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Prevalence of Extended-spectrum B-Lactemase and Carbapenemase producing Enterobacteriaceae among adult patients complaining UTI at South West Shoa, Lemman Hospital and Kersa Malimma Woreda Health Facility, Ethiopia

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This is to certify that the thesis prepared by Dirriba Diyana, entitled

Prevalence of Extended -spectrum B-Lactamase and Carbapenemase producing Enterobacteriaceae among adult patients complaining UTI at South West shoa, Lemman Hospital and Kersa Malima Woreda Health Facility, Ethiopia and submitted in partial fulfillment of the requirements for Masters of Sciences Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standard with respect to originality and quality.

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Abstract

Background: Infection caused by Extended-spectrum beta-lactamase- (ESBLs) and Carbapenemase producing Enterobacteriaceae (CPE) are serious problems of the world. Enterobacteriaceae are the most important producers of ESBLs and Carbapenemase enzymes.

Objective: To determine the magnitude of Extended -spectrum B-Lactemase and Carbapenemase producing Enterobacteriaceae patients complaining Urinary tract infection(UTI) at South West shoa, lemman Hospital and kersa malima woreda health facility, Ethiopia

Methods: Across-sectional study was conducted from a total of278 symptomatic UTI suspected adult patients fromSeptember1/2020 to December 30/ 2021. A convenient sampling technique was used to enrolled patients in this study .Socio demographic data and other information was collected using requisition form. Midstream urine samples were collected from each patient and inoculated onto Macconkey and Sheep blood agar media. Inoculated plates were incubated at 37^0C for 18-24 hours aerobically. The identification of Enterobacteriaceae and gram positive bacteria were carried on based on number of colonies, type of colonies, morphological appearance, and Gram reaction after growth on culture media. Carbapenemase and ESBLs producing Enterobacteriaceae were performed by using combination disk. Finally, data were analyzed using analytical binary regration SPSS version 24.

Results. The overall prevalence of Enterobacteriaceae isolates from urine cultures was 16.9 %(47/278).The highest isolated bacteria were Escherichia coli 78.7% (37/47) and Klebsiella pneumoniae were 8.5% (4/47). Most of them 51.1% (24/47) developed multidrug resistance (MDR≥3 drugs) to most commonly used antibiotics. Prevalence of ESBL producing and Carbapenems resistance Enterobacteriaceae was 44.7% and 0% respectively. The overall prevalence of ESBLs was 44.7% (95%CI; OR=2.68,P=O.582) among all isolates and 18/21 (85.7%) of ESBLs isolates were identified from females and 42.9 % (9/21) isolates were also indicated from patients with age group from 25-34 years.

Conclusion. ESBL β-lactamases producing Enterobacteriaceae were highly susceptible to Carbapenemase, piperacillin/tazobactam and more susceptible Nitrofurantoin. Consequently, the argent of ESBLs producing *Enterobacteriaceae* requires continuous testing& reviewing of antimicrobial strategy in hospitals and health facility in this area are increased. All carbapenems strains are sensitive (susceptible) for all Enterobacteriaceae isolates.

Keywords: Carbapenemase, ESBLs producing, *Enterobacteriaceae* and symptomatic urinary tract infection

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ABBREVIATIONS

AMR	**Antimicrobial Resistance**
AST	**Antimicrobial susceptibility Test**
ATCC	**American Type Culture Collection**
CLSI	**Clinical and Laboratory Standards Institute**
ECDC	**European Center of Diseases Control**
EPHI	**Ethiopian public Health Institute**
ESBL	**Extended-Spectrum Beta-Lactemase**
GNB	**Gram-Negative Bacilli**
IPC	**Infection prevention and control**
KPC	**Klebsiella Producing Carbapenemase**
MAC	**Macconkey**
MBL	**Metallo-Beta-Lactemase**
MDR	**Multi-Drug Resistance**
MHA	**Mueller-Hinton Agar**
MHT	**Modified Hodge Test**
MLST	**Multi-locus Sequence Typing**
NDM	**New Delhi metallo**
UTI	**Urinary Tract Infection**
WHO	**World Health Organization**

1. Introduction

1.1Background

Urinary tract infections (UTIs) is an infection of any part of the urinary system; UTIs involves the upper or lower urinary tract and are second most common type of infection in the body[1].Women is more likely to develop UTIs than men due to anatomical differences. *Enterobacteriaceae* such as *Escherichiacoli*, *Klebsiella pneumoniae, Klebsiella Ozanae*, *Klebsiella oxytoca* and *Enterobacter spps* and other non *Enterobacteriaceae* are the most important causes of serious urinary tract infection and public acquired microbial infections in humans. *Enterobacteriaceae* resistance to antimicrobial agents *are* become important public health problem [2].

Recently, there is a global spread of Multidrug Resistance (MDR) strains of Enterobacteriaceae that are usually producing to cephalosporin's producing ESBLs and Carbapenemase such as Klebsiella Producing Carbapenems(KPC) and Carbapenems Resistance *Enterobacteriaceae(CRE)*(3).ESBLs are the most common used antibiotics used against microbial infections. The predominant mechanism of Enterobacteriaceae producing to these groups of drug is the production of β-lactamases. B-lactamases can be increased when Enterobacteriaceae gene mutate continuously in response to overdose or misappropriation of β-lactam antibiotics. The most Carbapenems drugs of resistance antibiotics including imipenem, meropenem, doripenem, and ertapenem which are considered as last line therapy and first line agents for treatment of serious contagions caused by multidrug resistance *Enterobacteriaceae [3]*.

In Enterobacteriaceae, Carbapenems resistance increases for two main mechanisms: (i) gaining of Carbapenemase inheritable factor that encode for enzymes accomplished of degrading Carbapenems, or (ii) a reduction in the uptake of antibiotics by high or low deficiency of poring expression in association with over expression of b-Lactemase that possess very weak affinity for Carbapenems(4). ESBLs are divided in to four classes depending on their functional groups (groups 1–4, with many subgroups) [5]. ESBL and Carbapenemase are the best common types of beta-lactamases produced by *Enterobacteriaceae* which are usually multidrug Resistance Enterobacteriaceae [6].

Infections caused by ESBL producing *Enterobacteriaceae* are treated with Carbapenems which are last resort antibiotics [7].In the recent past, Carbapenems were potent against all multiple drug resistance (MDR) Enterobacteriaceae and in combination with their negligible toxicity to the host, Carbapenems became the preferred last resort antibiotics for the management of MDR Enterobacteriaceae infections. Development of Carbapenems resistance in *Enterobacteriaceae* is of great concern because there is no obvious next line of antibiotics to use against Carbapenems Resistance *Enterobacteriaceae* (*CRE) [8]*.

The most important role used in the infection control and prevention of treatment failure are rapid and accurate detection of β-lactamase producers[9].Infection caused by CRE most commonly happened among patients who are receiving treatment for other conditions. Ability to produce enzymes by Enterobacteriaceae causes multiple antibiotic producing resulting in treatment failure, increased morbidity and mortality. Thus accurate and timely detection of these resistant mechanisms is helpful to determine the prevalence of enzymes and their co-production so as to formulate a policy that will aid empirical treatment. The present study was determining the magnitude of ESBL and Carbapenems resistance Enterobacteriaceae and its antimicrobial susceptibility profile to recommend effective use of antibiotics and infection control policy [10].

Enterobacteriaceae spread easily between humans by hand carriage as well as contaminated food and water and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons, which are most important factors, for emergence of MDR among these bacteria. The increase rate of antibiotic resistance among Enterobacteriaceae has posed challenges in choosing empiric regimens, especially when the infections are caused by multidrug resistant Enterobacteriaceae (MDRE)[11]. Previously, the emergences of MDR among Enterobacteriaceae were mainly due to the production of enzymes, such as pencillinases, Cephalosporinases, and extended spectrum β-lactamase (ESBL) [11, 12].

However, recently Carbapenems resistance is one of the main mechanisms in the occurrence of drug resistance in the family of Enterobacteriaceae. Carbapenems Resistance Enterobacteriaceae (CRE) is a family of organisms that are difficult to treat because they have high levels of resistance to antibiotics. *Klebsiella pneumoniae* and *E. coli* are members of Enterobacteriaceae, which capable of break down all β-lactam agents including carbapenems and make it ineffective. Carbapenems such as imipenem, meropenem, ertapenem, &doripenem are considered as the last resort antibiotics to treat ESBL producing Enterobacteriaceae [12, 13].

1.2 Statement of the Problem

Multidrug Resistance (MDR) producing Enterobacteriaceae are the most common caused growing threat in the globally. Morbidity and mortality are infections caused by Enterobacteriaceae. ESBL and Carbapenems resistance Enterobacteriaceae are a serious community health problem [12].Enterobacteriaceae producing due to production of enzyme especially ESBLs is a particular problem for Enterobacteriaceae infections, which results multidrug resistance species. Enterobacteriaceae that produce both ESBLs and Carbapenems are increasing in the worldwide. Since β-Lactemase producing Enterobacteriaceae not always detected in routine antimicrobial susceptibility tests, these organisms usually show multidrug resistance. The absence of methods to detect such producing is a serious challenge facing clinical laboratories and this became a main factor in the dissemination of ESBL producers and treatment failures [13, 12].

Their ability to easily increase with high speed locally, locally and internationally through individual contacts, poor sanitation, travel, food chain is additional challenge [14].Carbapenems Resistance *Enterobacteriaceae* conducted in Ethiopia at different areas indicated that identifying factors such as, patient hospital and health facility stay and well-adjusted use of drugs that encourage Carbapenems resistance is essential to control spread of CRE within the public. The higher percentage MDR producing Enterobacteriaceae isolates conducted in Ethiopia such in Addis Ababa, Jimma and Gonder. The magnitude of multidrug CRE in Ethiopia is of the highest priority (15v, 13).

The magnitude of MDR Enterobacteriaceae is essential to understand their epidemiological, the disease burden and to strengthen hospital infection control strategy to prevent the increase of these Enterobacteriaceae. In Ethiopia the magnitude of ESBL and CRE has been less conducted to detect the extent of drug resistance of these separates or isolates due to ESBLs production. Combination disk were rapid and accurate method for identification of Enterobacteriaceae and antimicrobial susceptibility test. The most important accessible scarce information with regard to the increasing of multidrug producing of β-lactamase Enterobacteriaceae in Ethiopia in general the study site in specific. Emerging Resistance to Carbapenems and their increases all over the world emphasizes the need to evaluate the burden of Carbapenems resistance Enterobacteriaceae [16].

1.3 Significance of the Study

The etiology of UTI and the antibiotic resistance of Uropathogens have been changing over the past years, both in community and health care associated infections. Current knowledge on the burden and antimicrobial susceptibility pattern of the Enterobacteriaceae isolates is essential for appropriate therapy, since those groups of bacteria are the main cause of UTIs and possess several mechanisms to dismantle currently available antibiotics including carbapenems.

