ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES WITH SPECIAL EMPHASIS ON ENTEROBACTERIACEAE AMONG CHILDREN SUSPECTED FOR SEPTICEMIA AND URINARY TRACT INFECTION IN TIKUR ANBESSA UNIVERSITY HOSPITAL, ADDIS ABABA, ETHIOPIA.

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY SPECIALTY)

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This is to certify that the thesis prepared by Melese Hailu which is entitled with ***Prevalence and antimicrobial resistance of bacterial isolates with special emphasis on enterobacteriaceae among children suspected for septicemia and urinary tract infection in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.*** –And submitted in partial fulfillment of the requirements for the degree of Master of Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) 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

AAU----------Addis Ababa University

AST----------Antibiotic Susceptibility Test

ATCC ----------American Type Culture Collection

BD----------Becton Dickinson Company

BSIs----------Blood Stream Infections

CDC----------Communicable Disease Control

CDT----------Combination disk test

CLSI----------Clinical and Laboratory Standards Institute

CONS----------Coagulase negative Staphylococcus

CRE----------Carbapenem resistant enterobacteriaceae

DDST----------Double disk synergy test

EMA----------Ethiopian Medical Association

EMLA ----------Ethiopian Medical Laboratory Association

EPHA ----------Ethiopian Public Health Association

ESBL----------Extended spectrum beta-lactamase

EUCAST----------European Committee of Antimicrobial Susceptibility Testing

G3CR ----------Third generation cephalosporin-resistant

HIV ----------Human Immunodeficiency Virus

KPC----------*Klebsiella pneumoniae* carbapenemase

MDR----------Multidrug resistance

MHA----------Muller Hinton Agar

MHT----------Modified Hodge test

ML----------Milliliter

NCCLS---------- National Committee for Clinical Laboratory Standards

NDM-type ----------New Delhi Metallo-ß-lactamase–type

OPD----------Outpatient department

QC----------Quality control

SB ----------Significant Bacteriuria

SOPs ----------Standard Operating Procedures

SPSS ----------Statistical Package for the Social Sciences

USA----------United state of America

UTI----------Urinary Tract Infection

WHO ----------World Health Organization

Abstract

Background: Blood stream and urinary tract infections are a major cause of mortality and morbidity of the pediatric population. Extended spectrum beta-lactamase (ESBL) producing and carbapenem resistant enterobacteriaceae is the challenge for controlling now days. Assessing the prevalent bacteria and their antibiotic resistance helps to provide effective therapies, develop rational prescription programs and make policy decisions.

Objective: To determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on enterobacteriaceae among children suspected for septicemia and urinary tract infection in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Method: A cross sectional study was conducted from January 10 to March 30/2014 at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. A total of 322 study participants who were suspected for septicemia and/or UTI were recruited. All blood and urine samples were cultured on Blood and MacConkey agar. All culture positives were characterized by colony morphology, Gram stain and biochemical tests using the standard procedure. Significant bacteriuria was determined for all culture positive urine samples. Antimicrobial susceptibility test was performed for all bacterial isolates using Kirby-Bauer method. ESBL was detected using combination disk & double disk synergy methods on Muller Hinton agar. Carbapenemase were detected by Modified Hodge method using Meropenem. All demographic & laboratory data were entered to EPI INFO & exported to SPSS version 20 for analysis.

Result: The overall prevalence of bacteria isolates from blood and urine cultures was 17.1%. From 177 blood samples 13.0% (n=23/177) and from 145 urine samples 22.1% (n=32/145) were culture positives. *Coagulase negative Staphylococci* & *Klebsiella ozaenae* were the predominate bacteria isolated in blood and urine cultures respectively. Most of them 89.1% (n=49/55) developed multidrug resistance (MDR≥2 drugs) to most commonly used antibiotics. Multiple resistances were observed in 71.42% of Gram positive and 95.11% Gram negative isolates. Prevalence of ESBL producing and carbapenem resistant enterobacteriaceae was 78.57% and 12.12% respectively.

Conclusion: The choice of drugs in the treatment of bacteria isolates from blood and urine is quite narrow today due to the wide scale resistance to common antibiotics. The emergence of MDR calls for continuous monitoring & reviewing of antimicrobial policy in hospitals and the country at large.

Key terms: Septicemia, UTI, Bacterial isolates, antimicrobial resistance pattern, ESBL, Carbapenem resistance, Tikur Anbessa University Hospital, Addis Ababa, Ethiopia

1. Introduction

1.1. Background

Infectious diseases remain a major cause of debility and death around the world and are responsible for worsening of living conditions of millions of people. Bacterial infections continue to be an important cause of morbidity and mortality [1]. Bloodstream infection (BSI) caused by bacteria is one of the most important causes of morbidity and mortality throughout the world. The disease may be short and self limiting or may result in death or serious morbidity including admission to intensive care or prolonged hospital stay [2]. Continuous or transient presence of microorganism within the blood stream is Bacteremia while its dissemination throughout the body with evidence of systemic responses towards microorganism with variable severity is Septicemia [3]. Patient with septicemia present with fever, difficulty in breathing, tachycardia, malaise, refusal of foods or lethargy. It is a medical emergency that requires urgent rational antibiotics therapy [4].

Approximately 200,000 cases of bacteremia occur annually with mortality rates ranging from 20-50% worldwide [2]. Bacteremia is a common cause of morbidity and mortality in children worldwide which continues to be a serious problem that needs immediate attention and treatment [5]. In sub Saharan countries including Ethiopia septicemia is an important cause of illness and death in children, the mortality rate approaches 53% which makes it a significant health problem in developing countries [6]. The organisms responsible for bacteremia vary across geographical boundaries [7]. *E. coli* and *S. aurous* are the two commonest clinically significant causes of blood stream infection in the USA and Europe in contrast to *S. aurous, Klebsiella* species and *Salmonella* species in Sub-Saharan Africa [8]. Organisms like *E. coli, Klebsiella* species, S. *aureus, Coagulase negative staphylococci*, *Pseudomonas* species, *Salmonella* species and *Acinetobacter* species are potential pathogens in bacteremia because of their frequent isolation and multi-drug resistance which has reached worrying levels [9, 10]. Respiratory, genitourinary tract and intra-abdominal foci are identifiable sources of blood stream infections [11]. There is striking increase in incidence of bacteraemia caused by members of enterobacteriaceae since early 1950s and *E .coli* which was reported to be the commonest in the past is being replaced by other multidrug resistant bacteria like *Klebsiella, Enterobacter, Salmonella, Citrobacter,* etc [12].

Urinary tract infection (UTI) due to bacteria is another serious health problem and frequently encountered serious morbidity afflicting all segments of human population [13]. It involves proliferation of bacteria in the urinary tract resulting in infection of one or more parts of the urinary system such as the kidney, ureters, bladder or urethra [14].

Symptomatic UTI could be accompanied with a variety of clinical signs including dysuria, pyuria, strong urge to urinate frequently, even immediately after the bladder is emptied, painful burning sensation, discomfortable pressure and bloody urine, which may have a strong smell [15]. It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually [16]. The urinary tract is a common site of infection in the pediatric population. By seven years of age, 8 percent of girls and 2 percent of boys will have at least one episode [17].

Unlike the generally benign course of UTI in the adult population, UTI in the pediatric population is well recognized as a cause of acute morbidity and chronic medical conditions such as hypertension and renal insufficiency in adulthood [18]. Etiologic agents of UTI are variable and usually depend on time, geographical location and age of patients [19]. Common uropathogens include *E. coli, Klebsiella, Proteus, Enterobacter, Citrobacter, S. saprophyticus, S. agalacticus and Enterococci* [20].

Comparing to other groups of bacteria enterobacteriaceae are incriminated in virtually any type of infectious disease such as UTIs, BSIs, pneumonia peritonitis, cholangitis, and other intra-abdominal infections [1, 12, 21]. Immunocompromised patients are highly susceptible to environmental strains or following invasive such as catheterization, bronchoscopy, colposcopy or surgical biopsies [22].

Enterobacteriaceae infections resistant to extended-spectrum β-lactams are an emerging problem in children. Antibiotic resistance in Gram-negative bacteria has increased at an alarming pace over the last 2 decades particularly the emergence of enterobacteriaceae resistant to third-generation cephalosporins and aztreonam [23]. This resistance profile is commonly associated with the expression of extended-spectrum β-lactamases (ESBLs), a family of enzymes first identified in the mid-1980s that confer resistance to nearly all β-lactam antibiotics like ceftazidime, cefotaxime, monobactam-azteronam and related oxyimino β-lactams [24].

Carbapenems (e.g. ertapenem, imipenem, meropenem, and doripenem) are often the antimicrobials of last resort to treat infections due to extended-spectrum beta-lactamase (ESBL) or plasmid-mediated

AmpC (pAmpC)-producing organisms of the Enterobacteriaceae family. Carbapenems are crucial for the management of life-threatening healthcare-associated infections [25]. However, clinical utility of this group of antibiotic is under threat due to the recent emergence and spread of imipenem/meropenem-resistant enterobacteriaceae throughout the world. Resistance to carbapenems is mediated by mechanisms like loss of outer membrane proteins and production of carbapenemases that are capable of hydrolyzing the carbapenems. The growing incidence and also the diversity of carbapenemase producing strains is therefore a major concern [26].

There is often regional variability of pathogens and their susceptibility patterns, and these are capable of changing over time. Thus, determining the etiological agents and their antibiotic sensitivity patterns is needed to help in empirical treatment. A continuous review of antibiograms is also necessary to track changes in etiological agents and antimicrobial patterns [19].

1.2 Statement of the problem

Blood stream infections cause significant morbidity and mortality throughout the world [28]. Microorganisms present in circulating blood whether continuously or intermittently are a threat to every organ in the body [28] and associated with longer hospitalization and elevated cost [29]. Despite important progress in treatment and prevention of infectious diseases, they are considered as leading cause of death and disability [30].

Urinary tract infection is the most common infectious presentation in hospital acquired and community acquired infections since long time [31]. It is the most commonly encountered infectious diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million [32]. Nosocomial UTI is common following instrumentation namely, catheterization and cystoscopy [33]. Every woman has a 60% lifetime risk of developing bacterial cystitis, which develops mostly before the age of 24. By contrast, men have a lifetime risk of only 13%. In children approximately 5% of girls and 1% of boys have a UTI by 11 years of age [34].

It is well known that isolation of microorganisms is a gold standard for accurate detection of etiological agents of infectious diseases. Furthermore, early detection of bloodstream infections could prevent implantation of microorganisms into vital organs such as the brain, heart or kidneys [35]. Clinical assessment using a combination of symptoms and signs is a useful guide for the provisional diagnosis of septicemia. But bacteriologic culture to isolate the offending pathogen remains the mainstay of definitive diagnosis of septicemia [36]. The same is true for the diagnosis of urinary tract infections [37]. Due to constantly evolving antimicrobial resistant patterns there is the need for constant antimicrobial sensitivity surveillance [38]. Testing is required not only for therapy but also to monitor the spread of resistant organisms or resistance genes throughout the hospital and community [39].

Extended spectrum beta-lactamase producing microorganisms exhibit high level resistance to benzylpenicillins and narrow-spectrum cephalosporins such as ceftazidime, cefotaxime and ceftriaxone [40]. ESBL-producing pathogenic enterobacteriaceae poses a serious antibiotic management problem, as these genes are easily transferred from one organism to the other via plasmids. Many ESBL-producing bacteria are also resistant to other antimicrobial agents, namely, aminoglycosides, trimethoprim, and the quinolones [41]. This problem is more pronounced in areas where there is no

adequate infection control program, periodic surveillance and multidrug resistant bacteria detection laboratory facility [25, 26].

Carbapenem-resistant Enterobacteriaceae (CRE) due to carbapenemase production is being increasingly seen in clinical practice and has been reported worldwide over the past few years [25]. CRE are emerging as a major problem from the health care epidemiology stand point. Antimicrobial resistance in Gram negative bacilli is of increasing concern because of the lack of newer antibiotics to treat these infections [42].

Some studies have been conducted in Ethiopia on bacterial isolation and drug susceptibility patterns from different clinical samples at different area and showed that continuous assessment of the prevalent bacteria from different groups and testing their drug susceptibility is crucial to reduce their burden especially enterobacteriaceae which are the most frequent pathogens and the most important group which show high resistance to most antibiotics used [43, 44, 45, 46].

As the frequency and antibiotic sensitivity pattern to common pathogen has been changing day by day, choice of appropriate antibiotics depends on the knowledge of common organisms and their antimicrobial susceptibility pattern in local scenario [38]. Mainly information on the multidrug resistance pattern especially ESBL producers and carbapenem resistant enterobacteriaceae isolates is insufficient.

Hence the aim of this study was to determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on enterobacteriaceae among children suspected for septicemia and urinary tract infection in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

1.3 Significance of the study

As clearly indicated in the background and statement of the problem of this study bacteriological isolation is the mainstay to diagnose patients suspected for septicemia and urinary tract infections. Due to the increase in antimicrobial resistance especially multidrug resistance like enterobacteriaceae producing ESBL and carbapenemase choice of appropriate antibiotics depends on the knowledge of common organisms and their antimicrobial susceptibility pattern in local scenario.

This study provides current information on the prevalence of bacteria that cause septicemia and UTI among children. Most importantly this study indicates the current picture of antimicrobial resistance patterns on bacterial isolates from blood and urine cultures. This will help clinicians provide safe and effective empirical therapies, develop rational prescription programs and make policy decisions and finally assess the effectiveness of all.

Superiorly this study provides information on the prevalence of extended spectrum beta-lactamase producing and carbapenem resistant enterobacteriaceae. This will help for policy makers to amend or develop new infection control programs in local situations.

2 Literature Reviews

A bloodstream infection is a life-threatening condition that may be complicated by septic shock and death. BSI due to bacteria is an important cause of morbidity and mortality. Gram-negative bacteria have been associated with more deaths than Gram-positive bacteria. Mortality due to septic shock can be as high as 60% despite treatment. A better understanding of the spectrum of pathogens causing BSI is crucial for prompt management of patients, as antimicrobial therapy greatly influences the outcome of patients with BSI [47].

