Addis Ababa University

College of Health Sciences

School of Allied Health Sciences

Department of Medical Laboratory Sciences

Extended Spectrum Beta-lactamase producing *E. coli* and *K. pneumoniae* carriage among under five years children in Addis Raey public health center, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Mekdes Alemu Tola, entitled: **Extended Spectrum Beta-lactamase producing *E. coli* and *K. pneumoniae* carriage among under five years children in Addis Raey Public Health Center, Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Abbreviations

AST: Antimicrobial Sensitivity Test

CLSI: Clinical and Laboratory Standard Institute

ESBL: Extended Spectrum Beta-lactamases

ESBL-E: Extended Spectrum Beta-lactamases *Enterobacteriaceae*

ESBL-PE: Extended Spectrum Beta-lactamases Producing *Enterobacteriaceae*

MDR: Multidrug resistance

OPD: Outpatient Department

TASH: Tikur Anbessa Specialized Hospital

Operational Definition

Extended Spectrum β-Lactamases (ESBLs): was defined as enzymes produced by bacteria that are able to hydrolyze extended spectrum cephalosporin and which are inhibited by β-lactamase inhibitors such as clavulanic acid.

ESBL Carriage: was defined as detection of ESBL producer isolates in feces without the identification of correlating signs or symptoms of infection.

Previous antibiotic use: was defined as use of any type of antibiotics, either parentrally or orally, with in the past twelve months.

Hospital visit: was defined as having previous history of hospital visit but not admitted within the past twelve months.

Hospital admission: was defined as having previous history of hospital admission within the past twelve months.

Previous surgery: was defined as having previous history of surgery within the past twelve months.

Non-susceptible: A bacterial isolate was considered non-susceptible to an antimicrobial agent when it tested resistant or intermediate when using clinical breakpoints as interpretive criteria provided by the Clinical and Laboratory Standards Institute (CLSI).

Multidrug resistance: was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.

Abstract

Background: Extended-spectrum β-lactamase (ESBL) producing bacteria present an ever-growing burden in the hospital and community settings. Infections due to ESBL-containing pathogens continue to be associated with significant morbidity and mortality worldwide. Carriage of ESBL producing bacteria in the gut may serve as a reservoir of resistance genes that may subsequently be acquired by strains that cause clinically significant infection.

Objective: The objective of this study was to determine the prevalence of ESBL producing *E. coli* and *K. pneumoniae* carriage and associated factors among under five years children at Addis Raey public health center, Addis Ababa, Ethiopia.

Methods: A facility based cross sectional study was conducted from April to May 2017. Socio demographic and risk factors data were collected using questionnaires. Stool/rectal swab specimens collected from voluntary participants were inoculated on MacConkey agar. Bacteria identification, antimicrobial susceptibility testing and ESBL test were performed by VITEK 2 Compact (BioMe´rieux, France). Data was entered by EPI INFO version 7.2.1.0 and analyzed by SPSS version 20. The results were summarized and presented by tables.

Results: A total of 269 under five years children were recruited in the study. The mean and standard deviation of age were 22.48 and 15.83 months respectively. The overall prevalence of ESBL-producing *Escherichia coli* and *Klebsella pneumoniae* were 17.1% (46/269; 95% CI: 12.9%–22.7%). Of which, 83.0% were *E. coli* and 17.0% were *K. pneumoniae.* ESBL producing *E. coli* and *K. pneumoniae* isolates were showed high levels of MDR (93.6 %) and high rates of co-resistance to aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole. However, all ESBL producing isolates were susceptible to carbapenems (ertapenem and imipenem). Children of mother with lower educational level (primary school) (OR: 2.472, 95% CI: 1.323-4.618, P=0.0062) and children used tap water for drinking (OR: 1.714, 95% CI: 1.001-3.659, P=0.048) were factors significantly associated with higher ESBL carriage.

Conclusion: High prevalence of ESBL carriage in this study population was mainly Community-acquired and suggests a need for antimicrobial susceptibility surveillance and infection control measure.

Keyword: Extended-spectrum β-lactamase, *E. coli, K. pneumoniae,* carriage, children.

1 Introduction

1.1 Background

Antimicrobial resistance among bacterial strains is an emerging problem worldwide (1) with serious consequences on the treatment of infectious diseases (2). Beta-lactam drugs like penicillins, cephalosporins, carbapenems and aztreonam are common antibiotics used to combat most bacterial infections (3). Indiscriminate use of third generation cephalosporins to treat gram negative bacterial infections is partly responsible for the emergence of resistance to beta-lactam antibiotics (4), which subsequently led to the emergence of Extended Spectrum Beta-Lactamases (ESBL) producing organisms (3).

ESBL are enzymes produced by gram-negative bacteria that mediate resistance to penicillins, cephalosporins and monobactams (5). There is no consensus of the precise definition of ESBL. A commonly used working definition is that the ESBL are β-lactamases capable of conferring bacterial resistance to the penicillins, first, second, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid (6,7).

ESBLs recognized in 1980s in *Klebsiella* species and later in Escherichia *coli* and other gram-negative bacilli are currently spreading rapidly amongst other members of *Enterobacteriaceae*, largely due to genes located on plasmids that can distribute across species barriers (8). E. *coli* and *K. pneumoniae* are common species of *Enterobacteriaceae* that both have pathogenic potential and that frequently incorporate ESBL-encoding genes. The Infectious Diseases Society of America has listed them as two out of six pathogens for which new drugs are urgently needed in order to combat resistance development (9).

ESBL were initially associated with nosocomial outbreaks caused by single enzyme-producing strains, but recent studies have revealed the existence of more complex situations, with significant increases in the frequency of community isolates (10). ESBL determinants have been detected not only in clinical isolates but also in commensal bacteria from humans and animals, and isolates from products of the food chain and sewage;- revealing distribution and suggesting the presence of environmental reservoirs for these resistance determinants (11).

Several studies have been conducted to assess the risk factors associated with colonization of ESBL producing organisms including: prior exposure to antibiotics, hospitalization, surgical operation, foreign travel, international adoption and intra familial transmission of ESBL-producing bacteria between household members are more commonly being described (12–19).

In general knowledge about ESBL detection in commensal bacteria is limited and data on ESBL carriage are scarce in Ethiopia. The aim of this study was to determine the prevalence of ESBL producing *E. coli* and *K. pneumoniae* carriage and associated factors among under five years children at Addis Raey public health center, Addis Ababa, Ethiopia.

1.2 Statement of the problem

Infections caused by ESBL-producing gram negative bacteria are associated with increased morbidity and mortality (20). Majority of ESBL associated infections are resistant to various antibiotics, leaving only limited compounds as a therapy. Presently, carbapenems become the first line antimicrobials for the treatment of such infections (21). Which is cumbersome because these antibiotics are for parenteral use only and thus are difficult to administer and often not widely available in low-resource countries like Ethiopia, where the incidence of Extended Spectrum Beta-lactamases *Enterobacteriaceae* (ESBL-E) infections is particularly high (22).

Unfortunately, ESBL-producing bacteria in children have come to the forefront of emerging antibiotic resistant bacteria. Options for treatment of multidrug resistant (MDR) gram negative bacterial infections are generally limited, and given that fewer antibiotics are approved for use in children, as well as the perpetual dearth of pediatric drug trials, the problem is critically important to address (23). ESBL-producing organisms are associated with infections that result in poor clinical outcomes, delayed initiation of appropriate antibacterial therapy, increase in durations of illness, longer hospital stays, greater hospital expenses and more economic burden to families (24–26).

A threatening epidemiological problem is the dissemination of ESBL-producing organisms to healthy people in the community, which might depend on the frequency of ESBL fecal carriage as well as on the presence of ESBL-producing organisms in the food chain (27). The digestive tract is the main reservoir from which *Enterobacteria*ceae originate, whatever the type

(community or hospital acquired) of infection. It is also a melting pot where exchanges of resistance genes occur and antibiotic treatments select for the overgrowth of resistant bacteria (22). Colonization in the intestinal compartment by ESBL-producing isolates has been associated with a high risk for developing infection due to ESBL producers (12). It may also serve as a reservoir for ESBL resistance genes that can undergo horizontal transmission to other *Enterobacteriaceae* (16).

Despite their widespread distribution, the prevalence of ESBL-producing organism remains underestimated because a large number of laboratories do not perform routine tests that specifically detect ESBLs (4). Nowadays, infections due to ESBL-producing gram negative bacteria are an emerging problem in children. So, it is crucial to detect ESBL-producing bacteria in feces to understand the colonization rate with those bacteria, which is important to predict the risk of infection. In Ethiopia, data on the prevalence of ESBL carriage remain scarce and to the best of our knowledge, there was no study focusing children particularly in community settings. Therefore, this study was aimed to determine the prevalence and risk factors associated with ESBL producing *E. coli* and *K. pneumoniae* carriage among children under five years.

1.3 Significance of the Study

Screening for intestinal ESBL producing gram negative bacteria carriage is crucial to predict the risk of ESBL infection, as the colon serves as a reservoir for extra-intestinal pathogenic infection. As no data was available on the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* carriage among under five years children in Ethiopia particularly in community settings, this study will provide base line information on the magnitude of ESBL carriage and associated risk factors among children under five years.

This study, also heighten awareness for health professionals regarding ESBL-producing gram negative bacteria and help in the appropriate choice of empirical antimicrobial agents for infection caused by those bacteria. Evidence based data is also useful to implement an antimicrobial drug policy and to adopt best-practice infection control measures to prevent the spread of those organisms in health care facilities as well as in the community.

2 Literature Review

Infections with Extended-Spectrum β-Lactamase producing *Enterobacteriaceae* (ESBL-PE) were first reported during the late 1980s (28). ESBL-producing organisms were first detected in Europe. Although the initial reports were from Germany and England, the vast majority of reports in the first decade after the discovery of ESBLs were from France (6).

Fecal carriage of ESBL-E in the community was first reported in Spain and Poland, in 2001 and 2002 (22). Since then, rates of colonization by ESBL producing organisms have increased dramatically worldwide (27). Strikingly, over 1.1 billion ESBL-E carriers appear to be present in the community populations of Southeast Asia. The Western Pacific and Eastern Mediterranean regions rank second and third, with 280 and 180 million carriers, respectively, ahead of Africa, where 110 million carriers are estimated to be present. America and Europe appear to be far behind, with 48 and 35 million carriers, respectively. This ranking suggests that poor access to drinking water, poverty, and a high population density are extremely efficient driving forces for ESBL-E dissemination, as is the case for any fecal-orally transmitted diseases (22).

Asymptomatic fecal carriage of ESBL-producing bacteria in the community has been reported from several countries and continents with wide differences in carriage rates between geographic areas and study population characteristics. Very high fecal prevalence rates have recently been reported from Thailand (66%), Egypt (63%) and China (50%) (29). In Turkey, the prevalence of fecal carriage was 30% [173 of 576], recent use of antibiotics, hospitalization and surgical operation, diabetes, crowded household populations and old age were associated with higher carriage rates (14). ESBL-E carriage rate was 28.2% in intensive care unit (ICU) of Korea (28). ESBL-PE strains were detected in 12% [6 out of 50] of fecal samples collected from the inpatients of a Japanese pediatric hospital. The proportion of carriage of ESBL producers was higher among patients who had received antibiotics within the past 3 months and among those who had cardiologic diseases (30).

