing *Enterobacteriaceae* among hospitalized patients has been shown to vary between countries and the type of specimen examined. It has been reported a higher prevalence of ESBL in stool samples than in other specimens. There is also a trend of increasing prevalence resistance mediated by ESBL from time to time. ESBL-PE particularly (class A and D carbapenemase) are becoming common in Africa (Storberg, 2014).

2.2. General Mechanisms of drug resistance in bacteria

Antibiotics work by interacting with specific bacterial targets, inhibiting bacterial cell-wall synthesis, protein synthesis, or nucleic acid replication. To do this, the antibiotic must have access to and bind to its bacterial target site. Whether antibiotic resistance is intrinsic or acquired, the genetic determinants of resistance encode specific biochemical resistance mechanisms that include limiting uptake of a drug; modifying a drug target; inactivating a drug, and active drug efflux (Reygaert, 2018, Munita and Arias, 2016, Mulvey and Simor, 2009).



Adopted from (Mulvey and Simor, 2009)

Figure 1:Sites of action and potential mechanisms of bacterial resistance to antimicrobial agents

2.3. Mechanisms of Drug resistance in *Enterobacteriaceae*

Antimicrobial resistance in *Enterobacteriaceae* results from the expression of antibiotic-inactivating enzymes and nonenzymatic mechanisms. Both may be intrinsically expressed by a given species (chromosomal genes), or acquired by a subset of strains as a consequence of two distinct not mutually exclusive genetic events. Mutations in chromosomal genes resulting in an increase in the expression of intrinsic resistance mechanisms either by antibiotic-inactivating enzymes or efflux pumps or permeability alterations by loss of outer membrane porins, or target modifications; horizontal transfers of mobile genetic elements (MGEs) carrying resistance genes, most notably plasmid-encoding beta-lactamases, aminoglycosides-modifying enzymes (AMEs), or non-enzymatic mechanisms such as Qnr for fluoroquinolone resistance in *Enterobacteriaceae* (Ruppe *et al.*, 2015, Iredell *et al.*, 2016).

In *Enterobacteriaceae*, the main mechanism of resistance to beta-lactam antibiotics include the following: enzymatic inactivation of the antibiotic by chromosome- and/or plasmid-

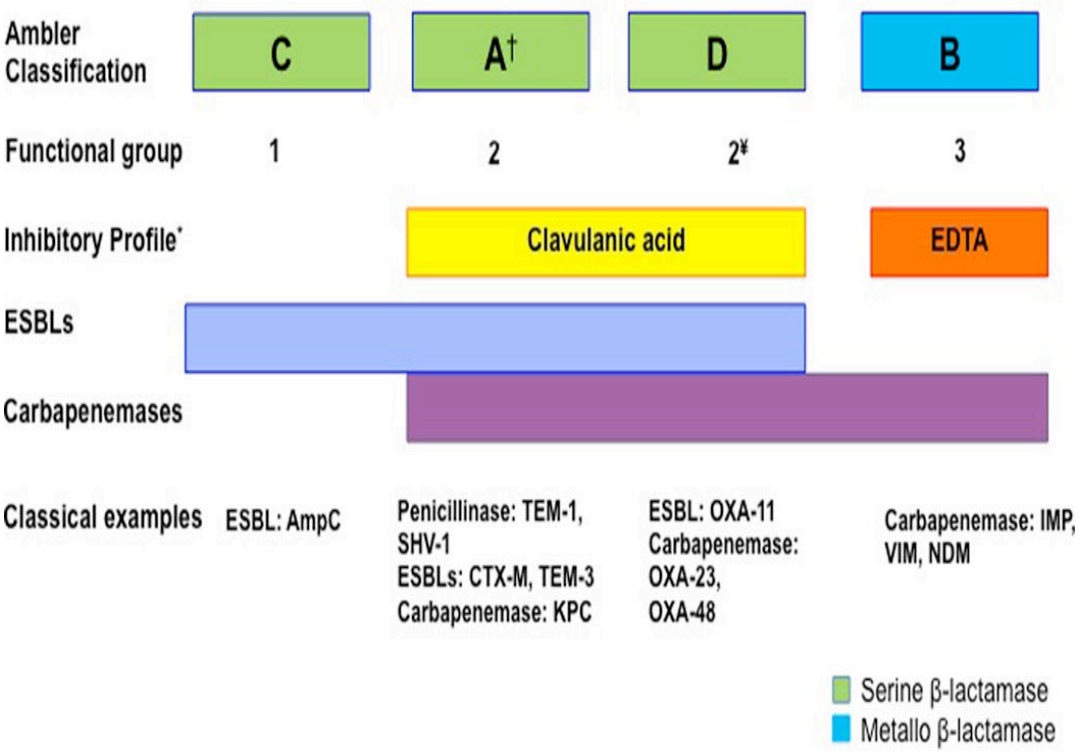
encoded enzymes possessing hydrolytic activity against beta-lactam molecules; decreased outer membrane permeability through the production of modified porins, loss of porin expression, or a change in the sorts of porins located in the outer membrane; and efflux of the antibiotic to the outside of the bacterium through the production of an efflux pump(Nordmann et al., 2012a).

Beta-lactamases produced by *Enterobacteriaceae* are diverse enzymes that hydrolyze the beta-lactam antibiotics. Based on their activity beta-lactamases can be penicillinase, cephalosporinase, ESBL, and carbapenemase. These enzymes destroy the amide bond of the beta-lactam ring, rendering the antimicrobial ineffective (Mohamudha et al., 2010, Nordmann et al., 2012a).

2.4. Classification of beta-lactamases

According to Ambler beta-lactamase is classified as A, B, C, and D based on amino acid sequence and Bush-Jacoby classified them into 4 categories based on their biochemical function, mainly based on substrate specificity. AmpC beta-lactamases are grouped under class C or group I cephalosporinases (Bush and Jacoby, 2010, Bush, 2013). The classification is summarized in Figure 3.

The molecular classification of B-lactamases follows the Ambler classification. Correlation with the main functional group of the Bush and Jacobi classification is also shown. Of note, the latter classification has several sub-groups that are not shown. Representative examples of each group of enzymes are provided.



Adopted from: (Munita and Arias, 2016)

Figure 2: Schematic representation of beta-lactamases

† Class A enzymes are the most diverse and include penicillinases, ESBLs, and carbapenemase.

¥ Ambler class D enzymes belong to the functional group/subgroup 2d.

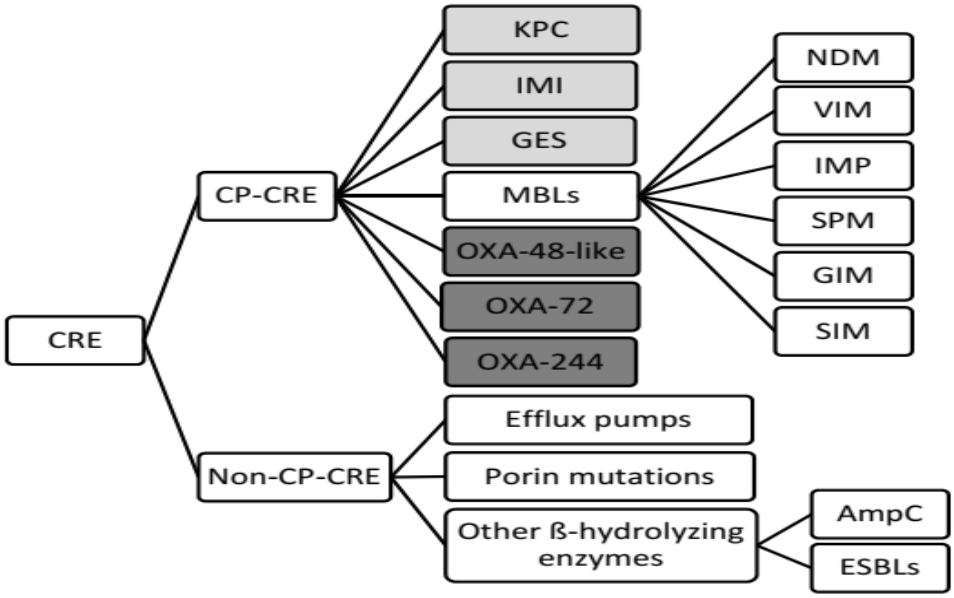
* Class A enzymes belonging to the subgroup 2br are resistant to clavulanic acid inhibition. EDTA, ethylene diamine tetraacetic acid; ESBLs, extended-spectrum β-lactamases

2.5. Mechanism of carbapenems resistance in *Enterobacteriaceae*

Carbapenem-resistant *Enterobacteriaceae* (CRE) is a group of bacteria that are resistant to carbapenem antibiotics. There are two types of CRE the one which carbapenemase-producing CRE and the other none carbapenemase-producing CRE. Carbapenemase-producing CRE genes are located on mobile genetic elements. There are five types of carbapenemase which include: *Klebsiella pneumonia* carbapenemase (KPC), class A serine-based β-lactamases, class B, New Delhi Metallo-β-lactamases (NDM), Verona integrin encoded Metallo-β-lactamase (VIM), class D, OXA or OXA-48-like carbapenemase and IMP, active on imipenem. *Enterobacteriaceae* species that have

intrinsic resistance to imipenem include *Morganella morganii*, *Proteus* spp. And *Providencia* spp (Breijyeh *et al.*, 2020).

Enterobacteriaceae become resistant to carbapenems either of the following mechanisms which include enzyme production, efflux pumps, and porin loss (Breijyeh et al., 2020, Suay-García and Pérez-Gracia, 2019).



Adopted from (Suay-García and Pérez-Gracia, 2019)

Figure 3: Classification of the different mechanisms of drug resistance in CRE

2.6. Laboratory detection methods for ESBL and carbapenemase-producing *Enterobacteriaceae*

ESBL production can be detected in different methods using conventional and automated techniques. The detection of ESBLs in the laboratory comprises two steps. The first is a screening test with an indicator cephalosporin which looks for resistance or reduced sensitivity. The Clinical and Laboratory Standards Institute (CLSI) has proposed disk diffusion and dilution methods for screening of ESBLs producing *Enterobacteriaceae*. Growth at or above the screening antibiotic susceptibility cut-off value is suspicious of ESBLs production and is an indication for the bacteria to be tested by a phenotypic confirmatory test (Rawat and Nair, 2010, CLSI, 2019).

The second is a confirmatory test that looks for synergy between cephalosporin and clavulanic acid to distinguish ESBLs enzyme-positive isolates from those resistant to other causes. Combination disks test (CDT), double-disk synergy test (DDST), broth

microdilution assay, three-dimensional test, E-test, inhibitor-potentiated disk-diffusion test, and disk approximation test are some of the phenotypic detection methods that have been developed to confirm ESBL production by *Enterobacteriaceae*. There are also other commercially available methods for detection of ESBL such as Phoenix 100, Vitek-2, and Micro Scan(Garrec H, 2011, Rawat and Nair, 2010). CLSI recommended CDT methods as a phenotypic confirmatory test for ESBLs producing *Enterobacteriaceae* (CLSI, 2019).

Phenotypic detection of carbapenemase production can be done by using the modified Hodge test, combined disk method, rapid Carba NP test, and modified carbapenemase inactivation methods (CLSI, 2019, Nordmann *et al.*, 2012b).

Genotypic methods such as polymerase chain reaction (PCR) and sequencing are also used for the detection of carbapenemase genes such as KPC, OX-48, VIM, NDM, and IMP, and ESBL genes (SHV, TEM, and CTX-M) (Asthana *et al.*, 2014).

2.7. Epidemiology of fecal carriage of ESBL and carbapenemase-producing *Enterobacteriaceae* in different countries

A hospital-based descriptive study done by Mulki *et al*., 2017 (Mulki et al., 2017) in India of 60 patients in intensive care units, 39 (65%) had fecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae.* Out of which, 22 (56%) were ESBL-producing *E. coli* and 17 (43%) *Klebsiella spp*. Another study in the same country by Babu *et al* described that the prevalence of fecal carriage rate of ESBL-PE was 62.7%. The highest ESBL-PE was *E. coli* which was 70.3% followed by *K. pneumoniae* 26.8%. ESBL-PE showed the highest sensitivity to carbapenems 85%, followed by amikacin (67.2%). Ciprofloxacin was found to have a high level of resistance (Babu et al., 2016).

A study in South Korea among hospitalized patients reported that from a total of 97 patients analysed from rectal swab 37 patients were ESBL producing *Enterobacteriacea*e carriers. Among these 37 patients, 17 patients (17.5%) had isolates of *E. coli,* 14 patients (14.4%) had isolates of *K. pneumoniae,* and 2 patients (2.1%) had isolates of *K. oxytoca*. Four patients (4.1%) had both *E coli* and *K pneumoniae*(Ko *et al.*, 2013).

According to a survey-based study conducted in Spain reported that the most isolated ESBL-producing micro-organisms at admission were *E. coli* n=655 (77.7%), followed by *K. pneumoniae* n=174 (20.64%,), with other species *E.cloacae* 0.59%; *C.freundii* 0.36%; *E. aerogenes* 0.24%; *K.oxytoca* 0.12%). Among ESBL *E. coli* isolates, 1.83% were simultaneously ESBL and carbapenemase producers (n=12). Among ESBL *K. pneumoniae*

isolates, 43.10% were simultaneously ESBL and CP producers (n=75). Of the ESBL typed, 52.15% belonged to the cefotaximases (CTX-M-15) type and 91.38% of the CP were oxacillinase (OXA-48) type% (Pérez *et al.*, 2019).

A study conducted in Hungary on comparison of rates of fecal colonization with extended-spectrum beta-lactamase-producing *enterobacteria* among patients in different wards, outpatients, and medical students showed that the overall prevalence of fecal carriage of ESBL producers among inpatients was 7.4%, whereas the prevalence of carriage was 3.1% and 2.6% in outpatients and medical students, respectively. Adults were found to have significantly higher carriage rates of ESBL producers (12.0%, than paediatric patients (4.1%; $P < 0.001$). When inpatients were grouped by ward type, the highest prevalence was found in rehabilitation (27.1%, followed by ICU patients (9.3%,). The lowest prevalence was recorded in non-ICU patients (6.1%)(Ebrahimi *et al.*, 2016).

According to the study conducted in Northern Cyprus by Ruh *et al.*, 2019 (Ruh et al., 2019) a total of 500 volunteers were recruited, from those 107 (21.4%) of 500 individuals were found to be colonized with ESBL-PE. *E. coli* accounted for the most ESBL cases n = 101 (94.4%), while *K. pneumoniae* (n = 3; 2.8%), *E. cloacae* (n =1; 0.9%), *E. aerogenes* (n = 1; 0.9%), and *P. rettgeri* (n = 1; 0.9%) were detected at low rates. Six (1.2%) out of 500 participants were colonized with CIRE. These isolates were identified as *E. coli* (n = 2/6; 33.3%), *K. pneumoniae* (n = 2; 33.3%), *K. oxytoca* (n = 1; 16.7%) and *E. aerogenes* (n = 1; 16.7%).

A study conducted in Algeria by Medboua-Benbalagh *et al* 2017 showed that of the 171 children studied, 93 (54%) were ESBL carriers. A total of 103 ESBL-PE isolates were found among these *K. pneumoniae* and *E. coli* were the predominant isolate (Medboua-Benbalagh *et al.*, 2017).

A study conducted in Chad by Mahamat *et al.*, 2019 (Mahamat et al., 2019) reported ESBL-PE rate among hospitalized patients was (51%). Antimicrobial susceptibility testing showed that few isolates were resistant to carbapenems (ertapenem and imipenem). A similar study conducted in Burkina Faso found that the prevalence of subjects with fecal ESBL-PE was 42% among hospitalized patients. ESBL-PE were mostly *E. coli* (78%). Among the sixty ESBL-producing *E. coli* strains (Ouedraogo *et al.*, 2017).