Urinary tract infection is most common which occurs in humans of all age groups in both genders as well as in hospital and community settings. It causes many urinary disorders as urosepsis, renal scarring and progressive kidney damage that lead to a high health risk with high mortality, morbidity and economic loss. Infections of urinary tract caused by microbial pathogens are classified primarily on the basis of infection site involved as cystitis in case of bladder, pyelonephritis in case of kidney and bacteriuria in case of urine. A wide range of microorganisms are involved in causing UTI, however, the most common among them is *E. coli* and other members of Enterobacteriaceae family which are estimated approximately 75% of the isolates [48].

A study conducted in India by Devanand *et al* [49] on antimicrobial susceptibility pattern of human pathogenic bacteria related to Enterobacteriaceae family causing urinary tract infection showed that the most common pathogens were *E. coli* (42.71%), *K. pneumoniae* (23.96%), *Proteus* species (19.79%) and *Enterobacter* species (13.54%). 90.24% *E. coli* showed resistance to Nalidixic acid, however, Amikacin showed 100% sensitivity to isolated *E. coli*. Ciprofloxacin and imipenem showed 69.57% resistance in *K. pneumonia*, however, Levofloxacin showed 100% sensitivity. Nitrofurantoin showed 92.30% resistance in *Enterobacter species* and most quinolones and carbenicillins was susceptible to *Enterobacter* species. *Proteus* species was 100% resistant against Third generation cephalosporin; however, carbepenems was highly sensitive to isolated *Proteus* species. Meropenem (90.63%) was most sensitive among all isolated UTI pathogens and Nalidixic acid showed 67.71% sensitivity among all isolates.

According to the retrospective study conducted in Pakistan by El-Jadba *et al* [50] on neonatal Septicemia in Gaza City Hospitals by El-Jadba etal showed that from 328 neonates out of 2487 cases had positive blood cultures with infections rate of 13.2%. *Coagulase negative Staphylococci, E. coli,*

and Klebsiella species were the most common isolated pathogens. These isolates were most sensitive to meropenem, amikacin, vancomycin, chloramphenicol, ciprofloxacin and third generation cephalosporine.

In Poland a study done by Katarzyna *et al* [51] on antibiotic susceptibility of bacterial strains isolated from urinary tract infections showed the most prevalent etiological agent was *E. coli* (73.0%), followed by *Proteus* species (8.9%) and other species of Enterobacteriaceae (9.6%). Few community infections were caused by Gram-positive bacteria (2.2%). Gram-positive cocci were isolated more frequently from a hospital setting (14.1%) and the most common were *Enterococci species* (8.5%). *P. aeroginosa* was found only among hospital isolates and was responsible for 0.7% of infections

Based on the study conducted by Sultan *et al* [26] on 2013 a total of 7192 enterobacteriaceae were screened for carbapenemase. Of these, 100 (1.39%) isolates were prospectively included in the study: Klebsiella pneumoniae 63 (63%); Escherichia coli 32 (32%); others 5 (5%). Out of the 100 isolates, 93 (93%) showed positive polymerase chain reaction results for New Delhi-Metallo-beta-lactamase (NDM-1) gene, and 69 (69%) isolates showed positive Modified Hodge Test. Four (5.8%) polymerase chain reaction negative isolates were found positive by Modified Hodge Test (false positive), which showed sensitivity of 42.8%, and specificity of 69.8% with a positive predictive value of 94.2% and a negative predictive value of 9.6%.

Based on the study conducted in Jordan by Mohammad [52] out of 4475 tested blood samples 378 isolates were recovered from blood cultures. The male to female isolate ratio was (1.26:1.0). The most frequent pathogen found was *S. aureus* (86.2%), followed by *Klebsiella* species (9%), *E. coli* (1.9%), *Streptococcus species* (1.9%), *Pseudomonas species* (0.8%), and *Acinetobacter* species was found in only one culture (0.3%).

Another study conducted in Saudi Arabia [53] showed that the most frequently isolated Gram-negative species were *E. coli* (9.1%), *Pseudomonas* species (8.7%), *Acinetobacter* species (7.8%), and *Klebsiella* species (6.7%). The results showed that 981 cases (60.3%) were male and 645 (39.7%) were female. The most age groups affected with septicemia were in infants and patients above 50 years old. The study also showed that the 3 departments generating the greatest number of positive cultures were; the intensive care unit with 585 out of 1626 (36%) cultures, the pediatric ward with 329 out of 1626 (20.2%) and the male medical ward with 272 out of 1626 (16.7%).

Based on the study conducted in Egypt by Al-Agamy *et al* [54] Imipenem, meropenem, colistin, tigecycline, and fosfomycin were the most active agents (susceptibility: 100%). Out of the 152 strains, 50% were resistant to piperacillin/tazobactam, 51.97% to ciprofloxacin, and 41.44% to gentamicin. The resistance rates for cefotaxime, aztreonam, cefepime, and amikacin were 22.36%, 21.71%, 21.71%, and 27.63%, respectively. Over 96%, 85.5% and 71.7% of the isolates were non-susceptible to amoxicillin/ clavulanic acid, piperacillin and Cefoxitin, respectively. Of the 152 E. coli isolates, 31 (20.4%) were confirmed to be ESBL producers.

According to the study conducted in Nigeria by Akingbade *et al* [4] the highest number of bacteria was found among patient age ≤ 10 years. *E. coli* accounted for 12(46.2%) of the bacteria isolated while *P. aeroginosa, Klebsiella* species *and S. pneumoniae* accounted for 6(23.1%), 6(23.1%) and 2(7.6%) respectively. *E. coli* were susceptible to Ceftazidime (83.3%), levoxin (66.7%), but were 100% resistance to cloxacillin, cotrimoxazole and tetracycline. The *Pseudomonas aeroginosa* isolates were 100% resistance to ampicillin, amoxicillin, cloxacillin, cotrimoxazole and tetracycline but 66.7% resistance were recorded to augmentin, ceftriaxone, ciprofloxacin, erythromycin and gentamycin (66.7%). *Klebsiella sp* were resistance to Ampicillin, Amoxicillin, Augmentin, Cefuroxime, Cloxacillin, Cotrimoxazole, Erythromycin, Gentamycin, Streptomycin and Tetracyline (100%) and were highly sensitive to Cefuroxime, Ceftazidime and Ofloxacin (66.7%).

In Ethiopia based on the study conducted by Mulat *et al* [6] showed that out of 390 bloods culture results, 71 (18.2%) were culture positives. The predominant bacteria isolated from blood culture were Coagulase negative *staphylococci* 30 (42.3%), followed by *S. aureus 17 (23.9%) and Klebiesella* species *9 (12.9%), E. coli 5 (7.0%), P. aeroginosa 4 (5.6%) and Salmonella* species 3 (4.2%). The Gram positive and Gram negative bacteria constituted 49 (69%) and 22 (31%) of the culture isolates; respectively. The finding indicates that the isolates showed high rates of resistance to most antibiotics tested. And the study also showed that the range of resistance for Gram positive and Gram negative were from 23.5% – 58.8%, and 20%– 100% respectively.

Another study conducted in Gondar by Yitayal *et al* [55] showed that from the 975 UTI suspected patients, 250(25.6%) was positive for significant bacteriuria. *E. coli* was the most predominant Gram negative bacteria and *S. aureus* from Gram positive bacteria. Chloramphenicol (62.3), ciprofloxacin (50.9%) and Norfloxacin (50%) were effective drugs for *E. coli* and ciprofloxacin (61.9%) and ampicillin and Norfloxacin (57.1%) for *S. aureus* compared to other tested drugs.

Based on another study conducted in Gondar by Amare *et al* [56]on bacterial profile and drug susceptibility patterns of neonatal septicemia, 58 of them showed positive blood culture for bacteria with infection rate of 32.1% from a total of 181 neonates (99 male and 82 female). The most common bacterial isolate was *S. aureus* (29.3%) followed by *K. ozaenae* (17.2%), *E. coli* (10.3%), non lactose fermenters Gram negatives (10.3%), *K. pneumonia* (8.6%) and coagulase negative *staphylococci* (6.8%). *K. ozaenae* showed extremely high level of resistance against amoxicillin (10, 100%), chloramphenicol (10, 100%), ampicillin (9, 90%) and tetracycline (7, 70%). Multiple drug resistance was observed in 92.1% of Gram negative isolates. There is exceedingly high rate of resistance of bacterial isolates to different antibiotics commonly prescribed.

In Dessie based on the study conducted by Mulugeta *et al* [57] the overall prevalence of the uropathogens was 319 (22.7%). And majority of the pathogens were isolated from females with isolation rate of 27.1% and 14.1% (95% CI=0.14) were from males. In the age group between 26 to 44 years of age *E. coli* was the most predominant pathogen isolated from urine samples with prevalence of 203 (63.6%). *Klebsiella* species, *Proteus* species, *Pseudomonas* species, *coagulase negative staphylococci* (CONS), *S. aureus, Enterobacter* species and *Citrobacter* species accounted for 36.4% of the isolates. Erythromycin had the highest overall resistance of 85.6%, followed by amoxicillin (83.9%) and tetracycline (76.7%). Nitrofurantoin, gentamycin and ciprofloxacin had overall resistance rates of 5.5%, 24.3% and 29.4%, respectively. The majority (96.2%) of *E. coli* isolates were obtained susceptible to nitrofurantoin with resistance rate of 3.8%. Other isolates were sensitive to gentamycin and ciprofloxacin with resistance rates of 24%-35% and 0%- 40%, respectively.

According to the study conducted in Jimma by Tizazu *et al* [58] on invasive bacterial pathogens and their antibiotic susceptibility patterns in Jimma Hospital showed from the total of 260 blood specimens 23(8.8%) were positive to seven different types of bacteria. The isolated bacteria were *Coagulase negative staphylococci* 6(26.1%), *S. aureus* 5 (21.7%), *S. pyogens* 3 (13.0%), *E. coli* 4(17.4%), *K. pneumoniae* 3(13.0%), *Salmonella* species 1(4.3%), and *Citrobacter* species 1(4.3%). The isolates showed high rates of resistance to most antibiotics tested. The range of resistance for Gram positive bacteria were 0% to 85.7%, and for Gram negative from 0% to 100%. None of the isolates were resistance to ciprofloxacin and ceftriaxone.

Another study conducted in Jimma by Getenet *et al* [59] on bacterial uropathogens in urinary tract infection and their antibiotic susceptibility pattern indicated that significant bacteria were detected from

9.2% of the total patients. The most common pathogens isolated were *E. coli* (33.3%), *K. pneumoniae* (19%) and *S. saprophyticus* (14.3%). *E. coli* and *K. pneumoniae* showed the highest percentage of resistance to ampicillin and amoxicillin (100%) however, all isolates of *E. coli* and *K. pneumoniae* were susceptible to ciprofloxacin. *S. saprophyticus* and *S. aureus* were resistant to ampicillin (100%) and amoxicillin (66.7%). For all UTI isolates, least resistance was observed against drugs such as ceftriaxone, gentamycin and chloramphenicol.

The study conducted in Addis Ababa by Shitaye *et al* [60] *showed* that out of the 302 neonates, 135(44.7%) were positive for blood culture. The most common isolated organisms were *Klebsiella spp*. (39.2%) and *Staphylococcus aureus* (22.2%). Gram positive bacteria were susceptible to most antimicrobial agents tested. On the other hand Gram-negative bacteria showed high-level resistance to ampicillin, ceftriaxone, cephalothin, chloramphenicol, and gentamycin. Multiple resistances (resistance to two or more drugs) were observed in 45.7% and 84.2% Gram positive and Gram negative bacteria respectively ($p < 0.05$).

According to the study conducted in Ethiopia by Seid *et al* [61] on the occurrence of extended-spectrum beta lactamase enzymes in clinical isolates of *Klebsiella species* from Harar region of the 57 *Klebsiella* isolates, in the 19(33.3%) isolates ESBL production was detected. The result also showed that ESBL producers where multidrug resistant (95%) than non producers (53%).

3 Objective

3.1 General Objective

- To determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on Enterobacteriaceae among children suspected for septicemia and urinary tract infection in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

3.2 Specific Objectives

- To assess the prevalence of bacterial isolates among septicemia and UTI suspected children.
- To determine antimicrobial resistance pattern of bacterial isolates among septicemia and UTI suspected children to the commonly used antibiotics.
- To determine the prevalence of extended spectrum beta-lactamase (ESBL) producing enterobacteriaceae by comparing double disk synergy and combination disk methods.
- To determine the prevalence of Carbapenem resistant enterobacteriaceae (CRE) among septicemia and UTI suspected children.

4 Materials and Methods

4.1 Study area

The study was conducted at Tikur Anbessa University Hospital which is found in Lideta Sub-city, Addis Ababa, Ethiopia. In 1998 it was handed to Addis Ababa University by the Ministry of Health as a teaching hospital for the medical faculty. The faculty is the largest and the oldest among health training institutions in the country, staffed with the most senior specialists. It is now the main teaching hospital for both clinical and preclinical trainings of most disciplines and it is where specialized services are rendered. The Hospital provides tertiary level referral treatment and is known to be open 24 hours for emergency services. The hospital is estimated to also offer diagnosis and treatment for approximately 370,000-400,000 patients yearly with 800 beds, 130 specialists and 50 non teaching doctors. The emergency department sees around 80,000 patients a year.

4.2 Study Design and Period

A Hospital based cross-sectional study was conducted from January 10/2014 to March 30/2014.

4.3 Source Population

- All children who seek medical service at Tikur Anbessa University Hospital during the study period.

4.4 Study Population

- All children suspected for septicemia and/or UTI and who visit the study site during the study period was the study population.

4.5 Inclusions and Exclusions criteria

4.5.1 Inclusions Criteria

- All children less than 15 years during the study period.
- All children who gave blood and/or urine sample and their parents or guardian volunteer to give consent to participate on the study.

4.5.2 Exclusion Criteria

- Patients who took antibiotics currently within the last10 days.

4.6 Sample Size

The sample size was calculated based on single sample size estimation as shown below [62]. The value of p was taken as 25.6% (0.256) from the study conducted by Yitayal *et al* 2013 on antimicrobial susceptibility pattern of bacteria isolates from urine of urinary tract infection patients in Northwest Ethiopia [55].

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion (P = 0.5), and d = precision (d = 0.05), Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

$$\frac{(1.96)^2 \times 0.256(1-0.256)}{(0.05)\,2} = \frac{292.67.}{} = 293.$$ The 10 % non response rate is 29.3.

Therefore the sample size will be: 322

4.7 Sampling Method and procedure

A total of 322 study participants were recruited using convenient sampling technique.