Several studies have identified international travel as a risk factor for colonization with ESBL. Study among international travelers in New York City, seven of 28 travelers (25.0%) acquired a new ESBL-PE during travel (16). ESBL-PE was present in 50.7% (107/211) of the returned to Berlin, Germany, after international travel. The proportion was highest for returnees from India (72%) and mainland Southeast Asia (59%), and comparatively lower for Africa (33%) and Central America (20%) (17). ESBL-E carriage rate was 30.5% among healthy participating travelers from the Netherlands (15). Study in the south-east of Sweden, ESBL-PE was detected in the post-travel samples from 68 (30%) travelers. The most important risk factor for acquiring ESBL-PE during travel was the geographical area visited, with the Indian subcontinent showing the highest risk [OR 24.8, P<0.001], followed by Asia (excluding the Indian subcontinent) [OR 8.63, P<0.001] and Africa north of the equator [OR 4.94, P<0.002]. Age and gastrointestinal symptoms also affected the risk significantly (18). In Swiss travelers, the overall acquired colonization rate with ESBL-producing *E. coli* was 69.4% [95% CI 62.1-75.9%], being highest in travelers returning from India [86.8%; 95% CI 78.5-95.0%] and lowest in travelers returning from Sri Lanka [34.7%; 95% CI 22.9-48.7%] (31).

Household contacts of ESBL-PE-infected patients are subject to increased risk of colonization by ESBL-PE. In Spain study in household contacts of infected community patients with ESBL, 28 out of 40 index cases (IC) patients [70%; 95% CI, 53.4 to 83.4] and 9 out of 54 household contacts [16.7%; 95% CI, 6.7 to 26.1] presented fecal carriage of ESBL producing *E. coli* strains. Moreover, 9 IC patients out of 29 had at least one colonized household contact [31.0%; 95% CI, 14.2 to 47.9]. This figure increased [42.1%; 95% CI, 19.9 to 64.3] in the subset of household contacts of IC patients with fecal carriage [8 of 19]. In contrast, only one household contact [1 of 10] [10%; 95% CI, 0 to 28.6] within the subset of those from households with IC patients with negative fecal carriage results presented an intestinal colonization of an ESBL-producing *E. coli* strain. Pulsed-field gel electrophoresis (PFGE) analysis revealed indistinguishable patterns among ESBL-producing *E. coli* isolates from IC patients (clinical sample or fecal sample) and their corresponding household contacts for 66% [6 of 9] of the isolates (27).

Study in Northern Ireland, 40% of 294 residents in the nursing homes had MDR *E. coli* in their feces. In a multivariate logistic regression model, days of fluoroquinolone use [OR: 51.33, 95% CI 1.04–1.69, P=0.02] and a history of urinary tract infection [OR 52.56, 95% CI 1.37– 4.78, P=0.003] were the only variables independently associated with the risk of carrying MDR *E. coli* (32). Similar study in north Lebanon among nursing home residents, 76.5% of the recruited residents were at least one-time carriers, recent antibiotic intake was found to be the only independent risk factor associated with the fecal carriage of MDR *Enterobacteriaceae* (13).

ESBL carriage among children shows large variation in several studies conducted in different countries. The overall colonization prevalence was 24.0% (30/125 children) in stool samples from 8- to 16-month-old healthy children in Northern Spain (33). In 2011 study on French children aged from 6 to 24 months, 4.6% ESBL-PE was found in a rectal sample. Recent third-generation oral-cephalosporin exposure was associated with a higher risk of ESBL carriage (OR=3.52, 95% CI [1.06-11.66], p=0.04) (12). Another Study in 2013, in three different pediatric clinics in Lebanon showed that 24.8 % healthy children aged from 1 to 5 years carried ESBL-PE. regular consumption of meat and chicken were significantly associated with high carriage rate of ESBL-PE (34).

Study at an orphanage in Mali, where 63% of the adults and 100% of the children were found to carry ESBL- PE that showed extensive co-resistance to other antibiotics (35). Study in pediatric emergency department in Guinea-Bissau in 2010, 32.6% children <5 years of age attending a pediatric emergency ward were carriers at least one ESBL-producing *E. coli* or *K. pneumoniae* strain. There was no association between age and colonization with ESBL-producing *E. coli* or *K. pneumoniae* (p-value 0.71) and even in the youngest age group of 0–3 months 27% of the children were colonized). Sharing bed with one or more children under the age of five was associated with increased risk of carriage (p = 0.04) of ESBL-producing *E. coli* or *K. pneumoniae*. There was no association with previous usage of antibiotics or recent hospitalization (9).

The prevalence of ESBL carriage was 59% (79/134) in rectal swabs from healthy young children aged 0-59 months in the Central African Republic. (36). ESBL-E fecal colonization prevalence was 23.2% [95% CI 19.1%–27.6%] in children attending pre-school childcare facilities in the Lao People's Democratic Republic. Use of antibiotics in the 3 months prior to sampling was identified as the only risk factor that remained significantly associated with ESBL-E colonization in the multivariate analysis (37).

Among 107 street children dwelling in Mwanza city, Tanzania, 34 [31.8%, 95% CI; 22.7±40.3] were colonized with at least one ESBL-producing *E. coli* or *K. pneumoniae* isolate (5). Study by Tellevik MG et al. showed that the overall prevalence of ESBL carriage was 34.3% (207/ 603) in children below 2 years of age in Dar es Salaam, Tanzania, including healthy community children and children hospitalized due to diarrhea or other diseases (38).

ESBL-E prevalence was 13.7% [95% CI 8.9–19.7] among HIV-infected children attending an HIV outpatient department in Harare, Zimbabwe. Participants admitted to hospital for a chest infection in the previous 12 months had 6.8 times the odds of carrying ESBL-E [95%CI : 0.9–50.6; P=0.033]. There was no association between ESBL-E carriage and sex, age, CD4 count or viral load (39). Fecal carriage of ESBL-PE was 4.7% (14/300) in children aged 4-6 years from the community in KwaZulu-Natal, South Africa (7).

Study in Madagascar showed that 10.1% (49/484) of patients attend to three health centers were carrier of ESBL-PE. The prevalence of rectal carriage of ESBL-PE in the community was not related to age or sex, but was significantly dependent on socio-economic status: poverty was the main risk factor (10). Another Study in a pediatric unit in Madagascar reported a prevalence of fecal ESBL carriage in 21.2% [54/ 244] of infants on admission and 57.1 % (88/154) on discharge, after more than 48 hours of hospitalization ($p < 0.001$). Significant risk factors for ESBL-PE carriage on admission were prior hospitalization in the last 30 days [adjusted OR = 7.4, 95%CI: 2.9-18.3] was the only independent risk factor for ESBL-PE carriage. Significant risk factors for ESBL-PE hospital acquisition was antibiotic therapy [adjusted OR = 4.1, 95%CI: 1.8-9.4] (40).

In Ethiopia, a study conducted in Tikur Anbessa Specialized Hospital (TASH) reported that 52% [95%CI; 46%–58%] of hospitalized patients were carriers of ESBL-E. In this study Prevalence of colonization with ESBL-E was significantly (p = 0.02) higher in neonates (74%) followed by children (59%) than in adults (46%). Logistic regression analysis indicated that having lower age as a significant risk factor for colonization by ESBL-E (p = 0.02). Five children (1.9%, 5/267) were colonized with ESBL positive and carbapenem resistant Enterobacteriaceae (ESBL-CARBA) (41). From the above review we have noticed that study on ESBL carriage is both at community setting and hospital setup. Except the TASH which is hospital based study, no study addresses the magnitude of ESBL-E carriage in community setting in Ethiopia. Hence, it is high time to fill such gaps.

Conceptual frame work

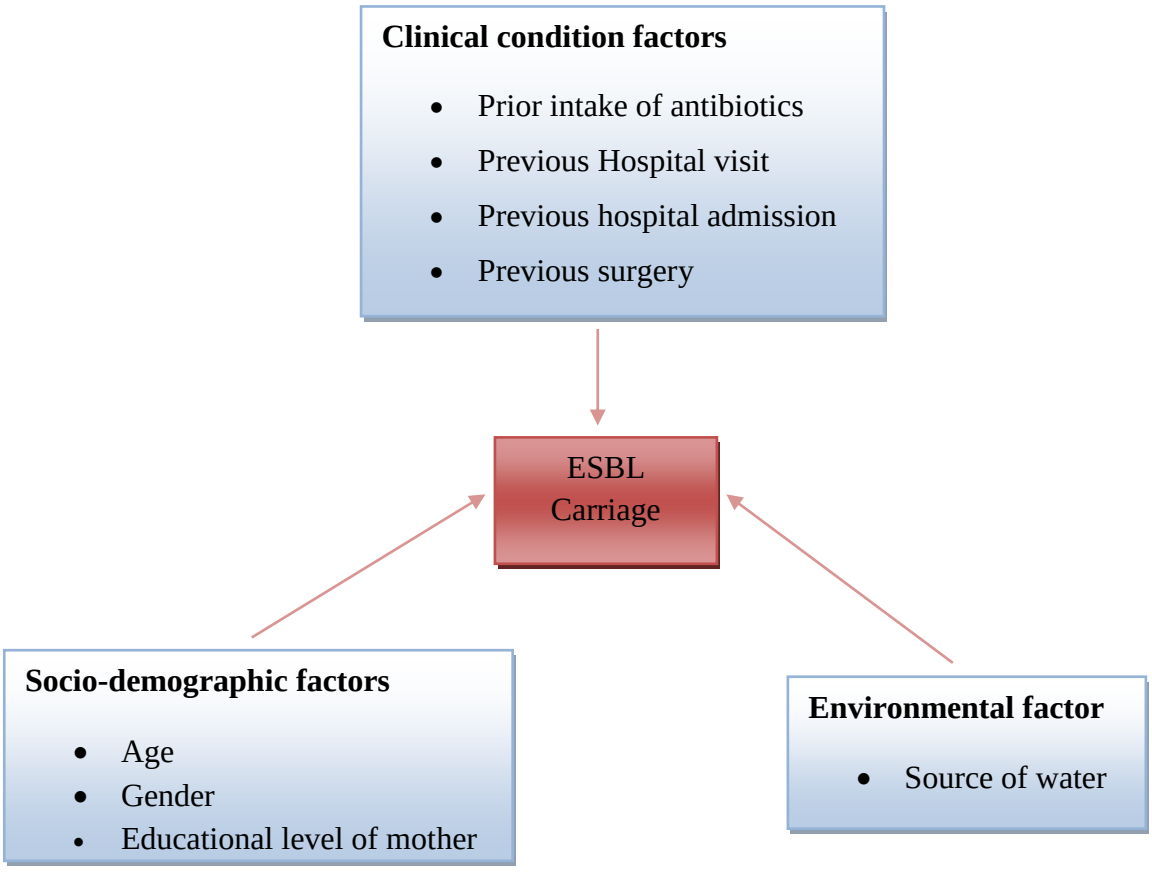

Figure 1. Conceptual framework for ESBL carriage.

3 Objectives

3.1 General Objective

The objective of this study was to determine the prevalence of ESBL producing *E. coli* and *K. pneumoniae* carriage and to assess associated factors among under five years children at Addis Raey public health center, Addis Ababa, Ethiopia.

3.2 Specific Objectives

1. To determine the prevalence of ESBL producing *E. coli* and *K. pneumoniae* carriage.
2. To determine the antimicrobial susceptibility pattern of ESBL and non-ESBL isolates.
3. To assess risk factors associated with ESBL producing gram negative bacteria carriage.

4 Hypothesis

“The prevalence of ESBL producing gram negative bacteria carriage is similar with previous study conducted at TASH (41).”

5 Materials and Methods

5.1 Study Setting

This study was conducted in Addis Raey public health center, Addis ketema sub city, Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia located almost in the center and in altitude of about 2,400 meters above sea level; with 3,100,425 a population size according to the 2015 population census (42). The city is divided into ten sub-cities. Addis Ketema is one of the 10 sub cities in Addis Ababa city administration with total population of 271,503. Under this sub city there are 10 health centers. Addis Raey public health center is found under woreda 07 administration and provides service for 3,540 (annually), 295 (monthly) and 15(daily on average) under five children outpatient department (OPD). The laboratory analysis was conducted at Ethiopian Public Health Institute (EPHI) in Clinical Bacteriology and Mycology National Reference Laboratory, which is a referral laboratory in the country and accredited by Ethiopian National Accreditation office (ENAO).