A prospective observational cohort study conducted in paediatric wards at two tertiary government hospitals in Harare, Zimbabwe among hundred and sixty-four paediatric inpatient admissions (median age = 1.0 year, IQR = 0.2–2.2years) were enrolled. On

admission, 68 (41%) patients had both ESBL and gentamicin-resistant *Enterobacteriaceae* detected, 18 (11%) had ESBL only, 17 (10%) had gentamicin resistance only and 61 (37%) had a negative screening for both forms of resistance. During hospitalization, 32 (20%) patients were found to have a type of resistant organism which was not present in their admission sample (Magwenzi *et al.*, 2017).

Another prospective cross-sectional study conducted by Tellevik *et al.*, 2016 (Tellevik *et al.*, 2016) in Tanzania reported that the overall magnitude of ESBL carriage rate was 34.3%. The frequency of ESBL carriage among hospitalized children was (50.4%). Their finding revealed *E. coli* and *K. pneumoniae* isolates have a high incidence of multidrug resistance (94%). Carbapenem resistance was not found. HIV-positive children (89.7%) had considerably greater ESBL prevalence than HIV-negative children (16.9%).

According to the prospective observational study conducted in Morocco on the rectal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized neonates revealed that from the rectal screening cultures of 164 neonates, 169 strains of *Enterobacteriaceae* were identified. The predominant species identified were *K. pneumoniae* (n = 83) and *E. coli* (n = 67. Of the 169 isolates, 98 (58.0%) were ESBL-producers, including *K. Pneumoniae* 65 (66.3%) and *E. coli 19* (19.4%). *E.cloacae*, *C. freundii*, *K. oxytoca*, and *P. mirabilis* represented lower rates of 5.1%, 4.1%, 3.1%, and 2.0%, respectively. The majority of ESBL-PE isolates were multi-resistant to a variety of antibiotics. Of the 98 ESBL-PE, 97 (99.0%) were resistant to gentamicin, 59 (60.2%) to SXT and 3 (3.1%) to ertapenem. A substantial level of resistance to quinolones was observed. 61 isolates (62.2%) were resistant to nalidixic acid and 57 isolates (58.2%) were to norfloxacin and ciprofloxacin. None of the isolates were resistant to amikacin and Fosfomycin (Arhoune *et al.*, 2017).

A cross-sectional study conducted in Egypt Abdallah *et al*., 2017 (Abdallah *et al.*, 2017) revealed that 64.61% n=73 were ESBLs isolates and five isolates showed carbapenemase producer. The highest isolate was *K. pneumoniae.*

In Ethiopia, a cross-sectional descriptive point surveillance study by Desta *et al*., 2016 at Tikur Anbesa Specialized Hospital showed a high carriage ESBL-PE of which was 52%. ESBL-*E. coli* and *K. pneumoniae* showed 68% and 32% respectively. The highest fecal ESBL-E carriage rate was in neonates (74 %) and the lowest carriage was in adults (46%). The rate of ESBL-*E. coli* colonization in neonates, children, and adults was 11%, 42%, and 42% respectively. *E. coli* strains were the leading isolated from adults, children, and

neonates, 44%, 49%, and 22% were ESBL positive respectively. The magnitude of ESBL-*K. pneumoniae* carriage in neonates, children, and adults was 68%, 22%, and 7% respectively. The overall carrier rate of CRE was 2% (Desta et al., 2016). Another facility-based cross-sectional study in Araba Minch Hospital reported overall gastrointestinal carriage of ESBL and carbapenemase-producing *Enterobacteriaceae* was 33.25% and 1.43% respectively. This study also indicated that the predominant ESBL producer was *E. coli* (42.46% and *K. pneumoniae* (41.09%)(Aklilu et al., 2020). A study in Gondar reported that the carriage rate of ESBL producing *Enterobacteriaceae* among HIV-positive children was 19.9% and all ESBL producing isolates were *E. coli* (16.2%) and *K. pneumonia* (3.7%)(Bayleyegn *et al.*, 2021).

2.8. Factors associated with fecal carriage extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae*

A study conducted in Turkey revealed that recent use of antibiotics, hospitalization and surgical operation, diabetes, crowded household populations, and old age were associated with higher carriage rates of ESBL-PE(Erdoğan *et al.*, 2017).

Another study conducted in Cyprus showed that higher socioeconomic status, antibiotic use, presence of gastrointestinal symptoms, traveling abroad were identified as the risk factors for fecal carriage of ESBL-PE(Ruh et al., 2019). Furthermore, a study conducted in Korea reported prolonged hospitalization and prolonged intensive care unit as independent risk factors for ESBL-PE(Ko et al., 2013).

A study conducted in Algeria reported that previous antibiotic use in the last 3 months and hematological malignancy were associated with fecal carriage of ESBL-PE(Medboua-Benbalagh et al., 2017).

A study done in Arba Minch showed that Chronic illness, a long history of hospitalization, the administration of oral antibiotics after admission, living in a rural region, and sex were all statistically significant factors (Aklilu et al., 2020).

A point prevalence survey in Vietnam revealed that treatment with carbapenem and duration of hospital stay were the independent risk factors for fecal carriage of CRE(Tran *et al.*, 2019). Another study in India found that length of ICU stays, usage of a ventilator, and using aminoglycosides were all risk factors for CPE carriage (Mittal *et al.*, 2016). Another study from the same country also showed the presence of any indwelling device

and nasogastric tube as independent risk factors for acquiring gut colonization (Mohan *et al.*, 2017).

A case-control study done in China showed that hospital readmission, sickbed changes, and invasive procedures were independently associated with CRE colonization(Zhao *et al.*, 2014).

2.9. Prevention and control strategies against ESBL and carbapenem-resistant infections

The first strategy to fight against drug resistance is effective infection prevention and control practices. This covers both professional and patient education. Professionals mustn't succumb to patient pressure(G, 2011).

Antibiotics should only be prescribed or administered if they are really necessary, and only after they have tested and confirmed which antibiotics their patients should be taking. Physicians should take a specimen and submit it to a clinical microbiology laboratory for culture and susceptibility testing whenever possible. In addition, clinicians should prescribe an inexpensive, narrow spectrum and an appropriate amount of antibiotics whenever the laboratory results demonstrate that such a drug effectively kills the pathogen(WHO, 2017c).

The World Health Organization (WHO) published a comprehensive multimodal guideline for the prevention and control of ESBL -PE, and CRE in healthcare settings. The first guideline is to practice good hand hygiene, which is critical in reducing the colonization and infection caused by resistant bacteria. Secondly, surveillance for ESBL and carbapenem-resistant Gram-negative bacilli infections, as well as asymptomatic colonizers. Thirdly, contact precaution entails ensuring appropriate patient placement, preventing patient transportation, wearing personal protective equipment, using disposable or dedicated equipment, and prioritizing patient room cleanliness and disinfection. Fourthly, the patients should be isolated in a dedicated room, either alone or with other patients who have been colonized with resistant gram-negative bacteria. Finally, the patient's environment should be cleaned. referred to as the patient zone(WHO, 2017a).

3.Hypothesis

The fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital is different from the previous study done in Addis Ababa.

4.Objectives

4.1. General objective

- To determine the fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, North Shoa, Amhara Regional State, Ethiopia from November 2020 to March 2021.

4.2. Specific objectives

- To determine the magnitude of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* among hospitalized patients.
- To assess the prevalence of carbapenemase-producing *Enterobacteriaceae* among hospitalized patients.
- To determine the antimicrobial resistance pattern of *Enterobacteriaceae* isolated from fecal specimens of hospitalized patients.

5.Materials and Methods

5.1. Study area

The study was conducted at Debre Berhan Comprehensive Specialized Hospital which is found in Amhara Regional State, Central Ethiopia. Debre Berhan is located 130 Km far from the capital city of the country, Addis Ababa. The hospital was first established as a health facility in 1937 by Italians to serve their soldiers during the 2nd Italian attempt to colonize Ethiopia. After that, it has served the local community for many decades and was upgraded to a Referral Hospital in 2010. It is the only hospital in North Shoa Zone/Amhara/ that serves as a Referral center for seven governmental district Hospitals and two private Hospitals. Moreover, it provides health services for over two million people of Amhara, Afar, and two woredas of Oromia regions with more than 200 beds. It has a range of departments including paediatrics, surgery, gynaecology, psychiatry, ophthalmology, HIV care, and outpatient clinics. Debre Berhan is situated at an altitude of 2840 m above sea level with a mean annual temperature that ranges from 10 to 16 °C. The weather condition of the town and surrounding areas is relatively cold, dry, and windy with two distinctive seasons summer and winter (Zemene and Shiferaw, 2018).

5.2. Study design and period

A hospital-based cross-sectional study was conducted from November 2020 to March 2021 at Debre Berhan Comprehensive Specialized Hospital, Amhara Regional State, Ethiopia.

5.3. Source population

All patients who were admitted at Debre Berhan Comprehensive Specialized Hospital during the study period.

5.4. Study population

All patients who were admitted to medical, paediatrics, surgery, neonatal intensive care unit, adult intensive care unit, gynaecology and obstetrics, and ophthalmology wards for ≥ 48 hours at Debre Berhan Comprehensive Specialized Hospital during the study period.

5.5. Inclusions and Exclusions Criteria

5.5.1. Inclusions criteria

All hospitalized patients including neonates, infants, children, and adult patients who were admitted for ≥ 48 hours and who gave informed consent or parent/guardian consent and assent were included in the study.

5.5.2. Exclusions criteria

- Patients admitted for less than 48 hours.
- Critically ill patients and unable to give a specimen

5.6. Study variables

5.6.1. Dependent variables

- The magnitude of fecal carriage of extended-spectrum beta-lactamase
- Antimicrobial resistance pattern of *Enterobacteriacea*e from fecal specimen
- The magnitude of fecal carriage of carbapenemase-producing *Enterobacteriacea*e

5.6.2. Independent variables

- Age
- Gender
- Admission ward
- Hospital stays
- History of antibiotic exposure

5.7. Sample size calculation and sampling method

5.7.1 Sample size determination

A single population proportion formula was used to determine the sample size.: n = Z2α/2 P (1- P)/ d2. The expected prevalence was taken as (Desta et al., 2016) p=52% (0.52) which is obtained from a previous study done in Addis Ababa by Desta et al., 2016 . The study used a 95 % confidence interval and hence Zα/2=1.96. d is the margin of error of 0.05.

$$n = Z2\alpha/2^2 \, P \, (1- P)/ \, d^2$$

$$n = (1.96)^2 \, 0.52 \, (1- 0.52)/ \, (0.05)^2 = 383$$ was the total sample size.

5.7.2. Sampling technique

The sample was stratified by wards of admission and then the sample size from each ward was proportionally determined. Based on the previous three-month report a total of 1994 patients were admitted to different wards. Based on this report the sample size was proportioned as 104 patients from medical,92 patients from surgical, 63 patients from paediatric, 39 patients from neonatal intensive care unit, 53 patients from gynaecology and obstetrics,25 patients from ophthalmology, and 7 patients from adult intensive care unit wards were included. From each ward, the study participants were selected conveniently.

5.8. Data collection

5.8.1. Sociodemographic data

Socio-demographic and clinical data were collected using structured questionnaires after obtaining informed consent or assent from the patient and their parents or guardians. Clinical data of the patients such as history of hospitalization in the past 12 months, history of antibiotic use in the past 3 months, and other clinical data were collected from the medical record book. For participants who cannot read and write, the information sheet was read to them and a witness signed before data collection. All this information was collected by the principal investigator.

5.8.2. Stool sample/rectal swab sample collection

A stool sample was collected from children and adults using a sterile stool cup. After collection, the stool was immediately taken to the Microbiology laboratory for analysis. A rectal swab was collected from neonates and patients that cannot give stool 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 test tube until it reached to Microbiology laboratory for bacteriological analysis.

5.8.3. Stool culture and identification

Each stool sample/rectal swab was first inoculated onto MacConkey agar (SRL. Pvt. Ltd. India). After inoculation, each plate was incubated aerobically at 37°C for 18 to 24 hrs. Then, each culture plate was examined for the growth of *Enterobacteriaceae.* Lactose fermenter and non-lactose fermenters were characterized on MacConkey agar, then non-lactose fermenter colonies were inoculated on Xylose-Lysine Deoxycholate (XLD) agar

(HiMedia. India) to observe further characteristics. Finally, pure colonies were taken for identification. As necessary, re-subculture onto MacConkey agar (SRL. Pvt. Ltd. India) was performed to get pure colonies. All isolated *Enterobacteriaceae* were further characterized and identified by colony characteristics, Gram staining, and conventional biochemical tests namely, indole, citrate utilization, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, and mannitol fermentation.

5.8.4. Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2019). After using 0.5McFarland turbidity standard for the preparation of the inoculum; Mueller Hinton agar (MHA) (Hi-Media: India) plates were inoculated and antimicrobial disks were applied on the plate. The antibiotic disks used in this study were ampicillin (AM:10µg), cefoxitin (FOX:10µg), gentamicin (GM: 10µg), ciprofloxacin (CIP: 5µg), cotrimoxazole (COT:25µg), imipenem (IMP: 10µg), meropenem (MEM: 10 µg), amoxicillin-clavulanic acid (AMC: 30µg), cefotaxime (CTX:30 µg), ceftazidime (CAZ:30µg), ceftriaxone (CRO:30µg), tetracycline (TE:30µg), cefepime (FEP:30µg), and chloramphenicol (CL:30µg) were applied for susceptibility testing. All antibiotic disks were Oxoid, United Kingdom.

5.8.5. Screening of ESBLs and carbapenemase

Enterobacteriaceae that showed an inhibition zone size of ≤ 22 mm with ceftazidime (30 µg), and or ≤ 27mm with cefotaxime (30 µg) were considered as potential ESBL producers and were further investigated for phenotypic confirmation of ESBL production. On the other hand, all *Enterobacteriaceae* that showed a zone of inhibition ≤ 19 mm for imipenem or meropenem were suspected as carbapenemase producers (CLSI, 2019).

5.8.6. Phenotypic confirmation of ESBLs with combination disk test

Ceftazidime (30 µg) and cefotaxime (30 µg) alone, as well as their combination with Clavulanic acid (30 µg g/10 µg) 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. *Enterobacteriaceae* that showed an increase in the inhibition zone diameter of ≥ 5mm for a combination disks versus ceftazidime or cefotaxime disk alone were confirmed as ESBL

producers. In all other cases, the results were considered negative for ESBL producers (CLSI, 2019).

5.8.7. Phenotypic confirmation of carbapenemase production

Enterobacteriaceae isolates that showed a zone of inhibition ≤ 19mm for imipenem or meropenem were confirmed by rapid Carba NP test kit (BioMérieux, Marcy l'Étoile, France) for the presence of carbapenemase(Nordmann et al., 2012b). It is hydrolyzed-based assay for rapid detection of carbapenemase-producing *Enterobacteriaceae*. *Enterobacteriaceae* isolates that showed red colour 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 colour on the control well (well d) and red colour on the test well (well e) or orange colour on the control well (well d) and orange on the test well were confirmed (well e) as negative for carbapenemase production.