4.8 Study Variables

4.8.1 Dependent Variables

- Prevalence of bacterial isolates
- Antibiotic Susceptibility pattern of bacterial isolates
- Prevalence of extended spectrum beta-lactamase producing enterobacteriaceae.
- Prevalence of Carbapenem resistant enterobacteriaceae

4.8.2 Independent Variables

- Age
- Gender
- Non-hospitalization (Outpatient)
- Hospitalization (inpatient)

4.9 Data Collection

4.9.1 Socio-demographic data

After obtaining informed consent socio-demographic data of children were collected from their parents or guardians.

4.9.2 Sample Collection

Blood and urine samples were collected from children after informed consent was obtained from their parents or guardians.

I. Blood Sample

A three to five ml of blood collected from the study participant then added to blood culture bottle containing brain heart infusion (Oxoid, England) by physician or nurse and sent to laboratory was used.

II. Urine Sample

Mid-stream first morning urine samples collected from suspected cases of UTI using sterile wide mouth container and sent to laboratory were used. The study participants‘ parents or guardians were given appropriate instruction before providing urine samples.

4.9.3 Culture and identification

Blood culture bottle was incubated at 37^{o}C and was inspected daily for the sign of bacterial growth. Turbid blood samples before the seventh day and the none-turbid blood samples on the seventh day from blood culture bottle were sub-cultured on blood agar (Oxoid, England) and MacConkey agar (BD, USA) at 37^{o}C for 24 hours.

All urine samples were inoculated to blood agar (Oxoid, England) and MacConkey agar (BD, USA) and were incubated at 37^{o}C for 24 hours. Colony type count was done on blood agar, and then significant bacteriuria (SB) was determined on MacConkey agar.

All positive cultures from blood and urine samples were characterized by colony characteristics, Gram stain and biochemical tests as outlined in Annex….I

4.9.4 Drug Susceptibility Patterns

The disk diffusion method was performed and after 16-18hours of incubation at 37^{o}C zone of inhibition was measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI) [63]. Using a sterile wire loop, 3-5 pure colonies were picked from blood for Gram positive and MacConkey agar for Gram negative and emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland was swabbed onto Muller-Hinton agar (dispensed on 100mm plate). Drug susceptibility testing of all bacteria isolates was performed using disk diffusion method incubating at 37^{o}C for 24 hours against Amoxicillin (30µg, BD), Amoxicillin-Clavulanic acid (30µg, BD), Chloramphenicol (30µg, BD), Gentamycin (10µg, BD), TMP-SXT (1.25µg, BD), Cefotaxime (30µg, BD), Cefoxitin (30µg, Oxoid), Tetracycline (30µg, BD), Nitrofurantoin (300µg, BD) and Norfloxacin (5µg, BD). Additionally all Gram positive and Gram negative isolates were tested for oxacillin (5µg, BD) and Carbapenem drugs (Imipenem (10µg, Oxoid) and Meropenem (10µg, Oxoid)) respectively. The zone of inhibition was measured to the nearest millimeter and isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI [63]. High, intermediate and low level of resistance was interpreted when the percentage of resistance was >80%, 60-80% and<60% respectively [55].

4.9.5 Extended spectrum beta-lactamase detection

Initial screening for ESBL was done by the diameters of zones of inhibition produced by Ceftazidime (30µg), Ceftriaxone (30µg) and Cefotaxime (30µg) found to be within the CLSI screening criteria. These breakpoints indicative of suspicion for ESBL production are: for CAZ≤22mm, CRO, ≤ 25 mm and for CTX≤ 27mm. Phenotypic detection of ESBL production was confirmed by double disk synergy test and combination (double disk potentiate) test according to EUCAST [64] and CLSI [63] guidelines respectively.

4.9.5.1 Combined disk (double disk potentiate) Test(CDT)

A Ceftazidime (30 µg) disk and Cefotaxime (30µg) dick were used alone and their combination with Clavulanic acid (30 µg/10 µg) for phenotypic confirmation of the presence of ESBLs. A ≥5 mm increase in zone diameter for either of the Cephalosporin disks and their respective Cephalosporin/Clavulanate disk was interpreted as ESBL producer. This method (according to CLSI) was used as reference phenotypic method for comparing double disk synergy method.

4.9.5.2 Double Disk Synergy Test(DDST)

The organism to be tested was spread onto a Mueller–Hinton agar plate. The antibiotic disks used were Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Aztreonam (30µg) and Amoxicillin/ Clavulanic acid (20/10 µg). The four antibiotics were placed at distances of 20 mm (edge to edge) from the Amoxycillin/Clavulanic acid disk that was placed in the middle of the plate. After 24-h incubation, if an enhanced zone of inhibition between either of the cephalosporin antibiotics and the Amoxicillin/Clavulanic acid disk occurred, the test was considered positive.

4.9.6 Carbapenem Resistant Detection

4.9.6.1 Modified Hodge test (MHT)

All the Carbapenem (imipenem or Meropenem) resistant or intermediate isolates were checked for the presence of carbapenemase using MHT, also known as the clover leaf test as per the EUCAST and CLSI. Presence of indentation indicates a positive test and the isolate is a carbapenemase producing strain. No growth of the ATCC *E. coli* 25922 along the organism growth streak indicates a negative test and the isolate is not a carbapenemase producer.

4.9.7 Quality Control

Standard Operating Procedures (SOP) were strictly followed verifying that media meet expiration date and quality control parameters per CLSI. Visual inspections of cracks in media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination was performed. QC was performed to check the quality of medium. Each new lots were quality controlled before use by testing the *E. coli* ATCC 25922 and/or *Staphylococcus aurous* ATCC 25923 standard strains. For ESBL, ESBL-positive *K. pneumoniae* ATCC 700603 and ESBL negative *E. coli* ATCC 25922 control strains were used in this study. *E. coli* ATCC 25922 control strains was used as negative control for Carbapenem resistant detection.

4.10 Statistical analysis and interpretation

The data was entered in to EPI INFO and double checked before analysis. Then the data was exported to SPSS version 20 for analysis. The descriptive statistics (mean, percentages or frequency) was calculated. The bi-variant logistic regression analysis was used to see the relation between dependent variable and independent variables. The association was assessed by using the Chi square test. Variables that showed a significant association were selected for further analysis using multiple logistic

regression models with a p-value < 0.05 as statistically significant. Finally, the results were presented in words, charts, graphs and tables.

4.11 Data quality Assurance

Socio-demographic characteristics of the patient were collected appropriately after getting consent form which was prepared carefully. Samples were collected in accordance with SOPs and were going soon for analysis. Culture results were recorded carefully before entry to statistical tool. Before analysis the data was double checked.

4.12 Operational definitions

i. **Significant Bacteriuria (SB)** is defined as the presence of more than 10^5 bacteria per milliliter of urine obtained by sterile technique.

ii. **Multidrug Resistance(MDR)** is a bacterium that is simultaneously resistant for two or more antimicrobials belonging to different chemical classes

4.13 Ethical clearance

The study was approved by ―Department Research and Ethical Review Committee(DRERC)" of the Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. Permission letter was also obtained from the study site. The purpose and procedures of the study was explained to the study participants' parents or guardians within the study period. Those children whose parents or guardians gave informed consent were selected and enrolled as the participants of the study. A patient result was communicated to the attending physicians.

5 Results

5.1 Socio-demographic characteristics

Three hundred twenty two (n=322) septicemia and urinary tract infection suspected children were investigated during the study period. One hundred seventy seven (55%) of them were suspected for septicemia and 145(45%) were suspected for UTI. Of these patients, 53.4% (n=172/322) were males and 46.6% (n=150/322) were females with males to females ratio 1.15:1. The majority of patients 98(30.4%) were between 1-6 years of age as shown in figure 5.1 and the mean (std. deviation) ages of patients were 3.22(1.229) with age range of 0- 15 years. 38.5% (n=124/322) were out patients while the remaining 61.5% (n=198/322) were inpatients. Socio-demographic characteristics of patients have shown in table 5.1.

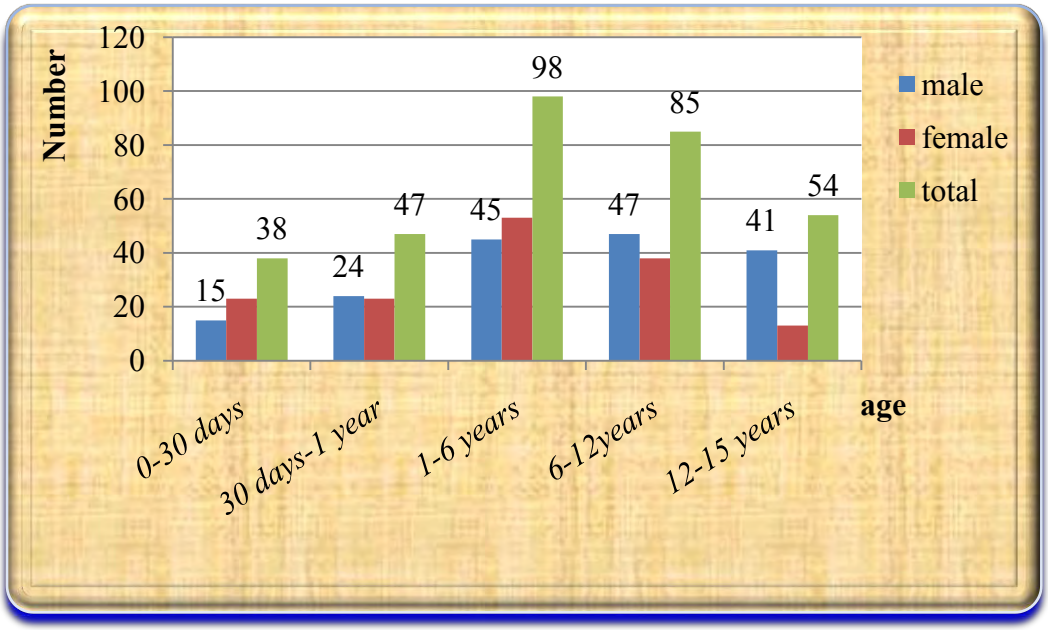


Figure 5.1 A bar graph showing age and sex distribution of septicemia and UTI suspected children at Tikur Anbessa University Hospital from January 10/2014 to March 30/ 2014.

Table 5.1 Socio-demographic characteristics of septicemia and UTI suspected children and their culture results at Tikur Anbessa University Hospital from January 10 to March 30/ 2014.

Variable		Culture results					
		Septicemia (n=177)			UTI (n=145)		
		Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**
Gender	Male	15(14.7)	87(85.3)	102(100)	16(22.9)	54(77.1)	70(100)
	Female	8(10.7)	67(89.3)	75(100)	16(21.3)	59(78.7)	75(100)
	Total	23	154	177	32	113	145
Age in Year	0-30days	5(15.6)	27(84.4)	32(100)	3(50.0)	3(50.0)	6(100)
	30days-1	5(20.8)	19(79.2)	24(100)	5(21.7)	18(78.3)	23(100)
	1-6	6(12.5)	42(87.5)	48(100)	9(18.0)	41(82.0)	50(100)
	6-12	4(9.3)	39(90.7)	43(100)	11(26.2)	31(73.8)	42(100)
	12-15	3(10.0)	27(90.0)	30(100)	4(16.7)	20(83.3)	24(100)
	Total	23	154	177	32	113	145
Patient Type	Outpatient	1(2.1)	46(97.9)	47(100)	10(13.0)	67(87.0)	77(100)
	Inpatient	22(16.9)	108(83.1)	130(100)	22(32.4)	46(67.6)	68(100)
	Total	**23**	**154**	**177**	**32**	**113**	**145**

OPD= Outpatient department

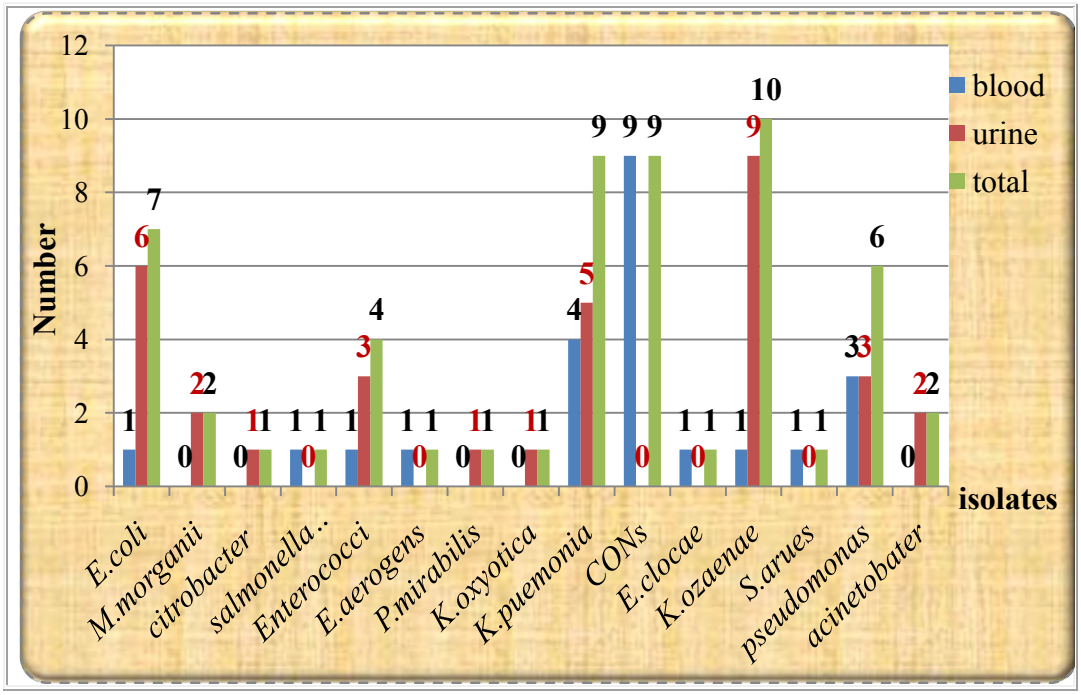
5.2 Prevalence of bacterial Isolates from septicemia and UTIs suspected children

The overall prevalence of bacteria isolates from blood and urine cultures of septicemia and UTI suspected children were 17.1% (n=55/322). From the 55% (n=177/322) of blood samples 13.0% (n=23/177) were culture positive while from the 45% (n=145/322) urine samples 22.1% (n=32/145) were culture positive. 56.36% (n=31/55) of the culture positives were from males and 43.64% (n=24/55) were from females. Gram positive and Gram negative isolates constitutes 25.5% (n=14/55) and 74.5% (n=41/55) respectively with Gram positives to Gram negatives ratio of 0.34:1. The predominant bacteria isolated from cultures were *Klebiesella ozaenae* 18.2% (n=10/55), *CONS* 16.4% (n=9/55) and *Klebiesella pneumoniae* 16.4% (n=9/55).