5.2 Study Design and period

A facility based cross-sectional study design was used and the study was conducted from April to May, 2017.

5.3 Study population and subject

5.3.1 Source Population

All under five years children attending OPD service in Addis Raey public health center.

5.3.2 Study Subject

Under five years children came for OPD service at Addis Raey public health center that fulfill the inclusion criteria during the study period.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

All under five children attending under five OPD service.

5.4.2 Exclusion criteria

Under five years children received antibiotic treatment for the last one week (7 days) prior to data collection time and unable to consent to participate in this study.

5.5 Study Variables

5.5.1 Dependant variable

- Prevalence of ESBL carriage
- Antimicrobial resistance patterns

5.5.2 Independent variable

- Socio demographic Factors: age, sex, source of water for child and mother Educational level
- Prior intake of antibiotics
- Hospital visit
- Previous hospital admission
- Previous surgery

5.6 Measurement and Data collection

5.6.1 Sample Size Determination

The sample size was calculated using single population proportion to determine the prevalence of ESBL producing gram negative bacteria carriage based on the following assumptions:

- Prevalence of ESBL carriage 23.2 % (37) was taken to estimate the sample size in study area.
- Significant level was calculated at 95% confidence interval

- Margin of sampling error tolerable was assumed to be [5%]

$$n = \frac{[Z_{\alpha/2}]^2 \, p \, [1-p]}{d^2}$$

$$n = \frac{[1.96]^2 \, 0.232 \, [1-0.232]}{[0.05]^2}$$

n=276

Where:

n = Minimum sample size

$Z_{\alpha/2}$ = Z value at 95% CI [1.96]

p = Estimated prevalence rate

d = Margin of error tolerated was 5%

5.6.2 Sampling method

Convenient sampling technique was used to collect the stool specimens from the study participants.

5.6.3 Data Collection Procedure

Data was collected using structured pretested questionnaires to obtain information on socio demographic and associated risk factors. Information concerning previous antibiotics usage (for the last 12 months) was obtained through medical records review. Verbal informed consent was taken from all each parents or guardians on the behalf of the children. All the interviews were carried out in a quiet and convenient place after the child gets the service. Recruitment of the study participants were facilitated by the under five OPD service providers. The interview was conducted in Amharic. (See more on annex 1-8).

5.7 Laboratory Methods

5.7.1 Specimen Collection and transportation

Stool specimens/rectal swabs were collected from children consented to participate in this study. All collected Stool/rectal swab specimens were transported by Cary-Blair transport medium to EPHI, Clinical Bacteriology and Mycology National Reference Laboratory for laboratory analysis (see more on annex 9).

5.7.2 Culture

Fresh colonies were obtained by inoculating and incubating the stool/rectal swab specimens on MacConkey agar plate for 18-24 hours at 37 ^{o} C. The culture was examined for growth; bacteria isolation was based on their colon characteristics and lactose fermentation. The pure colonies were used for subsequent identification, antimicrobial susceptibility testing (AST) and ESBL test. Bacteria identification, AST and ESBL test were performed by VITEK 2 Compact (bioMe´rieux, France).

5.7.3 Principle of VITEK 2 compact system

The VITEK 2 compact system is an automated microbiology bacterial identification and antimicrobial susceptibility system. Uses advanced colorimetry technology to determine individual biochemical reactions contained in a variety of microbe identification cards. After inoculation with a standardized suspension of the unknown organism, each self-contained cards is incubated and read by the instrument's internal optics. Comparison of results to known species specific reactions in the VITEK 2 database yields organism identifications. A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present (user manual).

5.7.4 Suspension preparation

- Aseptically transfer 3.0 ml of sterile saline (0.45% to 0.5% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12mm x 75mm).

- Use a sterile stick or swab to transfer a sufficient number of morphologically similar colonies (pure culture) to the saline tube prepared in step 1.
- Prepare a homogenous organism suspension with a density equivalent to the appropriate McFarland standard (0.50 to 0.63) using a turbidity meter called the DensiChek^{TM}. NOTE: the age of the suspension before loading the instrument for AST testing must be less than 30 minutes.
- In a second tube containing 3.0ml of saline, transfer 145ul (for AST-GN cards) of the suspension prepared in step 2. Then place this tube in the cassette with a susceptibility card (AST-GN86 cards). The tube with the initial bacterial suspension can also be used for inoculation of an identification card (ID-GN).
- Fill in a cassette worksheet with the test card and specimen information for the cassette. Bar Code Scanner was used for data entry.
- Place the test cards and specimen test tubes in their appropriate slots.
- Load the cassette into the Filler Station (filling and sealing the test cards).
- Transfer the cassette to the VITEK 2 Compact cassette loading station within 10 minutes. (Test cards incubated and analyzed automatically)
- Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. (Accommodate up to 30 or up to 60 cards).
- All card types are incubated at 35.5 +1.0 ^{0}c.

5.7.5 Identification of bacteria

GN cards were used for identification of gram-negative bacteria. The GN card is used for the automated identification of 135 taxa of the most significant fermenting and non-fermenting gram-negative bacilli. The reagent cards have 64 wells contain an individual test substrate. Substrates measure metabolic activities such as acidification, alkalization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of card allows for the appropriate level of oxygen transmission. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and unique identifier that can be linked to the sample either before or after loading the card onto the system.

5.7.6 Antimicrobial Susceptibility Testing

The VITEK®2 Antimicrobial Susceptibility Test is intended to be used with the VITEK®2 System for the automated quantitative or qualitative susceptibility testing of isolated colonies for most clinically significant aerobic gram-negative bacilli, *Staphylococcus spp*., *Enterococcus spp*., *Streptococcus agalactiae*, and *S. pneumoniae*. AST-GN86 cards were used to perform AST. AST-GN86 cards have 18 drugs for antimicrobial susceptibility test which includes; ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, cefazolin, cefuroxime, cefuroxime axetil, ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem, gentamicin, tobramycin, ciprofloxacin, levolfloxacin, tetracycline, nitrofuratoin and trimethoprim/sulfemethoxazole. The results of the susceptibility test were interpreted as sensitive, intermediate and resistance based on Clinical and Laboratory Standards Institute (CLSI) guideline (43). VITEK 2 also performs the Minimum Inhibitory Concentration (MIC). Multidrug resistance is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (44).

5.7.7 Extended-Spectrum Beta-Lactamase Detection

VITEK®2 ESBL Test is a confirmatory test to detect the presence of extended-spectrum beta-lactamase (ESBLs) in *Escherichia coli, Klebsiella pneumoniae,* and *Klebsiella oxytoca*. AST-GN86 cards have drugs for ESBL confirmation.

Concentrations:

Cefepime (1 µg/ml)	Cefepime/Clavulanic Acid (1/10 µg/ml)
Cefotaxime (0.5 µg/ml)	Cefotaxime/Clavulanic Acid (0.5/4 µg/ml)
Ceftazidime (0.5 µg/ml)	Ceftazidime/Clavulanic Acid (0.5/4 µg/ml)

The ESBL analysis for the VITEK 2 system is based on monitoring organism activity (growth) in seven different wells on the test card. One well is a control containing only growth media. The other six are cefepime, cefotaxime, and ceftazidime, each with and without clavulanic acid.

Organism activity is monitored in the control well to determine whether sufficient activity is present to complete the analysis and to determine the length of incubation. No ESBL result is reported unless the organism reaches predetermined growth thresholds. Once the organism reaches the exponential phase, incubation is extended a set amount of time to evaluate the activity in the antimicrobial wells with and without clavulanic acid.

Test Principle:

The detection of an ESBL is based on the inhibition of activity in the presence of clavulanic acid. The VITEK® 2 analysis looks for growth patterns that exhibit activity in the well containing the antimicrobial without clavulanic acid and limited activity in the corresponding antimicrobial well containing clavulanic acid. Each of the three pairs of wells is evaluated independently. If any one of the three pairs demonstrates the expected growth pattern (difference in activity with and without clavulanic acid) a positive test result is reported.

Result interpretation:

Negative – Strain does not produce ESBLs.

Positive – Strain produces ESBLs. Test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam.

5.8 Data Quality Assurance

To maintain the quality of data the questionnaire was pre-tested and collected data was checked carefully on spot and daily basis for their completeness, accuracy, and clarity. To assure the quality of laboratory results the standard operating procedures (SOPs) was followed in all steps of the pre analytical, analytical and post analytical. Quality control was done for all reagent used for this study. Culture media were tested for sterility and performance. In addition, quality control with a non-ESBL-producing *E. coli* American Type Culture Collection (ATCC) 25922 (negative control) and an ESBL-producing *K. pneumonia* ATCC 700603(positive control) were also performed as recommended by the CLSI guideline (43).

5.9 Data Analysis Procedures

The data entry was done by using EPI INFO version 7.2.1.0. Data cleaning and analyses was done by Statistical Package for Social Science (SPSS) version 20. A simple frequency was used to describe the study population in relation to socio-demographic, clinical condition and other relevant variables. Chi-square analysis was used to explore risk factors associated with the prevalence of ESBL carriage by using odds ratio with 95% confidence limit. A value of $p < 0.05$ was considered to be statistically significant. The data was summarized and presented by tables.

5.10 Ethical Issues

This study was approved by the Department Research and Ethical Review Committee of the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. Permission letter was obtained from the study site. Individual informed verbal consent was obtained after brief explanation of the purpose and benefits of the study for each parents or guardians on behalf of the children. All results were kept confidential. The participants were given the full right to refuse from participating in the research.

5.11 Dissemination and Utilization of the Result

The study will be presented to Department of Medical Laboratory Sciences School of Allied Health Sciences College of Health Sciences Addis Ababa University as partial fulfillment of Masters Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Track). We will send a manuscript for publication in peer reviewed journal and presentations shall be made in scientific conferences.

6 Results

6.1 Socio Demographic Characteristics

From the total of 269 under five children 155(57.6%) were infants (1-23 months) and 114 (42.4%) were children (24-59 months). The median age of the study participants was 18 months and range from 1 to59 months. One hundred thirty nine (51.7%) were female and130 (48.3%) were male. Most of study participants (76.6%) had family size (members) of 2 to 5. Majority of mothers (82.9%) were within age group 15-34. The study participant's mother educational status showed that 130 (48.3%) of them attended primary school and 61 (22.7%) were attended secondary education (Table 1).

Majority of the study participant's parent income were > 2000.00 birr (48.0%) and the minority were < 500.00 birr (4.5%). Two hundred sixty (96.7%) of study participants were from Addis Ababa. The majority house conditions were private rental 135 (50.2%). Concerning the source of water for the child, the majorities were used tap water 128 (47.6%) and bottled water 113 (42.0%). Majority of child family 236 (87.7%) were using communal latrine (Table 1).

Table 1. Socio demographic characteristics of under five years children at Addis Raey public health center, Addis Ababa, Ethiopia, 2017 (n=269).

Characteristics		Frequency	Percent
Age of children	29days-23months	155	57.6
	24-59 months	114	42.4
Gender	Male	130	48.3
	Female	139	51.7
Family size	2-5	206	76.6
	6-9	57	21.2
	10-13	6	2.2
Age groups of mother	15-24	80	29.7
	25-34	143	53.2
	35-44	30	11.2
	45-54	1	0.4
	Don't know	15	5.6
Mother Educational level	Illiterate/cannot read and wrote/	53	19.7
	Illiterate /able to read and write/	3	1.1
	Primary	130	48.3
	Secondary	61	22.7
	College Graduate	10	3.7
	Don't know	12	4.5
Parent Income	<500 birr	12	4.5
	500-1000birr	46	17.1
	1000 -2000birr	82	30.5
	>2000birr	129	48.0
Place of resident	Addis Ababa	260	96.7
	Out of Addis Ababa	9	3.3
House condition	Private	47	17.5
	Government rental	87	32.3
	Private rental	135	50.2

Continue in next page

Characteristics			Frequency	Percent
Source of water for the child	Tap Water	Yes	128	47.6
		No	141	52.4
	Boiled & Cooled Water	Yes	26	9.7
		No	243	90.3
	Treated Water	Yes	34	12.6
		No	235	87.4
	Bottled Water	Yes	113	42.0
		No	156	58.0
	Filtered Water	Yes	1	0.4
		No	268	99.6
Toilet use for family		Private	33	12.3
		Communal	236	87.7

6.2 Clinical condition of study participants

Nutritional status of under five children indicated that 14(5.2%) had moderate acute malnutrition (MAM) and 3(1.1%) had sever acute malnutrition (SAM). Majority of children 257 (95.5%) were born in health facilities (health center, hospital and clinic). About 220 (81.8%) of the study participants were born by vaginal delivery (Table 2).