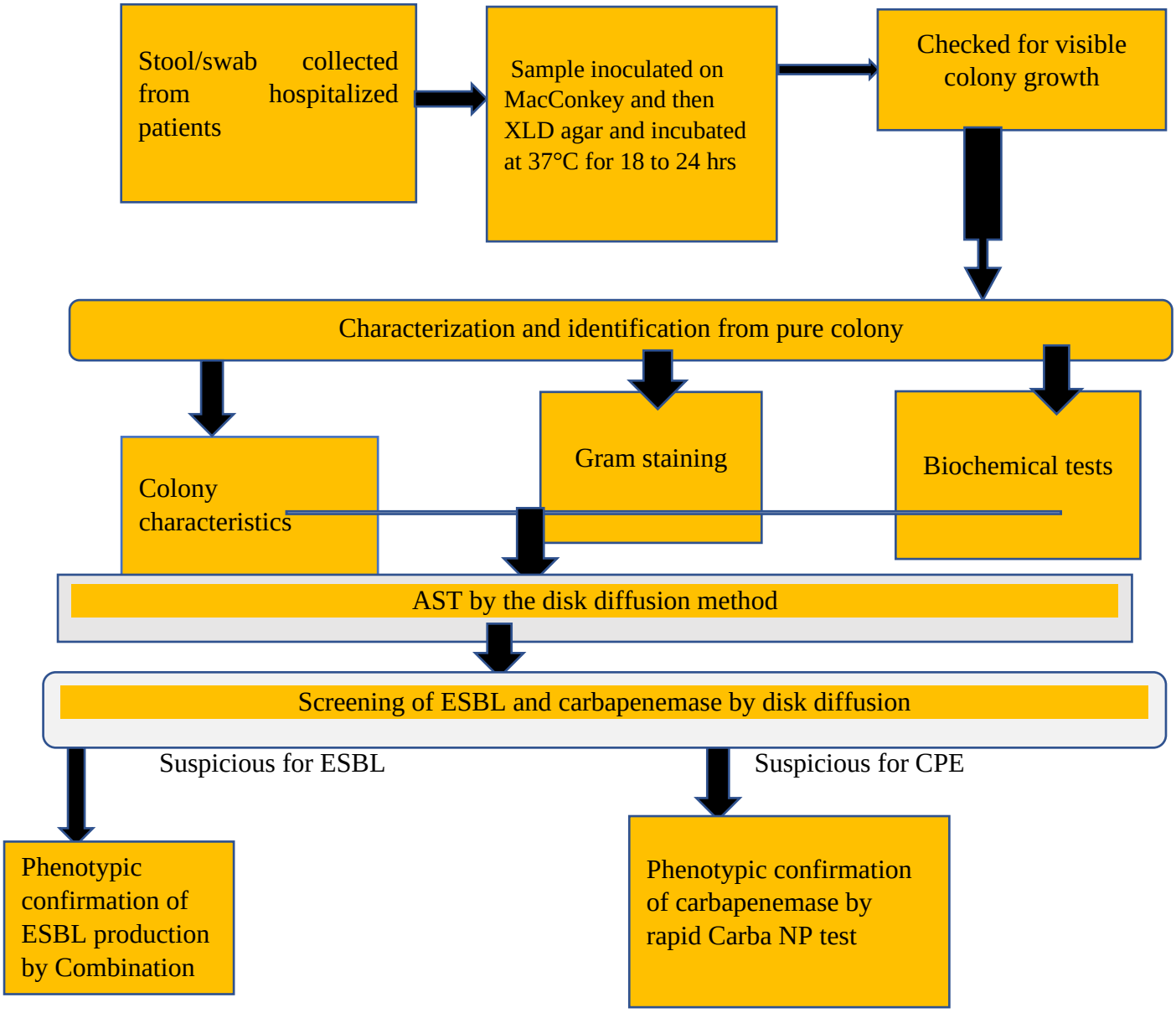


Figure 4: Flow chart for identification, susceptibility testing, ESBL and carbapenemase screening and phenotypic confirmation of *Enterobacteriaceae* among hospitalized patients at Debre Berhan Compressive specialized Hospital.

5.9. Quality control

Standard Operating Procedures (SOP) were strictly followed for each procedure. The stool specimen was processed and transported soon after receipt as possible. If there is a delay in processing the specimen, it was placed in the refrigerator. Before using the media, reagents, and antibiotic disks, the expiration dates were checked. Following sterility testing, the culture media was visually evaluated for cracks and thickness, as well as the presence of freezing, bubbles, and contaminants. Quality control for the new batch was performed using control strain. The glycerol (15%) with TSB (Trypticase Soy Broth) was QC tested for growth of *E. coli* ATCC 25922 standard strain before being used for storage. For ESBLs confirmatory test, ESBLs positive *K. pneumoniae* ATCC 700603 and ESBLs negative *E. coli* ATCC 25922 control strains were used. *K. pneumoniae* ATCC BAA-1705 was used as a positive control, and *K. pneumoniae* ATCC BAA-1706 was used as a negative control for carbapenemase detection. Finally, the isolated *Enterobacteriaceae* was stored at -20^{0}C using 15%TSBS.

5.10. Data entry and analysis

The data were entered into Epi Data version 3.1 and double-checked and cleaned before analysis. Then the data was exported to Statistical Package for Social Sciences (SPSS) version 25 for analysis. The descriptive statistics (median, percentages, or frequency) were calculated. The bivariant logistic regression analysis was used to observe the relationship between the dependent variable and independent variables. Variables that showed P-value $\leq$ 0.25 bivariant logistic regression analysis were selected for further analysis using multivariable logistic regression models. Any variables that showed a p-value $\leq$ 0.05 by multivariable logistic regression models were considered as statistically significant. Finally, the results were presented in words, graphs, and tables.

5.11. Operational definitions

I. **Multidrug Resistance (MDR**): Bacteria is resistant to one or more antibiotics in three or more classes of antimicrobials agents (Magiorakos *et al.*, 2012).

II. **ESBL producing *Enterobacteriacea*e (ESBL-PE)**: an enzyme produced by *Enterobacteriaceae* that hydrolyze penicillins, first, second, third cephalosporins and monobactam, except for cephamycins, clavulanate, and carbapenems(Ghafourian et al., 2015).

III. **Carbapenemase-producing *Enterobacteriaceae (*CPE*)***: an enzyme produced by *Enterobacteriaceae* that hydrolyze all the beta-lactams including carbapenems(Suay-García and Pérez-Gracia, 2019).

5.12. Ethical consideration

The study was reviewed and approved by the Departmental Research and Ethics Review Committee (DRERC) of Microbiology, Parasitology, and Immunology, School of Medicine, College of Health Sciences; Addis Ababa University. A written permission letter was also obtained from the Debre Berhan Comprehensive Specialized Hospital. The purpose and procedures of the study were explained to the study participants and parents or guardians during the study period by providing all information about the study in an information sheet. For participants who cannot read and write, the information sheet was read to them and a witness signed that the process had been conducted appropriately. Those patients and parents or guardians who give informed consent were enrolled in this study. The confidentiality of all study participants was maintained.

5.13. Dissemination of the result

The result of this study will be submitted to the Department of Microbiology, Parasitology and Immunology, Addis Ababa University. In addition, the result will be disseminated to Debre Berhan Comprehensive Specialized Hospital and will be presented at different scientific and research conferences. Finally, the full manuscript will be published in an International or National peer-reviewed journal.

6.Results

6.1. Socio-demographic characteristics of study participants

A total of 383 hospitalized patients were included in this study. Of which 72.8% (n=265/383) adults (age between 15 to 85 years median =41 years old) ,17% (n=65/383) children (age between 1.6 months to 13 years old median=1.25 years) and neonates 10.2% (n=39/383) (age between 4 to 22 days median =7 days) were included. Of those, nearly half of the study participants 50.4% (n=194/383) were females. The majority of the study participants had rural origin 61.9% (n=237/383). Among the study participants, 64% (n=245/383) were both married and illiterate. It was found that 28.7% (n=110/383) of the study participants were unemployed (Table 1).

Table 1: Sociodemographic characteristics of study participants at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021.

Variables	Categories	Number (n=383)	Percent (%)
Sex	Male	190	49.6
	Female	193	50.4
Age group	Neonate	39	10.2
	Children	65	17.0
	Adults	279	72.8
Residence	Rural	237	61.9
	Urban	146	38.1
Adults Marital status	Married	245	64.0
	Single	31	8.1
	Divorced	3	0.8
Educational status	Illiterate	245	64.0
	Primary	69	18.0
	Secondary	36	9.4
	Higher and above	33	8.6
Adults occupational status	Unemployed	7	28.7
	Government employed	33	8.6
	Daily labour	3	0.8
	Farmer	65	17.0
	Housewife	82	21.4
	Others	90	23.5
Family income	<1000 ETB	77	20.1
	1000-2000 ETB	115	30.0
	>2000 ETB	191	49.9

6.2. Clinical profile of the study participants

From a total of 383 study participants, 27.9%(n=107/383) of the study participants had a history of antibiotic usage in the past 3 months while 23.8 % (n=91/383) had a history of hospitalization in the past twelve months. Among 383 study participants, 8.9%(n=34/383), 3.4%(n=13/383) and ,5.7% (n=22/383) were admitted due to sepsis, urinary tract infections and pneumonia respectively. Only 12.0%(n=46/383) of the study participants had a history of invasive procedures in the past three months. More than one-fourth of the participants 27.2%(n=104/383) were admitted to the medical ward. It was found that 22.2%(n=85/383) had chronic diseases. Of them, 6.0% (n=23/383) had diabetes and 5.2% (n=20/383) were HIV positive (Table 2).

Table 2: Frequency of clinical profile of the study participants at Debre Berhan Comprehensive Specialized Hospital November 2020 to March 2021.

Variables	Categories	Frequency	Percent (%)
History of antibiotic use in the past 3 months	Yes	107	27.9
	No	276	72.1
History of hospitalization in the past 12 months	Yes	91	23.8
	No	292	76.2
Previous history of hospital stays	3-7 days	22	5.7
	8-14 days	48	12.5
	>14 days	21	5.5
Previous ICU stay	Yes	1	0.3
	No	382	99.7
Number of bed/rooms	2-4	48	12.5
	5-8	335	87.5
Number of patient/rooms	2-4	140	36.6
	5-8	243	63.4
Reason for admission	Sepsis	34	8.9
	UTI	13	3.4
	Pneumonia	22	5.7
	Meningitis	10	2.6
	Malnutrition	21	5.5
	Heart failure	28	7.3
	Peptic ulcer disease	16	4.2
	Others	239	62.4
History of invasive procedure in the past 3 months	Yes	46	12.0
	No	337	88.0
Types of invasive procedure	Catherization	32	8.4
	Surgery	14	3.6
Admission ward	Neonatal ICU	39	10.2
	Paediatric	63	16.4
	Medical	104	27.2
	Surgery	92	24.0
	Adult ICU	7	1.8
	Gynaecology and obstetrics	53	13.8
	Ophthalmology	25	6.5
Ward stays	3-7 days	326	85.1
	8-14 days	44	11.5
	>15 days	13	3.4
Chronic disease	Yes	85	22.2
	No	298	77.8
Types of chronic disease	Diabetes	23	6.0
	HIV	20	5.2
	Hypertension	31	8.1
	Haematological malignancy	1	0.3
	Tuberculosis	6	1.5
	Kidney disease	1	0.3
	Heart disease	3	0.8
Diarrhoea	Yes	0	0.0
	No	383	100
International travel within 6 months	Yes	0	0.0
	No	383	100

6.3. Prevalence of *Enterobacteriaceae*

A total 347 *Enterobacteriaceae* were isolated in this study. The most predominant isolates were *E. coli* 63.7% (n=221/347) followed by *K. pneumoniae* 26.5% (n=92/347), *E. cloacae* 3.5% (n=12/347) and *K. oxytoca* 2.6% (n= 9/347) respectively (Figure 5).

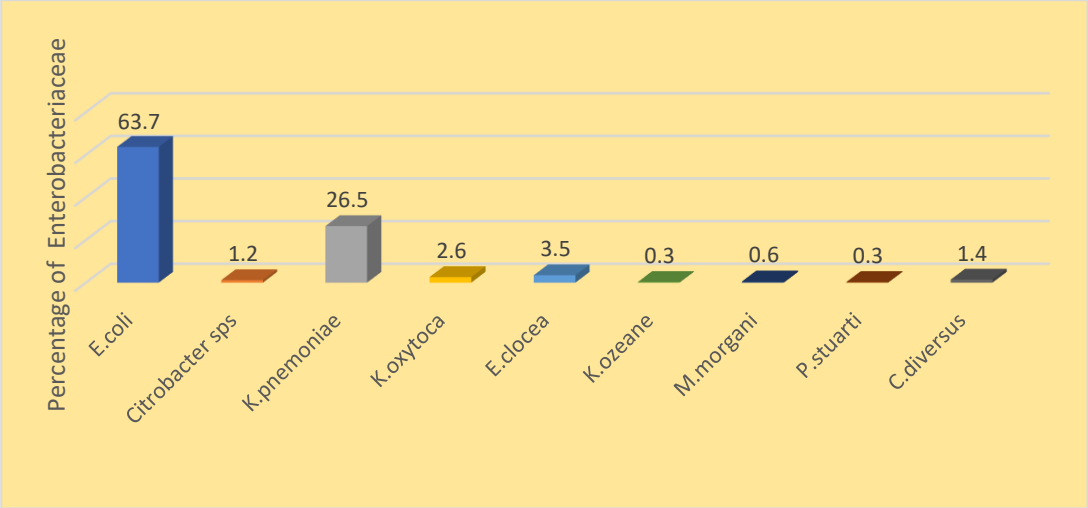


Figure 5:: Frequency of *Enterobacteriaceae* isolated from Debre Berhan Comprehensive Hospital from November 2020 to March 2021.

6.4. Antimicrobial resistance pattern of *Enterobacteriaceae*

In this study, antimicrobial susceptibility testing was done for all the isolated *Enterobacteriaceae* against fourteen selected antibiotics. The highest level of resistance was observed to ampicillin (98.3%) followed by gentamicin (80.7%), tetracycline (73.3%), and cotrimoxazole (64.8%) respectively. A low level of resistance was recorded against carbapenems (imipenem (6.3%) and meropenem (6.9%)) followed by chloramphenicol (15.9%) (Table 3).

E. coli isolates showed the highest resistance to ampicillin (97.3%) followed by gentamicin (73.3%), tetracycline (67.9%), and cotrimoxazole (54.8%). Among *K. Pneumoniae* isolates about (15.2 %) were resistant to meropenem **(**Table 3).

Among the isolated *Enterobacteriaceae* resistance against cefoxitin was not observed in *K. ozaenae and P. stuarti*i. Whereas, *Citrobacter* spp, *K. pneumoniae, K. oxytoca, E.cloaceae, K. ozonae, M. morganii, P. stuartii,* and *C. diversus* were 100% resistance against ampicillin **(**Table 3**)**.