The spectrum of BSI and UTI varies with the age of patients (table 5.2). 20.3% of BSI and UTI were found in infants who share the highest proportion of sepsis and UTI patients. However there was no significant association between age of patient and culture result (OR=0.84, 95%CI=0.314-2.65, P = 0.695). In this study 80% (n=44/55) of bacteria were isolated from hospitalized patients while the remaining 20% (n=11/55) were from those who attended outpatient department; and there was a significant association between being out patient or inpatient with blood and urine culture results (OR=2.935, 95%CI=1.452-5.934, P = 0.003).

Coagulase negative Staphylococcus 39.1% (n=9/23) was the most isolated from blood cultures followed by *k. pneumonia* 17.4% (n=4/23). Other isolates in blood cultures were *S. aureus* 4.3% (n=1/23), *salmonella* species 4.3% (n=1/23), *Enterobacter cloacae* 4.3% (n=1/23), *K. ozaenae* 4.3% (n=1/23), *Enterococci* 4.3% (n=1/23) and *Enterobacter aerogens* 4.3% (n=1/23). While in urine cultures *K. ozaenae* 28.1% (n=9/32) was the dominant isolated bacteria followed by *K. pneumonia* 15.6% (n=5/32). Other UTI isolates were *Pseudomonas* 9.4% (n=3/32), *Enterococci* 9.4% (n=3/32), *Morganella morganii* 6.3% (n=2/32), *Acinetobacter* 6.3% (n=2/32), *citrobacter* 3.1% (n=1/32), *Enterobacter cloacae* 3.1% (n=1/32), *K. oxyotica* 3.1% (n=1/32), and *Proteus mirabilis* 3.1% (n=1/32).

Among the bacteria isolates the family enterobacteriaceae were the most frequent bacterial group isolated 60% (n=33/55) both from blood and urine cultures. *K. ozaenae* 30.3% (n=10/33) was the dominant enterobacteriaceae isolated followed by *K. pneumonia* 27.27% (n=9/33).



CONs-----------*coagulase negative staphylococci*

Figure 5.2 A bar graph showing frequency and types of bacterial isolates from septicemia and UTI suspected children at Tikur Anbessa University Hospital from January 10/2013-March 30/2014.

Table 5.2 Association of independent variables with culture results among children at Tikur Anbessa Hospital from January 10-March 30/2014

Variables		Culture Results							
		Positive	**Negative**	**P-value**	**OR**	**95%CI**	**P-value**	**AOR**	**95%CI**
Gender	Male	31(18.0)	141(82.0)	0.631	0.87	[0.483-1.554]			
	Female	24(16.0)	24(84)	NA					
Age in Year	0-30days	8(21.1)	30(78.9)	0.305	0.56	[0.18-0.1700]			
	30days-1	10(21.3)	37(78.7)	0.269	0.55	[0.191-1.587]			
	1-6	15(15.3)	83(84.7)	0.695	0.84	[0.314-2.65]			
	6-12	15(17.6)	70(82.4)	0.462	0.7	[0.263-1.834]			
	12-15	7(13.0)	47(87.0)	1					
Patient Type	**Outpatient**	11(8.9)	113(91.1)	1					
	Inpatient	44(22.2)	154(77.8)	0.003	2.94	[1.452-5.934]	0.003	3.012	1.469-6.200

OR---------Odds ratio, CI----------Confidence Interval, AOR---Adjusted odds ratio,

NA-------Not applicable

5.3 Antibiotic Resistance patterns

Among the total isolates (n=55) multidrug resistance (MDR ≥2 drugs) were recorded in 49(89.1%) of all bacterial isolates. Gram positive and Gram negative isolates showed 71.42% (n=10/14) and 95.11% (n=39/41) multiple drug resistance respectively.

Antimicrobial resistance level for the Gram positive isolates causing blood stream and urinary tract infections range from 0-100% with multiple drug resistance of 71.42% (n=10/14). Among the Gram positive bacteria the predominant isolate *coagulase negative staphylococci* 69.2% (n=9/14) showed 66.67% (n=6/9) of MDR. It demonstrated intermediate level of resistance to amoxicillin (77.8%), Cefotaxime (77.8%), Sulphamethoxazole-Trimethoprim (77.8%), and Tetracycline (66.7%). Better susceptibility can be achieved using Nitrofurantoin (87.5%), Norfloxacin (77.8%), Amoxicillin-clavulanic acid (77.8%), oxacillin (75%) and Cefoxitin (71.4%) compared to other tested drugs.

In the same manner the resistance patterns of Gram-negative organisms causing blood stream and urinary tract infection were ranging from 0 to 100% and they showed 39(95.11%) multiple drug resistance. All isolates showed high level of resistance (>80%) for amoxicillin, Sulphamethoxazole-Trimethoprim and Cefotaxime, intermediate level of resistance (60-80%) to Amoxicillin-clavulanic acid, chloramphenicol, gentamycin, Tetracycline and low level of resistance for Cefoxitin, Norfloxacin and Nitrofurantoin, Imipenem and Meropenem. Among the Gram negative bacteria the predominant isolates *k. ozaenae* 24.39% (n=10/41) demonstrates high level of resistance to Cefotaxime (100%), amoxicillin (90%), Amoxicillin-clavulanic acid (90%), Sulphamethoxazole-Trimethoprim (80%), Gentamycin (80%) and Tetracycline (90%). It showed 9(90%) of multidrug resistance level. Better susceptibility can be achieved using Norfloxacin (80%), Imipenem (80%), Meropenem (70%), Cefoxitin (50%), and Nitrofurantoin (50%) compared to other tested drugs (table 5.3).

In this study from the members of Enterobacteriaceae isolated *K. ozaenae* 30.3% (n=10/33), *K. Pneumoniae* 27.3% (n=9/27), *M. morganii* 6.1% (n=2/33), *citrobacter* 3% (n=1/33), *E. aeroginosa* 3% (n=1/33) and E. *cloacae* 3% (n=1/33) showed 100% resistance to Cefotaxime (third generation cephalosporin) while *E. coli* 6(18.2%) showed intermediate level of resistance to Cefotaxime (66.7%). *Morganella morganii* were 100% (n=2/2) resistant to Imipenem but Meropenem contrarily showed 100% (n=2/2) sensitivity. In addition *K. ozaenae* showed relatively high resistance to Carbapenem drugs of Imipenem 20% (n=2/10) and Meropenem 30% (n=3/10) compared to other enterobacteriaceae isolates (Table 5.3).

Table 5.3 Antimicrobial resistance levels of bacterial isolates from blood and urine cultures among children at Tikur Anbessa University Hospital from January 10/2014-March 30/2014.

Organism isolated	Tested	Antimicrobial resistance level in no.(%)of bacterial isolates in blood and urine												
		AML	**AMC**	**SXT**	**FOX**	**C**	**CTX**	**GE**	**NOR**	**TE**	**FN**	**OX**	**IMI**	**MEM**
E. coli	6	6(100)	6(100)	6(100)	3(50)	4(66.7	4(66.7	4(66.7)	4(66.7	5(83.3)	0(0)	NA	0(0)	0(0)
M. morganii	2	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	NA	2(100)	0(0)
Citrobacter	1	1(100)	1(100)	I(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	NA	0(0)	0(0)
Salmonella	1	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	NA	0(00	0(0)
Enterococci	4	2(50.0	2(50)	2(50)	2(50)	4(100)	3(75)	2(50)	2(50)	2(50)	2(50)	2(50)	NA	NA
E. aerogens	1	1(100)	1(100)	1(100)	1(100)	0(100)	1(100)	1(100)	1(100)	1(100)	0(0)	NA	0(0)	0(0)
P. mirabilis	1	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	NA	0(0)	0(0)
K. oxyotica	1	1(100)	1(100)	1(100)	1(100)	0(00)	1(100)	0(0)	0(0)	1(100)	1(100	NA	0(0)	1(100
K. pneumoniae	9	9(100)	8(88.9)	8(88.9)	2(22.2)	6(66.7)	9(100)	9(100)	2(22.2)	5(55.6)	5(55.6)	NA	0(0)	0(0)
CONS	9	7(77.8)	2(22.2)	7(77.8)	2(28.6)	4(44.4)	7(77.8)	3(33.3)	2(22.2)	6(66.7)	2(12.5)	2(25)	NA	NA
E. cloacae	1	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	NA	0(0)	0(0)
K. ozaenae	10	9(90)	9(90)	8(80)	5(50)	5(50)	10(10 0)	8(80)	1(10)	9(90)	4(40)	NA	2(20)	3(30)
S. aureus	1	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	1(10 0)	NA	NA
Pseudomonas	6	6(100)	6(100)	6(100)	5(83.3)	5(83.3)	5(83.3)	5(83.3)	3(50)	5(83.3)	4(80)	NA	0(0)	1(16.7)
Acinetobacter	2	2(100)	2(100)	2(0)	1(50)	1(50	2(100)	2(100	0(0)	1(50)	2(100	NA	1(50	1(50)
Total	**55**	**49(89. 1)**	**41(74. 5)**	**46(83. 6)**	**25(48. 1)**	**34(61. 8)**	**47(85. 5)**	**39(70.9)**	**19(34. 5)**	**40(72.7)**	**21(39. 6)**	**5(38. 5)**	**5(12.2)**	**6(14.6)**

NA --Not applicable: AML--Amoxycillin, AmC--Amoxycillin-Clavulanic acid, SXT—Sulphamethoxazol-trimethoperem, FOX--Cefoxitin, C--Chloramphenicol, CTX--Cefotaxime, GM--Gentamycin, TE--Tetracycline, FN—Nitrofurantoin, NOR--Norfloxacin, OX--Oxacillin, IMI--Imipenem, MEM--Meropenem

Table 5.4 Multidrug resistance pattern of bacteria isolates from blood and urine among children at Tikur Anbessa University Hospital from January 10-March 30/2014

Gram negatives	**Organism isolated**	**Number Tested**	**(MDR≥2 drugs) No.(%)**
	E. coli	6	6(100)
	M. morganii	2	2(100)
	Citrobacter	1	1(100)
	Salmonella	1	1(100)
	E. aerogens	1	1(100)
	P. mirabilis	1	0(0)
	K. oxyotica	1	1(100)
	K. pneumoniae	9	9(100)
	E. cloacae	1	1(100)
	K. ozaenae	10	9(90)
	Pseudomonas	6	6(100)
	Acinetobacter	2	2(100)
	Total	**41**	**39(95.11%)**
Gram positives	*CONS*	9	6(66.67)
	Enterococci	4	3(75.0)
	S. aureus	1	1(100)
	Total	**14**	**10(71.48)**
Total		**55**	**49(89.1)**

CONs------*Coagulase negative Staphylococci,* MDR-----multidrug resistance

5.4 Extended spectrum Beta-lactamase producing Enterobacteriaceae

Thirty three (33) enterobacteriaceae have been isolated from 322 septicemias and UTIs suspected children. These isolates were *K. ozaenae* 30.3% (n=10/33), *K. pneumonia* 27.27% (n=9/33), *E. coli* 18.2% (n=6/33), *Morganella morganii* 6.1% (n=2/33), *Enterobacter aerogens* 3.0% (n=1/33), *Enterobacter cloacae* 3.0% (n=1/33), *Citrobacter* 3.0% (n=1/33), S*almonella* 3.0% (n=1/33), *K. oxyotica* 3.0% (n=1/33), and *Proteus mirabilis* 3.0% (n=1/33). However *Morganella morganii* 6.1% (n=2/33), *Enterobacter aerogens* 3.0% (n=1/33), *Enterobacter cloacae* 3.0% (n=1/33), and *Citrobacter* 3.0% (n=1/33) were excluded from further screening for ESBL though they were suspected because as these methods were not validated for these groups. To do so cefepime alone and with clavulanic acid combination is required as the guideline recommends it but was not available.

Therefore 28 enterobacteriaceae isolates were included however 3 of them were not suspected for ESBL which were *E. coli* (n=1), *Salmonella* (n=1) and *P. mirabilis* (n=1). Hence 25 enterobacteriaceae were tested for ESBLs using the phenotypic confirmatory combination disk method according to CLSI [63]. Double disk synergy procedure was done according to EUCAST [64]. Distribution of enterobacteriaceae isolated in blood and urine sample in relation to patient type has shown in table 5.5 below.

Table 5.5 Frequency of enterobacteriaceae isolated from blood and urine accordingly patient type at Tikur Anbessa Hospital from January 10-March 30/2014

		Enterobacteriaceae										
		K. ozaenae	***K. pneumoniae***	***E. coli***	***K. oxyotica***	***M. morganii***	***E. aerogens***	***E. cloacae***	***Citrobacter***	***Salmonella***	***P. mirabilis***	Total
Sample	**Blood**	1	4	1	0	0	1	1	0	1	0	9
	Urine	9	5	5	1	2	0	0	1	0	1	24
	Total	10	9	6	1	2	1	1	1	1	1	33
Patient	**Outpatient**	4	1	4	0	0	0	0	0	0	0	9
	Inpatient	6	8	2	1	2	1	1	1	1	1	24
	Total	**10**	**9**	**6**	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**33**

5.4.1 Combination (Disk Potentiating) Disk method

The overall prevalence of ESBL producing enterobacteriaceae was 78.57% (n=22/28). Among the suspected 25 isolates 88% (n=22/25) were phenotypically confirmed for ESBL using combination disk method. *K. ozaenae* 90% (n=9/10), *K. pneumonia* 77.8% (n=7/9), *E. coli* 100% (n= 5/5) and *K. oxyotica* 100% (n=1/1) were positive for ESBL (figure 5.5). For result interpretation we use this result as the CLSI recommend this technique as reference for other phenotypic methods. We also use this test result to compare the findings of double disk method.