This study is important to provide baseline and crucial information regarding to MDR and CRE isolates among UTI patients. Hence policy makers, health administrators and other stake holders can be benefited to design and implement appropriate intervention mechanisms to combat the incidence of infections caused by resistant strains in particular. Furthermore, as a result of this finding health care providers will enforce to follow empirical treatment rules, adopt and utilize standard protocols in the identification of CRE as routine activities.

Moreover, there are few studies conducted in the continent of Africa, based on the knowledge of literature review no published findings were found on the etiology and resistance pattern of community and hospital acquired UTIs caused by CRE in Ethiopia. Therefore, the purpose of this study was to determine the prevalence know the magnitude of Carbapenems resistance or MDR in these organisms at the study site and help physician in the selection of better drugs for treatment of their patients and also save patient from additional cost. Furthermore the result of this finding may help to initiate other large scale epidemiological study on Carbapenems Resistance Enterobacteriaceae.

2. Literature Review

2.1 The Global overview of antibiotic Resistance to Enterobacteriaceae

Across the world, first reports of Carbapenems occurred in the 1980s. Its subsequent spread raised a number of concerns, and these have coincided with years in which no new antimicrobial agents against Enterobacteriaceae have been developed. The occurrence of antimicrobials resistance in *Enterobacteriaceae* is increasingly reported worldwide and has become a one of major health problems (CDC, 2012). Multidrug resistant Enterobacteriaceae have also a major public health threat in developing countries. According to study in Nepal in 2012 among 202 Enterobacteriaceae isolates, 40.1% were MDRE, *Citrobacter* spp were the principal MDR isolate (72%) followed by *E. coli*(38.2%)(19). However, in a 2013 another study from Nepal showed that increased prevalence of MDRE (64.04%)was reported. *E.coli* (74%) and *K.pneumoniae* (44%) were the predominant MDR isolates (20). Besides, in Mozambique, 88.2% of isolates of Enterobacteriaceae were found to be MDR strains (21). A multicenter study in Senegal demonstrated that increased prevalence of antimicrobial resistance observed among Enterobacteriaceae Uropathogens. The overall resistance rates of ampicillin, amoxicillin, amoxicillin-clavulanic acid, naldixic acid, fluoroquinolones and Trimoxazole were77.3%, 34.7%, 14.7%, 13.3% and 55%, respectively. Among member of Enterobacteriaceae, 89% of drug resistances were implicated in *E. coli* and *K. pneumonia* 18].

Countries such as Israel, Greece and Colombia have outbreaks of KPC through their existing local conditions and others mainly by import for occurrence, in Canada, Australia and New Zealand. Following worldwide spread of *Klebsiella pneumoniae*ST258 type strain and throughout the USA in particular, MLST, a specific molecular tool was used to find out the global epidemiology linkages and help understanding the dissemination of KPC genes. There are fresh fears and challenges on-going and under discussion by researchers as to how to control the spread of Carbapenems resistance across the globe for epidemiological reasons. Detection has been difficult due to differences in their genetic makeup within and across countries [19].

Investigators have observed different producing patterns from CREs in Europe, the United States and South America. Previous CRE infections, particularly KPC, were confined to hospitals in New York. Between 2000 and 2010, the infection amount had increased from 1% to 12% in 42 States respectively with a 50% associated mortality rate [19,20].Thirty-nine states of the USA, including Puerto Rico, were success by the threat of KPC positive Enterobacteriaceae within the next few years. Strict control measures were instituted, such as short-stay in acute-care settings, made the CRE infections infrequent and contained the percentage below 5% as compared with 8% in long-term acute-care hospitals monitored by the CDC Network [21].The occurrence resulted in much other KPC-positive *Klebsiella*

pneumoniae identification to be categorized while the majority has been found in *Pseudomonas aeruginosa, Pseudomonas putida* and others in the Enterobacteriaceae family. Currently, 39 countries in the European Union including France and the United Kingdom have come together to fight the problem of CREs in their respective countries. The study is being coordinated by the European Centre for Disease Prevention and Control in Stockholm to keep epidemiological data of patients who test positive for such Enterobacteriaceae in their health-care organizations [22].There were scattered increases in numbers as the KPC-positive identification continued to be identified by the UK national public reference laboratory in 2008 (4 hospitals 5 isolated) and in 2009 (12 hospitals 13 isolated) with *Klebsiella pneumoniae*ST258 the most frequent strain to be taken by MLST (10 out of 12). The source was traced to Greece, Cyprus or Israel, with patients having travelled to those countries in the time prior to admission. Between 2010 and the first six months of 2012, the epidemiological pattern quickly changed in terms of numbers identified, 231 KPC-positive strains in 2010, 368 in 2011 and 293 for the first six months in 2012 were referred for more confirmation at the national reference laboratory [23,12].

Rapid spread of CREs across Europe was observed in late 2005 when an identified KPC isolate in Israel was later found to be genetically related to a type strain in New York. France acquired its first KPC-2-positive *Klebsiella pneumoniae* in 2005 from a patient who had 3 months admission in a New York City (NY, USA) hospital. From 2009 to 2012, there have been several CPEs examined. In a recent study, five cases out of 20 were confirmed and linked their sources to countries such as Kuwait, China, Italy and Israel. Community acquired strains were found to be rare and most of the documented cases in France were from persons colonization with the strains [24]. Carbapenems became a community health concern in 2007 when a total of 1275 CRE infections were identified across their health care centers. KPC-positive organisms found its way in to other countries including Italy, Colombia and the United Kingdom [25 16]. In early 2008, Sweden was the next country to identify multidrug-resistant KPC-positive isolates, this time with a different metallo-lactamase enzyme. More cases of this type of enzyme were seen within three years in the USA and in the UK. Other Enterobacteriaceae, such as Escherichia coli were harboring NDM, exhibiting more resistant traits than KPC-positive *Klebsiella* isolates [16]. Studies later exposed that Enterobacteriaceae carrying the NDM enzyme found its way outside the boundary of hospital settings into community water and sewage environments with some epidemiological link to parts of Southern Asia[26,19].

Various research findings claimed that increased trends in the prevalence of MDR among Enterobacteriaceae also a major concern in Ethiopia. Particularly in Gondar the prevalence of MDRE increased from time to time, i.e. 2002 the prevalence of MDR strains were 68%(23), the magnitude increased to 85.5% (2007)(24) and 93.5% (2013)(25).

Moreover; tremendous Prevalence of MDR strains also reported in other parts of Ethiopia, in Dessie (74.6%) (26), Bahirdar (95.6%)(27), and Jimma (100%) (28).In all studies *E. coli* and *K.pneumoniae* were the principal MDR isolates among Uropathogens. In the recent years, there is an increase trend of resistance to carbapenems among Enterobacteriaceae in clinical isolates. According to data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) showed that, the rates of CPE (*K. pneumoniae*) increased in most European countries. Greece, Cyprus and Italy reported resistance rates of 43.5%, 17.0% and 1.3% respectively (29-31). Likewise, based on center for disease control and prevention report on health care associated infections the overall prevalence of CPE (KPC) rising from less than 1% in 2000 to 8% in 2007 (15). Particularly, in France 6, 26 and 13 CRE episodes were reported in 2009, 2010 and the first four months of 2011, respectively (32)[29]. Carbapenems resistances Enterobacteriaceae are the principal clinical isolates from patients with UTI. A four year study from Ontario, Canada; demonstrated that from known 73 CRE clinical isolates, 46.6%, 32.9%, 5.5%, 2.75%, 2.75%, 2.75%, 1.4%, 1.4%, & 4.1% were recovered from urine, rectal swab, wound, blood, sputum, intraperitoneal fluid, bone, skin swab & other body samples respectively (34). Besides, similar isolation rate of Carbapenemase producing *K. pneumoniae* detected from patients with UTI in Italy and it was 47.5% (35).

According to a study, from 4564 screened Enterobacteriaceae isolates, 158 (3.5%) were carbapenems resistance strains. *K.pneumoniae, E.coli, oxytoca* and *Enterobacter* spp had found expressing different class of carbapenems (36). Moreover, comprehensive epidemiological study in United Kingdom, India and Pakistan showed that about 4.5% of isolates were CRE (37). In another study the overall prevalence of CRE had been found 10.9%, *K. pneumoniae* (90%) and *E.coli* (10%) was most important isolates (38). A finding from United States of America, the prevalence of CRE was found to be 21% (39).

Recently, China has launched a national action plan for combating various antimicrobial producing threats including CREs, which promises to make a substantial impact on public health, both locally and globally. Carbapenems resistance is increasingly reported among the species of Enterobacteriaceae in Saudi Arabia. Increased prevalence of CRE producers and the distribution of Carbapenems- resistance genes are of particular health concern to the health care providers and the Ministry of health in Saudi Arabia. Most reports related to CRE came from the central part of the country, Riyadh. On the other hand, only few studies describing the susceptibility pattern of Enterobacteriaceae against Carbapenems with few data on their molecular characteristics were reported from other regions of Saudi Arabia [33].In recent study, Al-Zahrani and Alasiri studied the molecular characteristics of 54carbapenemase non-susceptible *K. pneumoniae* isolates obtained from clinical specimens in two of the largest hospitals in the Southern province of Saudi Arabia. The other data from Libya indicated the Emergence of Carbapenems resistance *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. A total, of 49 clinical isolate in

which P. *aeruginosa* constitute 24and A. *baumannii* 25 which was collected from Burn and plastic Surgery Hospital of Libya which showed the high prevalence of resistance to Carbapenems. As revealed by the researchers *Pseudomonas aeruginosa* showed 79% resistance to meropenem and 87% imipenem and also *Acinetobacter baumannii* showed 92% and 88% resistance to meropenem and imipenem respectively [34,22]

2.2Frequency of ESBL and Carbapenems Resistance Enterobacteriaceae in various countries

The degree of Carbapenems Resistance Enterobacteriaceae (CRE) among specimen isolates varies greatly international and within geographic areas. A systematic review in African countries showed that about 83 studies conducted in Africa which presented that the frequency of Carbapenems manufacturer isolates was from 2.3% to 67.7% in North Africa and from 9% to 60% in sub-Saharan Africa. Oxacillinases especially blaOXA-48 was the predominant type of Carbapenems in Africa [35, 37].The manufacture of β-lactamase is the main mechanism for producing Enterobacteriaceae. Study in India done by Doddaiah *et al* [38] to control the prevalence of three common enzymes produced by Enterobacteriaceae from urine specimens indicated that out of 378 Enterobacteriaceae one or more β-lactamases were observed in 197 isolates. Of these, 33.86%, and 18.25% were ESBL and Carbapenems individually [39, 40].