Table 3: Antimicrobial resistance pattern of *Enterobacteriaceae* isolated at Debre Berhan Comprehensive Specialized from November 2020 to March 2021

	Antimicrobial resistance level in no. (%) of bacterial isolates													
Isolates(number) n(%)	**AMP**	**FOX**	**GM**	**CIP**	**COT**	**IMP**	**MER**	**AMC**	**CTX**	**CAZ**	**CRO**	**TE**	**FEP**	**C**
***E. coli* (n=221)**	215(97.3)	42(19.0)	162(73.3)	66(29.9)	121(54.8)	6(2.7)	6(2.7)	103(46.6)	94(42.5	66(29.9)	93(42.)	150(67.9)	35(15.8)	19(8.6)
***Citrobacter sps* (n=4)**	4(100.0)	2(50.0)	4(100.0)	1(25.0)	3(75.0)	0(0.0)	0 (0.0)	2(50.0)	3(75.0)	2(50.0)	3(75.0)	4(100.0)	0(0.0)	0(0.0)
***K.pneumoniae* (n=92)**	92(100.0)	3(38.0)	88(95.7)	54(58.7)	78(84.8)	12(13.0)	14 (15.2)	68(73.9)	63(68.5)	50(54.3)	65(70.7)	74(80.4)	34(37.0)	25(27.2)
***K.oxytoca* (n=9)**	9(100.0)	4(44.4)	8(88.9)	6(66.7)	7(77.8)	4(44.4)	4(44.4)	7(77.8)	9(100.0)	9(100.0)	9(100)	7(77.8)	5(55.6)	2(22.2)
***E. cloaca* (n=12)**	12(100.0)	6(50.0)	10(83.3)	5(41.7)	11(91.7)	0(0.0)	0(0.0)	8(66.8)	8(66.7)	7(58.3)	8(66.7)	12(100.0)	4(33.3)	7(58.3
***K. ozaenae* (n=1)**	1(100.0)	0(0.0)	1(100.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)	1(100.0)	1(100.0	1(100.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)
***M. morganii* (n=2**	2(100.0)	2(100.0)	2(100.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	2(100.0)	2(100.0)	2(100.0)	0(0.0)	2(100.0)	0(0.0)	2(100).0)
***P. stuartii* (n=1)**	1(100.0)	0(0.0)	0(0.0)	0(0.0)	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(0.0)	0(0.0)
***C. diversus* (n=5)**	5(100.0)	1(20.0)	5(100.0)	0(0.0)	3(60.0)	0(0.0)	0(0.0)	5(100.0)	4(100.0)	3(60.0)	2(40.0)	5(100.0)	0(0.0)	0(0.0)
Total resistance (n=347)	341(98.3)	92(26.5)	280(80.7)	133(38.3)	225(64.8)	22(6.3)	24(6.9)	196(56.5)	184(53.0)	140(40.3)	181(52.2)	255(73.3)	78(22.5)	55(15.9)

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: C: chloramphenicol

6.5. Multi-drug resistance pattern of *Enterobacteriaceae*

Among the total *Enterobacteriaceae* isolates (n=347) multi-drug resistance (resistance to at least 3 antibiotics in a different class) was observed in 87.6% (n=305/347). Surprisingly 100% MDR was recorded in *K. oxytoca, E. cloacae, Citrobacter* spp, *M. morganii, K. ozaenae, and C. diversus.* MDR was not observed in *P. stuartii* (Table 4).

Table 4: Multi-drug resistance patterns of *Enterobacteriaceae* isolates at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021.

	Antibiotic resistance level (n (%)								
Isolates(number)	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**≥R7**	**Total MDR Isolates (R≥3)**
E. coli (n=221)	1(0.5)	12(5.4)	27(12.2)	30(13.6)	32(14.4)	27(12.2)	21(9.5)	71(32.2)	181(81.9)
Citrobacter sps (n=4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	2(50.0)	4(100.0)
K. pneumoniae (n=92)	0(0.0)	0(0.0)	1(2.2)	7(7.6)	1(1.0)	10(9.7)	9(9.7)	64(69.5)	91(98.9)
K oxytoca (n=9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(11.1)	8(88.9)	9(100.0)
E. cloacae (n=12)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(16.6)	2(16.6)	8(66.6)	12(100.0)
K. ozaenae (n=1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	1(100.0)
M. morganii (n=2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100.0)	2(100.0)
P. stuartii (n=1)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
C. diversus (n=5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1((20.0)	2(40.0)	2(40.0)	5(100.0)
Total (n=347)	1(0.3)	13(3.7)	28(8.1)	37(10.6	33(9.5)	41(11.8)	36(10.3)	158(45.5)	305(87.8)

Note: R0: resistance to no antibiotics, R1-7: resistance to 1, 2, 3, 4, 5, 6, and 7 antibiotics; ≥R3: resistance to 3 or more antibiotics from different classes.

6.6. Magnitude of extended-spectrum beta-lactamase-producing *Enterobacteriaceae*

Of the total 347 *Enterobacteriaceae,* 52.1% (n=181/347) were suspected of ESBL production. Out of 181 suspected *Enterobacteriaceae*, ESBL production was confirmed in 90.6% (n=164/181) using a combination disk test (Figure 6).

The overall magnitude of ESBL producing *Enterobacteriaceae* was 47.3% (n=164/347) which accounts for *E. coli* 25.9% (n=90/347) followed by *K. pneumoniae* 15.9% (n=55/347) and other *Enterobacteriaceae* 5.5%(n=19/347).

The distribution of ESBL producing *Enterobacteriaceae* was varied among species. The highest ESBL-PE was observed in *K. oxytoca* 88.9% (n=8/9) followed by *K. pneumoniae* 59.8% (n=55/92) and *E.cloaceae* 50.0% (n=6/12) respectively. No ESBL production was observed in *M. morgani*i and *P. stuartii* (Figure 7).

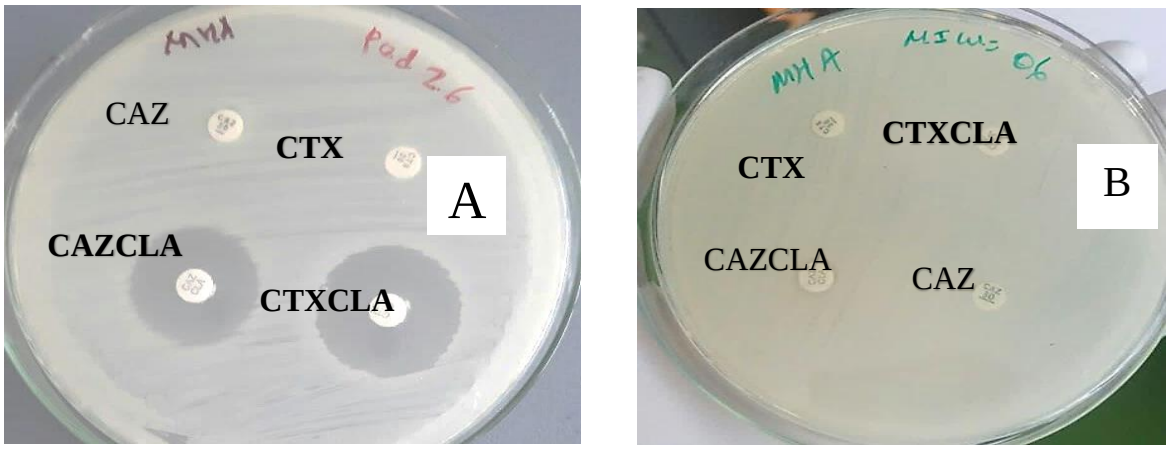


Figure 6:ESBL positive (A) and ESBL negative (B) *Enterobacteriaceae* using combination disk method from stool sample at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021.

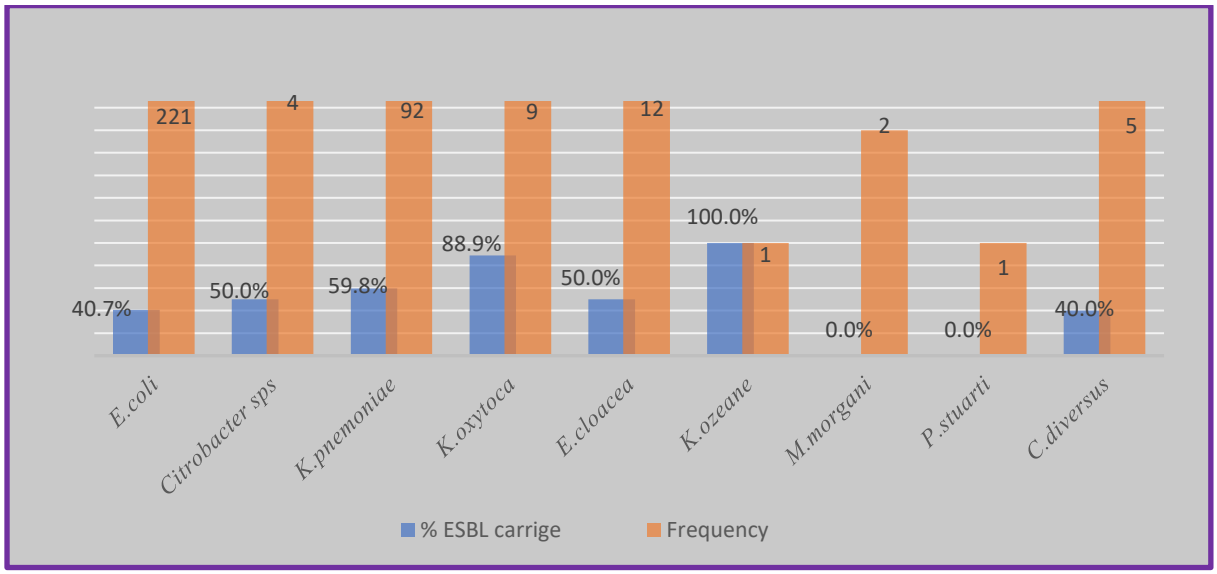


Figure 7: Frequency of ESBL producing *Enterobacteriaceae* at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021

6.7. Magnitude of carbapenemase-producing *Enterobacteriaceae*

Out of the 347 *Enterobacteriaceae*, 21 were suspected of carbapenemase production. Of 21 suspected, 90.5% (n=19/21) were confirmed as carbapenemase producers using the Carba NP test (Figure 8). The overall magnitude of carbapenemase-producing *Enterobacteriaceae* was 5.5% (n=19/347). The highest percentage of carbapenemase producer was *Klebsiella pneumoniae* which was 3.5% (n=12/347) followed by *K. oxytoca* 1.2% (n=4/347) and *E. coli* 0.9% (n=3/347**)** (Figure 9).

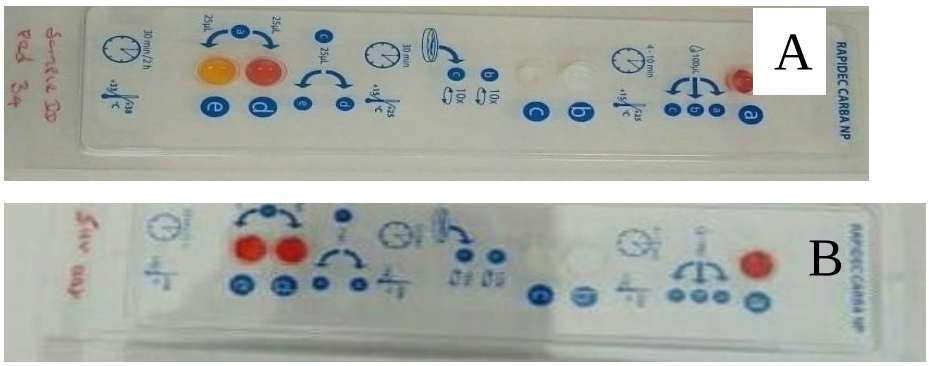


Figure 8: Carbapenemase positive (A) and carbapenemase negative (B) using Carba NP test from the fecal sample at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021.

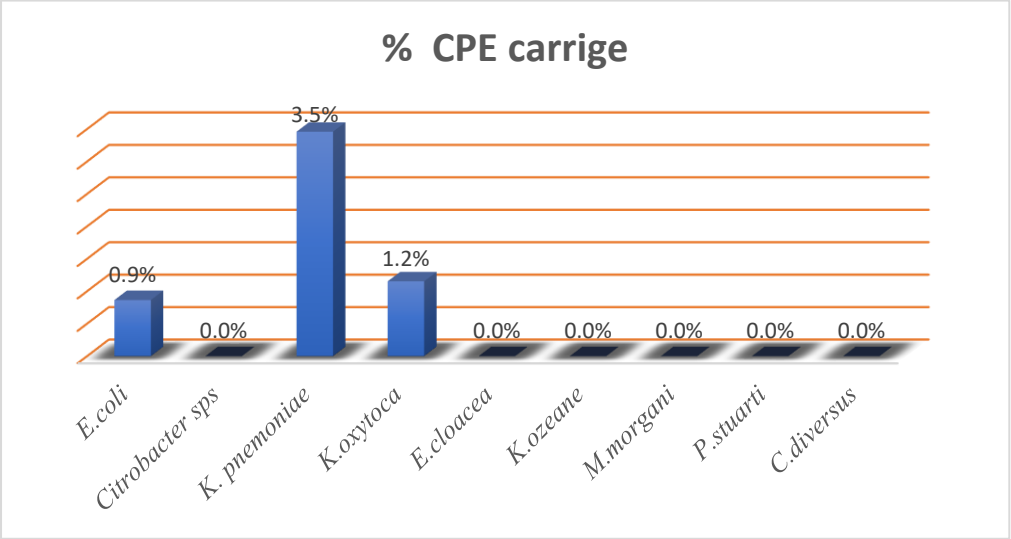
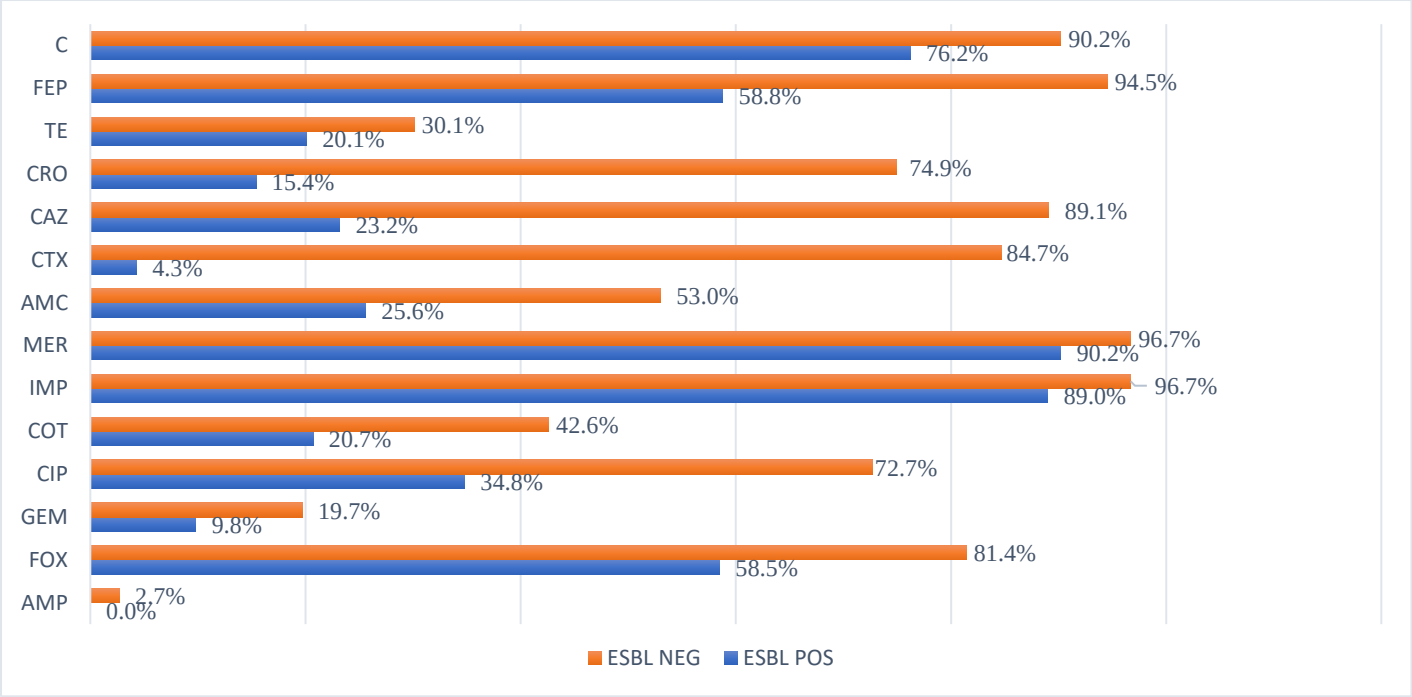


Figure 9: Frequency of carbapenemase-producing *Enterobacteriaceae* from a fecal sample at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021.