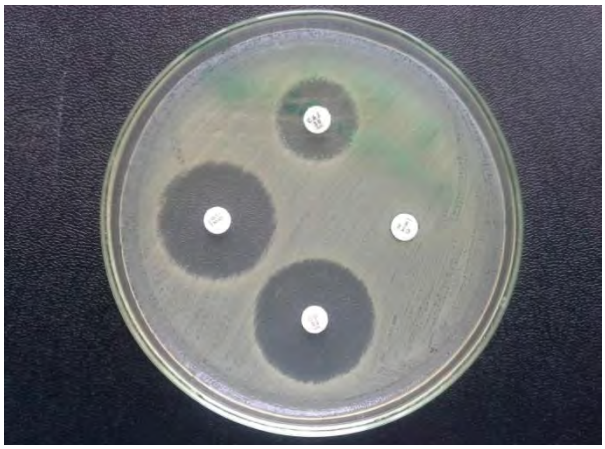


Figure 5.3 ESBL positive enterobacteriaceae using combination disk method isolated from children suspected for septicemia and UTI at Tikur Anbessa Hospital from January 10-March 2014.

5.4.2 Double Disk Synergy Test (DDST)

All isolates (n=25) were further tested for ESBL production by double disk synergy procedure, another phenotypic confirmatory method. The double disk method indicated 84% (n=21/25) were confirmed for ESBL with an overall 75% prevalence of ESBL producing enterobacteriaceae. From the 100% (n=22/22) which were positive by the reference (CDT) method, 90.90% (n=20/22) were positive by this method while only 9.1% (n=2/22) were negative. *E. coli* 100% (n=5/5), *K. ozaenae* 8/10(80%), *K. pneumonia* 77.8% (n=7/9), and *K. oxyotica* 100% (n=1/1) were ESBL positive by this method. Only16% (n=4/25) of the enterobacteriaceae were confirmed as ESBL negative by this method from the ESBL suspected enterobacteriaceae (figure 5.6).

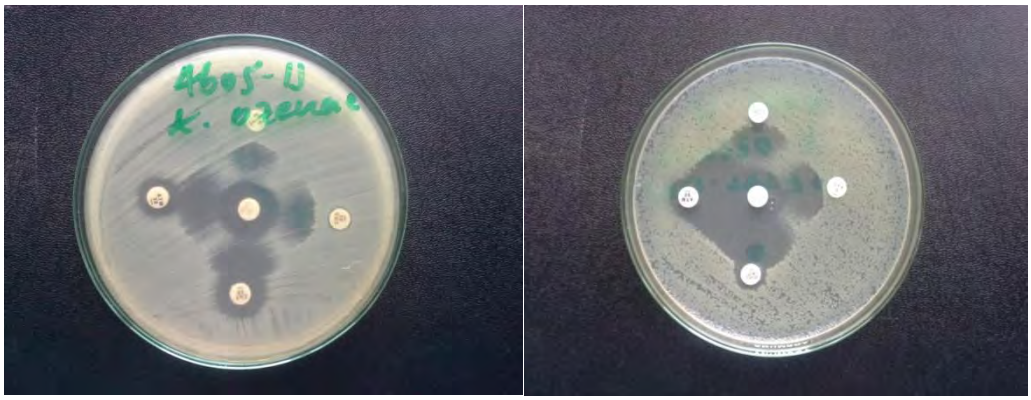


Figure 5.4 ESBL positive enterobacteriaceae using double disk synergy method isolated from children suspected for septicemia and UTI at Tikur Anbessa Hospital from January 10-March 2014.

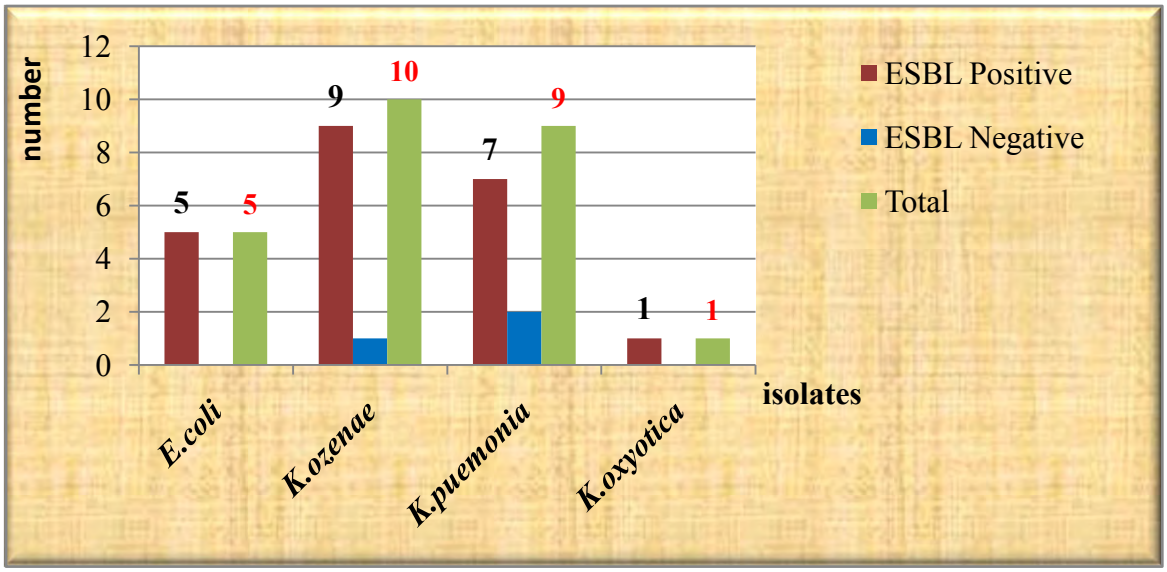


Figure 5.5 A bar chart indicting frequency of ESBL positive enterobacteriaceae from blood and urine cultures among children by Combination disk method at Tikur Anbessa Hospital from January 10-March 2014.

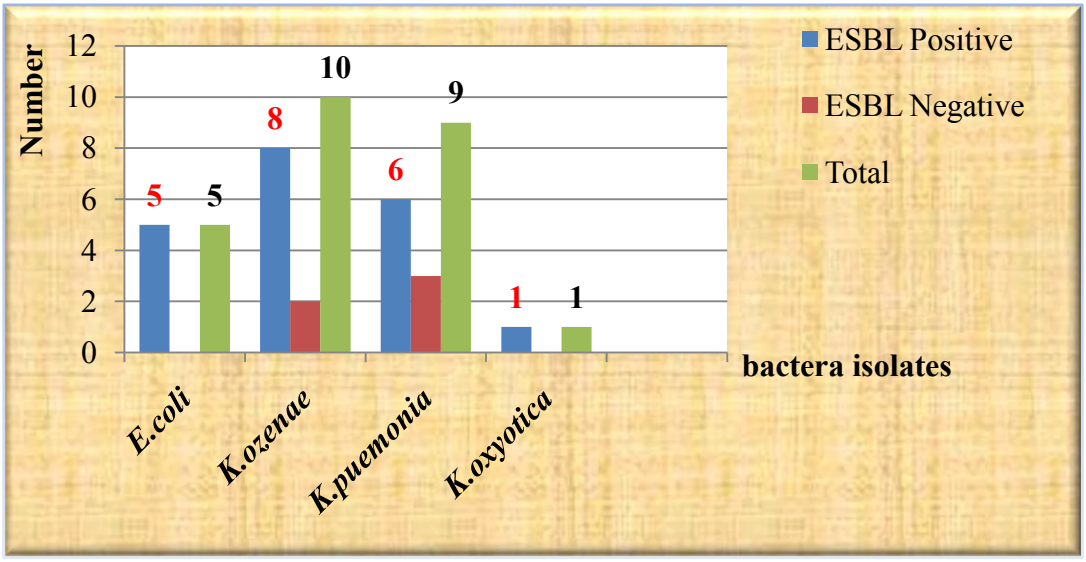


Figure 5.6 A bar chart indicating frequency of ESBL positive enterobacteriaceae from blood and urine cultures among children by Double Disk Synergy method at Tikur Anbessa Hospital from January 10-March 2014.

5.5 Carbapenem resistant enterobacteriaceae

Among the 33 isolated enterobacteriaceae 18.2% (n=6/33) of them showed intermediate or resistance to imipenem and/or Meropenem then they were suspicious for Carbapenem resistance. They were subjected to be confirmed phenotypically by using Modified Hodge Test (MHT). All bacteria were isolated from urine samples of UTI suspected inpatient children and 66.67% (n=4/6) of them were from male and 33.33% (n=2/6) where from female patient. *K. ozaenae* 50% (n=3/6), *Morganella morganii* 16.67% (n=2/6), *K. oxyotica* 16.67% (n=1/6) were carbapenem resistance suspected isolates. All isolates except *Morganella morganii* (suspected but not confirmed) were positive for ESBL as confirmed phenotypically by using combination disk. Norfloxacin was sensitive for all *K. ozaenae* and *K. oxyotica*. However *Morganella morganii* showed susceptibility only to one of the Carbapenem drugs one for Imipenem and the other one for Meropenem.

Out of a total 6 isolates which were showing resistant or intermediate zone for Meropenem or imipenem 66.7% (n=4/6) were positive for carbapenemase production by Modified Hodge Test having a general prevalence of 12.12% (n=4/33) from the total enterobacteriaceae isolated. The predominant Carbapenemase producing organisms in this study were *Klebsiella species* 9.09% (n=3/33) which were *K. ozaenae* 20% (n=2/10) and *K. oxyotica* 100% (n=1/1). The second carbapenemase confirmed enterobacteriaceae were *Morganella morganii* 3.03% (n=1/33).

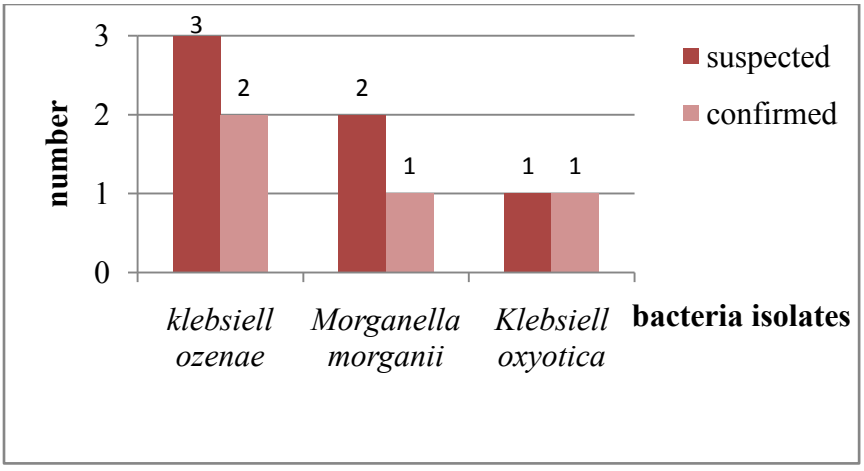


Figure 5.7 A bar graph indicating frequency of carbapenem resistant enterobacteriaceae isolated from blood and urine culture among children at Tikur Anbessa University Hospital from January 10-March 2014.

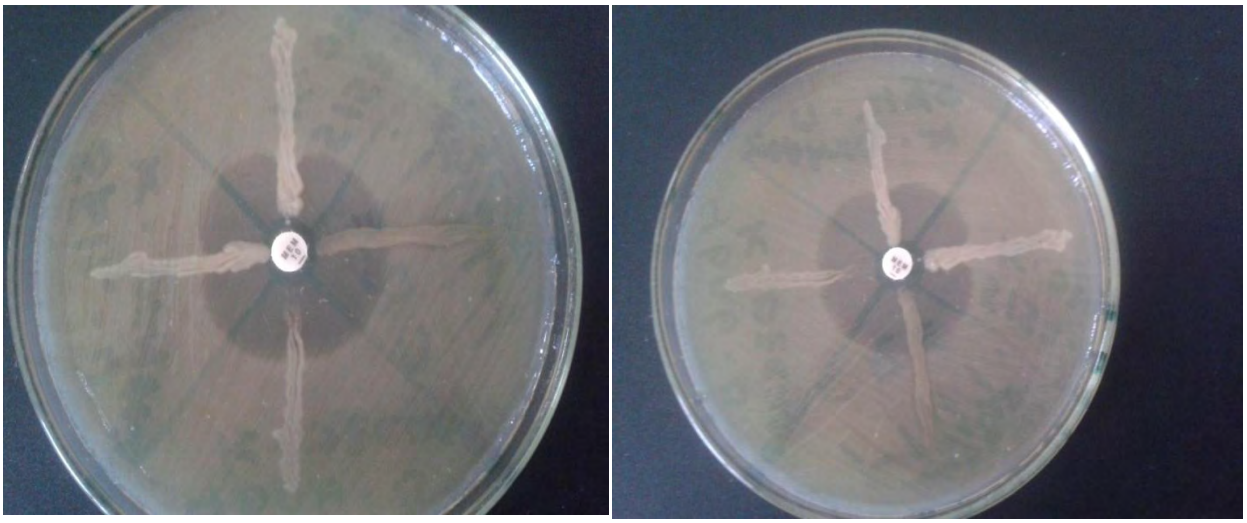


Figure 5.8 Carbapenem resistant enterobacteriaceae using Modified Hodge method isolated from children suspected for septicemia and UTI at Tikur Anbessa University Hospital from January 10-March 2014.

6 Discussion

6.1 Prevalence of bacteria isolates among septicemia and UTI suspected children

Infectious diseases remain a major cause of debility and death around the world and are responsible for worsening of living conditions of millions of people. Bacteremia and UTIs are common causes of morbidity and mortality in children worldwide which continues to be a serious problem that needs immediate attention and treatment [5, 13, 18]. The prevalence of bacteria that cause septicemia and UTI varies across geographical boundaries [7]. Due to the rising antimicrobial resistance of many bacteria treatment as well as control is becoming very difficult now days [24, 25]. Hence this study was undertaken and results demonstrated the profile of microbial isolates causing septicemia and UTI with their susceptibility pattern to most commonly used antimicrobial agents and also ESBL producing and Carbapenem resistant enterobacteriaceae.