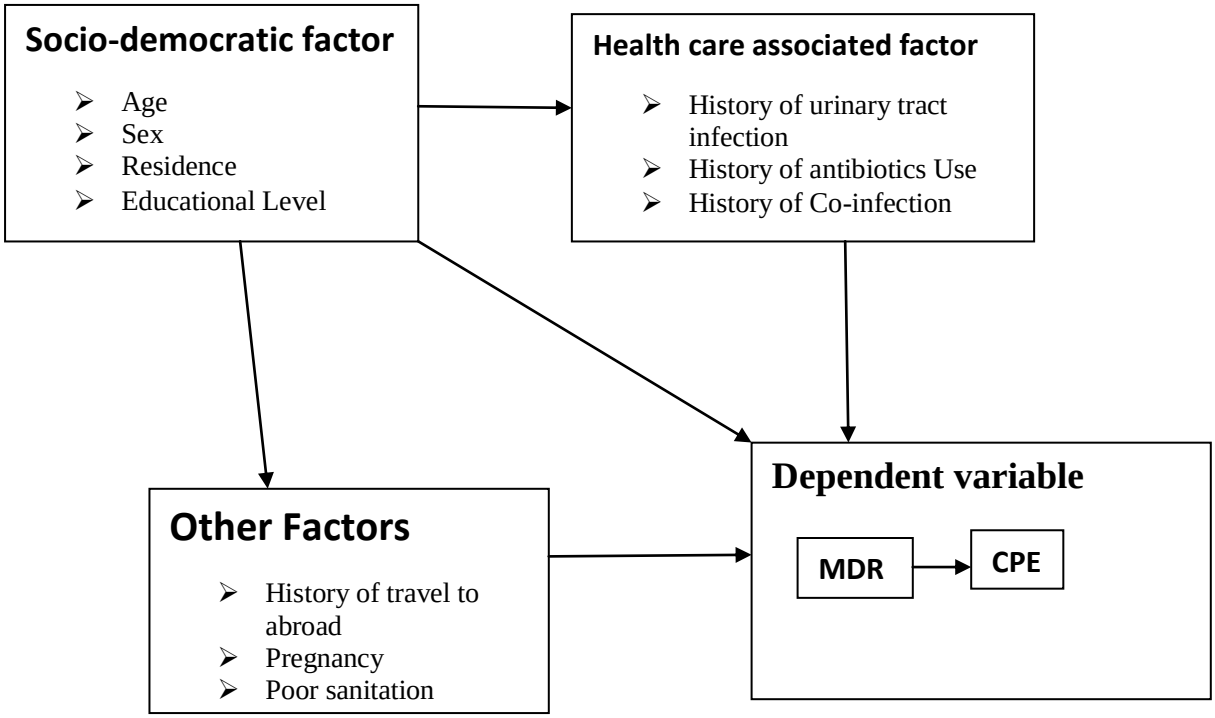
Other study showed by Ouedraogo *et al* [40] in Burkina Faso on increase frequency of ESBLs producing *Enterobacteriaceae*. ESBL producing E*nterobacteriaceae* was identified using combination disk method and ESBL was 58%. *E. coli* (67.5 %) and *K. pneumoniae* (26 %) were the major ESBL producing isolates. Meropenem, imipenem and amikacin were thebest effective drugs against ESBL producing Enterobacteriaceae.The study was conducted in Sierra Leone from December 2013 to April 2014 by Leski *et al* [45] antimicrobial susceptibility testing result confirmed that 85.7% of *Enterobacteriaceae* isolates were MDR where as 64.3% produced an ESBL. The highest percentage of *K. pneumoniae and E. coli* were MDR. Imipenem monitored by piperacillin/tazobactam and amikacin the lowest rate of producing. The study conducted in Morocco [46]from May 2009 to December 2010 on *Enterobacteriaceae* that resistance Carbapenems showed *E. coli* was the frequent separate monitored by *Klebsiella spps* and *Enterobacter spps* and Carbapenems resistance *Enterobacteriaceae* seen in (2.8%) of the isolates. ESBLs and Carbapenems were screened from total of 550 clinical isolates of *Enterobacteriaceae*. *Enterobacter spps* (12.5%) highest rate of co-production was seen. According to study done by Iraq out of 91 Enterobacteriaceae isolated the total prevalence of ESBLs producers are 74.7%[47]. Another, study done by Chittagong Bangladesh out of 120 out patients the prevalence of ESBLs producers are 53.03% and *Klebsiella spps* as most prevalent ESBLs producers [48].

Another study in Nigeria conducted to determine Carbapenems resistance between urine specimens of *Enterobacteriaceae* the overall frequency of Carbapenems resistance *Enterobacteriaceae* was 12.4%.Among a total of 27 Carbapenemase no susceptible isolates, (12.4%) were Carbapenemase producers but (2.8%) showed Carbapenems resistance for the cause of ESBL producing. Another study conducted in Uganda by Andrew *et al* [50] showed that from 100 identified *Enterobacteriaceae;* the most important *Enterobacteriaceae* was *E. coli* (44%). Among the 100 tested *Enterobacteriaceae* isolates, (89%) were identified as ESBL producing pathogens especially *the* most predominated ESBLs producers are *Klebsiella spps[50,49]*.

The study conducted in Ghana2013 indicated that from the total of 300 *Enterobacteriaceae* isolates 49.3% were ESBLs producers. Similarly, ESBLs producing isolates had highest producing to gentamicin, co-trimoxazole, ciprofloxacin and amikacin but all isolates were susceptibly to meropenem.The study conducted in Sudan by Dahab *et al* on detection of Carbapenems Resistance *Enterobacteriaceae* isolated from patients in Khartoum city. The most major *Enterobacteriaceae* isolates was *E. coli* (54.4%), examined by *Klebsiella Spps* (29.5%). The highest percentages isolates were Carbapenems resistance and these isolates were confirmed by MHT then 56% of the producing was Carbapenems resistance [51]. Another Study conducted by Eshetie *et al* in Gondar showed the high prevalence of MDR *Enterobacteriaceae* was (87.4%). Among of this isolates(2.73%) of the isolates were Carbapenems resistance and *Klebsiella pneumonia* and *Escherichia coli* were predominant isolates [52].

Like other developing countries study done in different part of the country shows antibiotic producing was increasing from time to time in Ethiopia. Study done in Eastern Ethiopia at Dire Dawa, Dil Chora Referral Hospital, Northern Ethiopia, University of Gondar teaching hospital and Addis Ababa Tikur Anbessa Specialized Hospital among urinary tract infection of pregnant women indicated an increased patterns of multi drug resistance *Enterobacteriaceae* [31,33]. As the data from the study sites shows the highest MDR isolate were reported from Dil Chora Referral Hospital by Derese B et al, in 2015. In this study MDR was noted in all of the isolated *Enterobacteriaceae* where *Klebsiella spps* .and *P. aeruginosa* showed more resistance [22.24].

Figure 1: Conceptual framework for factors associated with MDRE and CRE



3. Objective

3.1 General objective

To determine the prevalence of Extended-spectrum B-Lactamase and Carbapenemase producing *Enterobacteriaceae* among adult patients complaining UTI at South West shoa, lemman Hospital and kersa malima woreda health facility.

3.2. Specific objectives

- To determine the magnitude of bacterial isolates from UTI at south west shoa, Lemman Hospital and Health facility
- To determine the antimicrobial susceptibility patterns of bacterial isolates
- To determine the magnitude of ESBLs producing Enterobacteriaceae
- To determine the magnitude of Carbapenemase Resistance *Enterobacteriaceae*

4. Hypothesis

There is no difference in the prevalence of Carbapenems resistance in *Enterobacteriaceae* from previous studies.

5. Material and method

5.1 Study area

The study was conducted at leman hospital and kersa malima woreda health center facility laboratory in January 2020 to May 2020. Leman hospital and kersa malima woreda health center facility located in under south west shoa Lemman sub- city, Butajira main road which was established in 2008. Lemman Hospital Works on six thematic areas which includes, Public Health Emergency Management, Public Health Laboratory Service, Adult and pediatric OPD service, Delivery service, Neonatal intensive care unit, Surgical ward and Leadership with 133 total staffs, which constitutes, 12 Medical Doctor, 2 Specialist(MD), 7 Laboratory, 9 pharmacy,40 clinical nurse,9 Midwifery and 54 permanent and contract administrative. The primary objectives of the Institute are conducting on priority areas of health and nutrition problems and also provide communicable and non-communicable service on different wards. In Lemman Hospital and kersa malima woreda health facility suspected UTI may be done 8-12 per a day and 40-60 per a week's may be done.

5.2 Study design and period

A cross-sectional study was conducted from September1/ 2020 to December 30/ 2020

5.3 Population

5.3.1Source population

Source of population was all patients with suspected UTI seeking at leman hospital and kersa malima woreda health facility for diagnosis during the study period.

5.3.2Study Population

Patients with symptomatic suspected UTIs, who were accessed at the time of study period at in Leman hospital and kersa malima woreda health facility during the period was enrolled.

5.4 Inclusion criteria

All patients whose was sought symptomatic for UTIs and willingness to participate in the study.

5.5 Exclusion criteria

- Patients who are on antibiotic treatment and patients repeatedly to the study site and if the same bacteria is isolated
- Patients with asymptomatic UTI and patient on antibiotics (since the last 7 days) were excluded from the study.

5.6 study variables

5.6.1 Dependent variable

- Frequency of Enterobacteriaceae isolates from UTIs
- Magnitude of ESBLs producing Enterobacteriaceae
- Prevalence of MDR, Carbapenemase

5.6.2 Independent variable

Age, sex, history of travel to abroad, prior antibiotic use in the past 6 months, history of UTI, marital status, Educational level

5.7 Measurement and Data collection

5.7.1Sample size calculation

The sample size was determined by using single population proportion formula: n = Z2α/2 P (1- P)/ d2. The estimated prevalence, p=12% (0.12) which was taken Almugadam**,***et al.,* 2017 study in Kosti City, Sudan [1]. The study used 95 % confidence interval and hence Zα/2=1.96,*d* is the margin of error 0.04.

n = Z2 α/2 P (1- P)/ d2 n = (1.96) 2 *0.12(1- 0.12)/ (0.04) 2 =253, though the calculated sample size was 253 and 10% of 253 =25 though the calculated sample size was 253+25=**278** and

The estimated prevalence of ESBLS, P=79% (0.79) which was taken from Addis Ababa, et al,2017 study Addis Ababa(19).The study used 95% confidence interval and hence Zα/2=1.96, *d* is the margin of error 0.05.

n = Z2 α/2 P (1- P)/ d2 n = (1.96) 2 *0.79(1- 0.79)/ (0.05) 2 = 255,though the calculated sample size was 255 and 10% of 255=25though the calculated sample size was 255+25=280

5.7.2 Sample Technique

A convenient sampling method was implemented from September1/ 2020 to December 30/ 2020

5.7.3Data collection technique

The socio-demographic data of patients was recorded using questioner. Midstream urine specimens were collected from Patients in leman hospital and kersa malima woreda health facility and gave written informed consent and assent to participate in the study and consent was given by the participant or guardian and patients' socio demographic data and other variables were filled by the principal investigator. The completeness of requisition form was checked and urine specimen collected immediately processed following standard procedures.