6.8. Antibiotic susceptibility patterns of ESBL producing *Enterobacteriaceae*

In this study, non-ESBL producing *Enterobacteriaceae* were more sensitive to antibiotics than ESBL producing *Enterobacteriaceae*. Meropenem, imipenem, and chloramphenicol were active antibiotics for ESBL producing *Enterobacteriaceae* with a sensitivity of 90.2%, 89.0%, and 76.2% respectively. In addition, 34.8%, 25.6%, and 20.7% of ESBL producing *Enterobacteriaceae* were sensitive to ciprofloxacin, amoxicillin-clavulanic acid, and cotrimoxazole respectively. On the other hand, non- ESBL producing *Enterobacteriaceae* were 96.6%,94.5%, and 90.6% sensitive to both meropenem and imipenem, cefepime, and chloramphenicol respectively. The antibiotic susceptibility of ESBL producing *Enterobacteriaceae* is displayed in Figure 10**.**

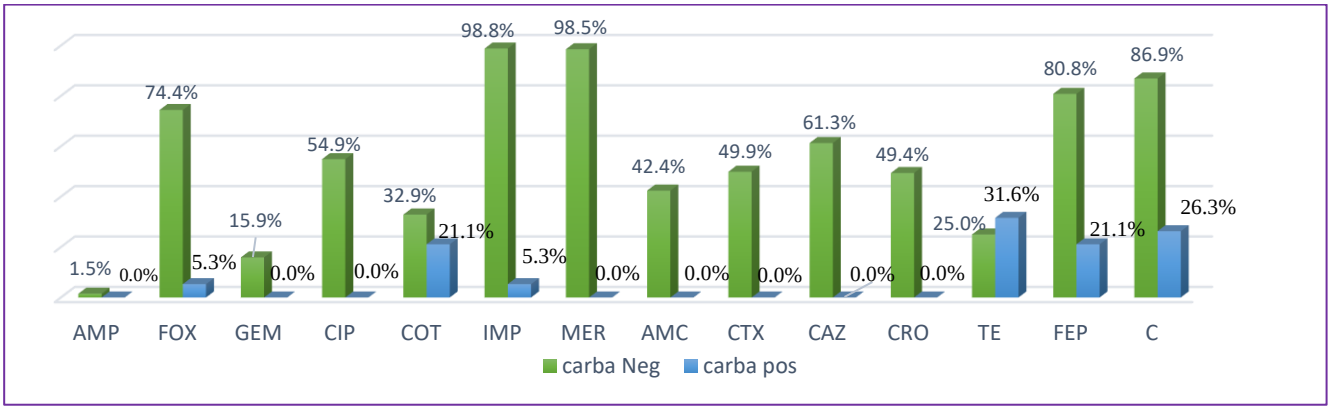


Note: AMP: ampicillin, FOX: cefoxitin, GM: gentamicin, CIP: ciprofloxacin, COT: cotrimoxazole: IMP: imipenem, MER: meropenem, AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, CRO: ceftriaxone: TE: tetracycline, FEP: cefepime: C: chloramphenicol

Figure 10: Antibiotics susceptibility pattern of ESBL positive and ESBL negative *Enterobacteriaceae* at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021.

6.9. Antibiotic susceptibility pattern of carbapenemase-producing *Enterobacteriaceae*

In the present study, carbapenemase-producing *Enterobacteriaceae* showed no susceptibility to the beta-lactam group of antibiotics except cefoxitin, cefepime, and imipenem which showed the sensitivity of 5.3%,21.1%, and 5.3% respectively. Surprisingly, tetracycline showed the highest sensitivity for carbapenemase-producing *Enterobacteriaceae* which was 31.6% (Figure11). Moreover, chloramphenicol showed a sensitivity of 26.3%, followed by cotrimoxazole 21.1%. On the other side, non-carbapenemase-producing *Enterobacteriaceae* showed high sensitivity to imipenem, meropenem, chloramphenicol, and cefepime with a sensitivity of 98.8%. 98.5%, 86.9%, and 80.8% respectively (Figure11).



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: C: chloramphenicol

Figure 11: Antibiotic susceptibility pattern of carbapenemase positive and carbapenemase negative *Enterobacteriaceae* at Debre Berhan Comprehensive Specialized Hospital from November 2020 to March 2021.

6.10. Distribution of beta-lactamase-producing *Enterobacteriaceae* and MDR

In the current study, a total of 305 MDR *Enterobacteriaceae* were isolated. Among 305 *Enterobacteriaceae* 53.3%(n=163/305) were ESBL positive. From the total 305 *Enterobacteriaceae*, 93.5%(n=286/305) were carbapenemase negative. Among the total 181 MDR *E. coli* 49.2%(n=89/181) were ESBL positive (Table 5**)**.

Table 5: Distribution of ESBL-PE and CPE and MDR isolates at Debre Berhan Comprehensive Hospital from November 2020 to March 2021.

Isolates(number)	ESBL +VE n(%)	ESBL -VE n(%)	Carbapenemase +VE n (%)	Carbapenemase -VE n (%)	Total MDR n%)
E. coli (n=221)	89 (49.2)	92(50.8)	3(1.65)	178(98.35)	181(59.3)
Citrobacter sps (n=4)	2(50.0)	2(50.0)	0(0.0)	4(100)	4(1.3)
K.pneumoniae (n=92)	55 (61.1)	35(41.1)	12(13)	79(86.8)	91(29.8)
K oxytoca (n=9)	8 (88.9)	1(11.1)	4(44.4)	5(55.6)	9(2.9)
E. cloacae (n=12)	6(50.0)	6(50.0)	0(0.0)	12(100)	12(3.9)
K. ozaenae (n=1)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	1(0.0)
M. morganii (n=2)	0(0.0)	2(100)	0(0.0)	2(100)	2(0.6)
P. stuartii(n=1)	0(0.0)	1(100)	0(0.0)	1(100)	0(0.0)
C. diversus (n=5)	2(40.0)	3(60)	0(0.0)	5((100)	5(1.63)
Total(n=347)	163(53.4)	142(46.6)	19(6.3)	286(93.7)	305(87.8)

6.11. Association of independent variables with fecal carriage of ESBL producing *Enterobacteriaceae*

In bivariate logistic regressions analysis, all independent variables including socio-demographic and clinical data were assessed to determine whether they were contributing factors or not for fecal carriage of ESBL producing *Enterobacteriacea*e. Admission in NICU ward[AOR= 4.86, 95%CI: (1.24-18.96)], history of antibiotic use in past 3 months [AOR= 4.68, 95%CI: (2.28-9.58)] and presence of chronic disease [AOR= 3.65, 95%CI: (1.87-7.13)] showed statistical significance for fecal carriage of ESBL producing *Enterobacteriaceae* (Table 6).

Table 6: Factors associated with fecal carriage of ESBL producing *Enterobacteriaceae* at Debre Berhan Comprehensive specialized Hospital from November 2020 to March 2021**.**

Variables	Categories	ESB carriage n (%)	COR (95% CI)	P- value	AOR (95% CI)	P -value
Age group	Neonates	23 (6.6)	3.92(1.69-9.09)	0.001	1.65(0.9-4.79)	0.67
	Children	31(8.9)	1.69(0.945-3.02)	0.077	1.50(0.28-8.09)	0.63
	Adults	110(31.7)	1			
History of antibiotic use in the past 3 months	Yes	80(23.1)	7.35(4.25-12.71)	0.000	4.68(2.28-9.58)	**0.000**
	No	84(24.2)	1			
History of hospitalization in the past 12 months	Yes	62(17.9)	4.03(2.36-6.86)	0.000	1.02(0.48-2.14)	0.960
	No	102(24.4)	1			
History of invasive procedure in the past 3 months	Yes	37(10.7)	5.63 (2.63-12.09)	0.000	2.28(0.91-5.74)	0.078
	No	127(36.6)	1			
Admission ward	NICU	23(6.6)	6.11(1.91-19.55)	0.02	4.86(1.24-18.96)	**0.023**
	Pediatric	31(8.9)	2.84(1.01-7.77)	0.039	1.15(0.38-3.52)	0.81
	Medical	39(11.2)	1.45(0.57-3.69)	0.432		
	Surgery	39(11.2)	1.69(0.66-34.32)	0.273		
	Gynecology and obstetrics	17(4.9)	1.24(0.44-3.49)	0.676		
	Ophthalmology	8(2.3)	1			
Chronic disease	Yes	60(17.3)	4.45(2.55-7.75)	0.000	3.65(1.87-7.13)	**0.000**
	No	104(30.0)	1			

Note: CI = Confidence interval, COR= Crude odd ratio and AOR = Adjusted odd ratio 1 =reference

6.12. Association of independent variables with fecal carriage of carbapenemase-producing *Enterobacteriaceae*

Firstly, all independent variables were assessed in bivariate logistic regressions analysis to observe significant association for fecal carriage of carbapenemase-producing *Enterobacteriaceae.* History of hospitalization in the past 12 months [AOR= 4.47, 95%CI: (1.26-15.76)] was statistically significant for fecal carriage of carbapenemase-producing *Enterobacteriaceae* (Table 7).

Table 7: Factors associated with fecal carriage of carbapenemase-producing *Enterobacteriaceae* at Debre Berhan Comprehensive specialized Hospital from November 2020 to March 2021.

Variables	Categories	Carbapenemase carriage n (%)	COR (95% CI)	P -value	AOR (95% CI)	P -value
History of antibiotic use in the past 3 months	Yes	10(2.9)	2.89(1.13-7.35)	0.026	1.04(0.32-3.39)	0.938
	No	9(2.6)	1			
History of hospitalization in the past 12 months	Yes	12(3.5)	0.17(0.45-0.65)	0.000	4.47(1.26-15.76)	**0.020**
	No	7(2.0)	1			
History of invasive procedure in the past 3 months	Yes	6(1.7)	3.23(1.19-9.23)	0.021	1.17(0.35-3.95)	0.796
	No	13(3.7)	1			
Chronic disease	Yes	9(2.6)	3.20(1.25-8.17)	0.015	1.63(0.55-4.81)	0.376
	No	10(2.9)	1			

Note: CI = Confidence interval, COR= Crude odd ratio and AOR = Adjusted odd ratio 1=reference

7.Discussions

Gastrointestinal carriage of ESBL and carbapenemase producing *Enterobacteriaceae* become a major challenge for hospitalized patients worldwide. Infections caused by ESBLPE and carbapenemase-producing *Enterobacteriaceae* usually multi-drug resistance makes the treatment option challenging (Abdallah et al., 2017). Even without infection colonization with ESBL-PE and CPE are the main threat that can lead to cross-transmission and self-infection among hospitalized patients (Kibwana *et al.*, 2020). Therefore, conducting research on the carriage rate of ESBL and carbapenemase-producing commensal is very important to prevent nosocomial infection and the spread of antimicrobial-resistant bacteria in the hospital setting.

7.1. Prevalence of *Enterobacteriaceae*

A total of 347 *Enterobacteriaceae* were isolated in this study. Among these, *E. coli* accounted for the highest proportion 221(63.6%) followed by *K. pneumoniae* 92(26.5%). The result was comparable with the previous study done in Addis Ababa, Ethiopia which showed that *E. coli* (79.7%) was the most common isolate followed by *K. pneumoniae* (19.7%) (Desta et al., 2016). Similarly, a study from Gondar, Ethiopia showed that *E.coli* (59.7%) and *K. pneumoniae* (16.1%) as frequent identified *Enterobacteriaceae* (Bayleyegn et al., 2021). Additionally, a study from Turkey also showed *E. coli* (94.5%), *K. pneumoniae* (5.1%) (Hazirolan *et al.*, 2018) as predominant isolates. Other species commonly isolated following *E. coli* and *K. pneumoniae* in this study were *E. cloacae.* This finding indicates *E. coli* and *K. pneumoniae* were the commonest normal flora that colonizes the gastrointestinal tract like other studies reported previously.

7.2. Antimicrobial resistance patterns of *Enterobacteriaceae*

Among the fourteen antibiotics used in this study, a high level of resistance was observed to ampicillin (98.3%) followed by gentamicin (80.7%), tetracycline (73.3%), and cotrimoxazole (64.8%) respectively. A low level of resistance rate was recorded against imipenem (6.3%) and meropenem (6.9%). Comparable results were also reported from Arba Minch, Ethiopia (Aklilu et al., 2020), Tanzania (Kibwana et al., 2020), Egypt (Abdallah et al., 2017), and Morocco (Arhoune et al., 2017). The reason for the high level of resistance to ampicillin might be being cheap and the first line of treatment makes these antibiotics to be highly misused.

E. coli isolates showed the highest resistance to ampicillin (97.3%) followed by gentamicin (73.3%), tetracycline (67.9%), and cotrimoxazole (54.8%). This was in close agreement with the study conducted in Addis Ababa, Ethiopia, Gondar, Ethiopia (Bayleyegn et al., 2021)) and Tanzania (Kibwana et al., 2020).

In *K. pneumoniae* the highest rate of resistance was recorded against ampicillin (100%), followed gentamicin (95.7%), cotrimoxazole (84.8%), and tetracycline (80.4%). This was comparable with the findings done in Arba Minch, Ethiopia (Aklilu et al., 2020), Tanzania (Kibwana et al., 2020), and Morocco (Arhoune et al., 2017). This high resistance pattern among the isolates may be due to inappropriate prescription of antibiotics, and self-medication practice. The increase of AMR is a threat for many developing countries; since the absence of detection methods due to lack of resources in developing countries. In addition, if there is poor hand hygiene these resistant bacteria can spread from one patient to another via healthcare worker's contaminated hands that predispose the patients to infection by antibiotic-resistant bacteria.

In this study, the overall carriage rate of MDR-*Enterobacteriaceae* (MDR-E) was 87.8%. This finding was comparable with the studies reported in Arba Minch, Ethiopia (71%) (Aklilu et al., 2020), and Tanzania (94%) (Tellevik et al., 2016). However, this finding was higher than studies done in Addis Ababa, Ethiopia (43%) (Desta et al., 2016), Gondar, Ethiopia (38.7%) (Bayleyegn et al., 2021), and Morocco(42.8%) (Girlich *et al.*, 2014). This inconsistency might be due to indiscriminate use of antibiotics, poor hygienic practice in the study area, the increase of MDR strain through time as a result of selective pressure, and differences in the study population.

In the present study,100% MDR carriage rate was seen in *K. oxytoca, E. cloacae, Citrobacter spp, M. morganii,* and *C. diversus.* Being colonized by such multidrug-resistant bacteria is recognized to be a cause of infection and cross-transmission. Therefore, good hygienic practice and careful infection prevention should be implemented in the study setting.

7.3. Magnitude of ESBL producing *Enterobacteriaceae*

The overall magnitude of ESBL producing *Enterobacteriaceae* (ESBL-PE) in this study was 47.3% 95% CI (42.0%-52.2%). This result was comparable with the reports done in Addis Ababa, Ethiopia (52%) (Desta et al., 2016), Chad (46%) (Mahamat et al., 2019), Madagascar (49%) (Andriatahina *et al.*, 2010), Burkina Faso (42%) (Ouedraogo et al., 2017), and India (43%) (Chaudhary *et al.*, 2018).

However, it was lower than compared to the studies done in Egypt (65%) (Abdallah et al., 2017), Tanzania (60%) (Kibwana et al., 2020), Algeria (54%) (Medboua-Benbalagh et al., 2017), Morocco (58%) (Arhoune et al., 2017), and India (63%) (Babu et al., 2016). In contrast, the current finding was higher than the studies done in Gondar, Ethiopia (16%)(Bayleyegn et al., 2021) Arba Minch, Ethiopia (33%) (Aklilu et al., 2020),Zimbabwe(41%) (Magwenzi et al., 2017) ,Spain (7.69%) (Pérez et al., 2019), Cyprus (21.4%) (Ruh et al., 2019), Turkey (34%) (Hazirolan et al., 2018).This variation might be due to the difference in the study population, inappropriate use of antibiotics, variation in antibiotic resistance prevention measures, and variation in the method of ESBL detection.