The overall prevalence 17.1% (n=55/322) of bacteria isolated from blood and urine culture of septicemia and UTI suspected children was almost similar with what had been previously reported in Ethiopia (18.2%, 22.7%) [6, 57] and Nigeria (21.7%) [4]. However, this finding was relatively lower than studies done in Gondar (25.6%, 32.1%) [55, 56] and much lower than studies done previously in Addis Ababa Ethiopia (44.7%) [60]. On the other hand, our finding was higher than studies done at another part of Ethiopia in Jimma and also in Gondar which were 8.8% and 9.2% [58, 59] and in Pakistan was 13.2% [50]. The possible explanation for the difference could be the difference in methodology used, the study design, nature of patient population, epidemiological difference of the etiological agents, and seasonal variation.

In this study 80% (n=44/55) of bacteria were isolated from hospitalized patients while the remaining 20% (n=11/55) were from those who attended outpatient department; and there was a significant association between being out patient or inpatient with culture results (OR=2.935, 95% CI =1.452-5.934, P = 0.003). This is somehow supported by the result from Jimma which showed relatively higher prevalence of bacterial isolated from hospitalized patients than patients attending outpatient department [6]. Multivariable analysis showed that hospitalization was an independent risk factor for blood stream and UTI infections. In this study inpatients are 3 times more likely to develop blood stream and UTI infections than outpatients (Adjusted OR, 3.012; 95% CI, 1.469-6.200, P=0.003).

In the present study 25.5% (n=14/55) of infections were caused by Gram-positive and 74.5% (n=41/55) by Gram-negative bacteria with Gram positives to Gram negatives ratio of 0.34:1. The predominant bacteria isolated from cultures were *Klebiesella ozaenae* 18.2% (n=10/55), *CONS* 16.4% (n=9/55) and *Klebiesella pneumoniae* 16.4% (n=9/55) in the present study. This study has established that septicemia affects nearly all targeted (0-15years) groups but it was observed that infants were more vulnerable than other pediatric groups and has an agreement with other study [53]. This is may be due to they accounted for the majority (20.8%) of the patients that had culture proven septicemia in this study.

The predominant etiological agents in the blood cultures in our study were Gram positive organisms. It conforms to other studies [36, 50, 56]. *Coagulase negative staphylococci* 39.1% (n=9/23) were the most commonly isolated bacteria in the blood cultures and this has been also found in other studies [6, 50, 58, 59]. Reports from Gondar [6] revealed that (42.3%) *CONS* were isolated; which is similar to our finding. To the contrary, this finding was higher than other studies done in Gondar, Ethiopia (6.8%), Jimma Ethiopia (26.1%) [56, 58] and India (12.3%) [36]. *CONS*s have been long regarded as non pathogenic but their important role as pathogens and their increasing incidence in different infections like bacteremia related to indwelling devices, central nervous systems shunt infections, native or prosthetic valve endocarditis and UTIs had been reported and it is also an important cause of morbidity and mortality in immunocompromised individuals [58].

In the present study, we observed a gradual but definite rise in isolation of frequency of *Klebsiella pneumoniae* 4(17.4%) among Gram negative bacilli in blood stream infections which is similar to other studies [5, 36, 52, 58]. This finding is also in line with the previous study done in Addis Ababa [60]. However this showed disagreement with study done in Nigeria, the predominant bacteria are *E. coli* and *S. aureus* [8]. *S. aureus* 4.3% (n=1/23) has been isolated in low frequency contradicting to other studies as it was reported as the common isolated bacteria in BSIs [2, 4, 12]. *E. coli* were reported to be the most common Gram-negative organisms isolated from blood stream infections in many studies [8] however we found lowered prevalence of *E. coli* 4.3% (n=1/23). This is indicated by other studies that showed *E. coli* is replaced by other Gram negative bacilli like *Klebsiella species* [6, 12]. The high occurrence of non-lactose fermenters (*Pseudomonas* and *Acinetobacter species*) was often concern as these bacteria are associated with high degree of resistance to many antibiotics (MDR=100% (n=8/8)). Other isolates in blood cultures were *salmonella species* 4.3% (n=1/23), *Enterobacter cloacae* 4.3%

(n=1/23), *K. ozaenae* 4.3% (n=1/23), *Enterococci* 4.3% (n=1/23) and *Enterobacter aerogens* 4.3% (n=1/23).

From the 45% (n=145/322) urine samples 22.1% (n=32/145) were culture positive which is in line with the findings of other study in Gondar (22.7%) [57]. And also it was a similar result with other findings of 25.6% [55] however it was higher than the study conducted in Jimma which recorded 9.2% [59]. The present finding was lower than other studies in different parts of the world (36.68%, 71.72%, 84.12%) [13, 16, 34]. In our study the spectrum of UTI varies with the age of patients and age groups of 1-6years were the most UTI suspected group in this study however higher frequency 50% (n=3/6) was seen in age group of 0-30days. UTI in neonates may be non-specific and with no localization.

In urine culture the predominant etiological agents in our study were Gram negative organisms 90.6% (n=29/32) while Gram positive accounts small proportion 9.4% (n=3/32). It showed similarity with other studies [51, 55, 57, 59]. This could be due to the presence of unique structure in Gram negative bacteria which help for attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion – resulting in invasive infection and pyelonephritis [58].

The predominant bacteria isolated in urine were *K. ozaenae* 28.1% (n=9/32). This finding disagrees with other studies in which *E. coli* were the predominant bacteria and *Klebsiella species* hold the second rank in urine culture [16, 49, 51, 55]. Difference in identification methods are known to influence the relative prevalence of bacteria which makes comparison difficult [57]. Bacterial etiologies of UTI can show geographic variation and may even vary over time within a population [57] and our finding could be signal as the most UTI causing organism *E. coli* is replaced by another enterobacteriaceae *Klebsiella species*(*K. ozaenae*). The second dominant isolates were *E. coli* 15.6% (n=5/32) and *K. pneumonia* 15.6 (n=5/32%) in equal proportion from urine cultures. There are also similar reports from the different parts of the world [37]. Other UTI isolates were *Enterococci* 9.4% (n=3/32), *Pseudomonas* 9.4% (n=3/32), *Morganella morganii* 6.3% (n=2/32), *Acinetobacter* 6.3% (n=2/32), *citrobacter* 3.1% (n=1/32), *Enterobacter cloacae* 3.1% (n=1/32), *K. oxyotica* 3.1% (n=1/32) and *Proteus mirabilis* 3.1% (n=1/32).

6.2 Antimicrobial resistance patterns of bacterial isolates

Antibiotic resistance is a major clinical problem in treating infections which has increased over the years and resistance rates vary from place to place even [2, 31]. Antimicrobial resistance among septicemia and UTI isolated pathogens to the commonly used antibiotics become increasing that make clinicians with very few choices of drugs for the treatment of sepsis and urinary tract infections [55]. Table 5.3 showed the result of antimicrobial resistance pattern of the isolate.

A study of in vitro antimicrobial susceptibility profile of the aetiological agents of septicemia and UTI has revealed that there is a growing emergence of multidrug resistant microbes (MDR≥2 drugs). Table 5.4 showed the pattern of multidrug resistance level of Gram positives and Gram negatives. Overall multidrug resistance were recorded in 89.1% (n=49/55) of all bacterial isolates. Our finding is relatively lower than what has been recorded in Gondar which is 95.2% [55]. However the present result was higher than compared to other study in our country from Dessie that report 47.85% of MDR bacteria isolates [57].

In our study Gram positive and Gram negative bacteria showed 71.48% (n=10/14) and 95.11% (n=39/41) multiple drug resistance respectively. It showed similarity with the study done in Gondar, Ethiopia [55]. The present finding is different from the result of previous study at Tikur Anbessa University Hospital in which 45.7% and 84.2% multiple drug resistances were recorded in Gram positives and Gram negatives bacteria respectively [60]. This showed that antimicrobial resistance is changing unexpectedly to higher level which raises serious concern. Therefore the reasons for this alarming phenomenon might suggest a very high resistance gene pool due to gross misuse and inappropriate usage of the antibacterial agents [6], inappropriate and incorrect administration of antimicrobial agents in empirical therapies and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organisms in the community [55].

Antimicrobial resistance levels for the Gram positive and Gram negative isolates range from 0-100% most of them showing multiple drug resistance which is comparatively similar with the study findings in Jimma which is 0-85.7% for Gram positive and 0-100% for Gram negative isolates[58]. However a study done in Gondar shows a lower and narrow range of antimicrobial resistance for Gram positives (23.5%-58.8%) and a relative narrow range of antimicrobial resistance for Gram negative 20%-100% [6]. Most of the Gram negatives showed high to intermediate resistance level for most of tested drugs

which were Amoxicillin (89.1%), Cefotaxime (85.5%), Sulphamethoxazole-Trimethoprim (83.6%), Amoxicillin-clavulanic acid (74.5%), Tetracycline (72.7%), Gentamycin (70.9%) and chloramphenicol (61.8%). Lower resistance level was seen for Cefoxitin (48.1%), Nitrofurantoin (39.6%), Norfloxacin (34.5%), Meropenem (14.6%) and Imipenem (12.2%).

Amoxicillin (89.1%) showed the highest level of resistance for all blood and urine isolates. This is almost a similar finding with a study in Gondar (93.5%) [44]. It is also supported by the record of amoxicillin resistance 83.9% from Jimma [57]. However a lower resistance level was observed in other study from Gondar (64%) [55] and India (69%) [34]. In our study all most all the predominant bacteria amazingly develop 100% resistance level for amoxicillin which were *Klebsiella pneumonia* 100% (n=9/9), *Pseudomonas* 100% (n=6/6), *E. coli* 100% (n=6/6) and *Klebsiella ozaenae* 90% (n=9/10) follows.

Cefotaxime (85.5%) is the second drug that showed high level of resistance following amoxicillin in this study. This result has an agreement with other studies [20] with resistance of 83%. However a lower resistance level was observed in India (37.5%, 46.15%) [32,36]. In the same manner to amoxicillin all most all the predominant bacteria develop 100% resistance level for Cefotaxime which are *Klebsiella ozaenae* 100% (n=10/10), *Klebsiella pneumonia* 100% (n=9/9), *Pseudomonas* 100% (6/6) , *Acinetobacter* 100%(n=2/2) and *E. coli* 4(66.7%) follows. Though this is a little bit higher finding it is supported by other study of which cefotaxime resistance for *Klebsiella* and *E. coli* was 88% and 87% respectively in India [39]. 100% resistance of *Klebsiella species* to cefotaxime is also recorded in Nigeria [4]. This is mainly due to the selective pressure created to the third generation cephalosporin agents [61].

Among the common drugs Norfloxacin (63.9%) and Cefoxitin (50%) have a better susceptibility for most of multidrug resistant bacteria isolated in our study as compared to other drugs tested. Nitrofurantoin (47.3%) was also an option which has lowered resistance level of 39.6%. There is concordance with other studies [19, 32, 37, 55]. However a lower resistance to Norfloxacin and nitrofurantoin was reported in India 27.27% and 18.88% respectively [34].

Carbapenem drugs (Imipenem and Meropenem) have resistance level of 12.2% and 14.69% respectively with 2.4% of intermediate level for each. Higher imipenem resistance 18.75% and 22.5% than our result were recorded in studies from India and Saudi Arabia respectively [49, 65]. However this imipenem resistance level is a higher finding as compared to other reports from different countries, other study in

India where the resistance of imipenem is 0% [31] and in Brazil [29], resistance of imipenem was 8.2%. In cases of Meropenem (14.69%) the present result is higher than as compared to other study as 0% resistance recorded [54]. However this result is lower than what had been found in other study which was 26.08% [38]. Since these drugs were not sold in our country and also they were expensive, this probably had restricted their procurement and indiscriminate use, therefore making the organisms susceptible to it.

However their resistance level showed that antimicrobial resistance is becoming our concern today as they are the last resort for the treatment of enterobacteriaceae even though the resistance level was lowered as compared to other drugs. Still these drugs are excellent for treatment of MDR enterobacteriaceae however they were expensive and not accessible for most patients in our country for patient diagnosis. Meanwhile knowing the resistance level of these drugs is important for epidemiological purpose.

Among the Gram negative bacteria the predominant isolates *k. ozaenae; showed* 90% (n=9/10) of MDR, demonstrates high level of resistance to Cefotaxime (100%), amoxicillin (90%), Amoxicillin-clavulanic acid (90%), Sulphamethoxazole-Trimethoprim (80%), Gentamycin (80%) and Tetracycline (90%). It has an agreement with the study done in Gondar, Ethiopia [56] and in Nigeria [4]. Better susceptibility can be achieved using Norfloxacin (80%), Imipenem (80%), Meropenem (70%), Cefoxitin (50%), and Nitrofurantoin (50%) compared to other tested drugs. From the Gram positive bacteria the predominant isolate *CONs; showed* 66.67% (n=6/9) of MDR, demonstrates intermediate level of resistance to amoxicillin (77.8%), Cefotaxime (77.8%), Sulphamethoxazole-Trimethoprim (77.8%), and Tetracycline (66.7%). Better susceptibility can be achieved using Nitrofurantoin (87.5%), Norfloxacin (77.8%), Amoxicillin-clavulanic acid (77.8%), oxacillin (75%) and Cefoxitin (71.4%) compared to other tested drugs. Similar result has been documented in other studies [58].

The present study showed that most of isolated pathogens were highly resistant to most commonly used antibiotics. It is quite serious problem that most of the organisms included in the study from blood and UTI found resistant to multiple drugs. This ineffectiveness of drugs against these organisms in our study could be the reflection of frequent prescription and also the misuse of these drugs by the physicians. The findings have no doubt there is an urgent need for constant monitoring of susceptibility of pathogens in different populations to commonly used anti-microbial agents.

6.3 Prevalence of ESBL producing enterobacteriaceae

Extended-spectrum beta-lactamases (ESBLs) are a heterogeneous group of plasmid-mediated bacterial enzymes that confer significant resistance to oxyimino-cephalosporin and monobactam antimicrobials [66]. In recent time‘s emergence of ESBL-producing pathogenic enterobacteriaceae poses a serious antibiotic management problem, as these genes are easily transferred from one organism to the other via plasmids [41].

ESBL-producing microorganisms exhibit high level of resistance to benzylpenicillins and narrow-spectrum cephalosporins such as ceftazidime, cefotaxime and ceftriaxone. Many ESBL-producing bacteria are also resistant to other antimicrobial agents, namely, aminoglycosides, trimethoprim, and the quinolones [40]. ESBL infections are concerning for many reasons, including increased hospital costs, length of stay, and mortality rates. Among children isolation of third generation cephalosporin-resistant (G3CR) and presumed ESBL-producing enterobacteriaceae is becoming more common across patient settings and pediatric age groups [31, 40, 41, 66].