5.7.3.1 Isolation of Enterobacteriaceae from Urine sample

Midstream urine Specimens collected from UTI complaining patient was inoculated onto primary isolation culture media such as Blood agar and Macconkey agar. The inoculated media was incubated aerobically at 37°C and if growth is observed gram stain was done and then sub-cultured onto the specific media if sub-cultures fail to yield any Enterobacteriaceae growth, Blood agar was incubated at 37^0C in CO_2 incubator for 24 hours while Macconkey agar was incubated at the same temperature and period but aerobically. Agar culture plates with no bacterial growth in the above incubation conditions were further incubated for another 24 hours. Culture media and reagents used in this study were prepared as per the procedure of the manufacturer [56].

5.7.3.2Enterobacteriaceae identification

Enterobacteriaceae pathogen were characterized by colony morphology and number and Enterobacteriaceae was identified by employing conventional biochemical methods such as urease test, glucose fermentation test, motility test, in dole test, oxidize test, citrate utilization test hydrogen sulphide production test motility test and fermentation of different carbohydrates. The tests are based on bacteriological utilization and degradation of specific substrates detected by several indicator systems.

Acid making is specified by a change in phenol red indicator when an isolate is able to utilize a carbohydrate substrate. Enterobacteriaceae that utilize a specific carbon source decrease the resazurin-based indicator [57].

5.7.3.3 Antimicrobial Susceptibility Test

Antimicrobial Susceptibility testing was performed on isolates according to the criteria of Clinical and Laboratory Standards Institute (CLSI,2019) on Muller-Hinton Agar. A homogeneous Enterobacteriaceae suspension was prepared by mixing a loop full of pure bacterial colony in 5ml of 0.85% saline solution. After adjusting the 0.5 McFarland standard of the suspension, it was evenly inoculated on Muller Hinton agar plate using sterile cotton swab and antibiotic disks were placed at 15mm and 24mm distance from the edge and from each other respectively. Susceptibility testing for12 antimicrobials namely ceftazidime, cefuroxime,ceftriaxone, Nitrofurantoin, amoxicillin/clavulanicacid, Cefazolin, Ertapenem, gentamicin, meropenem, ampicillin, sulfametoxazole-trimethoprim, piperacillin/ tazobactam was performed by using *combination disk, which also has selection for ESBL and Carbapenemase producing* Enterobacteriaceae. Constant quantities of changes to the indicator as well as Enterobacteriaceae turbidity are used in the identification of Enterobacteriaceae growth. Each AST panel configuration contains several antimicrobial agents. Enterobacteriaceae identification is used in the analysis of the disk distribution standards of each antimicrobial agent [58].

5.7.3.4 Storing and Isolate Holding

After Suspected beta-lactamase producing Enterobacteriaceae was isolate than sub cultured on 5% sheep blood agar o get fresh colonies for combination disk interpretation of ESBL and Carbapenems. After that isolate was analyzed it is stored at -80oC [58].

5.7.3.5 Confirmation of ESBLs with Combination Disc Test

Screening of ESBL production was done by using ceftazidime (CAZ) (30mg) and cefotaxime (CTX) (30mg) disks. Suspected ESBL producers were done by which Enterobacteriaceae were non susceptible to cefotaxime and/or ceftazidime. Acompact disk of ceftazidime (30 µg) and cefotaxime (30 µg) alone and ceftazidime + clavulanic acid (30 µg/10 µg) and cefotaxime (30 µg) + clavulanic acid (30 µg/10 µg) were placed at a distance of 25 mm, center to center, on a Muller Hinton agar plate inoculated with a Enterobacteriaceae suspension of 0.5 McFarland turbidity standards and incubated overnight (18 -24 hrs.) at 37°C. As recommended by the CLSI if the zone of inhibition (ZOI) of ceftazidime is ≤22 mm and the ZOI for cefotaxime is ≤ 27 mm, the isolate was considered as ESBL producer. Since ZOI is ≥ 5 mm for either anti Enterobacteriaeae agent in combination with clavulanic-acid acid (CA) versus its zone when tested alone confirmed ESBL. *E. coli*ATCC 25922 and *K. pneumoniae*ATCC 700603 were used as negative controls respectively [60].

5.7.3.6Screening of Carbapenemase making with MHT

Carbapenems resistance was differentiating by the MHT which is commended by CLSI. Confirmed MHT is seen when the test isolate products of the enzyme and agrees growing of a Carbapenems susceptible strain (*E. coli* ATCC 25922) towards a carbapenems disk.Screening of Carbapenems was done for the isolates which are producing to imipenem (IPM 10ug), meropenem (MEM10ug) and ertapenem based on CLSI break point. Verification of Carbapenems in Enterobacteriaceae was done by Modified Hodge Test (MHT), where Mueller-Hinton agar plate was inoculated with a 1:10 dilution of a 0.5 McFarland suspension of overnight sub-cultured E. coli ATCC 25922 and streaked for confluent growth using a swab. A 10ug imipenem disk was placed in the center, and each test isolate were streaked from the disk to the edge of the plate. A positive Modified Hodge Test (MHT) was indicated by clover leaf-like indentation of the *E. coli* ATCC 25922 growing the test Enterobacteriaceae growth streak within the disk distribution zone. Confirmation of Carbapenems in Enterobacteriaceae producing to Carbapenems drugs and negative by MHT was done by combination disk method, where two IPM disks (10µg), one containing 10µl of 0.1M (292µg) anhydrous EDTA, was placed 25mm apart from center to center. An increase in zone diameter of > 4mm around the IMP-EDTA disk compared to that of the IPM disk alone was considered positive for metalo-beta-Lactamase (MBL) [62].

5.8Quality Assurance of Data

Final Enterobacteriaceae identification and data controlling keep the quality of the work from isolate collection up to the standard operating procedure of laboratory examination was strictly monitored.

Pre-analyitical

Specimens were collected following SOPs and brought to clinical and mycology laboratory immediately for bacteriological culture, expiry date of Medias, antibiotic disk and reagents, Sterility and performance of Medias and antibiotics were checked by known standard strains such as *E. coli ATCC 25922*, and *Pseudomonas aeruginosa* ATCC 27853.

Analytical

The selected culture media was checked for sterility by incubating the five percent of prepared media for 24 hours and check for the presence of Enterobacteriaceae growth. Abilities of the prepared media supporting the growth of Enterobacteriaceae were checked by inoculating control strains *E. coli* (ATCC 25922) and other Enterobacteriaceae

Post analytical

Quality of the data was maintained by coding with unique number and finally all clinical isolate were preserved in deep freeze using 20% glycerol with Trypticase soya yeast (TSY) in case needed or for future further investigation.

5.9 Data analysis and interpretation

The data were collected, summarized; missed variables were checked to clean the data and analyzed using SPSS version 24. Frequency and Percentages of MDR, Carbapenems and ESBL producing gram-negative bacteria were calculated. Prevalence of MDR Carbapenems and ESBL producing Enterobacteriaceae was determined at 95% CI and P values < 0.05 was considered as statistically significant and Chi-square was used to measure the association between the variables.

5.10 Ethical considerations

The ethical clearance was obtained from the Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University and supportive letters were written to the study site for collaborative work. Appropriate safety precaution was followed to ensure safe handling and disposing of the wastes or clinical isolates, after completion of drug susceptibility test; all clinical isolate was stored in secured area at Lemman Hospital and kersa malima woreda health facility laboratory and appropriately disposed to prevent dissemination of these resistant strains in the laboratory environment and further to the community.

5.11 Dissemination of the result

Result of the study was submitted to the department and communicated to a scientific community in the form of thesis defense, presented in local and international conferences, and also the result was sent to known publication for journals.

5.12 Operational Definitions

I. Multidrug Resistance: The bacterium that was simultaneously resistant for three or more antimicrobials belonging to different classes of antibiotics tested.

II. β-lactam antibiotics: β-lactam antimicrobial agents that share a common, central, and fourmember's B lactam ring.

III. **Carbapenemase**: Carbapenemase are β-lactamases that hydrolyze penicillin, in most Cases cephalosporin's, and to various degrees carbapenems and monobactams.

IV. **Combined disk method:** Disks containing cephalosporin alone (cefotaxime, ceftazidime, and cefepime) and in combination with clavulanic acid are applied.

V.**ESBL**: ESBLs are enzymes that hydrolyze most penicillin and cephalosporin's, including Oxyimino-β-lactam compounds.

VI. None: means non-illiterate (uneducated)

6. Results

6.1 Socio-demographic characteristics

Among two hundred seventy eight (n=278) urinary tract infection suspected adult participant greater than fifteen (>=15) years investigated during the study period. From these patients 8.6% (n=28/278) were males and 89.9%(n=250/278) were females with males to females ratio 1.037:1.107.The majority of patients 85(30.6%) were between 25-34 years of ages and the mean (std.deviation) ages of patients were 36.49 and13.846) with age range of above fifteen years. The Socio-demographic characteristics of patients have shown Table .1

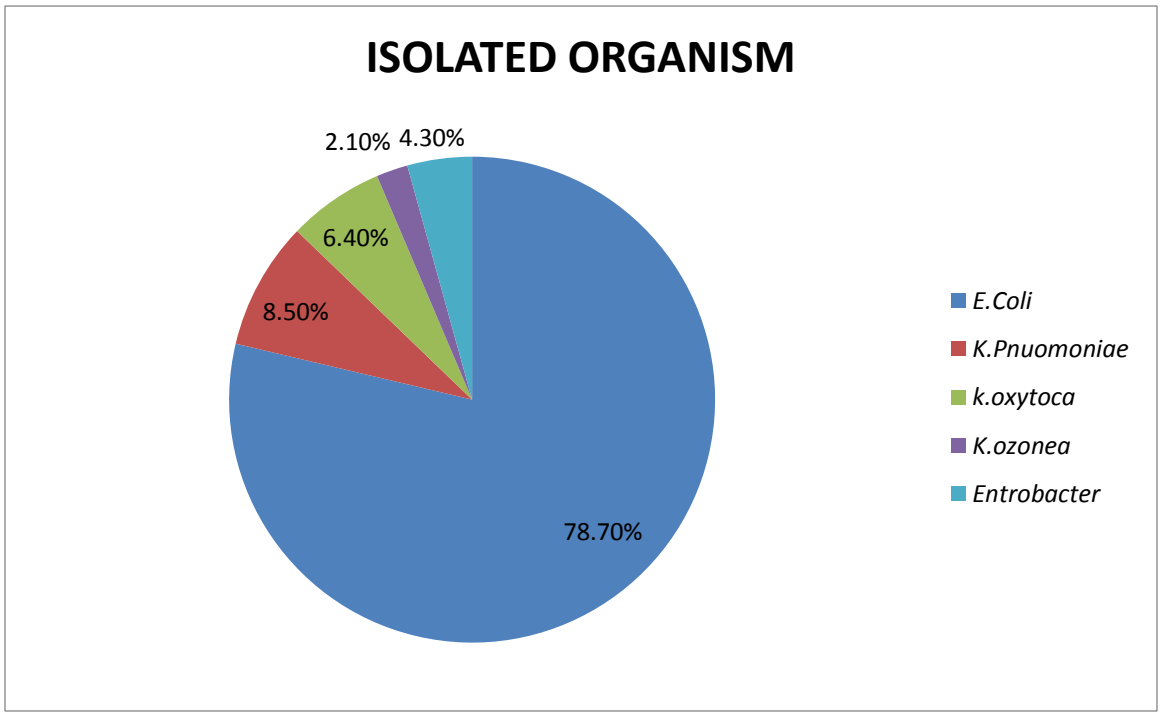
Table1. Socio-demographic characteristics of study participants and their culture result at south west shoa Lemman Hospital and Health facility from September 2020 to December 2020.