In the present study, *E. coli* 25.9% and *K. pneumoniae* 15.9% were predominant ESBL producer. This result was lower than the previous findings from Addis Ababa, Ethiopia *E. coli* (70%) the highest ESBL producer (Desta et al., 2016), Tanzania *E.coli* (68%) (Kibwana et al., 2020), Burkina Faso: *E. coli* (78%)(Ouedraogo et al., 2017) Cyprus *E. coli* (94.4%)(Ruh et al., 2019), and Spain *E. coli* (77.7%) (Pérez et al., 2019).

However, our finding was higher than the studies done in Gondar, Ethiopia *E. coli* (16.2%)(Bayleyegn et al., 2021) Morocco *E. coli* (19.4%) (Arhoune et al., 2017), and Korea *E. coli* (14.4%) (Ko et al., 2013). The potential reason for the difference in magnitude of ESBL among *Enterobacteriaceae* could be several factors such as variation in type and frequency of isolates, sample size, study participants, and geographical location.

7.4. Antimicrobial susceptibility patterns of ESBL producing *Enterobacteriacea*e

In this study, ESBL producing *Enterobacteriaceae* were highly susceptible to meropenem (90.2%) and imipenem (89.0%). The highest susceptibility of carbapenems was in close agreement with the studies conducted in Addis Ababa, Ethiopia (Desta et al., 2016), Zimbabwe (Wilmore et al., 2017), and India (Babu et al., 2016) where all reported a 100% susceptibility of carbapenems drugs. This highest susceptibility of ESBL-PE to carbapenems might be unavailability and high cost of carbapenems in healthcare settings and pharmacies of developing countries like Ethiopia and being last resort drug, limit the overuse and misuse of such antibiotics, consequently there will be low resistance against carbapenems.

In the present study, ESBL producing *Enterobacteriaceae* also resistant to multiple antibiotics including aminoglycosides, sulfonamides, tetracycline, and

other class antibiotics used. Similar findings were reported in Chad and Burkina Faso (Ouchar Mahamat *et al.*, 2019, Ouedraogo et al., 2017).

ESBL producers showed the highest resistance to gentamicin (91.2%) followed by tetracycline (79.9%) and cotrimoxazole (79.3%). ESBL producers also showed significant resistance to amoxicillin-clavulanic acid (74.4%), ciprofloxacin (65.2%), and cefoxitin (41.5%). This result was fairly similar to the findings reported in Arba Minch, Ethiopia (Aklilu et al., 2020) Morocco (Arhoune et al., 2017), Egypt (Abdallah et al., 2017), and Tanzania (Kibwana et al., 2020) that all revealed ESBL-PE showed the highest resistance to gentamicin, tetracycline, and cotrimoxazole. The possible justification for the high co-resistance to other classes of antibiotics used in this study might be irrational use of antibiotics, poor hygienic practice in the study area, and prescription of antibiotics without antimicrobial susceptibility testing in the study area. In addition, co-resistance to non-beta lactam antibiotics in this study could be explained by the fact that gene codes for ESBL production are usually found on the same mobile genetic elements: that may also carry resistance genes for non-beta-lactam antibiotics. This finding indicates high fecal carriage ESBL producing *Enterobacteriaceae* that were also resistant to most antibiotics. This poses a high risk for nosocomial infection, dissemination of resistance genes and thus resistant pathogen in the hospital.

7.5. Risk factor for fecal carriage of ESBL producing *Enterobacteriaceae*

In this study, all socio-demographic and clinical factors were analyzed as an independent risk factor for ESBL-PE carriage. Multivariable logistic regression identified 3 variables as a contributing factor for ESBL-PE carriage. Antibiotic use in the past 3 months, presence of chronic diseases, and admission in neonatal intensive care unit showed statistically significant association with fecal carriage of ESBL-PE.

Participants who had a history of antibiotic use in past 3 months were 4.68 times more likely carrier for ESBL producing *Enterobacteriaceae* than who had not to use (AOR 4.68, 95% CI (2.28-9.58). This was similar to the studies conducted in Gondar, Ethiopia (Bayleyegn et al., 2021), Tanzania(Kibwana et al., 2020), Algeria (Medboua-Benbalagh et al., 2017), Burkina Faso(Ouedraogo et al., 2017), and Cyprus(Ruh et al., 2019). This might indicate inappropriate use of

antibiotics by the study participants which may result in selective pressure in the bacteria. This finally could have a role for ESBL carriage.

Another factor that had associated with the fecal carriage of ESBL-PE was the presence of chronic disease. This was consistent with the studies done in Arba Minch, Ethiopia (Aklilu et al., 2020), and Algeria (Medboua-Benbalagh et al., 2017). This might be participants who had chronic diseases will have exposure to antibiotics, and frequent contact with health professionals, which may lead to ESBL-PE carriage.

The third factor that had contributed to the carriage of ESBL-PE was admission to a neonatal intensive care unit. Participants who had been admitted to NICU were 4.86 times more likely carriers for ESBL-PE than those who had been admitted to other wards. This finding is supported by the previous study conducted in Korea (Ko et al., 2013) and Turkey (Kiremitci *et al.*, 2011). This could be due to the overuse of broad-spectrum antibiotics in the neonatal intensive care unit (NICU) to treat serious infections. Eventually, patients with ESBL-PE carriage might be the risk for self and cross-transmission of resistance genes among the patients that result in untreatable nosocomial infection in the study area.

Other factors such as all socio-demographic factors, history of hospitalization in the past 12 months, and previous history of invasive procedure in the past 3 months were not significantly associated with fecal carriage ESBL-PE in this study. This finding is in contrast with the findings in Arba Minch(Aklilu et al., 2020), Tanzania(Kibwana et al., 2020), Burkina Faso(Ouedraogo et al., 2017), Turkey(Kiremitci et al., 2011), and Cyprus(Ruh et al., 2019). The reason for these observed variations might be due to differences in the study population, sample size, geographical location, and clinical condition of the study participants.

7.6. Magnitude of carbapenemase-producing *Enterobacteriaceae*

Although no nationwide study has been reported so far on the fecal carriage of carbapenemase-producing *Enterobacteriaceae* (CPE) in Ethiopia, two studies have been conducted in Ethiopia (Desta et al., 2016, Aklilu et al., 2020). In the present study, the overall carriage of CPE was 5.5% 95% CI (3.2%-7.2%) using the rapid Carba NP test. This result was in close agreement with the study done in Egypt (5%)(Abdallah et al., 2017) and China (6.6%)(Zhao et al., 2014).

However, higher than the studies done in Addis Ababa (2%)(Desta et al., 2016), Arba Minch, Ethiopia (1.43%) (Aklilu et al., 2020), and Morocco (1.8%)(Arhoune et al., 2017). The result from the current study was lower than the study conducted in China (8.5%)(Liu, 2019). The possible reason for this discrepancy might be the difference in carbapenemase detection method, methodological difference, sample size and study participants.

In the present study, the highest carbapenemase production was recorded in *K. pneumoniae* 3.5% followed by *K.oxytoca* 1.2% This result was comparable with the study conducted Arba Minch by Aklilu et al. *K. pneumoniae* 1.43% (Aklilu et al., 2020), Egypt 5% (Abdallah et al., 2017) and China 11.6% (Liu, 2019).

7.7. Antibiotics susceptibility pattern of carbapenemase-producing *Enterobacteriaceae*

In this study, carbapenemase-producing *Enterobacteriaceae* showed high resistance to most of the antibiotics used in the study. Our finding showed that CPE were 100% resistant to ampicillin, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, and amoxicillin-clavulanic acid. The highest sensitivity was observed for tetracycline 31.6%, followed by chloramphenicol 26.3% and cotrimoxazole 21.1% respectively. This finding agreed with the study done in Egypt: gentamicin (100%)(Abdallah et al., 2017) China: ampicillin (100%), ceftriaxone (100%), ciprofloxacin (81%), gentamicin (83%)(Liu, 2019), and Shanghai: cefotaxime (100%), ceftazidime(100%)(Pan *et al.*, 2019). Although colistin, tigecycline, and new antibiotics in clinical trials are the treatment option for carbapenem resistance *Enterobacteriaceae*, they are very toxic and costly. Therefore, careful selection of antibiotics and good hygiene practices should be implemented to prevent the dissemination of resistant strains in the hospital.

However, our finding is different from the findings in Egypt: resistance to cotrimoxazole(100%)(Abdallah et al., 2017) and China: cefoxitin (100%), and cefepime (100%)(Liu, 2019). This high resistance pattern in this study could be explained by variation in the study population, misuse of antibiotics in the study area, emergence of new resistance strain, absence of guidelines for the selection of antibiotics, and self-medication in the study area.

7.7. Associated risk factors for fecal carriage of carbapenemase-producing *Enterobacteriaceae*

In this study, we had tried to see the associations of carriage of CPE with different socio-demographic and clinical factors. According to the binary logistic regression analysis variables, like a history of antibiotic use in the last 3 months, the previous history of invasive procedure in the last 3 months, presence of chronic underlying disease seemed to be associated with carriage of CPE. But by multivariable logistic regression analysis model, only history of hospitalization, in the last 12 months showed statistically significant association. This association was consistent with studies conducted in different nations throughout the world including Brazil(Salomão *et al.*, 2017), China(Zhao et al., 2014), Japan(Asai *et al.*, 2018), and Turkey(Erdoğan et al., 2017). This can be explained by a long stay in the hospital and frequent contact with health professionals.

On the other hand, the study conducted in Lebanon, Japan, and Brazil showed previous antibiotic use was an independent risk factor associated with fecal carriage of CRE (Salomão et al., 2017, Asai et al., 2018, Dandachi *et al.*, 2016). Another study in China also showed invasive procedures and sickbed change were associated with CRE colonization(Zhao *et* al., 2014). Furthermore, a study done in India showed the presence of an indwelling device as an independent risk factor for acquiring gut colonization of CRE(Mohan et al., 2017). The discrepancy of association with our finding could be explained by the difference in the clinical condition of the study participant, sample size, methodological difference, and study setting.

8.Strength and limitation of the study

8.1. Strength of the study

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

8.2 Limitation of the study

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

9. Conclusion

In this study, the fecal carriage of multi-drug resistance *Enterobacteriaceae* and extended-spectrum- beta-lactamase-producing *Enterobacteriaceae* was high among hospitalized patients. The highest resistance was recorded against ampicillin followed by gentamicin and tetracycline. The predominant ESBL producing *Enterobacteriaceae* were *E. coli* and *K. pneumoniae*. ESBL producing *Enterobacteriaceae* showed a high level of resistance to gentamicin, tetracycline, and cotrimoxazole.

The overall magnitude of carbapenemase-producing *Enterobacteriaceae* was 5.5%. *K. pneumoniae* was the highest carbapenemase producer followed by *K. oxytoca*. Carbapenemase-producing *Enterobacteriaceae* were resistant to most of beta-lactam antibiotics.

Antibiotic use in the past 3 months, admission in neonatal intensive care unit, and presence of chronic disease showed statistically significant association with fecal carriage of ESBL-PE. Hospitalization in the past 12 months was independently associated with fecal carriage of CPE.

10.Recommendations

This high carriage of ESBL-PE and CPE among hospitalized patients is alarming. Therefore

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

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Annexes

Annex I: Laboratory procedures for sample collection, biochemical reactions, drug susceptibility testing (CLSI guidelines), ESBL and Carbapenemase detection.

1.Sample collection

A. Stool sample collection

1. Sterile clean cup that contains cotton tip applicator sticks with the container that contain transport medium given to the patient

2. Patient should pass the stool into a special container mounted on the toilet for this purpose.

3. a pea-sized amount of fecal specimen that displaces to line the Cary-Blair medium.

B. Rectal swabs

1. Pass the tip of a sterile swab beyond the anal sphincter.

2. Carefully rotate the swab to sample the anal crypts, and withdraw the swab.

3. Place the swab in Cary-Blair medium or buffered glycerol saline.

2.Culture and identification

A. Bacteria growth from the stool on MacConkey agar

Yes □ No □

B. Identification steps for the isolated colonies

a) Colony Characteristics

b) Lactose fermentation on MacConkey agar

□ Lactose Fermenter □ Non lactose fermenter

- Lactose non -fermenter will be inoculated on salmonella shigella agar or XLD

c) Gram stain: _____

C. Biochemical reactions

Identification of bacterial isolates involves the use of biochemical screening media. Indole, Urease, Triple sugar iron (TSI), Citrate, Motility, Lysine Decarboxylase, and Oxidase tests.

Pt ID:								
		Indo	Urea	TSI	Cit	Mot	LDC	Mannitol
Result	Positive							
	Negative							

Key: LDC = Lysine decarboxylase, Triple sugar iron (TSI), Ox = Oxidase test, Cit = Citrate test, Mot = Motility, Ind = Indole test, Urea = Urease, H2S = Hydrogen sulphide (blackening), R = Red-pink (alkaline reaction), Y = Yellow (acid reaction).

A. **Indole test:** Few colonies of the culture will be inoculated into peptone water and incubated at 37oC for 24 hours. Few drops of indicator (Kovac's reagent) will be added and gently shake to mix well. Color change will be then observed. If the layer of indicator reagent turns to red within 1 minute, it is Indole positive (positive result). If the layer of indicator reagent remains yellow within 1 minute, it is indole negative (negative result).

B. **Urease test (Christensen's (modified) urea broth):** Urea agars will be inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap will be loosened and then incubated at 37^{o}C for 3-12 hours. A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish red color of the Medium. Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.

C. **Triple Sugar Iron (TSI) Agar Slant:** Using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism. Incubate at 37◦C for 18 to 24 h.

- If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only.
- If alkaline slant–alkaline butt (red–red): glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

B. Citrate **utilization test using Simmon's citrate agar:** Simmons's citrate slopes will be prepared in bijou bottles as recommended by the manufacturer (stored at 2-8oC). And the slopes will be then stabbed and incubated at 37oC aerobically for 48 hours. Blue color indicates a positive reaction and if Simmons's citrate agar slopes remained as green in color indicate negative reaction.

C. **Motility Test** (using motility agars): Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35-37oC for 24 hours. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

D. **Lysine decarboxylase**: Decarboxylation of lysine can be detected by culturing bacteria in a medium containing the desired amino acid, glucose, and a pH indicator bromocresol purple. The acids produced by the bacteria from the fermentation of glucose will initially lower the pH of the medium and cause the pH indicator to change from purple to yellow. The acid pH activates the enzyme that causes decarboxylation of lysine to amines and the subsequent neutralization of the medium. This results in another color change from yellow back to purple. Bacteria that decarboxylate lysine turn the medium purple. In addition, bacteria that produce H2S appear as black colonies.

3.Procedure for performing the disk diffusion test

I. Direct colony suspension preparation

Prepare a saline suspension of the isolate from an overnight incubated agar plate (use a nonselective medium, such as nutrient agar) to obtain 0.5 McFarland turbidity. For visual comparison, look through the suspension in transmitted light against a white background with contrasting black stripes.

II. Inoculating test plates

Fresh Mueller Hinton agar (MHA) plates will be used the same day or stored in a refrigerator (2-8ºC); if refrigerated, they should be wrapped in plastic to minimize evaporation. Just before use, if excess moisture is visible on the surface, plates should be placed in an incubator (35ºC) or, with lids ajar, in a laminar-flow hood at room temperature until the moisture evaporates (usually 10 to 30 minutes).

Mueller Hinton agar (MHA) plate will be inoculated within 15 minutes after the inoculum has been adjusted. A sterile cotton swab is dipped into the suspension, rotated several times, and gently pressed onto the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab will then be streaked over the entire surface to the agar plate three times, with the plate rotated approximately 60o each time to ensure even distribution of the inoculum. A final sweep of the swab will be made around the agar rim. The lid may be left partly open for 3 to 5 minutes but no longer than 15 minutes to allow any excess surface moisture to be absorbed before the drug-impregnated disks are applied.