Most under five children 216 (80.3%) had exposure of previous antibiotics usage. However, majority of the study participants had no exposure of hospital visit 220(81.8%), previous hospital admission 248 (92.2%) and previous surgery 267 (99.3%). One hundred thirty (48.3%) had at least one episode of diarrhea for the last three month and 120 (44.6%) had GI (Gastroenteritis) symptom (Table 2). Concerning prior antibiotic use, most of the study participants used amoxicillin, cotrimoxazole and metrendazole (Figure 2).

Table 2. Clinical condition of under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017 (n=269).

Characteristics		Frequency	Percent
Nutrition Status	Normal	252	93.6
	MAM	14	5.2
	SAM	3	1.1
Place of birth	Home	12	4.5
	Health center	145	53.9
	Hospital	108	40.1
	Private Clinic	4	1.5
Mode of delivery	Vaginal Delivery	220	81.8
	Cesarean Section	49	12.2
Prior intake of antibiotics	Yes	216	80.3
	No	53	19.7
Hospital visit	Yes	49	18.2
	No	220	81.8
Previous hospital admission	Yes	21	7.8
	No	248	92.2
Previous surgery	Yes	2	0.7
	No	267	99.3
diarrhea for the last three month	Yes	130	48.3
	No	139	51.7
GI symptom	Yes	120	44.6
	No	149	55.4
Number of visit	First	56	20.8
	Second	63	23.4
	Third	87	32.3
	More than three	63	23.4

MAM: Moderate Acute Malnutrition **SAM:** Sever Acute Malnutrition **GI:** Gastroenteritis

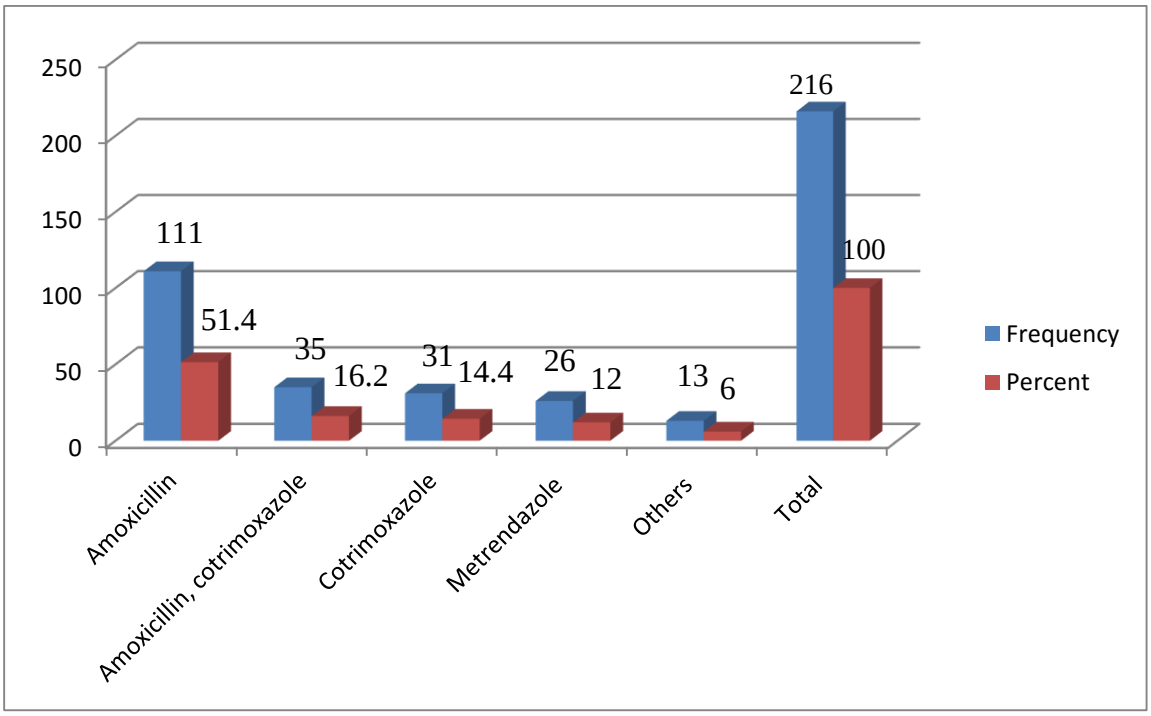

Figure 2. Previous intake of antibiotics by under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017 G.C. (n=269).

Others: Amoxicillin, cotrimoxazole, ampicillin, gentamicin, augmentin, cefalaxin, ceftriaxone, cloxacillin and tetracycline.

6.3 Bacteria Isolates

A total of 286 bacteria were isolated from 269 patients fecal/rectal swab samples. Of these, 224 (77.2 %) were *E. coli*, 39 (13.4 %) were *K. pneumoniae* and 1 (0.3 %) was *Klebsiell*a *oxytoca.* Among the total of 264 *E. coli* and *Klebsiella* species isolates, E. *coli* 224(84.8%) was the most commonly isolated bacteria followed by *K. pneumoniae* 39(14.8%) and *K. oxytoca* 1(0.4%). However, two bacteria remained unidentified and two samples had no bacterial growth (Table 3).

Table 3. Proportion of bacteria isolates from feces/rectal swabs of under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017.

Bacterial Isolates	Frequency	Percent
E. coli	224	77.2
K. pneumoniae	39	13.4
K. oxytoca	1	.3
Entrobacter cloacae ssp cloacae	5	1.7
Entrobacter cloacae ssp dissolves	3	1.0
Entrobacter asburiae	1	.3
Citrobacter freundi	3	1.0
Proteus mirabilis	1	.3
Shigella boydii	4	1.4
Cronobacter sakazakii group	2	.7
Raoultella orithinolytica	2	.7
Escherichia fergusonii	1	.3
Unidentified organism	2	.7
No growth	2	.7
Total	**290**	**100.0**

6.4 Prevalence of ESBL carriage

The overall gastrointestinal carriage rate of ESBL producing *E. coli* and *K. pneumonia* in under five children was 17.1% (46/269; 95% CI: 12.9%–22.7%). A total of 47 *E.* coli and *Klebsiella* species were ESBL positive, of which 83.0% (39/47) were *E. coli* and 17.0% (8/47) were *K. pneumoniae.* There was only one patient that colonized with both ESBL producing *E. coli* and *K. pneumoniae*. The highest intra-species frequency of ESBL production was seen among *K. pneumoniae* 20.5% (8/39) followed by *E. coli* 17.4% (39/224).

As seen in table 5, ESBL carriage proportion was higher among children in the age groups 29 days -23 months 19.4% (30/155) than children with age group 24-59 months 14.0% (16/114). About 17.7 % (23/130) of males and 16.5% (23/139) of females were ESBL carriers. ESBL carriage among MAM nutrition status 21.4% (3/14) was higher than among normal nutrition status 17.1% (43/252). Children who were born in home 25.0% (3/12) and private clinic 25.0% (1/4) had higher rate of ESBL carriage than those born in health center 16.6 % (24/145) and hospital 16.7% (18/108). ESBL carriage was high among Children delivered by vagina 17.3% (38/220) than in Cesarean Section 16.3% (8/49) (Table 4). .

ESBL carriage was low in children with history of prior intake of antibiotics 16.7% (36/216), previous hospital visit 14.3% (7/49) and hospital admission 14.3% (3/21) than their counterparts. Two children were undergoing a surgical operation in the last 12 months but none of them were ESBL carrier. ESBL carriage was also low among children who had at least one episode of diarrhea for the last three months 16.9% (22/130) and GI symptoms 15.8% (19/120) than their contrary. ESBL carriage was high among children that had first 19.6 % (11/56) and second 27.0% (17/63) health center visits than those who had third 10.3% (9/87) and more than three 14.3% (9/63) visit (Table 4).

Table 4. Fecal carriage of ESBL producing E. coli and K. pneumoniae among under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017 (n=269).

Characteristics		Total N=269	ESBL	
			Negative N=223 N (%)	Positive N=46 N (%)
Age	29days-23months	155	125(80.6)	30(19.4)
	24-59 months	114	98(86.0)	16(14.0)
Sex	Male	130	107(82.3)	23(17.7)
	Female	139	116(83.5)	23(16.5)
Nutrition Status	Normal	252	209(82.9)	43(17.1)
	MAM	14	11(78.6)	3(21.4)
	SAM	3	3(100)	0(0.0)
Place of birth	Home	12	9(75.0)	3(25.0)
	Health center	145	121(83.4)	24(16.6)
	Hospital	108	90(83.3)	18(16.7)
	Private Clinic	4	3(75.0)	1(25.0)
Mode of delivery	Vaginal delivery	220	182(82.7)	38(17.3)
	Cesarean Section	49	41(83.7)	8(16.3)
Prior intake of antibiotics	Yes	216	180(83.3)	36(16.7)
	No	53	43(81.1)	10(18.9)
Hospital visit	Yes	49	42(85.7)	7(14.3)
	No	220	181(82.3)	39(17.7)
Previous hospital admission	Yes	21	18(85.7)	3(14.3)
	No	248	205(82.7)	43(17.3)
Previous surgery	Yes	2	2(100.0)	0(0.0)
	No	267	221(82.8)	46(17.2)
diarrhea for the last three months	Yes	130	108(83.1)	22(16.9)
	No	139	115(82.7)	24(17.3)
GI symptom	Yes	120	101(84.2)	19(15.8)
	No	149	122(81.9)	27(18.1)
Number of visit	First	56	45(80.4)	11(19.6)
	Second	63	46(73.0)	17(27.0)
	Third	87	78(89.7)	9(10.3)
	More than three	63	54(85.7)	9(14.3)

MAM: Moderate Acute Malnutrition SAM: Sever Acute Malnutrition GI: Gastroenteritis

6.5 Antimicrobial susceptibility pattern

The susceptibility data of ESBL producing and non-ESBL producing *E. coli* and *K. pneumoniae* isolates are summarized in Table 5. The overall susceptibility patterns of *E. coli* and *K. pneumoniae* isolates were shows the highest level of resistance to ampicillin (77.2 %), followed by trimethoprim/sulfemethoxazole (60.8 %), tetracycline (57.4 %) and ampicillin/sulbactam (55.8 %). All ESBL and non-ESBL producing *K. pneumoniae* were 100% resistance to ampicillin. ESBL producing *E. coli* and *K. pneumoniae* isolates were shows higher percent of resistance to all cephalosporins compared to non-ESBL producer that shows low or no resistance (Table 5).

For ESBL producing *E. coli* and K. *pneumonia*, co-resistance to aminoglycosides, fluoroquinolones, tetracycline and trimethoprim-sulfamethoxazole was also observed. High level of resistance for trimethoprim/sulfemethoxazole (78.7%), followed by tetracycline (70.2%), ciprofloxacin (25.5%) and gentamicin (17%) were detected in ESBL producing *E. coli* and *K. pneumoniae*. High level of resistance for trimethoprim/sulfemethoxazole (82.1 %), tetracycline (74.4%), ciprofloxacin (25.6%), and gentamicin (10.3%) were observed for ESBL producing *E. coli*. For ESBL producing K. *pneumonia* the highest percent of resistance was detected in trimethoprim/sulfemethoxazole (62.5%), tetracycline (50%), gentamicin (50%) and ciprofloxacin (25%). All ESBL and non-ESBL producing E. *coli* and K. *pneumoniae* isolates were susceptible to carbapenems (ertapenem and imipenem) (Table 5).