Variable	**culture results from UTI(n=47)**			
		Positive	Negative	Total
	F(43)	43(15.5%)	207(74%)	250(89.9%)
	M(4)	4(1.4%)	24(8.6%)	28(10.1%)
	Total	47	231	278
Age in group	15-24	14(5%)	54(19.4%)	68(24.5%)
	25-34	18(6.5%)	67(24.1%)	85(30.6%)
	35-44	7(2.5%)	40(14%)	47(16.9%)
	45-54	5(1.8%)	40(14.4%)	45(16.2%)
	>=55	3(1.1%)	30(10.8%)	33(11.95)
	Total	47	231	278
Level of education	None-illiterate	15(5.4%)	99(35.6%)	114(41%)
	Primary	16(5.8%)	62(22.3%)	78(28.1%)
	Secondary	13(4.7%)	47(16.9%)	60(21.6%)
	College	3(1.1%)	23(8.3%)	26(9.4%)
	Total	47	231	278
Marital status	Married	41(14.7%)	156(56.1%)	197(70.9%)
	Unmarried	6(2.20%)	65(23.4%)	71(25.5%)
	Divorced	0(0)	10(3.6%)	10(3.6%)
	Total	47	231	278
Residence	Rural(171)	32(18.7%)	139(81%)	171(61.5%)
	Urban(107)	15(14%)	92(86%)	107(38.5%)
	Total(278)	47	131	278

6.2 Prevalence of Enterobacteriaceae Isolates from UTI suspected participants

From the study participants the overall prevalence of Enterobacteriaceae was 16.9% (47/278) patients, who had positive urine culture isolates of Enterobacteriaceae were identified and 8.5 %(n=4/47) of the culture positive were from male and 91.5 %(n=43/47) were from females. The majority of bacteria isolated from cultures were E.coli 78.7%(n=37/47), *Klebsiella pneumoniae* 8.5%(n=4/47), *Klebsiella oxytoca6.38%(n=3/47), Enterobacter 4.3%(n=2/47) and Klebsiella Ozanae 2.1%(n=1/47).* From a total of 47 isolated Enterobacteriaceae 46(97.9%) were resistance to three or more antibiotics, only 1(2.1%) isolated was no resistance to all antibiotics. In this study 100% (n=47/47) of Enterobacteriaceae were isolated from those who attended outpatient department; However there was no significant association between age of patient and culture result (OR=1.03, 95%CI=0.030-0.013, P =0.587).From the bacteria isolates the families Enterobacteriaceae were the most frequent bacteria group isolated from urine culture.

Figure 2. Frequency of Enterobacteriaceae isolates among study participants from south west shoa leman Hospital and health facility from September 1/2020 to December 30/2020.



6.3 Antibiotics Resistance Enterobacteriaceae

From the total isolates (n=47) multidrug resistance (MDR ≥3 drugs) were recorded in 24(51.1%) of all Enterobacteriaceae isolatesand multiple drug producing respectively. All isolates showed low level of resistance (<80%) for all antibiotics and ampicillin is intermediate level at 63.8%.Low level of resistance forCefoxitin,Gentamicin,sulfametoxazole-trimethoprim,Amoxicillin-clavulanicacidCefazolin, ceftriaxone, cefotaxime and ceftazidime. From Enterobacteriaceae isolates the predominant isolates *K.pneumoniae*8.5% (n=4/47) demonstrates high level of producing to ampicillin (100%)and k.oxytoca demonstrates high level of producing to ampicillin (100%), Cefazolin(100%), cefuroxime (100), ceftriaxone(100%), Amoxicillin-clavulanic acid(100%) and sulfametoxazole-trimethoprim(100%). Better susceptibility can be achieved using Meropenem(100%), ertapenem (100%), piperacillin/ tazobactam(100%) and Nitrofurantoin (93.6%) compared to other tested drugs.

In this study from the members of Enterobacteriaceae isolated *K. ozanae* 2.1% (n=1/47), *K. Pneumoniae*8.5% (n=4/47), *Escherichia. Coli*78.7% (n=37/47), *Enterobacter*4.3% (n=2/47), *and K.oxytoca* 6.4% (n=3/47) showed resistance to Cefotaxime and ceftazidime (third generation cephalosporin) and all isolates of Enterobacteriaceae were susceptible to piperacillin/ tazobactam ,Ertapenem and Meropenem contrarily showed 100% (n=47/47) sensitivity (**Table 2)**. In this study from the members of Enterobacteriaceae isolated there were relatively no resistance to Carbapenems drugs.

Table 2. Antimicrobial resistance levels of bacterial isolates from urine cultures among adults above fifteen years old at south west shoa, leman Hospital and health facility from September 1/2020-December30/2020.

ISOLATED ORGANISM	no	**Antibiotic Resistance pattern of Enterobacteriaceae %**											
Escherichia coli	37		AMP	CZ	CXM	CTR	CAZ	AMC	STX	GEN.	NIT	TZP	ERP/ MEM
		s	14 (37.8)	25 (67.6)	22 (59.5)	22 (59.5)	27 (73)	22 (59.5)	22 (59.5)	34 (91.9)	36 (97.3)	37 (100)	37 (100)
		R	23 (62.2)	12 (32.4)	15 (40.5)	15 (40.5	10 (21.3)	15 (40.5)	15 (40.5)	3 (8.1)	1 (2.7)	0 (0)	0 (0)
Klebsiella Pneumoniae	4	S	0 (0)	2 (50)	2 (50)	2 (50)	2 (50)	1 (25)	3 (75)	4 (100)	4 (100)	4 (100)	4 (100)
		R	4 (100)	2 (50)	2 (50)	2 (50)	2 (50)	3 (75)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
Klebsiella Oxytoca	3	S	3 (100)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	1 (33.3)	2 (66.7)	3 (100)	3 (100)
		R	1 (33.3)	3 (100)	3 (1oo)	3 (10)	2 (66.7)	3 (100)	3 (100)	2 (66.7)	1 (33.3)	0 (0)	0 (0)
Klebsiella Ozanae	1	S	0 (O)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1(100)
		R	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Enterobacter	2	S	0 (0)	2 (100)	2 (100)	1 (50)	1 (50)	0 (0)	2 (100)	2 (1OO)	1 (50)	2 (100)	0(0)
		R	2(100)	0(0)	0(0)	1(50)	1(50)	2(100)	0(0)	0(0)	1(50)	0(0)	2(100)
Total Isolated	47	S	17 (36.2)	30 (63.8)	27 (57.4)	26 (55.3)	32 (68.1)	24 (51.1)	28 (59.6)	42 (89.4)	44 (93.6)	47 (100)	47 (100)
		R	30 (63.8)	17 (36.2)	20 (42.6)	21 (46.7)	15 (31.9)	23 (46.9)	19 (40.2)	5 (10.6)	3 (6.4)	0 (0)	0 (0)

NOTE: AMP…Ampicillin,CZ….Cefazolin….CXM….Cefuroxime, CTR….Ceftriaxone, CAZ…..*Ceftazidime*, AMC…Amoxicillin-clavulanic acid, STX…. Sulfamexazole/trimethoprim,TZB…piperacillin/tazobactam, GEN…Genetamyicin,NI… Nitrofurantoin, ERP…Ertapenem,MEM…Meropenem

6.4Multi-drug Resistance of Enterobacteriaceae

From the total of 47 Enterobacteriaceae isolates 51.1% (24/47) of Enterobacteriaceae isolates were MDR. From Enterobacteriaceae isolates, 100% MDR were recorded in *K.oxytoca* and *K.pneomoniae*, Enterobacter *were recorded 50%*whereas the least resistance, 48.6%, was recorded against *E.coli*. Among the total of 47Enterobacteriaceae isolates only 27.7% (13/47) Enterobacteriaceae isolates were susceptible for all tested antibiotics (Table 3).

Table 3. Multidrug resistance of Enterobacteriaceae among study participants [28]

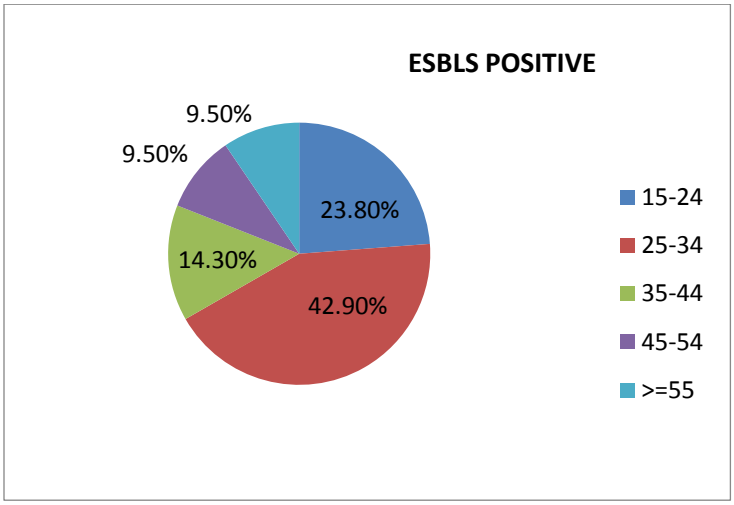
Enterobacteriaceae isolates	**Degree of antibiotics n (%)**								**MDR ISOLATES (≥3)**
	R0	R1	R2	R3	R4	R5	R6	≥ R7	
Escherichia coli(37)	12 (32.4%)	4 (10.8%)	3 **(**8.1%**)**	5 (13.5%)	1 (2.7%)	1 (2.7%)	5 (13.5%)	6 (13.5%)	**18 (48.6%)**
Klebsiella Pneumoniae(4)	0 (0%)	1 (25%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (25%)	**2 (50%)**
Klebsiella oxytoca(3)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33.3%)	2 (66.7%)	**3 (100%)**
Klebsiella Ozone a(1)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
*Enterobacter(*2)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	**1 (50%)**
Total (47)	13 (27.7%)	5 (10.6%)	5 (12.8%)	5 (10.8%)	1 (4.3%)	2 (10.6%)	7 (19.1%)	9 (6.4%)	**24 (51.1%)**

NOTE: Ro: resistance to no antibiotics, R1-6resistance to 1, 2, 3, 4, 5, 6 and ≥7resistance to 7, 8, 9 and above groups of antibiotics; ≥R3: resistance to 3 or more antibiotics from differentclasses.