Worldwide ESBL prevalence in community and hospital widely varies [31]. This new development should alert the clinical microbiologist to devise ways and means of readily identifying these ESBL-producing organisms and instituting appropriate therapy. This is all the more important because resistance to one of the extended-spectrum cephalosporins (ceftazidime, cefotaxime, or ceftriaxone), when mediated by an ESBL, means therapeutic resistance to all even when sensitivity test results may indicate otherwise [40, 66]. Over the past decade, several studies have assessed the occurrence of ESBLs among enterobacteriaceae recovered from the community and hospitalized patients [31, 65, 66].

In our study among the 55 isolated bacteria from 322 septicemia and UTI suspected children 33 of them were enterobacteriaceae. Theses isolates were *K. ozaenae* 30.3% (n=10/33), *K. pneumonia* 27.27% (n=9/33), *E. coli* 18.2% (n=6/33), *Morganella morganii* 6.1% (n=2/33), *K. oxyotica* 3% (n=1/33), *Enterobacter aerogens* 3.0% (1/33), *Enterobacter cloacae* 3.0% (n=1/33), *Citrobacter* 3.0% (n=1/33), S*almonella* 3.0% (n=1/33)and *Proteus mirabilis* 3.0% (n=1/33). However *Morganella morganii* 6.1% (n=2/33), *Enterobacter aerogens* 3.0% (n=1/33), *Enterobacter cloacae* 3.0% (n=1/33) and *Citrobacter* 3.0% (n=1/33) were excluded from further testing for ESBL though they were suspected because these methods were not validated for these groups. To do so cefepime alone and with clavulanic acid combination is required as the guideline recommends it but was not available. Therefore 28

enterobacteriaceae isolates were included for further ESBL testing however 3 of them were not suspected for ESBL which were *E. coli* (n=1), *salmonella* (n=1) and *P. mirabilis* (n=1). Hence 25 enterobacteriaceae were tested for ESBLs using the phenotypic confirmatory combination disk method according to CLSI [63].

Among the suspected 25 isolates, 88% (n=22/25) were phenotypically confirmed for ESBL using combination disk method while the remaining 12% (n=3/25) were confirmed as ESBL negative giving an overall prevalence of 78.57% (n=22/28). Relative agreement is seen with other study [67]. This is a higher result as compared to other study from India [36, 39] in which 32.14% and 58% of ESBL recorded and a study done in Ethiopia in which 33.3% of ESBL recorded [61].

In our study among the tested *E. coli* 100% (n=5/5) with ESBL phenotypes, all showed ESBL production. This is in line with the study from Saudi Arabia that found from the tested 31 *E. coli* isolates with ESBL phenotypes, all were positive for ESBL production [54]. However our finding was relatively higher than the report that showed 87% ESBL prevalence [39]. *K. ozaenae* 9/10(90%) was the second bacteria that showed high prevalence of ESBL among the tested *K. ozaenae (n=10)* in our study. It showed an agreement with the study done in Philippine [69].

In the present study *K. pneumonia* 77.8% (n=7/9) were the third bacteria that demonstrate high level of ESBL. It has an agreement with other study in Ahmadabad [67] in which the prevalence of ESBL producing *K. pneumonia* was 81.48%. However our result showed disagreement with other study done in our country in which ESBL producing *K. pneumonia* was 33.3% [61]. This typically indicates how much ESBL producing bacteria are growing rapidly overtime. To the contrary a higher result (88%) was reported in Pakistan [39]. *K. oxyotica* 100% (n=1/1) was another Klebsiella species that were positive for ESBL in this study. It showed disagreement with the study done in Sudan [70].

All of the ESBL positive as well as ESBL negative isolates showed high level of resistance (>80%) to amoxicillin, Sulphamethoxazole-Trimethoprim and Cefotaxime, intermediate level of resistance (60-80%) to Amoxicillin-clavulanic acid, chloramphenicol, gentamycin and Tetracycline. It means that the use of theses antibiotics for treatment of infection caused by ESBL production strains may result in failure in significant proportion of cases. Thus the problem of ESBLs is clinically important and yet remains relatively unappreciated by most clinicians [61].

The choice of antimicrobial agents effective against ESBLs producing species is currently limited and cause series therapeutic problems in the future [25]. In our study they were susceptible for some limited drugs and these were Norfloxacin (63.9%) and Cefoxitin (50%) as compared to other tested drugs. Nitrofurantoin (47.3%) was also an option which has lowered resistance level of 39.6% however it may not be satisfactory as needed since it has wide range of intermediate level (13.2%) with a tendency of becoming resistant. In addition if possible and if they are accessible ESBL positive strains can respond better for Carbapenem drugs Imipenem and Meropenem which have resistance level of 12.2% and 14.69% respectively.

Though many reasons can be responsible and mentioned for the occurrence of high level of ESBL inappropriate and incorrect administration of antimicrobial agents in empirical therapies, lack of appropriate infection control strategies which can cause a shift to increase prevalence of resistant organisms in the community and the selective pressure created by the use of third generation cephalosporin has been described as one of the most important factors in the appearance of ESBL producing strains [42, 54, 61].

6.3.1 Comparison of double disk synergy test(DDST) with the combination disk Test(CDT)

Organisms producing ESBLs are clinically relevant and remain an important cause for failure of therapy with cephalosporins and other classes of antibiotics throughout the world [25]. Therefore it is necessary to know the ESBL status of clinical isolates especially in tertiary care hospitals [41]. Various phenotypic ESBL detection methods such as disk diffusion, combination (disk potentiating), double disk synergy procedure, three dimensional test and E-test ESBL strip have been described for ESBL detection [67, 68] ; however each method has its own limitations. Hence in the present study, we compared double disk synergy methods with combination disk method for detection of ESBL to know if it is the best suitable phenotypic method in Ethiopian context for application in routine bacteriology laboratory. Since the main goal of ESBL detection is to achieve high sensitivity [71], statistical comparisons were evaluated among the two methods.

Double disk synergy method by any of the four disks with a disk spacing of 20 mm detected enhancement of zone in 90.9% (n=20/22) from the 100% (n=22) phenotypically confirmed ESBL producing isolates by CDT. This is a higher sensitivity as compared to other study done in Ahmadabad [67]; the sensitivity of DDST was 81.18%. However another study showed a relatively higher sensitivity

of DDST as compared to our findings which was 95.0% [68]. Using ceftazidime, ceftriaxone and cefotaxime with amoxicillin-clavulanic acid also showed 85% sensitivity [68].This showed significant sensitivity (90.9%) of DDST with specificity (66.7%), positive predictive value (95.2%) and negative predictive value (50%) which can be applied as routine ESBL detection method in bacteriology laboratory for general hospitals where technical resources and expertise may not be in abundant supply [41].

6.4 Prevalence of Carbapenem resistant enterobacteriaceae

Carbapenems are a group of β-lactam antimicrobial agents with an exceptionally broad spectrum of activity. They are used as a last resort against many multi drug resistant, Gram negative bacteria, and in cases of infections due to extended spectrum beta lactamase (ESBL) and Amp C enzyme producing *enterobacteriaceae.* Carbapenems are crucial for the management of life-threatening healthcare-associated infections [25].

However, clinical utility of this group of antibiotic is under threat due to the recent emergence and spread of imipenem/meropenem-resistant enterobacteriaceae throughout the world [42, 73]. Resistance to carbapenems is mediated by mechanisms like loss of outer membrane proteins and production of carbapenemase that are capable of hydrolyzing the carbapenems [42]. Carbapenem-resistant enterobacteriaceae (CRE) due to carbapenemase production is being increasingly seen in clinical practice and has been reported worldwide over the past few years. This jeopardizes the effective use of carbapenems and has led to the development of `superbugs ` [26]. Carbapenemase are the enzymes which are capable of hydrolyzing carbapenems and are a heterogeneous mixture of beta lactamase belonging to Ambler molecular class A (Penicillinases), class B (Metallo enzymes) and class D (oxacillinases) [25, 26].

Blood stream and urinary tract infections caused by these CRE are important cause of morbidity and mortality. Resistance to carbapenems has been reported in places worldwide, such as Africa [72], Argentina [25], India [42] and Netherland [73] with varying prevalence. In the present study, we investigated the prevalence of *carbapenemase* producing enterobacteriaceae isolates from blood and urine; were *Klebsiella species (K. ozaenae and K. oxyotica) and Morganella morganii*. In this study, the resistance of the isolates to Meropenem and Imipenem was 14.6% and 12.2%, respectively, carbapenemase producing strains is therefore a major concern [26]. Most of the isolates were resistant to

the routinely used antimicrobial agents as seen in almost all the studies done on CRE isolates. The frequent co-resistance to other classes of antibiotics seen in CRE isolates is mainly because of the simultaneous presence of other resistance determinants often carried on integrons [25, 42].

In our study we found an overall 12.12% (n=4/33) prevalence of carbapenem resistance enterobacteriaceae. Out of a total 6 isolates which were showing resistant or intermediate zone for Meropenem or imipenem 66.7%(n=4/6) of them were positive for carbapenemase production by Modified Hodge test. This study was in line with the study done in India which showed 12.26% of Carbapenem resistant enterobacteriaceae [74]. Our study has also showed an agreement with the study done in Nigeria [69] which showed 14.0% prevalence of CRE. However this study has showed a much lowered prevalence as compared to the study done in India [42] which showed 59.1% carbapenem resistant enterobacteriaceae by using MHT. The lower prevalence in our study may be attributed to the fact that carbapenem were not commonly sold in our country because of their high cost. The origin or source of these enzymes is unknown, but probably they were imported from abroad or they emerged locally and spread by gene transfer [72].

In our study all CRE were isolated only from urine samples which is supported by the study done in India where most of CRE isolates were from urine sample [74]. Additionally in the present study all the CRE isolates were obtained from inpatients, has an agreement with study done in India [42], which indicate that these carbapenem resistant isolates are predominantly nosocomial pathogens. The indiscriminate use of antibiotics as empirical therapy to treat the multidrug resistant pathogens may be responsible for the emergence of these carbapenemase producing isolates in the hospital settings [25, 72]. But one must also be aware of the fact that these multidrug pathogens can also be community acquired as CREs have also been isolated from the common water sources [42].

Carbapenemase producing organisms in this study were Klebsiella species 9.09% (n=3/33); they were *K. ozaenae* 20% (n=2/10) and *K. oxyotica* 100% (n=1/1), and *Morganella morganii* 3.03% (n=1/33). It showed disagreement in prevalence as well as type of carbapenem resistant bacteria isolated with the study done in Nigeria, identified CRE are *E. coli* (13.5%), *K. pneumoniae* (16.7%), *Proteus species* (16.0%) [48]. However a study done in Italy showed that the tested *K. oxyotica* 1(100%) were positive for CRE by MHT [75]. And this study also indicated a lower prevalence 15% (n=3/20) of carbapenem resistant *Klebsiella species* as compared to other study where the prevalence of CRE *Klebsiella species*

were 30% [76]. *Morganella morganii* 50% (n=1/2), which accounted 3.03% from the general CRE prevalence, were the second carbapenem resistant enterobacteriaceae identified in our study.

CRE isolates often exhibit an extensively drug resistant phenotype, resistant to most of the currently available antibiotics rendering them ineffective and the clinical data on the antimicrobial agents available for the treatment of these isolates remain sparse [25]. The multiple resistance capability of these bacteria may be due to multiple factors including uncontrolled antibiotic usage, inappropriate dosing regimens, wide spread of counterfeit and substandard antibiotics and local hospital practices concerning isolation of patients with multi-resistant pathogens which is poorly managed [73].

Strengths and limitations of the study

Strengths

- This study indicates the prevalence of ESBL positive enterobacteriaceae
- It suggests the very valuable and simply technique (double disk synergy method) which can be implemented in routine bacteriology laboratory to detect ESBL which is very important for controlling the spread of ESBL producing bacteria.
- It also showed the spectrum of carbapenem resistant enterobacteriaceae

Limitations

- Anaerobic cultures were not available to assess the prevalence of anaerobes

7 Conclusion

The overall prevalence of bacteria isolates from blood and urine cultures was 17.1% (n=55/322). From 177 and 145 blood and urine samples 13.0% (n=23/177) and 22.1% (n=32/145) were culture positives respectively. The finding of this study showed that bacteremia and UTIs in children were predominantly caused by *coagulase negative staphylococci* and *Klebsiella species* respectively, which develop multidrug resistance to commonly used antimicrobials.

The choice of drugs in the treatment of bacterial isolates in the blood and urine is quite narrow today due to the wide scale resistance that the common pathogens show to drugs which have been used previously. Multidrug resistance level reaches to 89.1%. Multiple resistances were observed in 71.42% of Gram positive and 95.11% Gram negative isolates. From the commonly used antibiotics amoxicillin (89.1%) and cefotaxime (85.5%) showed the highest level of resistance for all blood and urine isolates. Moreover, bacterial strains resistant to most classes of antibiotics arise which is our current challenge.

ESBL producing enterobacteriaceae reaches to worrying level 78.57%. The increasing frequency of ESBL-producing enterobacteriaceae among hospitalized patients is an important problem for both microbiologists and clinicians. Screening and confirming ESBL production using simple method like DDST as a routine procedure in clinical bacteriology laboratories gives valuable information to the clinician for appropriate selection of antibiotics. When detecting ESBL-positive strains suggestions of reliable therapeutic options for successful treatment of infected patients is needed from microbiology laboratories.

Bacteria that are carbapenem resistant mainly due to carbapenemase production being increasingly are alarming in clinical practice. This study shows a significant rate of carbapenem resistance among enterobacteriaceae (12.12%) isolated from hospitalized children. It provides a clearer picture of the current CRE scenario in the hospital setup and thus further emphasis is needed for control of CRE dissemination within the community. The need of the hour would be to have a strong antimicrobial stewardship program, which is followed by all concerned.

8 Recommendation

- Active surveillance and hospital infection control policies should be implemented not only in the acute health-care settings but also in the non-acute care facilities.
- The pattern of antimicrobial resistance pattern should be assessed at regular interval to control the spread of such resistant bacteria especially in case of multidrug resistance.
- Effective antibiotic policy; like antibiotic restriction, combination therapy and antibiotic cycling, and infection control programs combined with good medical practices can help in confronting the menace of antibiotic resistance to prevent the spread of multidrug resistant strains.
- There is a need for continuous screening and surveillance for ESBL producers in pediatric unit.
- In Ethiopian context double disk synergy method for detection of ESBL can be implemented in routine bacteriology laboratory as it is very simple and valuable to indicate better treatment options and control the spread of ESBL positive enterobacteriaceae.
- Routine testing of enterobacteriaceae isolated from clinical samples for possible carbapenemase activity may result in availability of data on such isolates for future control planning.