A total of 65.4% (172/263) MDR was detected in all ESBL producing and non-ESBL producing *E. coli* and *K. pneumoniae* isolates. Among the total MDR, *E. coli* was the predominant species with 86.6% (149/172) followed by *K. pneumonia* 13.4% (23/172). The overall proportion of MDR was 93.6% among ESBL producer and 59% in non-ESBL *E. coli* and *K. pneumoniae* isolates. The highest MDR was observed among ESBL producing *E. coli* 94.9 % (37/39) and *K. pneumoniae* 87.5 % (7/8) isolates than non-ESBL producing *E. coli* 60.5 % *(*112/185) and *K. pneumonia* 51.6 % (16/31) (Table 6).

Table 5. Antimicrobial resistance pattern of ESBL producing and non-ESBL producing E. coli and K. pneumoniae isolated from feces/rectal swab of under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017 G.C.

Antimicrobial Agents	**ESBL producing**			**Non -ESBL producing**		**Total (N=263)**
	E. coli (N=39)	*K. pneumonia (N=8)*	Total	*E. coli* (N=185)	*K. pneumonia (N=31)*	
	Resistance	Resistance		Resistance	Resistance	Resistance
Ampicillin	37(94.9)	8(100)	45(95.7)	127(68.6)	31(100)	203(77.2)
Amoxicillin/Clavulanic Acid	17(43.6)	2(25)	19(40.4)	50 (27.0)	3 (9.7)	72(27.3)
Ampicillin/Sulbactam	30(76.9)	6(75.5)	36(76.5)	101 (54.6)	10(32.3)	147(55.8)
Cefazolin	29(74.4)	7(87.5)	36(76.6)	23 (12.4)	1 (3.2)	60(22.8)
Cefuroxime	34(87.2)	7(87.5)	41(87.2)	16 (8.6)	0(0)	57(21.7)
Cefuroxime Axetil	37(94.9)	7(87.5)	44(93.6)	22 (11.9)	1 (3.2)	67(25.7)
Ceftazidime	28(71.8)	7(87.5)	35(74.5)	0 (0)	0(0)	35(13.3)
Ceftriaxone	29(74.4)	7(87.5)	36(76.6)	0(0)	0(0)	36(13.7)
Cefepime	29(74.4)	7(87.5)	36(76.6)	0(0)	0(0)	36(13.7)
Ertapenem	0(0.0)	0(0.0)	0(0.0)	0(0)	0(0)	0(0)
Imipenem	0(0.0)	0(0.0)	0(0.0)	0(0)	0(0)	0(0)
Gentamicin	4(10.3)	4(50.0)	8(17.0)	1 (0.5)	1 (3.2)	10(3.8)
Tobramycin	7(18.0)	5(62.5)	12(25.5)	2 (1.0)	1 (3.2)	15(5.7)
Ciprofloxacin	10(25.6)	2(25.0)	12 (25.5)	3 (1.5)	0(0)	15(5.7)
Levofloxacin	9(23.1)	1(12.5)	10(21.3)	3 (1.6)	0(0)	13(4.9)
Tetracycline	29(74.4)	4(50.0)	33(70.2)	105(56.7)	13 (41.9)	151(57.4)
Nitrofurantoin	8(20.5)	4(50.0)	12(25.5)	7(3.7)	1 1(35.5)	30(11.4)
Trimethoprim/Sulfemethoxazole	32(82.1)	5(62.5)	37(78.7)	110(59.5)	13(41.9)	160(60.8)

Table 6. Multidrug resistance pattern of ESBL producing and non- ESBL producing E. coli and K. pneumoniae isolated from feces/rectal swab of under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017 G.C.

	Isolates	Level of antibiotics resistance ((number (%))								MDR (≥R3)
		R_0	R_1	R_2	R_3	R_4	R_5	R_6	≥R_7	
ESBL positive	*E. coli* (n= 39)	0 (0.0)	1 (2.6)	1 (2.6)	3 (7.7)	3 (7.7)	10 (25.6)	10 (25.6)	11 (28.2)	37 (94.9)
	K. pneumonia (n=8)	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	0 (0.0)	1 (12.5)	0 (0.0)	5 (62.5)	7 (87.5)
ESBL negative	*E. coli* (n=185)	42 (22.7)	11 (5.9)	20 (10.8)	26 (14.1)	62 (33.5)	22 (11.9)	1 (0.5)	1 (0.5)	112 (60.5)
	K. pneumonia (n=31)	0 (0.0)	9 (29.0)	6 (19.4)	6 (19.4)	8 (25.8)	2 (6.5)	0 (0.0)	0 (0.0)	16 (51.6)
Total (N=263)		42 (16.0)	21 (8.0)	28 (10.6)	36 (13.7)	73 (27.8)	35 (13.3)	11 (4.2)	17 (6.5)	**172 (65.4)**

R0: resistance to no antibiotics, R1-6: resistance to 1, 2, 3, 4, 5, and 6, ≥R7: resistance to ≥**7** antibiotics; MDR: Multidrug resistance MDR **(**≥R3): resistance to 3 or more antibiotics from different classes.

6.6 Risk Factors associated with ESBL carriage

There was no significant association was found among age groups, sex, previous antibiotics usage, hospital visit, hospital admission and undergoing a surgical operation in the last 12 months and ESBL carriage (Table 7). However, Children of mother with lower educational level (primary school) and children used tap water for drinking have statistical significant association with higher ESBL carriage (Table 8).

Table 7. Analysis of factors associated with ESBL producing E. coli and K. pneumonia fecal carriage in under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017 (n=269).

Characteristics		Total N=269	ESBL		OR	95%CI		X^2	p-value
			Negative N=223 N (%)	Positive N=46 N (%)		Lower	Upper		
Age	29days-23months	155	125(80.6)	30(19.4)	1.379	0.791	2.406	1.311	0.252
	24-59 months	114	98(86.0)	16(14.0)					
Sex	Male	130	107(82.3)	23(17.7)	1.069	0.632	1.810	0.62	0.803
	Female	139	116(83.5)	23(16.5)					
Prior intake of antibiotics	Yes	216	180(83.3)	36(16.7)	0.883	0.469	1.663	0.145	0.703
	No	53	43(81.1)	10(18.9)					
Hospital visit	Yes	49	42(85.7)	7(14.3)	0.806	0.383	1.693	0.335	0.563
	No	220	181(82.3)	39(17.7)					
Previous hospital admission	Yes	21	18(85.7)	3(14.3)	0.824	0.279	2.432	0.127	0.721
	No	248	205(82.7)	43(17.3)					
Previous surgery	Yes	2	2(100.0)	0(0.0)	1.208	1.144	1.276	0.416	0.519
	No	267	221(82.8)	46(17.2)					

Table 8. Analysis of factors associated with ESBL producing E. coli and K. pneumoniae fecal carriage in under five years children at Addis Raey public Health Center, Addis Ababa, Ethiopia, 2017 (n=269).

Characteristics			Total N=269	ESBL		OR	95%CI		X^2	p-value
				Negative N=223 N (%)	Positive N=46 N (%)		Lower	Upper		
Mother Educational level	Illiterate/cannot read and wrote/		53	46(86.8)	7(13.2)	0.690	0.2901	1.644	0.405	0.5245
	Illiterate /able to read and write/		3	3(100.0)	0(0.0)	0.797	0.0392	1.618	0.318	0.5727
	Primary		130	105(80.8)	25(19.2)	2.472	1.323	4.618	7.487	**0.0062**
	Secondary		61	50(82.0)	11(18.0)	1.087	0.5152	2.295	0.000	0.9788
	College Graduate		10	8(80.0)	2(20.0)	1.222	0.2509	5.948	0.032	0.8573
	Don't know		12	11(91.7)	1(8.3)	0.428	0.0539	3.401	0.187	0.6650
Source of water for the child	Tap Water	Yes	128	100(78.1)	28(21.9)	1.714	1.001	3.659	3.927	**0.048**
		No	141	123(87.2)	18(12.8)					
	Boiled & Cooled Water	Yes	26	23(88.5)	3(11.5)	0.652	0.217	1.956	0.687	0.407
		No	243	200(82.3)	43(17.7)					
	Treated Water	Yes	34	31(91.2)	3(8.8)	0.482	0.158	1.469	1.881	0.178
		No	235	192(81.7)	43(18.3)					
	Bottled Water	Yes	113	94(83.2)	19(16.8)	0.971	0.569	1.658	0.11	0.915
		No	156	129(82.7)	27(17.3)					
	Filtered Water	Yes	1	1(100.0)	0(0.0)	1.207	1.143	1.275	0.376	0.540
		No	268	222(82.8)	46(17.2)					

7 Discussion

Different study conducted on ESBL carriage around the world shows large variation from country to country. This variation is due to difference in geographical location and study subjects. There was limited data on the carriage of ESBL-producing *E. coli* and *K. pneumoniae* from Ethiopia. This was the first study reported ESBL carriage among under five children from Ethiopia particularly from community perspectives using VITEK 2 instrument. In present study, 17.1% (46/269; 95% CI: 12.9%–22.7%) of the under five children were carriers of ESBL-producing *E. coli* and *K. pneumoniae*. The result of this study is comparable with the study by Stoesser N et al. (37) which reported that ESBL-E fecal colonization prevalence was 23.2% (95% CI 19.1%–27.6%) in children attending pre-school childcare facilities in the Lao People's Democratic Republic. This result is also consistent with the study in Spain which reported that 24.0% (30/125) of 8- to 16-month-old healthy children were colonized with ESBL-PE (33) and with the study in Lebanese from healthy children aged from 1 to 5 years reported that 24.8% of the children were carriers of ESBL-PE (34).

The prevalence in present study is lower than the report of study by Desta K et al. (41) which reported that 52% (95%CI; 46%–58%) of hospitalized patients admitted at TASH were carriers of ESBL-PE. It is also lower than the study by Isendahl J et al. (9) among children <5 years of age with fever or tachycardia attending a pediatric emergency ward in Guinea-Bissau in which 32.6% of the children were carriers of ESBL-producing *E. coli* or *K. pneumoniae*. Also lower than the report in Dar es Salaam, Tanzania, which showed that the overall prevalence of ESBL carriage was 34.3% (207/ 603) in children below 2 years of age including healthy community children and children hospitalized due to diarrhea or other diseases (38). This variation is due to the difference in study settings, which indicates that hospital admitted patients are at a higher risk of acquiring ESBL-PE than community patients.

On the other hand, the prevalence in this study is higher compared with Study in Madagascar, reported that 10.1% (49/484) of patients were carrier of ESBL-PE (10), fecal carriage of ESBL-PE was 4.7% (14/300) in children aged 4-6 years from the community in South Africa (7) and study in France showed that 4.6% children aged from 6 to 24 months carried ESBL-PE (12). This difference might be due to the difference in study participants and countries. The higher prevalence in our study is probably due to sick children attending OPD were included in the

study. However, in the first two study healthy individuals not received antibiotics or were hospitalized previously were included in the study and the last study was enrolled children during routine check-ups with normal findings.

In present study, the predominant ESBL producing isolate was *E. coli* (83%) followed by *K. pneumoniaen* (17%). Similarly, *E. coli* was the predominant ESBL-producing organism in Turkey study (14) and in Tanzania, of the 36 ESBL-producing isolates detected, 83.3% (30/36) and 16.7% (6/36) were *E. coli* and *K. pneumoniae*, respectively (5). Another study in the Lao People's Democratic Republic also showed that *E. coli* was the most common colonizers in which 78% of E. *coli* and 18% of *K. pneumoniae* were ESBL-producer (37). Study in Zimbabwe showed that 95.8 % (23/24) of E. *coli* and 4.2 % (1/24) of K. *pneumonia* were ESBL producer (39). Study in Madagascar (10), Korea (28) and Lebanese (34) also demonstrated that *E. coli* as the predominant ESBL-producing organism.