6.5 Extended spectrum Beta-lactamase producing Enterobacteriaceae

A total of 47 Enterobacteriaceae isolated from urine samples, 44.7% (21/47) were ESBLs producers. The overall prevalence of ESBLs was 44.7% (95%CI; 2.687-2.824%) among all isolates and 18/21 (85.7%) of ESBLs isolates were identified from females and 42.9 % (9/21) isolates were also indicated from patients with age group from 25-34 years **(figure 3)** and85.7% (18/21) were from female and 14.3% (3/21) were from male patients**.** From the total of 47Enterobacteriaceae isolates,21 ESBL screening for Enterobacteriaceae with MIC ≥2μg/ml for ceftazidime and/or for cefuroxime using, 44.7% (21/47) were confirmed as ESBLs producing Enterobacteriaceae using combination disk test**.** However *k.ozonea* 2.1 % (n=1/47) are excluded from further screening for ESBL.

Figure3. Frequency of ESBL producing Enterobacteriaceae against age category at south west shoa zone leman Hospital and health facility from September 1/2020 to December 30.2020



Therefore, 21 Enterobacteriaceae isolates were suspected for ESBL which were *E. coli* (n=15), *K.oxytoca* (n=3) and *K.pneomoniae* (n=2) and Enterobacter (n=1). The higher percentage of ESBL producing Enterobacteriaceae was recorded in *E .coli* 71.4% (15/21) and *K.oxytoca*14.3% (3/21) respectively. Hence 21Enterobacteriaceae were tested for ESBLs using the combination disk method according to CLSI.

6.5.1 Combination (Disk Potentiating) Disk method

From the total of 47 Enterobacteriaceae isolates,44.7% (n=21/47) were confirmed for ESBL using combination disk method, *K. oxytoca*100% (n=3/3), *K. pneumoniae*100(n=2/2), *E. coli* 100% (n=15/15) and *Enterobacter spps*100% (n=1/1) were positive for ESBL

6.6The magnitude of ESBLs producing Enterobacteriaceae Association of independent variables

The magnitude of β-lactamases producing Enterobacteriaceae data analysis of using logistic regression model showed that had no statistically significant association with age group and urine specimen (P>0.05). Enterobacteriaceae isolate that are isolated from age group >=55 year are (95%, AOR =1.251 (0.065-0.413), p = 0.582) the same as other age group and also in binary logistic regression analysis also magnitude of ESBLs production had no statistically significant association with other variables (gender, marital status, level of education and age group) in this study (Table.4).

Table4. Association of marital status, gender, level of education and age group with magnitude of extended spectrum β-lactamases producing Enterobacteriaceae at south west shoa zone leman hospital and health facility from September 1/2020 to December 30.2020 .

Variable(n)	ESBLs(+ve) N%	Bi-variable		Multi-variable	
		COR (CI)	P-value	AOR (95% CI)	P- value
Gender					
Female(43)	18(41.9)	0.982(0.504-2.499)	0.982	0.696(0.191-2.531)	0.582
Male(4)	3(75)	0.696(0.191-2.535	0.582	2.896(0.37622.352)	0.582
Age(years)					
15-24(14)	5(35.7)	0.394(0.204-1.489	0.170	1.381(0.241-7.218)	0.750
25-34(18)	9(50)	0.391(0.107-1.437)	0.157	1.977(0.402.9.727)	0.402
35-44(7)	3(42.8)	0.668(0.159-2.810)	0.582	1.012(0.119-6.435)	0.582
45-54(5)	2(40)	0.819(0.180-3.725)	0.796	0.763(0.101-5.7450	0.796
≥ 55(3)	2(66.7)	0.667(0.342-1.339)	0.697	1.812(1.332-2.465)	0.967
Educational level					
None(14)	8(57.1)	0.958(0.191-4.818)	0.959	0.497(0.087-2.842)	0.432
Primary(16)	8(50)	2.196(0.474-10.154)	0.315	0.959(0.175-5.268	0.962
Secondary(13)	3(23.1)	1(0.189-5.289)	1	0.646(0.098-4.267	0.650
Tertiary(1)	0(0)	0.632(0.089-4.623)	0.627	Ref*	Ref*
College(3)	2(66.7)	Ref*	0.999	Ref*	Ref*
Residence					
Rural(32)	15(46.8)	1.412(0.724-2.752)	0.34	1.324(0.381-4.595	0.659
Urban(15)	6(40)	1.655(0.619-4.421	0.315	1.250(0.354-2.924)	0.607

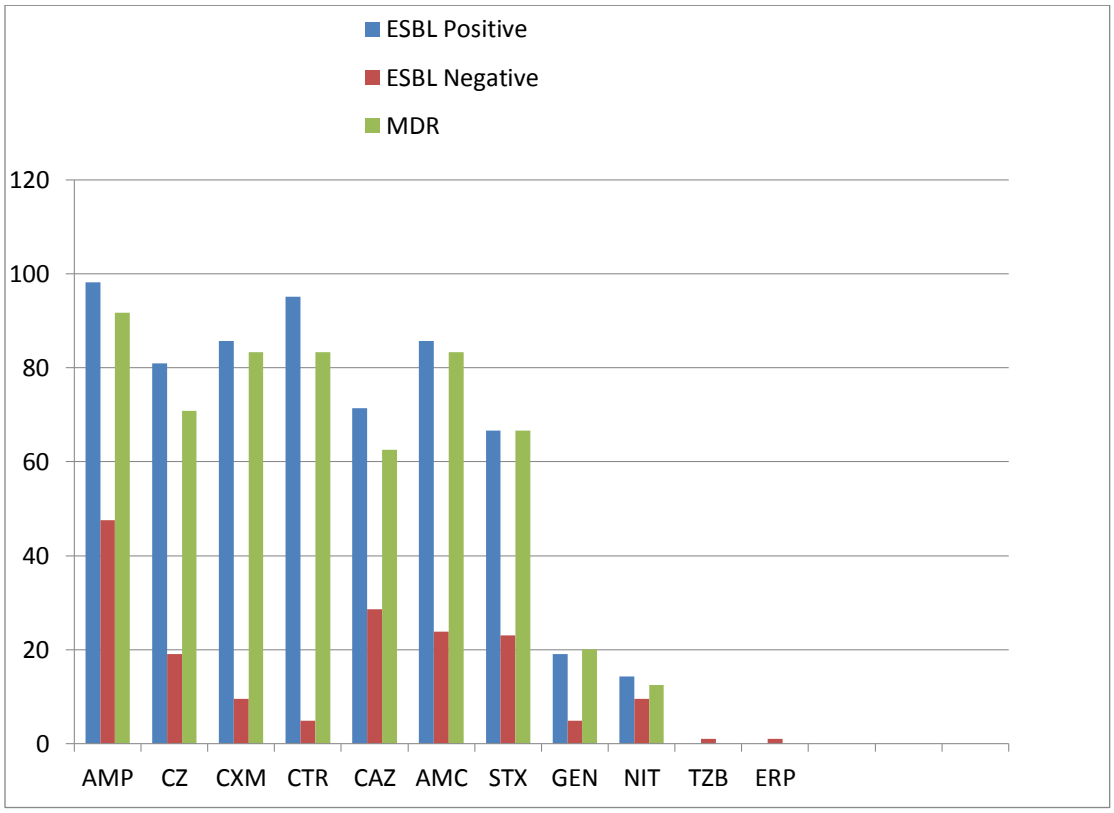
Ref* =Reference

COR=Crud odds ratio, AOR= Adjusted odds ratio, CI= Confidence Interval

6.7 ESBLs, Non-ESBLs and MDR Enterobacteriaceae producing against different classes of antibiotics

ESBLs producers Enterobacteriaceae showed higher producing antibiotics tested but non-ESBLs producers were more producing for cefuroxime, ceftriaxone, Nitrofurantoin and gentamicin. Multidrug resistance Enterobacteriaceae, for most of the antibiotics, showed higher producing ESBLs than non-ESBLs producers (Figure.4).

Figure 4. Antibiotics producing of ESBLs positive, ESBLs non-producing and MDR Enterobacteriaceae against different classes of antibiotics at South west shoa Lemman hospital and health facility, Ethiopia, from September 1/2020 to December 30.2020.



Note: AMP --- ampicillin, CZ-- Cefazolin, CXM -- cefuroxime, CTR -- ceftriaxone, CAZ-- ceftazidime, MC ---amoxicillin/clavulanic acid , SXT -- trimethoprim/sulfametoxazole, GEM --- gentamicinand NIT--- Nitrofurantoin

6.8 Effective antibiotics Drugs against ESBLs producing and MDR Enterobacteriaceae

Ertapenem and Meropenem are the best active drugs for ESBLs producing Enterobacteriaceae with sensitivity 100%, Nitrofurantoin and Gentamicin are more sensitivity of 85.7% and81% respectively. Trimethoprim/sulfametoxazole and Ceftazidime also low sensitivity with 33.3% and 28.6%.77.8% (7/9) of the antibiotics tested had less than 50% activity against MDR producer Enterobacteriaceae. The higher activity was seen against Nitrofurantoin with 87.5% sensitivity and Gentamicin79.2% sensitivity (Table 5).

Table 5. Antibiotic susceptibility patterns of ESBLs and MDR producing Enterobacteriaceae at south west shoa, leman hospital and health facility from September 1/2020 to December 30/2020.