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Annexes

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

1. Sample collection

A. **Blood Sample Collection**

Careful technique is important to avoid contamination of the blood culture media by normal skin flora during the process of collection. This is important because normal bacterial skin flora can cause systemic disease such as infective endocarditis, and in some circumstances blood culture contamination can make it difficult to distinguish between false positive results and true infection.

Aseptic blood collection and dispensing technique for culturing blood is as follows:

1. Using a pressure cuff, locate a suitable vein in the arm. Deflate the cuff while disinfecting the vein-puncture site.
2. Wearing gloves, thoroughly disinfect the vein puncture site as follows:
   - ✓ Using 70% ethanol, cleanse an area about 50 mm in diameter. Allow to air-dry.
   - ✓ Using 2% tincture of iodine and a circular action, swab the area beginning at the point where the needle will enter the vein. Allow the iodine to dry for at least 1 minute.
3. Lift back the tape or remove the protective cover from the top of the culture bottle(s). Wipe the top of the bottle using an ethanol-ether swab.
4. Using a sterile syringe and needle, withdraw about 10 ml of blood from an adult or about 2 ml from a young child
5. Insert the needle through the rubber liner of the bottle cap and dispense 10 ml of blood into the Brian Heart infusion culture medium bottle containing 90ml of broth or in proportion of 1ml of blood to 9ml of BHI broth containing bottle.
6. Using a fresh ethanol-ether swab, wipe the top of each culture bottle and replace the tape or protective cover(s). Without delay, mix the blood with the broth.
   - ❖ ***Important***: The blood must not be allowed to clot in the culture media because any bacteria will become trapped in the clot.
7. Clearly label each bottle with the name and number of the patient, and the date and time of collection.

8. As soon as possible, incubate the inoculated media. Protect the cultures from direct sunlight until they are incubated. Incubate at 35–37 °C for up to 7 days, examining and sub-culturing. A longer incubation period should be allowed when endocarditis is suspected.

Culture of blood from neonates

To reduce the risk of contamination, blood from neonates should be collected from a peripheral vein not from the umbilical vein. When only a small amount of blood is obtained, inoculate it into a bottle of diphasic culture medium. Organisms causing bacteraemia in young children are usually present in sufficient concentration to be detected in small volumes of blood (1–2 ml) of blood).

Prior inspection of culture media bottles

Do not use a bottle of culture medium if it shows signs of contamination, i.e. broth appears turbid. Do not use a bottle of thioglycollate broth if it appears oxidized, i.e. more than a third of the top of the medium appears pink when the indicator in the medium is resazurin, or more than 20 mm down from the surface of the medium appears green-blue when the indicator is methylene blue. When oxidation has occurred, the medium must be reduced by steaming before it is used.

B. Urine sample collection

The first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for culture, microscopy, and biochemical analysis.

Midstream urine (MSU) for microbiological examination is collected as follows:

1. Give the patient a sterile, dry, wide-necked, leak proof container and request a 10–20 ml specimen.
    - ❖ ***Important***: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a ‗clean-catch‘ specimen.

    A. ***Female patients***: Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.

    B. ***Male patients***: Wash the hands before collecting a specimen (middle of the urine flow).
        - ❖ ***Note***: When a patient is in renal failure or a young child, it may not be possible to obtain more than a few milliliters of urine.
2. Label the container with the date, the name and number of the patient, and the *time* of collection.

3. *As soon as possible*, deliver the specimen with a request form to the laboratory. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4–6 °C. When a delay in delivery of more than 2 hours is anticipated, add boric acid preservative to the urine.

2. Culture and identification

i. Bacteria growth from the blood and urine on MacConkey agar

Positive/present □ Negative/absent □

ii. Identification steps for suspected colonies

a) Gram stain result _____

b) Oxidase test positive □ /negative □

c) Colony morphology

d) Lactose fermentation from MacConkey agar

- Lactose Fermenter
- Late Lactose fermenter
- Non lactose fermenter

iii. Biochemical reactions

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

Biochemical reactions		Indo	Urea	Man	TSI	Cit	Mot	LDC	OX
Result	Positive								
	Negative								
Gram negative rods									

Key: LDC = Lysine decarboxylase, Man = Mannitol (mannite), Triple sµgar iron (TSI), Ox = Oxidase test, Cit = Citrate test, Mot = Motility, Ind = Indole test, Urea = Urease, H2S = Hydrogen sulphide (blackening), R = Red-pink (alkaline reaction), Y = Yellow (acid reaction), d = different strains give different results.

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

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

C. Triple Sugar Iron (TSI) Agar Slant: Using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism. Incubate at 37◦C for 18 to 24 h. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only. If alkaline slant–alkaline butt (red–red): glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

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

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

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

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

3. Antibiotics susceptibility result for Bacteria isolates

Isolated bacteria	Antibiotics		AML	AMC	SXT	FOX	C	CTX	GE	TE	NOR	FN	OX	IMI	MEM
	Drug Susceptibility pattern	S													
		I													
		R													

NOTE: AML--Amoxycillin, AmC--Amoxycillin-Clavulanic acid, SXT----Sulphamethoxazol-trimethoperem, FOX----Cefoxitin, C---Chloramphenicol, CTX----Cefotaxime, GM--Gentamycin, TE---Tetracycline, NOR---Norfloxacin, OX--- Oxacillin, IMI--- Imipenem, MEM--- Meropenem

Procedure for Performing the Disk Diffusion Test

Inoculum Preparation

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

Inoculation of Test Plates

- Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level.

- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.
- The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

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

Application of Disks to Inoculated Agar Plates

- The predetermined battery of antimicrobial disks is dispensed onto the surface of the inoculated agar plate. Each disk must be pressed down to ensure complete contact with the agar surface.
- The plates are inverted and placed in an incubator set to 37°C within 15 minutes after the disks are applied.

Reading Plates and Interpreting Results

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

4 Phenotypic extended spectrum beta-lactamase detection

4.1 Combination disk test (CDT)

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

4.2 Double-disk synergy test (DDST)

Disks containing cephalosporins (cefotaxime, ceftriaxone, ceftazidime, Aztreonam) are applied next to a disk with amoxicillin-clavulanic acid. Positive result is indicated when the inhibition zones around any of the cephalosporin disks are augmented in the direction of the disk containing clavulanic acid. The distance between the disks is critical and 20mm centre-to-centre has been found to be optimal for cephalosporin 30µg disks.

Note: procedures used for antimicrobial testing is applied here for both combination and double disk synergy test.

5 Carbapenemase detection using Modified Hodge Test

I. Prepare a 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of *E .coli* ATCC® 25922 (the indicator organism) in broth or saline, and dilute 1:10 in saline or broth. Inoculate an MHA plate as for the routine disk diffusion procedure. Allow the plate to dry 3 to 10 minutes. Place the appropriate number of ertapenem or meropenem disks on the plate.

II. Using a 10-µL loop or swab, pick 3 to 5 colonies of test or QC organism grown overnight on a blood agar plate and inoculate in a straight line out from the edge of the disk. The streak should be at least 20 to 25 mm in length.

III. Following incubation, examine the MHA plate for enhanced growth around the test or QC organism streak at the intersection of the streak and the zone of inhibition.

   a. Enhanced growth=positive for carbapenemase production.
   b. No enhanced growth=negative for carbapenemase production.
   c. Some test isolates may produce substances that will inhibit growth of *E. coli* ATCC® 25922. When this occurs, a clear area will be seen around the streak and the MHT is un-interpretable for these isolates.

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

2.1 Participant information sheet

Department of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title: Prevalence and antimicrobial resistance of bacterial isolates with special emphasis on enterobacteriaceae among children suspected for septicemia and urinary tract infections at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Blood stream infections are a major cause of mortality of children both developed and developing countries despite important progress in treatment and prevention of infectious diseases. Urinary tract infection is a serious health problem and frequently encountered serious morbidity and mortality of children and it is the most common infectious presentation in hospital acquired and community acquired infections since long time. Among the bacterial group Enterobacteriaceae are the most causative agents of septicemia and UTI and from the family *E. coli*, *Klebsiella, Enterobacter, Citrobacter, Proteus,* and *Salmonella* take the leading. In addition to their high prevalence the development of drug resistance is the challenge for controlling enterobacteriaceae now days. As the frequency and antibiotic sensitivity pattern to common pathogen has been changing day by day, choice of appropriate antibiotics depends on the knowledge of common organisms and their antimicrobial susceptibility pattern in local scenario. Mainly information on the multidrug resistance pattern especially ESBL producers and carbapenem resistant enterobacteriaceae isolates is insufficient.

Hence the aim of this study was to determine the prevalence and antimicrobial resistance of bacterial isolates with emphasis on enterobacteriaceae among children suspected for septicemia urinary tract infection in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Aim of the study

The purpose of the study is to determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on enterobacteriaceae among children suspected for septicemia and urinary tract infection at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, results will be given to their physician for treatment or to get counseling. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policy makers.

Risks and complication

There is no considerable risk(s) in participating in this study other than the possible minor bleeding from the site of vein-puncture when your physician or nurse collect blood sample for your requested bacteriological investigation. Vein-puncture is a routine clinical practice for blood sample collection and has minimal risk, and the amount of blood collected will be 3- 5 ml (1 to 2 tea spoon) blood only. No risk to give urine sample.

Confidentiality

In order to maintain the confidentiality of participants‘ information, the name will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. No personal information will be disclosed to third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Melese Hailu (PI): Signature: _____ Date: _____

Note: If you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

PI Address: Melese Hailu: Department of Medical Laboratory Sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: melerose85@gmail.com; Tel.: +251913705279

2.2 Informed consent

I have been informed about the study which plans to determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on enterobacteriaceae and associated risk factors among children suspected for septicemia and urinary tract infection in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my childrens‘ blood or urine for the mentioned study. I agreed that the specimen would be tested for enterobacteriaceae. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the analysis of blood and/or urine isolates of enterobacteriaceae will be given to the Doctor who follow my child and that I may ask the information if I want.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participant code:_____ Signature: _____ Date: _____

Annex III: Amharic Version of the participant information sheet and Consent form (የተሳታፊዎች መረጃ ቅጽ ና የፈቃደኝት ማረጋጫ)

3.1 የተሳታፊወች መረጃ ቅጽ

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮላጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

አርስት፡ በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ ና ለሽንት መሽንያ በሽታ ከታከሙት ስርጭቱን ማወቅ

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ።

መግቢያ

ሰፕቲሴሚያ ና የሽንት መሽኒያ በሽታ በተለያዩ ተህዋስያን የሚመጡ የጤና ችግር ሲሆኑ በዓለም ላይ የሚያደርሰት የሞት እና የኢኮኖሚ ቀውስ ከፍተኛውን ደረጃ ይይዛል፡፡ በተለይም በታዳጊ ሀግሮች ውስጥ ለሚገኙ ህፃናቶች የሞት እና ጉዳት ዋነኛ ምክንያት ነው፡፡ ለዚህም ዋነኛ መንስኤው ሁሉም አይነት ባክቴሪያዎች የሚከሰት ሲሆን በተለይም ኢንተሮባክተሪሴ የተባሉት ተህዋስያን በሚያመጡት በሽታ ምክንያት የሚሞቱት ሰዎች ከፍተኛውን ቁጥር ይይዛሉ፤ በተጨማሪም እነዚህ ባክቴሪያዎች ለመድሐኒቶች በከፍተኛ ደረጃ የግትርነት ባህሪ(resistance) እያሳዩ መሆኑ ችግሩን ውስብስብ አድረጎታል፡፡ ሥለሆነም ይህን በሽታ ከፍተኛ ጉዳት ከማስከተሉ በፊት በመከላከል የሕብረተሰቡን ሞት ለመቀነስ ያለውን ስርጭት እንዲሁም ለመድሃኒቶችም ያለዉን የግትርነት ማዎቅ በጣም አስፈላጊ ነው፡፡

የጥናቱ አላማ

በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ ና ለሽንት መሽንያ በሽታ ከታከሙት ሕጻናት ስርጭቱን ማወቅ ና ለመድሐኒት ያለውን የግትርነት ደረጃ ማሳየት፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም። ነገር ግን ዉጤታቸዉ ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ ለዚህ ጥናት የምንጠቀምበት የደም ናሙና ለታዘዘልዎ የባክቴሪዎሎጂ ምርመራ ሐኪመዎ ወይም ነርስዎ ከክንድዎ ከሚዎሰዱት ሲሆን መጠኑም ከ3- 5 ሚሊ ሊትር (ከ ፣ -2 የሻይ ማንኪያ) ነው፡፡ ይህም ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም አይነት ጉዳት አያደርስም፡፡ ለሽንት ምርመራ የሚሆን ሽንት በሚያመጡበት ስአትም ቢሆን ምንም የተለየ ስሜት የለዉም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ ሊያገኙ የሚችሉ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማ ብቻ የሚገለፅ ይሆናል፡፡

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የዋና ተመራማሪው አድራሻ፤

- **መለሰ ኃይሉ ለገሰ፤** የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስ አበባ፣ ኢትዮጵያ
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3.2 የፈቃደኝነት ማረጋገጫ ቅፅ

በአዲስ አባባ ከተማ፣ በጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ ና ለሽንት መሽንያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድሐኒት ያለውን የግትርነት ደረጃ ማሳየት በሚል ርዕስ ለይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ የደም ወይም የሽንት ናሙና ውጤት በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠዉ የደም ናሙና እና የሽንት ናሙና ለባክቴሪያዎች ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች ለተቋሞች እንደሚሰጥና ዉጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የደም ወይም የሽንት ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ____

Annex IV: Declaration

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

Name: Melese Hailu

Signature _____

Place_____

Date of submission _____

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

1. Name: Gebru Mulugeta (BSC, MSC)

   Signature _____

   Place _____

   Date of submission _____
2. Name: Daniel Asrat (MD, MSC, PHD)

   Signature_____

   Place_____

   Date of Submission_____