III. Antimicrobial disks application

Application of antimicrobial disks to an agar plate should be done within 15 minutes of inoculation of plates. The selected antimicrobial disks will be dispensed evenly onto the agar plate with the help of a forceps/sterile needle/surgical blade. Flame the tips of the applicator intermittently. Each disc must be pressed down to ensure complete contact with the agar surface.

1.Apply 12 disks on a 150 mm plate or 5 disks on a 100 mm plate, keeping at least a distance of 24 mm between disks. Dispensing too near to the edge of the plate should be avoided. Because some of the drugs diffuse instantaneously, a disc should not be relocated once it has come in contact with the agar surface.

2.Place disks that give predictably small zones like aminoglycosides, next to those disks that give larger zones like cephalosporins.

3.Disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.

4.Only those disks that have not reached the manufacturer's expiration date stated on the label will be used. Unused disks will be discarded on the expiration date.

5.Incubation– No longer than 15 minutes after disks are applied, the plates will be inverted and incubated at 35º ± 2ºC in ambient air.

IV Interpretation and reporting of AST results

Each plate will be examined according to the recommendation of CLSI after overnight incubation (16-18 hours), for confluent growth and circular zones of inhibition. The diameters of the zones of complete inhibition, including the diameter of the disk, will be measured to the nearest whole millimeter with calipers or a ruler. The measuring device is held on the back of the inverted Petridis, which is illuminated with reflected light located a few inches above a black, non-reflecting background. Zone margin should be considered the area showing no obvious visible growth detectable with the unaided eye. The faint growth of tiny colonies visible only by lens should be ignored. In case of the presence of discrete colonies within clear zone of inhibition, repeat the test with a subculture of a single colony/pure culture from the primary culture plate. If discrete colonies still appear, inner colony-free zone size will be measured. For Proteus spp., swarming should be ignored.

V. Breakpoint of drug susceptibility of *Enterobacteriaceae*

Pt Id Isolated organism ----------

Antimicrobial agent	Disk content (μg)	Zone of diameter (mm)			Test Result (mm)	Interpretation (S, I and R)
		S	I	R		
Ampicillin (AM)	10	≥17	14-16	≤13		
Cefoxitin (FOX)	10	≥18	15-17	14		
Gentamicin (GM	10	≥15	13-14	≤12		
Ciprofloxacin (CIF)	5	≥31	21-30	≤20		
Trimethoprim - sulfamethoxazole (SXT)	1.25/23 .75	≥16	11-15	≤10		
Imipenem (IMP)	10	≥23	20-22	≤19		
Meropenem (MEM)	10	≥23	20-22	≤19		
Amoxicillin - clavulanic acid (AMC)	20/10	≥18	14-17	≤13		
Cefotaxime (CTX)	30	≥26	23-25	≤22		
Ceftazidime (CAZ)	30	≥21	18-20	≤17		
Ceftriaxone (CRO)	30	≥23	20-22	≤19		
Tetracycline (TE)	30	≥15	12-14	≤11		
Cefepime (FEP)	30	≥25		≤18		
Aztreonam (ATM)	30	≥ 21	18-20	≤17		
Chloramphenicol (C)	30	≥18	13-17	≤12		

S= susceptible R= resistance I=Intermediate

VI. Combination disc test (CDT) for phenotypic ESBL detection

For each test disks containing cephalosporin alone (cefotaxime and ceftazidime) and in combination with clavulanic acid will be applied. The inhibition zone around the cephalosporin disk/tablet combined with clavulanic acid will be compared with the zone around the disk/tablet with the cephalosporin alone. The test is positive if the inhibition zone diameter is 5 mm and larger with clavulanic acid than cephalosporin alone. In all other cases, the test result is negative.

VII. Carbapenemase using RAPIDEC® CARBA NP

Principle of the test

RAPIDEC® CARBA NP test is based on the detection of carbapenem hydrolysis by carbapenemase-producing bacteria.

Hydrolysis acidifies the medium which results in change in colour of the PH of indicators. After bacterial lysis which enables the extraction of enzyme, the lysate is added to a detection solution containing

-a carbapenem: imipenem (carbapenemase substrate)

-a Phenol red (PH indicator)

- Zinc required for detection of Metallo dependent carbapenemase-producing strains

After incubating a maximum of 2 hours reading is performed visually by comparing a control well without imipenem to a reaction well-containing imipenem.

Contents of the kit

Kits for 10 tests

RAPIDEC® CARBA NP test strips (10)

• API® Suspension medium 2 ml (10)

• Incubation lids (10)

• 1 pack of stirring sticks

• 1 two- coloured (black and white) support

Composition of the strips

The function of each well is of the RAPIDEC® CARBA NP strips is given below.

Well	Reagents
a	Phenol red solution
b	Turbidity control
c	Lysis buffer
d	Control well without imipenem
e	Reaction well containing imipenem

Preparation of the test

- Open an ampule of API® Suspension medium(2ml).
- Dispense 100µlinto wells **a, b, c.**
- Place an incubation lid on the test strip.
- Leave for4-10 minutes at room temperature (15-25°C).
- Gently mix the contents of well **b** using a stirring stick.

Preparation of the inoculum and bacterial lysis

Procedure

1. Transfer 25 µL from well **c** to wells **d** and **e**
2. Transfer 25µL from well **a** to wells **d** and **e**
3. Place an incubation lid on the test strip and incubate for30 minutes at 33-38°C.
4. At the end of the 30minute incubation period, place the test strip on the two-coloured (black and white) support card and remove the incubation lid to perform the initial reading. If a colour change from red to yellow, light orange, orange or dark orange is observed in well **e**, the result is positive and the test is complete.
5. If no colour change is observed, continue the incubation at 33-38°C for up to 2 hours and perform a final reading.

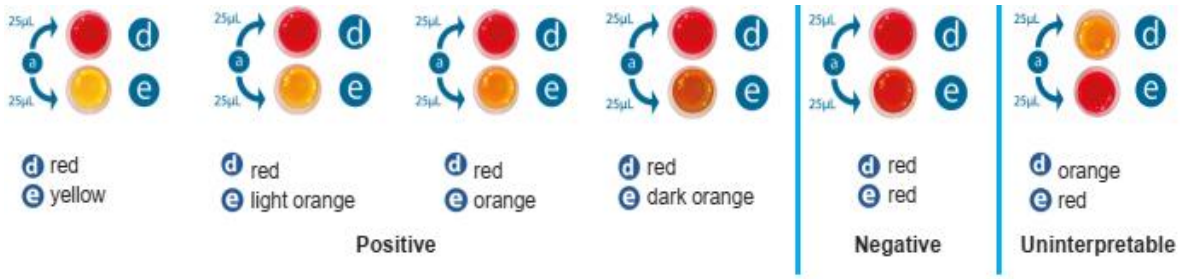
Reading and interpretation

Place the strip on the two-coloured (black and white) support. Position wells d and e on the white background to facilitate reading. Remove the incubation lid.

Reading is performed by comparing the colours in wells d and e ensuring that the strip is firmly flatten against the two-coloured (black and white) support.

A test is positive when a significant variation in colour is observed between the two wells.

Control well d	Test well e	Interpretation
red	red	Negative (absence of carbapenemase)
orange	orange	
red	yellow, light orange, orange, dark orange	Positive (presence of carbapenemase)
orange	yellow	
any color other than red or orange	Not applicable	*Uninterpretable
orange	red	


25µL
a
25µL
d
e
d red
e yellow
d red
e light orange
d red
e orange
d red
e dark orange
Positive
d red
e red
Negative
d orange
e red
Uninterpretable

Annex II: Information sheet for adult participant

Information sheet English version

Department of Microbiology, Immunology, and Parasitology School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title: Fecal carriage of extended-spectrum -beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, Amhara Regional State, Ethiopia

Principal Investigator: Demissew Shenkute

Introduction

You are invited to participate in research will be conducted by an MSc candidate, from Addis Ababa University. Your participation is voluntary. The research teams include principal investigator, advisors; from Addis Ababa University Microbiology, Immunology, and Parasitology department, and collaborators from Debre Berhan Comprehensive Specialized Hospital. Please take as much time as you need to read or listen to the information sheet.

Purpose of the study

The purpose of the study is to determine the fecal carriage of microorganisms that are resistant to most of the antibiotics that are currently in use among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, Amhara Regional State, Ethiopia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study. You can ask any question that is not clear to you and after that, you can give your consent. You will be asked to give a small amount of stool specimen in the container that we will provide to you.

Potential risks and discomforts

There is minimal risk associated with sample collection except for your time.

Confidentiality

To maintain the confidentiality of the participant's personal information, your name will be kept confidential and samples will be coded. No personal information will be disclosed to a third party or will not appear in any report from this study.

Potential benefits to participants and/or to the society

You will not receive any payment for your participation in this research study as compensation. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policymakers.

Participation and withdrawal from the Study

Participation is voluntary and you have the right not to participate in this study. You can withdraw at any time and place without consequences of any kind. You may also reject to give any sample. Your response to our request will not affect the service and care that you would normally get from the Hospital.

Contact information

If you have any questions about this study you can contact the following principal investigator for further information.

Demissew shenkute **Phone:** 092307609 **E-mail:**demissewshen@gmail.com

Information sheet for Adult participant Amharic version

ለአዋቂዎች ተሳታፊ የመረጃ ወረቀት

የማይክሮባዮሎጂ ፣ ኢመኖሎጂ እና ፓራሲታቶሎጂ የሕክምና ትምህርት ቤት ፣ የጤና ሳይንስ ኮሌጅ ፣ አዲስ አበባ ዩኒቨርሲቲ ፣ አዲስ አበባ ፣ ኢትዮጵያ

የጥናቱ ርዕስ ፡ ፌካል ካሬጅ ኦፍ ኢስቢል ኤንድ ካረባፐንሜስ ፕሮዱሲንግ እንትሮባክትሬያሴ አሞነግ ሆስፒታልዝድ ፔሼንትስ

መግቢያ

ከአዲስ አበባ ዩኒቨርሲቲ በ MSC እጩ በሚካሄደው ጥናት እንዲሳተፉ ተጋብዘዋል ። የእርስዎ ተሳትፎ በርስዎ ፈቃደኝነት የተመሰተ ነው ። የምርምር ቡድኖቹ ዋና ተመራማሪ ፣ እና አማካሪዎችን ፣ ከአዲስ አበባ ዩኒቨርሲቲ ማይክሮባዮሎጂ ፣ ኢሚኖሎጂ እና ፓራሲታሎጂ ዲፓርትመንት እና ከደብረብርሃን አጠቃላይ ስፔሻላይዝድ ሆስፒታል ባልደረባዎች ። በመረጃ ወረቀቱ ውስጥ ያለዉን መረጃ ለማንበብ ወይም ለማዳመጥ ትንሽ ጊዜ ይውሰዱ ።

የጥናቱ ዓላማ

የጥናቱ ዓላማ በአሁኑ ጊዜ በአማራ ብሔራዊ ክልል ደብረ ብርሀን አጠቃላይ ስፔሻላይዝድ ሆስፒታል በሆስፒታል ተኝተዉ በሚገኙ ታማሚዎች የሚጠቀሙትን አብዛኞቹን አንቲባዮቲኮችን የሚቋቋሙ ኢነተሮባክቴሪያሲዎችን ለማወቅ ነው።

የጥናቱ ሂደቶች እና ተሳታፊ ለመሆን የሚጠበቅበዎት

ለመሳተፍ ፈቃደኛ ከሆኑ የጥናቱን ዓላማ መረዳት ያስፈልግዎታል ። ለእርስዎ ግልፅ ያልሆነ ማንኛውንም ጥያቄ መጠየቅ ይችላሉ እና ከዚያ በኋላ ፈቃድዎን መስጠት ይችላሉ ። እኛ በምናቀርብልዎ መያዣ ውስጥ ትንሽ መጠን ያለው የሰገራ ናሙና እንዲሰጡ ይጠየቃሉ ።

ሊጋጥሙ የሚችሉ አደጋዎች እና ችግሮች

ጊዜዎን ሳይጨምር ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም።

የጥናቱ ምስጢራዊነት

የተሳታፊዎችን የግል መረጃ ሚስጢራዊነት ለመጠበቅ ስም በሚስጥር የሚጠበቅ ሲሆን ናሙናዎች ኮድ ይደረጋሉ ። ምንም ዓይነት የግል መረጃ ለሶስተኛ ወገን አይገለጽም ወይም ከዚህ ጥናት በየትኛውም ሪፖርት አይደረግም ።

ጥናቱ ለተሳታፊዎች ለተሳታፊዎች እና / ወይም ለህብረተሰቡ የሚሰገኘዉ ጥቅሞች

በዚህ የምርምር ጥናት ውስጥ በመሳተፍዎ ምንም ክፍያ የለዉም ። ከሁሉም በላይ ይህ ጥናት ለወደፊቱ እና ለበለጠ አገራዊ ጥናት መረጃን ወይም መረጃዎችን ለማቅረብ እና ለጤና ፖሊሲ አውጪዎች የጤና ፕሮግራሞችን ለማዘጋጀት አስተዋፅ ያደርጋል ።

ከጥናቱ ተሳትፎ እና መውጣት

ተሳትፎው በፈቃደኝነት ሲሆን እርስዎ በዚህ ጥናት ውስጥ ላለመሳተፍ መብት አልዎት ። ያለምንም መዘግየት በማንኛውም ሰዓት እና ቦታ መውጣት ይችላሉ ። እንዲሁም ማንኛውንም ናሙና ለመስጠት እምቢ ማለት ይችላሉ ። ለጥያቄያችን የሚሰጡት ምላሽ በፊት ከሆስፒታሉ በሚያገኙት አገልግሎት እና እንክብካቤ ላይ ተጽዕኖ የለውም ።

የግንኙነት መረጃ

ስለዚህ ጥናት ማንኛውንም ጥያቄ ካልዎት ለተጨማሪ መረጃ የሚከተሉትን ዋና ተመራማሪ አድራሻ በመጠቀም ያነጋግሩ።

ደምሰዉ ሸንቁጤ

ሞባይል፡ 0923076091

ኢሜል፡ demissewshen@gmail.com

Annex III. Consent form for adult participant

Card no…..……

Participants name…….

I had been informed that the objective of this study is to determine fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, Amhara Regional State, Ethiopia. For this study, a stool sample will be required. The aim and possible risk of the study were explained to me well. I was also informed that all the information contained in the questionnaire is to be kept confidential. Moreover, I have been informed that I have the right to withdraw from the study. It is therefore with the full understanding I gave the informed consent voluntarily to the researcher to use my information and specimen for this study.

I_____ hereby give my consent for providing the requested information and specimens for the investigator

Signature: _____

Date_____

Witness: _____signature_____

Adult consent form Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ ፌካል ካሬጅ ኦፍ ኢስቢል ኤንድ ካረባፕንሜስ ፕሮዱሲንግ እንትሮባክትሬያሴ አሞነግ ሆስፒታልዝድ ፔሼንትስ ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ሰገራ ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎችም ተረድቻለሁ፡፡

በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ፡፡በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ፡፡የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው የገንዘብ ክፍያ አለመኖሩን እና የጥናቱ ዉጤት ለፖሊሲ አዉጪዎች ፖሊሲዎቸዉን እንዲመርምሩ /እንዲያሻሽሉ እንደሚረዳቸዉ ተረድቻለሁ፡፡

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

Annex IV: Information sheet for parents/guardians

Organization: Department of Microbiology, Immunology and Parasitology School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Principal Investigator: Demissew Shenkute

We would like to conduct research which is entitled "Fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, Amhara Regional State, Ethiopia"

We are requesting you allow your child to voluntarily participate in this study. We are going to inform you about the purpose, responsibility of investigators to keep confidentiality, and how we are going to use the data.