Types of antibiotics	ESBLs producer Enterobacteriaceae		MDR producer Enterobacteriaceae	
	Total No.	Sensitivity (%)	Total No.	Sensitivity, n (%)
Ampicillin	21	1(4.8%)	24	2(8.3%)
Cefazolin	21	4(19%)	24	7(29.2%)
CEFUROXIME	21	3(14.3%)	24	4(16.7%)
CEFTRIAXONE/ CEFOTAXIME	21	1(4.8%)	24	4(16.7%)
CEFTAZIDIME	21	6(28.6%)	24	9(37.5%)
AMOXACILLIN/ CLAVULINIC ACID	21	3(14.3%)	24	4(16.76%)
TRIMETHOPRIM /SULFAMETOX AZOLE	21	7(33.3%)	24	8(33.3%)
GENENTAMICIN	21	17(81%)	24	19(79.2%)
NITROFURANT OIN	21	18(85.7%)	24	21(87.5%)

6.9CarbapenemsResistance Enterobacteriaceae

In the present study showed that out of forty seven isolated Enterobacteriaceae, there is no Carbapenems resistance Enterobacteriaceae. All Carbapenems strains are sensitive (susceptible) for all isolated. The lower prevalence in this area may be attributed to the fact that Carbapenems were not commonly sold in this area because of their high cost this site was rural area.

7. Discussion

Urinary tract infection(UTIs) are public sources of mortality and morbidity in worldwide which continues to be a serious problem that needs immediate attention and treatment [13].The prevalence of Enterobacteriaceae that cause UTI different across geographical boundaries [7].Hence this study was undertaken and results established the profile of microbial isolates causing UTI with their susceptibility pattern to most commonly used antimicrobial agents and also ESBL producing and Carbapenemase producing Enterobacteriaceae. In this study the overall prevalence 16.9% (n=47/278) of bacteria isolated from urine culture and UTI suspected patients was almost the same as with what had been previously reported in Ethiopia (18.2%, 22.7%) [14, 15] and Nigeria (21.7%) [45; 49]. However, this finding was relatively lower than studies done in Gondar (25.6%, 32.1%) [19, 52] and much lower than studies done previously in Addis Ababa Ethiopia (44.7%) [47].

In this study,from the total of 47Enterobacteriaceae Isolates, *E. coli* 78.7 % (37/47) was the highest followed by *K. pneumoniae* 8.5% (4/47). This finding was the same as studies carried out in Ethiopia : Bahir Dar, *E. coli*58.1% and *K. pneumoniae*23.3% [74], North west Ethiopia : *E. coli* 61.2% &*K. pneumoniae* 15.8% [22].

The highest antimicrobial resistance Enterobacteriaceae isolates was ampicillin 63.8%. The second predominant was amoxicillin with clavulanic acid (48.9%, cefotaxime (46.7%), cefuroxime (42.6%) and sulfametoxazole-trimethoprim (40.2).Therefore, the lowest level of producing to Cefazolin(36.1%) and gentamycin (10.6%). Similar result were reported in Ethiopia such as in Gondar: ampicillin (84.6%) and amoxicillin with clavulanic acid (79.5%) and gentamicin (35.9%) [22], Debre Markos: ampicillin (70.4%), amoxicillin with clavulanic acid (58.8%), cefuroxime(53.1%) [34].*K. pneumoniae* the second most producing Enterobacteriaceae isolates in this study were which was producing to most tested antimicrobials such as ampicillin (100%), amoxicillin with clavulanic acid (75%), cefuroxime,Cefazolin, ceftriaxone and ceftazidime (50%). This was the same as study in Gondar: Ampicillin (100%), sulfametoxazole-trimethoprim (72.2%) [52], in Bahirdar: sulfametoxazole-trimethoprim (61.0%) [74], in Harer: sulfametoxazole-trimethoprim (64.9%) [79], Tanzania: ampicillin (100%), amoxicillin with clavulanic acid (90.0%) [49], in Nepal: ampicillin (100%), ceftazidime (86.4%), sulfametoxazole-trimethoprim (51.3%) [62].

The overall prevalence of MDR among all Enterobacteriaceae isolate in this study was 51.1%. There were dissimilar results from studies conducted in Gondar (68.0%) [52], AddisAbaba (73.7)[22], Dessie (74.6%) [80], Debre Markos: (72.2%) [34] and Nepal (64.0%) [58]. However, this result was much lower than studies done in Gondar (87.4%) [22, 52], Bahir Dar (93.1%) [60, 74], Sierra Leone (85.7 %) [57], Nepal (96.8%) [40,62].The difference between of them in prevalence of MDR isolates might be due to empirical treatment, definition for MDR and patient condition. In addition, these results was

higher when compared to a previous study done in Jimma (59.3%) [23], Nepal (54.2%) [62], another study in Nepal by Lamichhane *et al* [79] reported (33.14).In addition, these Enterobacteriaceae are intrinsic and acquired producing to antimicrobial agents; this makes treatment difficult [58].

The overall frequency of ESBLs producing Enterobacteriaceae in the present study was 44.7%, which was very low level than study reported in Addis ababa78.57%[24],greater than study Harrer 33.3% [79], Ghana 49.3% [65], Nepal 34.5% [62], Spain 42.8% [76], India 44.0% [42,58].This is a higher result as compared to other study from India [42, 58] in which 32.14% and 58% of ESBL recorded and a study done in Ethiopia in which 33.3% of ESBL recorded [61].In this study among the tested E. coli 100%,k.pneumoniae 100%,k.oxytoca 10% and Enterobacteriaceae(100%and (n=15/15),(n=2/2),(n=3/3) and (n=1/1) with ESBL phenotypes, all showed ESBL production. These was the study done from Saudi Arabia that found from the tested 31 E. coli isolates with ESBL phenotypes, all were positive for ESBL production [54].

All of the ESBL positive isolates showed high level of Enterobacteriaceae producing (>80%) to ampicillin, Amoxicillin-clavulanic acid, Cefazolin, Ceftriaxone and Cefuroxime. Carbapenems resistance Enterobacteriaceae in this study were 0% there is no resistance all isolated at Enterobacteriaceae this study for Carbapenems groups. The lower prevalence in this area may be attributed to the fact that Carbapenems were not commonly sold in this area because of their high cost this site was rural area[55,19].

The maximum MDR isolates resistance to β-Lactam agents, 91.7, 83.3%, 83.3%and83.3%producingwasexhibitedagainstampicillin,cefuroxime,ceftriaxone/cefotaximeand amoxicillin with clavulanic acid respectively, the same findings of Esthete*et al*., in Gonder: (100% Vs 97.4%) [22, 52], Kaup *et al*, in South Karnataka (100% Vs 90.2%) [79] And in Sierra Leone 100%producing to ampicillin [44,69].In this study, MDR isolates showed low level of sensitivity to Nitrofurantoin (87.5%) and Gentamicin (79.2%) [53].

In this study, the highest susceptibility of ESBLs producing isolates was found against Nitrofurantoin and Gentamicin sensitivity of 85.7% and 81% individually, similarly findings of Esthete*et al*., in Gonder: (100% Vs 97.4%) [52], Kaup *et al* in South Karnataka (100% Vs 90.2%) [79] And in Sierra Leone 100%producing to ampicillin [44,57].The present study showed that ESBL producers had high levels of producing to ampicillin(95.2%), ceftriaxone/cefotaxime (95.2%), cefuroxime (85.7%) and amoxicillin/clavulanic acid (85.7%) respectively. Only Cefazolin and ceftazidime showed 81% and 71% activity from ceftriaxone and ceftazidime in our study. Similarly finding were reported from Harer; ceftriaxone 94.7%, Ghana; ampicillin 100%, cefuroxime 100%, ceftazidime 90%, India; ampicillin 96%, ceftriaxone 94.9%, Nepal; ampicillin 100% [62], ceftazidime 100%, ceftriaxone 100% [62].

8. Limitation and Strength of the Study

8.1 Strength of the study

This is a timely study, since antibiotic resistance becoming an alarming problem in both developed and developing world. Therefore, our study is perhaps the first one to identify drug resistance mechanisms via the production of carbapenems among Enterobacteriaceae urinary tract infections; which were not studied in this area. Susceptibility(sensitivity) testing for 12 antibiotics including β-lactams and. β-lactamase inhibitors like piperacillin/tazobactam was tested.

8.2 Limitation of the study

- Anaerobic cultures were not available to assess the prevalence of anaerobes
- Chocolate agar was not used

9. Conclusion

The overall prevalence of Enterobacteriaceae isolates from urine cultures was 16.9% (n=47/278).From the commonly used antibiotics Ampicillin (63.8%) and amoxicillin with clavulanic acid(46.9%) showed the highest level of resistance for all urine isolates. Additionally, Enterobacteriaceae strains resistant to most classes of antibiotics increase which is our current challenge. The prevalence of ESBLs producing Enterobacteriaceae isolates from urine culture was 44.7% in this study. The majority of patients 85(30.6%) were between 25-34 years of ages and the mean (std.deviation) ages of patients were 36.49 and13.846) with age range of above fifteen years. Among study participants, 47(16.9%) out patients, who had positive urine culture Enterobacteriaceae were identified and 8.5 %(n=4/47) of the culture positive were from male and 91.5 %(n=43/47) were from females.

10. Recommendations

Based on this finding the following recommendations are put forward to Governmental bodies(Hospital administrative and Health center Directors)

1.Should like direct responsibility to facilitate periodic surveillance to control and prevent the emergence of MDR strain.

2. Implementation of local microbiology laboratories for the detection of CPE should be promoted through introduction of new methodologies

Health professionals

1. Multidisciplinary approach is required in order to reduce patient hospital and health center stay and for appropriate prescription of antibiotics
2. Should pay special attention elderly and female patients, since these groups easily susceptible to acquire infections caused by MDR strain.

The Researchers

1. Specific scientific and medicinal research, especially on CRE is so significant, which includes using relatively standard method, large study population and geographical area should be considered.
2. Trends on the frequency and antibiotics producing Enterobacteriaceae should continually review through rigorous knowledgeable findings.
3. Detection of ESBLs producers need to be introduced as routine tests in microbiology laboratories for rapid detection of resistant isolates and to control their spread.
4. Routine testing of Enterobacteriaceae isolated from urine samples for possible Carbapenems activity may result in availability of data on such isolates for future control planning.

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ANNEX

Annex I: English version of participant information sheet and consent form

Participant information sheet

This is to invite you to participate in a study conducted by Dirriba Diyana, MSc student at Addis Ababa University, College of Health Science, and Department of Medical Laboratory Science. I am conducting a research on the prevalence of extended-spectrum B-Lactemase and Carbapenemase production in Enterobacteriaceae among patients complaining UTI. I am going to give you information to be part of this research. Please read or understand the following information and ask any doubt you have. However, you are not obligated to participate and you can refuse to answer any questions or withdraw from the interview at any time. You were able to receive the services if you do not want to participate. If you are interested to being part of this study, you have to sign on the consent form.

Procedure: - This questionnaire was taken about 15 minutes of your time. There are two parts. First, I was asked you about your demographic information and your pervious antibiotic consumption. Second, I was asked you for a urine sample and I need only small amount of samples.