In present study, the majority of children were often repeatedly exposed to amoxicillin and cotrimoxazole. The antimicrobial susceptibility patterns of isolates in present study also showed that majority of the isolates were resistant to ampicillin (77.2%) and trimethoprim-sulfamethoxazole (60.8%). The level of resistance reflects the usage of these antibiotics in the public health sector in Ethiopia. For instance, K. *pneumoniae* was 100% resistance to ampicillin in both ESBL and non-ESBL producer.

In this study, ESBL producing *E. coli* and K. *pneumonia* isolates showed high level of co-resistance for aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole. The level of resistant was high in trimethoprim/sulfemethoxazole (78.7%), followed by tetracycline (70.2%), ciprofloxacin (25.5%) and gentamicin (17%) were detected in ESBL producing *E. coli* and *K. pneumoniae*. Similar studies from different countries also reported the presence of co-resistance, in Tanzania, 36 (100%), 35 (97%), 25 (69%) and 16(44%) were resistant to tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin and gentamicin, respectively (5). In Madagascar, trimethoprim-sulfamethoxazole (91.3%), gentamicin (76.1%) and ciprofloxacin (50.0%) were resistant (40), in Spain, Nalidixic acid (64.7%), ciprofloxacin (32.4%), levofloxacin (32.4%) and trimethoprim-sulfamethoxazole (41.2%) were resistant (33). and in Lebanese, gentamicin (54.8 %), tetracycline (41.9%), trimethoprim-sulfamethoxazole (32.3%) and nalidixic acid (35.5 %) were susceptible (34).

In this study, high level of resistance for trimethoprim/sulfemethoxazole (82.1 %), tetracycline (74.4%), ciprofloxacin (25.6%), and gentamicin (10.3%) were observed for ESBL producing *E. coli*. For ESBL producing K. *pneumonia* the highest percent of resistance was detected in trimethoprim/sulfemethoxazole (62.5%), tetracycline (50%), gentamicin (50%) and ciprofloxacin (25%). This result is also comparable with study in Guinea-Bissau: *E. coli* was resistant to trimethoprim-sulfamethoxazole (94%), Quinolone (81.9%), gentamicin (43.4%) and tobramycin (71.1%) and *K. pneumoniae* was resistant to trimethoprim-sulfamethoxazole (91.2%), Quinolone (48.4%), gentamicin (93.4%) and tobramycin (94.5%) (9), in Madagascar study, ciprofloxacin was resistant in 34.5% of *K. pneumonia* and in 68.4% of *E. coli* (40) and in Tanzania, co-resistance to ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole was detected in about half of the *E. coli* and *E. cloacae* complex isolates and in about 14% of the *K. pneumoniae* isolates (38).

In our study, the overall prevalence of MDR was 93.6 % among ESBL producer *E. coli* and *K. pneumoniae* isolates. This result is in line with the findings of studies conducted in Tanzania (94%) MDR (38), in Guinea-Bissau nearly all isolates were MDR (9) and in Madagascar most ESBL-PE isolates are MDR strains (40). However, it was higher than a study conducted in Spain, 52.4% of the ESBL-PE were resistant to three or more antimicrobial classes (33). This difference might be due to the difference in study setting, previous antibiotic usage and definition for MDR.

ESBL-producing bacteria are frequently associated with co-resistance to non-beta-lactam antimicrobial agents as demonstrated in several studies (5,9,10,34), which may critically complicate the treatment of severe bacterial infections and leaves very few choices for treatment (limits the therapeutic choice to carbapenems). Interestingly, unlike to previous report of Desta et al. study in TASH which reported that 2% (5/267) of ESBL producing isolates were resistant to carbapenem and all detected in children (41) in present study, all ESBL producing *E. coli* and *K. pneumoniae* isolates were susceptible to carbapenems (ertapenem and imipenem). This result is in line with the studies conducted in Tanzania (38), in Guinea-Bissau (9), in Lebanese (34) and in Spain (33). The results of this study was also comparable with a studies conducted in two studies from Madagascar reported all isolates were susceptible to amikacin and imipenem (10,40) and in another study in Tanzania reported all isolates were susceptible to colistin, and

meropenem (5). Therefore, Carbapenems is the active drug to treat infection caused by these resistant isolates.

The finding from this study revealed that there was no association between age and ESBL carriage (OR: 1.379, 95% CI: 0.791-2.406, p= 0.252), similar to study in Guinea-Bissau (9)which reported that there was no association between age and colonization with ESBL-producing *E. coli* or *K. pneumoniae* (p-value 0.71) and with the study from Madagascar (10). However, this result is inconsistent with the report of Tellevik MG et al. (38)which reported that having age equal to or below 12 months was significantly associated with ESBL carriage (P = 0.012; OR = 1.82; 95% CI: 1.14± 2.91),this is different from French study reported the risk of ESBL-PE carriage was higher among children over 1 year old than in younger children (6.5% versus 2.5%, respectively; OR=2.69, 95% CI [0.95–7.61]) (12) . Our study also showed that there was no significant difference between male and female ESBL carriers (OR: 1.069, 95% CI: 0.632-1.810, P=0.801), this finding is similar with study by Herindrainy P et al.(10) and Erdoğan DC et al. (14) but different from the study by Hijazi SM et al. (34), which reported that males had a higher colonization frequency (33.9 %) than did females (15.9 %) (P = 0.09).

The risk analysis result of present study showed that there was no association between previous usage of antibiotics (in the last 12 months) and ESBL colonization (OR: 0.883, 95% CI: 0.469-1.667, P=0.703), which is in agreement with the study conducted in Guinea-Bissau (9), Tanzania (5) and Lebanese (34)which reported that there was no association with previous usage of antibiotics and ESBL colonization. However, it is different from several studies that indicated use of antibiotics as a risk factor for ESBL carriage. A study by Tellevik MG et al. (38) showed that Children who used antibiotics during the last 14 days prior to study enrollment were more likely to carry ESBL-producing strains than those who had not taken antibiotics (P = 0.022; OR = 1.61; 95% CI: 1.07±2.41). Another study in Democratic Republic also showed use of antibiotics in the 3 months prior to sampling was identified as the only risk factor that remained significantly associated with ESBL-E colonization in the multivariate analysis (37). Study in France children also shows recent third-generation oral-cephalosporin exposure (within 7 days to 3 months before enrolment) was associated with a higher risk of ESBL carriage (AOR=3.52, 95% CI [1.06-11.66], p=0.04) (12). This difference is due to the types and duration of antibiotic usage prior to sampling and also small numbers of children that did not use antibiotics. Recent

use of antibiotics (during the last 14 days and 3 months) may result in these resistant ,compared to our study that included children who used antibiotics in the last 12 months prior to sampling, while children with antibiotic treatment within 7 days before enrolment were excluded from the study.

In study by Erdoğan DC et al. (14), ESBL carriage rates were found to be significantly higher in those who were hospitalized in the past 6 months. Similarly, study in Lebanese from healthy children aged from 1 to 5 years, reported that subjects admitted to the hospital in the last 12 months, were significantly associated with high carriage rate 43.5 % (10/23) than their counterparts 20.6 % (21/102) with P = 0.019 (34). However, in the present study, we could not demonstrate a significant association between previous hospital admission (in the last 12 months) and high rate of ESBL carriage (OR: 0.824, 95% CI: 0.279-2.432, P=0.721), it is similar to the study conducted by Isendahl J et al. (9). Our finding also showed that hospital visits (OR: 0.806, 95% CI: 0.383-1.693, P=0.563) in the last 12 months was not associated with higher ESBL carriage rates. The study by Erdoğan D C et al. (14) also noted that undergoing a surgical operation (P = 0.005) in the last 6 months was associated with higher ESBL carriage rates. However, in present study two children were undergoing a surgical operation in the last 12 months but none of them were ESBL carrier. The difference may be due to the small sample size of children who were hospitalized and undergoing a surgical operation in the last 12 months.

In addition, this study demonstrated that children of mother with lower educational level (primary school) were significantly associated with higher ESBL carriage (OR: 2.472, 95% CI: 1.323-4.618, P=0.0062). Using tap water for drinking was also significantly associated with higher ESBL carriage (OR: 1.714, 95% CI: 1.001-3.659, P=0.048) in present study. Several studies identified the presence of ESBL producing bacteria in foods and drinks. Which suggest foods and drinks could serve as reservoirs for ESBL producing bacteria. Study in the Netherlands showed that 92 (94%) of the 98 samples (poultry and retail chicken meat) contained at least one *E.* coli isolate with an ESBL phenotype (45). Another Study by Shu'aibu I et al. (46) showed that 33.34% of food and drinks sold in Gombe Metropolis, Nigeria were found to be ESBLs positive. Similarly, eight isolates (5.3 %) were confirmed as ESBL producers from a total of 101 sachet-packaged water bags sold as drinking water in the streets of Kinshasa, the capital

of Democratic Republic of the Congo (47)**.** Study in Bahir Dar City, Northwest Ethiopia detected 9.4% ESBL-PE from drinking water sources (48).

In general, in this study the prevalence of ESBL carriage was high in infant than children but the difference was not statistically significant. This indicates that colonization with ESBL-producing *E. coli* and *K. pneumoniae* often occurs early in life in this population. High levels of MDR and co-resistance to aminoglycosides, fluoroquinolones, tetracycline and trimethoprim-sulfamethoxazole were also observed. All ESBL producing E. *coli* and K. *pneumoniae* isolates were susceptible to carbapenems (ertapenem and imipenem). The risk analysis result of demographic data revealed that children of mother with lower educational level (primary school) and children used tap water for drinking were significantly associated factor with higher ESBL carriage. This suggests that the majority of ESBL-producing isolates were community-acquired.

8 Strength and Limitation

8.1 Strength

- Used the advanced VITEK 2 Compact system which is an automated microbial identification and antimicrobial susceptibility system that provides highly accurate and reproducible results.

8.2 Limitation

- This study was limited to test ESBL production only in *E. coli* and *Klebsiella* species. Other isolates like *Enterobacter* species (n=9), *C. freundii* (n=3) and *P. mirabilis* (n=1) which are known as ESBL producer were not tested. This may probably lower the prevalence.
- A negative ESBL test result does not rule out the presence of an ESBL masked by an AmpC beta-lactamase.

9 Conclusion

This study was reported a high prevalence (17.1%) of ESBL carriage among under five children. The antimicrobial susceptibility pattern of ESBL producing isolates showed high levels of MDR (93.6 %) and high rates of co-resistance to aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole. However, all ESBL producing E. *coli* and K. *pneumoniae* isolates were susceptible to carbapenems (ertapenem and imipenem). This study also demonstrated that Children of mother with lower educational level (primary school) (OR: 2.472, 95% CI: 1.323-4.618, P=0.0062) and children used tap water for drinking (OR: 1.714, 95% CI: 1.001-3.659, P=0.048) were significantly associated factor with higher ESBL carriage. This indicates that the majority of ESBL-producing isolates were community-acquired. Therefore, colonization with ESBL organism is considered as a prerequisite for infection and a potential source for transmission of these resistant strains in to the community.

10 Recommendations

Based on the study findings, the following recommendations are forwarded.

- Since the diagnosis of infection caused by these resistant strain is based on their antimicrobial susceptibility pattern and it differs from strain to strain and from country to country. Therefore, antimicrobial resistance surveillance should be established to develop treatment guideline.
- Infection control measure should be implemented to prevent the widespread transmission of these resistant strains to the community.
- Further studies are needed for better understanding of the dissemination and magnitude of ESBL-producing organisms including their molecular characterization and associated risk factors.