Purpose of the study

The purpose of the study is to determine the fecal carriage of microorganisms that are resistant to most of the antibiotics that are currently in use among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, Amhara Regional State, Ethiopia.

Procedures and the expected participation

If you agree your child participate in this study and you sign the consent form the following will be done:

- ✓ We will ask for a small amount of stool specimen from your child
- ✓ Review your child medical history
- ✓ You will have an interview with us sometime about the child medical condition

Potential risks and discomforts

There is no risk associated with sample collection except your time on your child.

Confidentiality

To maintain the confidentiality of your child's information, your child's name will be kept confidential and samples will be coded. No personal information will be disclosed to a third party or will not appear in any report from this study.

Potential benefits to participants and/or to the society

Your child will not receive any payment for participation in this research study as compensation. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policymakers.

Participation and withdrawal from the Study

Your child's participation is voluntary and you have the right to refuse your child's participation in this study. You can stop your child to participate in the study at any time after giving your consent. You may also reject to give any sample from your child. Your response to our request will not affect the service and care that your child would normally get from the hospital.

Contact information

If you have any questions about this study you can contact the following principal investigator for further information.

Demissew shenkute **Phone:** 0923076091 **E-mail:**demissewshen@gmail.com

Information Sheet for Parents/Gurdians (Amharic Version)

የድርጅቱ ስም፤ የማይክሮባዮሎጂ ፣ ኢሚኖሎጂ እና ፓራሲታቶሎጂ የሕክምና ትምህርት ቤት ፣ የጤና ሳይንስ ኮሌጅ ፣ አዲስ አበባ ዩኒቨርሲቲ ፣ አዲስ አበባ ፣ ኢትዮጵያ

የጥናቱ ርዕስ፡ ፌካል ካሬጅ ኦፍ ኢስቢል ኤንድ ካረባፕንሜስ ፕሮዱሲንግ እንትሮባክትሬያሴ አሞነግ ሆስፒታልዝድ ፔሼንትስ

የጥናቱ ምንነት ፡እንትሮባክትሬያሴ የሚባሉት ባክቴሪያዎች የተለያዩ በሽታዎችን የሚያምጡ እና በመድሃኒት ግትርነት እያሰቸገሩ ያሉ ባክቴሪያዎች ናቸዉ፡፡ በመሆኑም የችግሩን ተባባሽነት ለመቀነስ በደብረ ብረሃን አጠቃላይ እስቴሻላይዝድ ሆሰፒታል ተኝተዉ በሚገኙ በሽተኞች ጥናት ማካሄድ አስበናል፡፡ በዚህ ጥናት ውስጥ ልጅዎ/የሚያሳድጉት/ጓት ልጅ እንዲሳተፍ/እንድትሳተፍ ከተስማሙ የሚከተሉትን ነገሮች እናደርጋለን፡፡

የሰገራ ናሙና ከልጆዎ እንወሰዳልን፡፡

ለጥናቱ የሚያሰፈልጉ የልጆዎን የህክምና መረጃ እንያለን እና ከርስዎ አንደበት እንጠይቃለን፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ከልጆዎ ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥማትም/ዎም ፡፡

የጥናቱ ሚስጥራዊነት

ስለ ልጆዎ የሰጡት ወይም እኛ የሰበሰብንዉ ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን ልጆዎ በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኘው/ኘዉ ጥቅም አይኖርም፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው?

በዚህ ጥናት ልጆዎ የሚሳተፈዉ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት በመሆኑ በማንኛውም ሰዓትና ቦታ ልጆዎን ከዚህ ጥናት የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ ልጆዎ ከጥናቱ በማግለልዎ ምክንያት የሚቀርብለት ምንም አይነት የሆስፒታል አገልግሎት አይቋረጥም ፡፡ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት፡፡ እርስዎ ስለ ልጆዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ልጆዎ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡

ደምሰዉ ሸንቁጤ

ሞባይል፡ 09-23-07-60-91

ኢሜል፡ demissewshen@gmail.com

Annex V: Consent form for parents/guardians

Code No. ----------

Name of study participant ----------

Name of the participant's family or Guardian ----------

I have been informed about the study which is aimed at "fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Compressive Specialized Hospital, Amhara Regional State, Ethiopia". For this study, a stool sample will be required. The aim and possible risk of the study were explained to me well. I was also informed that all the information contained in the questionnaire is to be kept confidential. Moreover, I have been informed of the rights to withdraw from the study. The study participant mentioned above who is not able to give informed consent himself because he/she is younger than 18 years not allowed to decide on him/herself. It is therefore with full understanding; by taking full responsibility I gave my assent voluntarily to the researcher to use his/her information and specimen for this study.

Participant's signature/Fingerprint ----------

Participant's family/Guardian signature ----------Name of data collector ---------- Sign ---------- Date ----------

Please contact us for any question or problems you may encounter during the study

Principal investigator: Demissew Shenkute

Phone number- 0923076091 E-mail:demissewshen@gmail.com

Consent form for parents/guardians Amharic version

የጥናቱ ተሳታፊ ለሆኑ ህፃናት ወላጅ/አሳዳጊ የስምምነት ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

የተሳታፊዉ ወላጅ/አሳዳጊ ስም ----------

እኔ ከዚህ በላይ ስሜ የተጠቀሰዉ የጥናቱ ተሳታፊ ህፃን ወላጅ/አሳዳጊ ፌካል ካሬጅ ኦፍ ኢስቢል ኤንድ ካረባፕንሜስ ፕሮዱሲንግ እንትሮባክትሬያሴ አሞነግ ሆስፒታልዝድ ፔሼንትስ በሚል ርዕስ ስለሚሰራዉ የምርምር ስራ አስፈላጊዉ መረጃ ሁሉ ተነግሮኛል። ለዚህ ጥናት የሰገራ ናሙና እንደሚያስፈልግ እና የምርምሩ ዓላማ ምን እንደሆነ እና በጥናቱ ምክንያት ሊከሰቱ የሚችሉ ጉዳቶች በዝርዝር ማብራሪያ ተሰጥቶኝ ተረድቻለሁ። በተጨማሪም ማንኛዉም ዓይነት ለዚህ ጥናት ስለ ልጄ የሰጠሁት መረጃዬ በሚስጥር ተጠብቆ እንደሚያዝልኝ እና በፈለግሁት ግዜና ሁኔታ በጥናቱ መሳተፍ ካልፈለግሁ ማቋረጥ መብቴ እንደሆነ በግልፅ ተነግሮኛል። ከላይ በስም የተጠቀሰዉ የጥናቱ ተሳታፊ ከ18 ዓመት እድሜ በታች በመሆኑና በራሱ ፈቃድ ለመሳተፍ ስምምነት መፈራረም ስለማይችል እኔ የእርሱ/ሷ ወላጅ/አሳዳጊ ቤተሰብ በመሆኔ የተሰጠኝን መረጃ መሰረት በማድረግ እና ዓላማዉንም በመረዳት በፈቃዴ የምሰጠዉን ልጄን የሚመለከት መረጃ እና የሰገራ ናሙና ለምርምር አገልግሎቱ እነዲያዉሉት ተስማምቼ መፍቀዴን በፊርማዬ አረጋግጠለሁ።

የተሳታፊዉ ፊርማ ----------

የተሳታፊዉ ወላጅ/አሳዳጊ ፊርማ----------

የመረጃ ሰብሳቢዉ ስም ---------- ፊርማ ----------

ቀን -----

ማንኛዉም ችግር ካጋጠመዎት በሚቀጥለዉ አድራሻዎች ያሳዉቁን

የጥናቱ ባለሙያ ፡ ደምሰዉ ሸንቁጤ

ስልክ ቁጥር ፡ 0923076091

ኢ-ሜይል ፡ demissewshen@gmail.com

Annex VI: Children assent for Age between 12-18 years

I, _____ understand that my parents /guardian have/has permitted for me to take part in research entitled" Fecal carriage of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* among hospitalized patients at Debre Berhan Comprehensive Specialized Hospital, Amhara Regional State, Ethiopia" I agree to give a sample for the research. I also understand the information is confidential and I can withdraw from the study at any time. Therefore, I give my Assent freely to participate in this study.

.Signature_____

Date_____

Signature of the interviewer _____ Date _____

Children Assent for Age between 12-18 years Amharic Version

እኔ----------የተባልኩ ልጅ ወላጆቼ /አሳዳጊዎቼ ፌካል ካሬጅ ኦፍ ኢስቢል ኤንድ ካረባፕንሜስ ፕሮዱሲንግ እንትሮባክትሬያሴ አሞነግ ሆስፒታልዝድ ፔሼንትስ በሚል ርዕስ ለሚካሄደዉ ጥናት እንድሳተፍ መስማማታቸውን ተረድቻለሁ፡፡ እኔም ናሙና ለመስጠት ተስማምቻለሁ፡፡ እንዲሁም የሚስበስበዉ መረጃዎች በሚስጥር እንደሚያዙ እና በማንኛዉም ሰዓት ከጥናቱ ማቋረጥ እንደምችል ተረድቻለሁ፡፡ ስለዚህ ሁሉንም በመረዳት በዚህ ጥናት ዉስጥ ለመሳትፍ ፍቃደኛ ነኝ፡፡

ፊርማ ---------- ቀን----------

ዉይይቱን ያከናወነዉ ባለሙያ ፊርማ---------- ቀን----------

Annexes VII: Questionnaire (English and Amharic version)

Table :8 English questioner

Participant's ID No. _____ _____	Date_____
Questions	**Response categories**
1. Sex	Male …….1 Female ……2
2. Age _____years	
3. Current residence:	Urban ……1 Rural…….2
4. Educational status	Illiterate ….1 Elementary ….2 Secondary …...3 Higher rand above….4
5. Occupational status?	Government-employed…….1 Merchant……..2 House wife .....3 Daily laborer …….4 Farmer……5 Other (specify).....6
6. Family monthly income	
7. History of antibiotic use in the past 3 months?	Yes ……1 No……2
8. History of hospitalization in the past12 months	Yes…….1 No……2
9. If yes for Q #8, how long	_____days/ months
10. ICU stays?	Yes…….1 No……2
11. If yes for Q #10 how long have you stayed?	_____day /month/s/
12. Number of beds in a single room?	_____
13. Number of patients in a single room during sample collection?	_____
14. Reason for hospital admission (diagnosis)	Sepsis ……1 Pneumonia …….2 UTI……3 Meningitis……4 Gastroenteritis……...5 malnutrition ……..……6 other…… …… 7
15. History of invasive procedure in the past 3 months	Yes…….1 No……2
16. If yes for Q #15 which type of invasive procedure	Surgery….1 Catheterization ….2 Transplantation .3 other specify….. 4
17. Ward in which the patient is admitted to	Pediatrics ……...1 Gny Obs…….…...2 Surgical……..…3 Medical ……..…4 Neonatology……5 ICU……6 Ophthalmology ……7
18. For how long have you been admitted for this case?	_____day/s
19. Chronic disease	Yes ……1 no ….2
20. If yes Q # 19 list them	Diabetes mellitus ……1 HIV/AIDIS……...3 Hypertension …….2 Hematological malignancy……4 Solid malignancy….5 Others specify ……6
21. Diarrhea	Yes….1 No …….2
22. International travel within 6 months	Yes…….1 No……2
23. If yes in ♯22	Specify the place

Table 9: Amharic questioner

	የተሳታፊው መለያ ቁጥር._____ ቀን_____	
	መጠይቅ	**መልስ**
1.	ፆታ	ወንድ ……..1 ሴት ……2
2.	ዕድሜ_____ አመት	
3.	አሁን የሚኖሩበት ቦታ-------	ከተማ ……1 ገጠር ……2
4.	የትምህርት ሁኔታ	ያለተማረ……1 የመጀመሪያ ደረጃ ……2 ሁለተኛ ደረጃ….3 ከፍተኛከዚያ በላይ……..4
5.	አሁን የሚሰሩት ስራ አይነት ምንድነዉ?	የተቀጠረ……1 ንግድ……..2 የቤት እመቤት .....3 የቀን ሰራተኛ …….4 ግብርና……..5 ሌላ ከነዚህ ዉጪ ከሆነ ይጥቀሱ.....6
6.	ወርሃዊ የቤተሰብ ገቢ	----------
7.	ባለፈዉ 3 ወር ዉስጥ መድሃኒት ወስደዋል?	አዎ……1 አልሰድኩም……2
8.	ባለፈዉ አንድ አመት ዉስጥ ሆስፒታል ገብተዉ ያዉቃሉ?	አዎ……1 አልገባሁም……2
9.	ለጥያቄ #8 መልሱ አዎ, ከሆነ ለምን ያህል ጊዜ ቆዩ?	_____ቀን/ ወር
10.	የፅኑ ህመማን ማቆያ ክፍል ተኝተዉ ያዉቃሉ?	አዎ……1 አልቆየሁም……2
11.	ከቆዩ ለምን ያህል ጊዜ ቆይተዉ ነበር?	_____ቀን/ወር
12.	በዋረዱ ዉስጥ ያሉት የአልጋ ብዛት	_____
13.	በዋርዱ ዉስጥ ያሉት የተማሚዎች ብዛት	----------
14.	ታማሚዉ ሆስፒታል የተኛበት ምክንያት	ሴብሲስ……1 የሳንባ ምች……..2 የሽንት ቧንቧ እንፌክሽን…..3 ማጅራት ገትር……4 የአንጅት እንፌክሽን…….5 የምግብ እጥረት.....6 ሌላ ካለ ይጠቅሱ……7
15.	ባለፈዉ 3 ወር ዉስጥ ከፈተኛ የህክምና ፕሮሲጀር አካሂደዋል	አዎ…….1 አላካሄደኩም……..2
16.	ለጥያቄ #15 መልሱ አዎ,የትኛዉን አይነት	ቀዶ ጥገና ….1 ካታተራዜሽን….2 ትራንስፕለዜሽን…….3 ሌላ……..4
17.	ታማሚ/ዋ የተኛበተ/ት ዋርድ	የሀፃናት ክፍል ……..1 የማህፀንና የፅንስ ክፍል……..2 ቀዶ ጥገና ክፍል……..3 የዉስጥ ዶዬ ክፍል……4 የጨቅላ ህፃናት ክፍል……5 የፅኑ ህመማን ማቆያ ክፍል……6 የአይን ህክምና ክፍል ……..7
18.	ታማሚ/ዋ ተኝቶ/ታ የቆየበት/ችበት ጊዜ	_____ቀን/ናት
19.	ተያያዥነት ያላችዉ በሽታዎች	አለ…….1 የለም…2
20.	ለጥያቄ 19 አዎ የትኞቹ	ስኳር ……..1 ኤች አይ ቪ ኤድስ……..2 ግፊት ……..3 የደም ካንሰር……..4 ከደም ዉጪ ያለ ካንሰር……5 ሌላ ከነዚህ ዉጪ ከሆነ ይጥቀሱ…6
21.	ተቅማጥ አለዎት	አዎ……1 የለብኝም
22.	በዚህ 6 ወር ዉስጥ የዉጪ ሀገር ጉዞ አለዎት	አዎ…….1 የለበኝም……2
23.	ለጥያቄ #19 መልሱ አዎ, ከሆነ	ቦታዉን ይጥቀሱ

Annex VII: Declaration

I, the undersigned candidate, declare that this M.Sc. thesis is my original work, that it has not previously been submitted for a degree at this or any other university, and that all sources of materials utilized in the thesis have been properly acknowledged.

M.Sc. candidate: Demissew shenkute

Signature: _____

Date: _____

Approval of the Advisors

Name of the advisor: Dr. Yimtubezinash Woldeamanuel

Signature _____

Date _____

Name of the advisor: Prof. Daniel Asrat

Signature _____

Date _____