Benefit: - This study was help you and other people who took different antibiotic for treatment of bacterial infection and I was use these results to decide whether there is resistance for the commonly used antibiotics for treatment of bacterial infections.

Risk:-There is no risk to you from answering the questions however; there might be a discomfort while we took samples from you for culture test.

Payment: - There is no cost to you for being part of the study.

Confidentiality: - All information collected during this study were kept private and was only be known by the investigators and your physician who order test and also only used for study purpose. I was not used any information that might identify you when I present or publish the study's results.

Annex II

Consent form

Participant Agreement I have read the information above, or he has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study to give my sample urine and be a participant in this study and understand that I have the right to withdraw from the study at any time.

1. Participant name_____ Gender M ☐ F ☐ Age ☐

2. Address: _____ Religion: _____

3. Marital status: Married ☐ Unmarried ☐ Divorced ☐ Separated ☐

4. Educational level: None ☐ Primary School ☐ Secondary School ☐ ary School ☐

5. Do you take antibiotics before two weeks: yes ☐ No ☐

Name of investigator _____Signature _____Date_____

በጥናትለመሳተፍየመረጃሺትናየስምምነትቅጽ

መለያቁጥር--------

በአዲስአበባዩኒቨርሲቲ፤የጤናሳይንስኮሌጅየህክምናላቦራቶሪትመህርትክፍልበሁለተኛድግሪተማሪየመመረቅያጥናትላይእንድሳተፉተገብዘ ዋል፡፡እባክዎቅጹንከመፈረዎበፊትከዚህቀጥሎየሚገኘዉንመረጃበጥሞናያንብቡ/ይረዱ፤ግልጽያልሆነነገርካገጠመዎትይተይቁ፡፡

መግቢያ

የጥናቱርዕስ፡የመድሃኒትብግርነትንስርጭትበባክቴርያየተጠቁትታካሚዎችላማከምየሚዉሉመድሃኒቶችበሕ/ጤ/ኢየሚሰራጥናት፡፡

የጥናቱአካሄድ፡- ይህቃለመጠይቅ 15 ደቂቃይወስዳል፡፡ቃለመጠየቁም

ሁለትክፍሎችአሉት፡፡የመጀመሪያውአጠቃላይእርሰወንየእድሜናከዚህበፊትየወሰዱትንመድሃኒትየሚገጠይቀዎትሲሆንሁለተኛውክፍል ሓኪመዎያዝዘለዎትንየ-------- ምርመራለማድረግየሚያስችለውንየናሙናዓይነትመወሰድነው፡፡

የጥናቱጥቅም፡ይህጥናትበባክቴርያየተጠቁትታማሚዎችየመድሃኒትብግረነቱንበመለየትለእርሰምሆነለሌሎችታካሚዎችመታዘዝያለበትን መድሃኒትበማወቅለታካሚዎችተገቢውንየመድሃኒትያይነትእንድያዙበማድረግካላስፈላጊወጪያድናል፡፡በተጨማሪምበአጭርጊዜእንድኑ በማድረግወደመደበኛስራቸውፈጥነውእንደመለሱበማድረግአሥተዋጾደርጋል፡፡በዚህጥናትበመሳተፈዎምንምዓይነትክፍያአይከፈለዎትም፡ **፡የሚያስከትለዉጉዳት**፡-
ይህንንጥያቄበመመለስዎየሚነዱትነገርአይኖርምይሁንናበተለይምየደምናሙናስንወስድልዎትንሽያለመመቾትሊሰማዎይችላል፡፡

ይህንንጥያቄበመመለስዎየሚነዱትነገርአይኖርምይሁንናበተለይምየደምናሙናስንወስድልዎትንሽያለመመቾትሊሰማዎይችላል፡

ሚስጥራዊነት፡-
የጥናቱውጤትምሆነመረጃውለህትመትሲዘጋጅበስመዎሳይሆንበመለያቁጥርነውየሚቀመጠውናሚታተመው፡፡ከዚህምበተጨማሪየዕርሰ ዎንመረጃማየትየሚችሉትከትትልየሚያደርግልዎትሃኪምናየጥናቱዋናአስተባበሪብቻናቸው፡፡

የተሳታፊስምምነት፡-
የጥናቱምንነትተገልጾልኛል፣ያልገባኝንምጠይቄእንደረዳምእድሉተሰጥቶኛል፡፡ሁሉምጥቄዎቼተመልሰውልኛል፡፡በዚህጥናትተሳታፊየሆን ሁትምበምርጫየነው፡፡በቃለመጠየቁመካከልምስምምነቴንበመተውየጥነቱአካልላለሆንእችላለሁ፡፡የተሳታፊስም----------

የስምምነትፊርማ---------- ሥ.ቁ.---------- ቀን----------የጥናቱዋናአስተባበሪስም----------ፊርማ----------ቀን----------

Annex III. Laboratory procedures for Enterobacteriaceae identification, drug susceptibility testing, ESBL and Carbapenems detection and isolate handling.

1. Urine collection and processing

Specimen collection: First morning specimens yield highest bacterial counts from overnight. Incubation in the bladder, and are the best specimens. Midstream urine (MSU) for microbiological examination is collected as follows:

Procedure for midstream urine for Enterobacteriaceae investigation

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

2. Give the patient suitable container

3. Instruct the patient before the collection, preferably with illustration.
4. Tell the patient not to touch the inside or rim of the container

- **Male**
  - If not circumcised, draw back the foreskin
  - Begin to urinate, but pass the first portion into the toilet.
  - Collect the mid-portion of urine into the container, and pass the excess into the toilet.
- **Female**
  - Squat over the toilet and separate the labia with one hand.
  - Avoid the first portion of urine into the toilet.
  - Collect the mid-portion of urine into the container and pass the excess into the toilet.

Processing: approximately 20ml of urine sample is required; the maximum time allowed for processing a urine sample is 2 hours from the time of collection. If delay is inevitable, should be refrigerated / preserved (boric acid). During culturing, the urine must be re-suspended and streak 1µl of the volume to blood agar. After overnight incubation plate count of 100,000 CFU/ml of pure culture should be considered positive and isolated organism should be identified and sensitivity test was performed.

3.Urine Culture and identification

The isolates were identified by standard microbiological laboratory methods such as colony appearance, the pure colony also used for bacterial identification as well as antibiotic susceptibility test. Isolates suspected of beta-lactamase production was sub cultured on sheep blood agar and Macconkey agar plate for confirmation using combination disk methods.

i. *Enterobacteriaceae* growth from the urine on Macconkey agar

Positive □ Negative□

ii. Identification steps for suspected colonies

a) Oxidase test positive_____ negative _____

b) Colony morphology

d) Lactose fermentation from Macconkey agar

- Lactose Fermenter
- Non lactose fermenter

iii. Biochemical reactions

Identification of *Enterobacteriaceae* isolates involves the use of biochemical screening Medias. Indole, Urease, Mannitol, Triple sugar iron (TSI), Citrate, Motility, Lysine Decarboxylase, and Mannolet and Oxidase tests.

Biochemical tests for Enterobacteriaceae								
ISOLATES	TSI	Gas.Prod.	H2S	Lysine Dec.	Citrate	motility	Urea	Indole
E.coli	A/A	+	_	+	_	+	_	+
K.pneumoniae	A/A	+	-	+	+	-	+	-
k.oxytoca	A/A	+	-	+	+	-	+	+
k.ozonea	A/A	+	-	+	+	+	-	-
Enterobacter spps	A/A	+	-	+	-	+	-	-

Key: LDC = Lysine decarboxylase, Man = Mannitol (mannite), Triple sµgar 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), d = different strains give different results.

A. **Indole test**: Few colonies of the culture was inoculated into peptone water and incubated at 37C for 24 hours. Few drops of indicator (Kovac‘s reagent) was added and gently shake to mix well. Colour change was 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 were inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap was loosened and then incubated at 37oC 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 hr. 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.

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

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

F. Lysine decarboxylase: Decarboxylation of lysine can be detected by culturing bacteria in a medium containing the desired amino acid, glucose, and a pH indicator bromcresol purple. The acids produced by the bacteria from the fermentation of glucose was 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 decarboxylases lysine turn the medium purple. In addition bacteria that produce H2S appear as black colonies.

G. **Oxidase test:** A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent was oxidized to a deep purple colour.

3. Antibiotics susceptibility result for *Enterobacteriaceae* isolates

Isolated bacteria	Antibiotics		AMP	CZ	CXM	CTR	CAZ	AMC	SXT	Gen.	F	TZP	ERT/MEM
	Drug Susceptibility pattern	S											
		R											

NOTE:AMP-- Ampicillin,CZ—cefazolin,CXM--- cefuroxime , CTX----cefotaxime, , CTR---ceftriaxone, AZ--- ceftazidime,AmC--Amoxycillin-Clavulanic acid,SXT----Sulphamethoxazol-trimethoperem, GM--Genetamyicin,NI---Nitrofurantoin,ERT---Erapenem,MEM---Meropenem,TZP--- peprizem/Tazobactam

Procedure for Performing the Disk Diffusion Test

Inoculum Preparation

- At least three to five well-isolated pure colonies of the same morphological type will be selected from Blood or Macconkey agar plate. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of tryptone soy broth.
- The turbidity of the broth culture will be adjusted with that of the 0.5 McFarland standards.

Inoculation of Test Plates

- Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level.
- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.
- The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Disks to Inoculated Agar Plates

- The predetermined battery of antimicrobial disks is dispensed onto the surface of the inoculated agar plate. Each disk must be pressed down to ensure complete contact with the agar surface.

Reading Plates and Interpreting Results

- After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the

disk. Zones are measured to the nearest whole millimeter, using sliding calipers which is held onthe back of the inverted plate.

4 .Extended spectrum beta-lactamase detection

4.1 Combination disk test (CDT)

For each test disks containing cephalosporin alone (ceftriaxone and ceftazidime) and in combination with clavulanic acid are applied. The inhibition zone around the cephalosporin disk/tablet combined with clavulanic acid is 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

Note: procedures used for antimicrobial testing is applied here for combination disk.

Annex IV: Declaration

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

M.Sc. candidate: Dirriba Diyana (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Kassu Desta (MSc, PhD fellow, AssociateProfessor)

Signature: _____

Date: _____

Place Addis Ababa, Ethiopia

Advisor: Melese Hailu (MSc, PhD fellow, Assistant Professor)

Signature: _____

Date: _____

Place Addis Ababa, Ethiopia

Advisor Degefu Beyena (BSC, MSc)

Signature:_____

Date: _____

Place Addis Ababa, Ethiopia

Advisor Dajene Shiferew (BSC, MSc)

Signature:_____

Date: _____

Place Addis Ababa, Ethiopia