11 References

1. Chander A, Shrestha CD. Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates in a tertiary care hospital in Kathmandu , Nepal. *BMC Research Notes*. 2013;6:487.
2. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases : Types , epidemiology and treatment. *Saudi J Biol Sci*. 2015;22(1):90–101.
3. Raut S, Gokhale S, Adhikari B. Prevalence of Extended Spectrum Beta-Lactamases among *Escherichia coli* and *Klebsiella* spp isolates in Manipal Teaching Hospital , Pokhara , Nepal. *Journal of Microbiology and Infectious Diseases*. 2015;5(2):69–75.
4. Akingbade O, Ogiogwa J, Okonko IO, Okerentungba PO, Innocent-Adiele HC,Nwanze JC, et al. Plasmid Profile of Isolated *Klebsiella* species in a tertiary Hospital in ogun State , Nigeria. *World Applied Sciences Journal*. 2013;21(3). 371-78. DOI: 10.5829/idosi.wasj.2013.21.3.65163.
5. Moremi N, Claus H, Vogel U, Mshana SE. Faecal carriage of CTX-M extended-spectrum beta-lactamase-producing *Enterobacteriaceae* among street children dwelling in Mwanza city , Tanzania. *PLoS ONE*. 2017; 12(9): 1–11. e0184592. https://doi.org/10.1371/journal. pone.0184592
6. Paterson DL, Bonomo RA. Extended-Spectrum β-Lactamases : a Clinical Update. 2005;18(4):657–86.
7. Mahomed S, Coovadia YM. Faecal carriage of Extended Spectrum Beta- lactamase producing *Escherichia coli* and *Klebsiella* pneumoniae in children from the community of. *Int J Infect Control*. 2014, v11:i3.
8. Singh T, Das S, Ramachandran VG, Saha R, Khan AM, Rai A. "Emergence of Extended Spectrum Beta Lactamases Producing Multi Drug Resistant Diarrheagenic *Escherichia coli* in Children Under Five Years". *Acta Scientifica International Journal of Medical Science*. 2015;1(1):1–9.
9. Isendahl J, Turlej-Rogacka A, Manjuba C, Rodrigues A, Giske CG, Nauclér P. Fecal Carriage of ESBL-Producing *E. coli* and *K. pneumoniae* in Children in Guinea-Bissau: A Hospital-Based Cross-Sectional Study. *PLoS One*. 2012;7(12):1–8.
10. Herindrainy P, Randrianirina F, Ratovoson R, Hariniana E, Buisson Y, Genel N, et al.

Rectal carriage of extended-spectrum beta-lactamase-producing Gram-negative bacilli in community settings in Madagascar. *PLoS One*. 2011;6(7).

11. Ogbolu DO, Alli OAT, Olanipekun LB, Ojo OI, Makinde OO. Faecal carriage of extended-spectrum beta-lactamase (ESBL) -producing commensal *Klebsiella pneumoniae* and *Escherichia coli* from hospital out-patients in Southern Nigeria. *International journal of medicine and medical sciences.* 2013;5(3):97–105.
12. Birgy A, Cohen R, Levy C, Bidet P, Courroux C, Benani M, et al. Community faecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in french children. *BMC Infect Dis.* 2012;12(1):315.
13. Dandachi I, Sokhn ES, Najem E, Azar E, Daoud Z. International Journal of Infectious Diseases Carriage of beta-lactamase-producing *Enterobacteriaceae* among nursing home residents in north Lebanon. *International Society for Infectious Diseases*; 2016;45:24–31.
14. Çakir Erdoğan D, Cömert F, Aktaş E, Köktürk F, Külah C. Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *klebsiella* spp. In a Turkish community. *Turkish J Med Sci*. 2017;47(1):172–9.
15. Paltansing S, Vlot JA, Kraakman MEM, Mesman R, Bruijning ML, Bernards AT, et al. Extended-Spectrum *Enterobacteriaceae* among Travelers from the Netherlands. *Emerging Infectious Diseases.* 2014;19(8).
16. Weisenberg SA, Mediavilla JR, Chen L, Alexander EL, Rhee KY, Kreiswirth BN, et al. Extended Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* in International Travelers and Non- Travelers in New York City. *PLoS ONE*.2012;7(9):2010–3.
17. Miranda IB, Ignatius R, Pfu R, Friedrich-ja B, Steiner F, Paland M, et al. High carriage rate of ESBL-producing *Enterobacteriaceae* at presentation and follow-up among travellers with gastrointestinal complaints returning from India and Southeast Asia. *Journal of Travel Medicin.* 2016;1–7.
18. Ostholm-Balkhed A, Tarnburg M, Nilsson M, Nilsson LE, Hanberger H, Hallgren. Travel-associated faecal colonization with ESBL-producing *Enterobacteriaceae*: incidence and risk factors. *J Antimicrob Chemother.* 2013;(68):2144–53. doi:10.1093/jac/dkt167.
19. Tande D, Boisrame S, Munick MR, He´ry-Arnaud G, Gouriou S, Jallot N, et al. Intrafamilial transmission of extended-spectrum- b -lactamase- producing *Escherichia coli* and *Salmonella enterica* Babelsberg among the families of internationally adopted

children. 2010;1–7. *J Antimicrob Chemother.* doi:10.1093/jac/dkq068.

20. Ogefere HO, Aigbiremwen PA, Omoregie R. Extended-Spectrum Beta-Lactamase (ESBL)– Producing Gram-negative Isolates from Urine and Wound Specimens in a Tertiary Health Facility in Southern Nigeria. *Tropical Journal of Pharmaceutical Research*. 2015;14(6):1089–94.
21. Kandeel A. Prevalence and risk factors of extended-spectrum β-lactamases producing *Enterobacteriaceae* in a general hospital in Saudi Arabia. *Journal of Microbiology and Infectious Diseases.* 2014;4(2):50–4.
22. Woerther P, Burdet C, Chachaty E. Trends in Human Fecal Carriage of Extended-Spectrum β-Lactamases in the Community : Toward the Globalization of CTX-M. *Clinical Microbiology Reviews.* 2013;26(4):744–58.
23. Lukac PJ, Bonomo RA, Logan LK. Extended-Spectrum β -Lactamase – Producing *Enterobacteriaceae* in Children : Old Foe , Emerging Threat. 2015;60:1389–97.
24. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of ESBL-producing *Enterobacteriaceae* in samples collected from patients in various wards of Mulago Hospital , Uganda. *BMC Pharmacology and Toxicology.* 2015; 16 (14): 14–9.
25. Chandramohan L, Revell PA. Prevalence and Molecular Characterization of Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* in a Pediatric Patient. *Antimicrobial Agents and Chemotherapy*. 2012;56(9):4765–70. doi:10.1128/AAC.00666-12.
26. Logan LK, Meltzer LA, Mcauley JB, Hayden MK, Beck T, Braykov P, et al. Extended-Spectrum β -Lactamase – Producing Enterobacteriaceae Infections in Children : A Two-Center Case – Case – Control Study of Risk Factors and Outcomes in Chicago , Illinois. *Journal of the Pediatric Infectious Diseases Society*. 2014;3(4):312–9.
27. Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Canto R, Cobo J. High Rate of Intestinal Colonization with Extended-Spectrum- β - Lactamase-Producing Organisms in Household Contacts of Infected Community Patients. *Microbiology Journal of Clinical*. 2008;46(8):2796–9. doi:10.1128/JCM.01008-08.
28. Kim J, Lee JY, Ph D, Kim S Il, Song W, Kim J, et al. Rates of Fecal Transmission of Extended-Spectrum β-Lactamase-Producing and Carbapenem-Resistant

Enterobacteriaceae Among Patients in Intensive Care Units in Korea. *Ann Lab Med*. 2014;20–5.

29. Sun Q, Tarnberg M, Zhao L, Lundborg CS, Song Y, Grape M, Zhao L, Sta C, Nilsson LE. Varying High Levels of Faecal Carriage of Extended- Spectrum Beta-Lactamase Producing *Enterobacteriaceae* in Rural Villages in Shandong , China : Implications for Global Health. *PLoS ONE*. 2014;9(11): e113121. doi:10.1371/journal.pone.0113121.
30. Minami K, Shoji Y, Kasai M, Ogiso Y, Nakamura T, Kawakami Y, et al. Proportion of rectal carriage of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in the inpatients of a pediatric tertiary care hospital in Japan. *Jpn J Infect Dis*. 2012;65(6):548–50.
31. Kuenzli E, Jaeger VK, Frei R, Neumayr A, Decrom S, Haller S, et al. High colonization rates of extended-spectrum β -lactamase (ESBL) -producing *Escherichia coli* in Swiss Travellers to South Asia – a prospective observational multicentre cohort study looking at epidemiology , microbiology and risk factors. *BMC Infectious Diseases*. 2014. 14 (528): 1-10 p.
32. Rooney PJ, Leary MCO, Loughrey AC, Mccalmont M, Smyth B, Donaghy P, et al. Nursing homes as a reservoir of extended-spectrum b -lactamase (ESBL) -producing ciprofloxacin-resistant *Escherichia coli*. 2009;64:635–41.
33. Fernández-Reyes M, Vicente D, Gomariz M, Esnal O, Landa J, Oñate E, et al. High rate of fecal carriage of extended-spectrum-β-lactamase-producing *Escherichia coli* in healthy children in Gipuzkoa, northern Spain. *Antimicrob Agents Chemother*. 2014;58(3):1822–4.
34. Hijazi SM, Fawzi MA, Ali FM, Abd El Galil KH. Prevalence and characterization of extended-spectrum beta-lactamases producing *Enterobacteriaceae* in healthy children and associated risk factors. *Ann Clin Microbiol Antimicrob*. *BioMed Central*; 2016;15(1):3.
35. Tandé D, Jallot N, Bougoudogo F, Montagnon T, Gouriou S, Sizun J. Extended-spectrum β-lactamase- producing *Enterobacteriaceae* in malian orphanage. *Emerg Infect Dis*. 2009;15(3):472–4.
36. Farra A, Frank T, Tondeur L, Bata P, Gody JC, Onambele M, et al. High rate of faecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in healthy children in Bangui, Central African Republic. *Clin Microbiol Infect*. doi: 10.1016/ j.cmi.2016.07.001.

37. Stoesser N, Xayaheuang S, Vongsouvath M, Phommasone K, Elliott I, Del Ojo Elias C, et al. Colonization with *Enterobacteriaceae* producing ESBLs in children attending pre-school childcare facilities in the Lao People's Democratic Republic. *J Antimicrob Chemother*. 2014;70(6):1893–7.
38. Tellevik MG, Blomberg B, Kommedal Ø, Maselle SY, Langeland N, Moyo SJ. High prevalence of faecal carriage of esbl-producing *Enterobacteriaceae* among children in Dar es Salaam, Tanzania. *PLoS One*. 2016;11(12):1–13.
39. Wilmore SMS, Kranzer K, Williams A, Makamure B, Nhidza AF, Mayini J, et al. Carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in HIV-infected children in Zimbabwe. *J Med Microbiol*. 2017;66(5):609–15.
40. Andriatahina T, Randrianirina F, Hariniana ER, Talarmin A, Raobijaona H, Buisson Y, et al. High prevalence of fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a pediatric unit in Madagascar. *BMC Infect Dis*. 2010;10:204.
41. Desta K, Woldeamanuel Y, Azazh A, Mohammod H, Desalegn D, Shimelis D, et al. High Gastrointestinal Colonization Rate with *Enterobacteriaceae* in Hospitalized Patients : Emergence of Carbapenemase-Producing *K . pneumoniae* in Ethiopia. *PLoS ONE*. 11(8): 2016;1–14.
42. Central Statistical Agency of Ethiopia, 2015.
43. CLSI. Performance standards for antimicrobial susceptibility testing. Vol. 26th ed., CLSI supplement M100S.Wayne, PA: Clinical and Laboratory Standards Institute. 2016.
44. Magiorakos A, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. bacteria : an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2011;18(3):268–81.
45. Hall MAL, Dierikx CM, Stuart JC, Voets GM, Munckhof MP Van Den. Dutch patients , retail chicken meat and poultry share the same ESBL genes , plasmids and strains. *Clin Microbiol Infect*. 2011; 17:873-80.
46. Shu'aibu I, Hadiza JA, Yusha M, Kabiru MY, Ahmad MM, Lawal G, et al. Assessment of Foods and Drinks for the Presence of Extended Spectrum Beta Lactamase (ESBL) Producing Bacteria in Gombe Metropolis , Nigeria. *Indian Journal of Science and Technology*. 2016;9(48):1–9. DOI: 10.17485/ijst/2016/v9i48/109624.

47. Boekck HD, Miwanda B, Lunguya-Metila O, Muyembe-Tamfum J, Stobberingh E, Glupczynski Y,et al. ESBL-Positive *Enterobacteria* Isolates in Drinking Water . *Emerging Infectious Diseases.* 2012;18(6):1019–20.

48. Abera B, Kibret M, Mulu W. Antibiogram in *Enterobacteriaceae* from Clinical and Drinking Water Sources from Bahir Dar City , Ethiopia. *PLoS ONE*. 2016;34: 11(11):1–10. e0166519. doi:10.1371/journal.pone.0166519.

12 Annexes

12.1 Annex I: General information for parent/ guardian in English

Date.....

Greetings!

Introduction

Hello, how are you?

My name is …… and I am MSc student of Addis Ababa University, School of Medical laboratory Sciences. I am doing a research entitled "Extended Spectrum Beta-lactamase producing *E. coli* and *K. pneumoniae* carriage among under five children in Addis Raey Health Center, Addis Ababa, Ethiopia".

Purpose of the study: The objective of this study is to determine the prevalence of ESBL producing *E. coli* and *K. pneumoniae* carriage and to assess associated factors among under five years children at Addis Raey public health center, Addis Ababa, Ethiopia.

Duration: The duration of this study depend upon the availability of study subjects. It might take about one month or more.

Risk associated with the specimen collection: There is no risk associated with the specimen collection since the specimen is stool.

Procedure of the study: If you agree to participate in the study, you will provide stool specimen.

Confidentiality: All the data obtained will be kept strictly confidential and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names.

Benefit: There will not be any payment or direct benefit for participating and you are not asked to pay for the laboratory examination. The result will be given to you and if your result is clinically significant, it will help you for further diagnosis and treatment.

Withdrawal rights: Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You are free to refuse to participate in the study or you can withdraw your consent at any time, without giving reasons and this will not involve any penalty or loss of benefits to which you are entitled such as proper care and treatment. Your access to treatment

will not be dependent on your participation in the study. If you are not comfortable please feel free to stop it at any level of the study. I appreciate your cooperation to a great extent. If you have any question regarding to this study, the address of the principal investigator is:

Principal Investigator: Mekdes Alemu Tel: +251-911083384

Advisor: Mr. kassu Desta (MSc, PhD Fellow, Assistant Professor)

Tel: +251-911107099

Address of school: Tel: +251-112 75 51 70

12.2 Annex II: General information for parent/ guardian in Amharic

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12.3 Annex III: Parental consent form in English

I_____ parent, after being fully informed about the purpose of this study, Study title: **"**Extended Spectrum Beta-lactamase producing *E. coli* and *K. pneumoniae* carriage among under five children**"** at Addis Raey health centre, Addis Ababa, Ethiopia.

I, the undersigned, have been told about this research. My child has to say to choose if I want to be in the study. I have been informed there is no harm related to giving specimen. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

_____	_____	____/____/___
Name of adult parent	signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)		Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

12.4 Annex IV: Parental consent form in Amharic

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12.5 Annex V: Guardian consent form in English

I_____ guardian, after being fully informed about the purpose of this study, Study title: **"**Extended Spectrum Beta-lactamase producing *E. coli* and *K. pneumoniae* carriage among under five children**"** at Addis Raey Health Center, Addis Ababa, Ethiopia.

I, the undersigned, have been told about this research. My guardian has to say to choose if I want to be in the study. I have been informed there is no harm related to giving specimen. I have been informed that other people will not know my guardian results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my guardian. By signing below I agree to let my guardian to participate in this research study.

_____	_____	____/____/___
Name of guardian	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)	Signature	Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

12.6 Annex VI: Guardian consent form in Amharic

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12.7 Annex VII: English version of the questionnaire

The title of this study is **"**Extended Spectrum Beta-lactamase producing *E. coli* and *K. pneumoniae* carriage among under five children" attending Addis Raey health centre, Addis Ababa, Ethiopia.

Interview

We are gratefully for your agreement to participate in this study. Now we are going to have an interview with you and the interview is about general socio demographic characteristics and clinical data. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore we respectfully ask you to give us the right response.

Questioner Code: _____ Date□ _____

Name of Health Facility: _____

Child Card Number: _____

Unique Child Id: _____

Name of Interviewer: _____

Sr.No	Question		Answer	Skip
	Part One: Sociodemographic Data			
101	Age of child		_____months	
102	Sex of child		1.Male 2.Female	
103	Family size		_____	
104	Age of parent/guardian		Mother_____ Father _____ Guardian_____	
105	Parent/mother Educational level		1. Illiterate/cannot read and wrote/ 2. Illiterate /able to read and write/ 3. Primary 4. Secondary 5. College Graduate	
106	Parent Income (What is the average income in the house hold?)		1. <500 birr 3. 1000 - 2000birr 2. 500-1000birr 4. >2000birr	
Continue in next page				
107	Place of resident		1. Addis Ababa 2. Out of Addis Ababa	
108	House condition		1. Private 2. Government rental 3.Private rental	
109	Source of water for the child	Tap water	1.yes 2.No	
		Boiled & cooled water	1.yes 2.No	
		Bottled water	1.yes 2.No	
		Treated water	1.yes 2.No	
		Filtered water	1.yes 2.No	
110	Toilet use for the family		1. Private 2. Communal	
	Part Two: Child Medical History			
201	Weight		_____Kg	
202	MUAC --------cm		1. Normal 2. Moderate Acute Malnutrition (MAM) 3. Sever Acute Malnutrition(SAM)	
203	Place of birth		1. Home 2. Health center 3. Hospital 4.Private Clinic	

204	Mode of delivery	1. Normal delivery 2. Cesarean section	
205	Prior intake of antibiotics	1.Yes Review medical records ___ 2.No	
206	Hospital visit	1. Yes 2. No	
207	Previous hospital admission	1.Yes 2.No	
208	Previous surgery	1.Yes 2.No	
209	Does the child have diarrhea for the last three month?	1.Yes 2.No	
210	Number of visit	1.First 3.Third 2.Second 4.More than three	

MUAC: mid upper arm circumference

We would like to express our respect and gratitude to you for your interest and motive to participate in this study.

12.8 Annex VIII: Amharic version of the questionnaire

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12.9 Annex IX: Laboratory test Procedures

A. Specimen Collection

Stool specimen collection:

- Labeled stool cup was given to each participant by providing advisory service on how to collect fresh stool sample.
- Fresh stool were collected in clean container.
- Using applicator swab, rotate to take the stool.
- Transfer the applicator swab into the Cary-Blair transport medium.
- Label with the patient's code number.

Rectal Sample Collection:

- Insert the sterile applicator swab through the rectal sphincter 2-3 cm and gently rotate.
- Remove and examine to make sure there is fecal material visible on the tip of the swab.
- Transfer the swab into the Cary-Blair transport medium.
- Holding the end of the swab shaft, place against the rim of the tube and bend it to break
- Discard the broken upper part of the swab shaft and tighten the cap.
- Label with the patient's code number and send the sample to the laboratory.

Transportation: Fecal/rectal swab in Cary-Blair transport medium were transported to Ethiopian Public Health Institute Clinical Bacteriology and Mycology National Reference Laboratory.

B. Preparation of Cary Blair transport medium

The medium is prepared from ready to use dehydrated powder, available from most suppliers of culture media. Its contents are Sodium thioglycollate, Disodium phosphate, Sodium chloride, Calcium chloride and Agar.

1. Prepare as instructed by the manufacturer.
2. When the medium has cooled to 50–55 ^{o}C, mix well and dispense aseptically in sterile 7 to 10 ml amounts in wide mouth screw capped bottles. Date the medium and give it a batch number.
3. Store the plates at 2–8 ^{o}C.

C. Preparation of MacConky agar

The medium is prepared from ready to use dehydrated powder, available from most suppliers of culture media. Its contents are Peptone, lactose, bile salts, sodium chloride, neutral red and agar.

The medium is usually used at a concentration of 5.2 g for 100 ml distilled water.

4. Prepare as instructed by the manufacturer. Sterilize by autoclaving at 121 ^{o}C for 15 minutes.
5. When the medium has cooled to 50–55 ^{o}C, mix well and dispense aseptically in sterile Petri dishes. Date the medium and give it a batch number.
6. Store the plates at 2–8 ^{o}C preferably in plastic bags to prevent loss of moisture.
    - Shelf-life: Up to 4 weeks providing there is no change in the appearance of the medium to suggest contamination or an alteration of PH.
    - PH of medium should be within the range pH 7.2–7.6 at room temperature.

D. Inoculation and isolation

- Stool specimens/rectal swabs were inoculated in to MacConky agar aseptically.
- Incubated aerobically at 35-37^{o}C for 18-24 hours.
- Examined the culture for growth; bacteria isolation was based on their colon characteristics and lactose fermentation. The pure colonies were used for subsequent identification, AST and ESBL test by VITEK 2 Compact (bioMe´rieux, France).

E. Suspension preparation and testing procedure by VITEK 2 Compact

- Aseptically transfer 3.0 ml of sterile saline (0.45% to 0.5% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12mm x 75mm).
- Use a sterile stick or swab to transfer a sufficient number of morphologically similar colonies (pure culture) to the saline tube prepared in step 1.
- Prepare a homogenous organism suspension with a density equivalent to the appropriate McFarland standard (0.50 to 0.63) using a turbidity meter called the DensiChek^{TM}. NOTE: the age of the suspension before loading the instrument for AST testing must be less than 30 minutes.
- In a second tube containing 3.0ml of saline, transfer 145ul (for AST-GN cards) of the suspension prepared in step 2. Then place this tube in the cassette with a susceptibility card (AST-GN86 cards). The tube with the initial bacterial suspension can also be used for inoculation of an identification card (ID-GN).

- Fill in a cassette worksheet with the test card and specimen information for the cassette. Bar Code Scanner was used for data entry.
- Place the test cards and specimen test tubes in their appropriate slots.
- Load the cassette into the Filler Station (filling and sealing the test cards).
- Transfer the cassette to the VITEK 2 Compact cassette loading station within 10 minutes. (Test cards incubated and analyzed automatically)
- Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. (Accommodate up to 30 or up to 60 cards).
- All card types are incubated at35.5 +1.0 ^{0}c. Data are collected at 15-minute intervals during the entire incubation period.
- A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum.
- A special algorithm is used to eliminate false readings due to small bubbles that may be present.
- Calculations are performed on raw data and compared to thresholds to determine reactions for each test. .
- Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa.
- An unknown biopattern is compared to the database of reactions for each taxon, and a numerical probability calculation is performed. Various qualitative levels of identification are assigned based on the numerical probability calculation.

Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged.

Name of the principal investigator: Mekdes Alemu (BSc, MSc candidate)

Signature_____

Date of submission: _____

This thesis has been submitted with my approval as University advisor.

Name of advisor: Mr. Kassu Desta (MSc, PhD fellow, Assistant Professor)

Signature _____

Date _____

Place: Addis Ababa, Ethiopia.