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Prevalence and Antimicrobial Susceptibility Pattern of *ESKAPE* Pathogen and Associated risk factors among Pediatric cancer patients suspected blood stream infection and urinary tract infection at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia.

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This is to certify that the thesis prepared by Meselech Moges, entitled: **Prevalence and antimicrobial susceptibility of ESKAPE pathogen among pediatric cancer patient suspected blood stream infection and urinary tract infection and associated risk factors at Tikur Anbessa Hospital, Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Table of Contents

Summary ..... **Error! Bookmark not defined.**
List Of Abbreviations and Acronyms ..... **Error! Bookmark not defined.**
Acknowledgments ..... **Error! Bookmark not defined.**
Introduction 7
1.1 Background 7
1.2 Statement of the problem 3
1.3 Significance of the study 5
Literature review 7
Prevalence of ESKAPE ..... **Error! Bookmark not defined.**
Antimicrobial resistance ..... **Error! Bookmark not defined.**
Objective 18
3.1 General Objective 18
3.2 Specific Objectives 18
4. Materials and methods 18
4.1 Study area 18
4.2 Study design and period 18
4.3 Population 18
4.3.1 Source population 18
4.3.2 Study population 19
4.4 Inclusion and exclusion criteria ..... **Error! Bookmark not defined.**
4.4.1 Inclusion criteria ..... **Error! Bookmark not defined.**
4.4.2 Exclusion criteria ..... **Error! Bookmark not defined.**
4.5 Study Variables 19
4.5.1 Dependent Variables 19
4.5.2 Independent Variables 19
4.6 Sample size calculation and sampling method 19
4.6.1 Sample size calculation 19
4.6.2 Sampling method 20
4.7 Measurement and Data Collection 20
4.7.1 Sample and Data Collection Procedure 20

4.7.2 Laboratory Analysis 20
4.7.2.1 Culture and Identification 20
4.7.2.1.1 Blood culture identification 20
4.7.2.1.2 Urine culture identification 21
4.7.2.2.1 Drug Resistance Patterns 21
4.8 Quality Assurance 21
4.9 Data Analysis and Interpretation 22
4.10 Ethical Consideration 22
5. Dissemination Of Results 22
5. Result 23
6. Discussion 35
8. Conclusion and recommendation 39
Conclusion 39
6 Work Plan ..... **Error! Bookmark not defined.**
Budget Breakdown ..... **Error! Bookmark not defined.**
Reference 40
Annex I 46
Annex II: Information sheet in English Version 46
Annex III: Informed consent form in English version 50
Annex III: Informed consent form in Amharic version 50
Annex IV: Assent form for less than 18 years old 51
Annex IV: Amharic versions of ascent form for participants less than 18 years of age 51
Annex VI: Questionnaire 52
Annex V: Procedure for specimen collection, processing, and result 56
Annex VII- Declaration 63

List of tables

Table 1 Socio-demographic characteristics of pediatric cancer patients active on chemotherapy at Tikur Anbessa Hospital, Addis Ababa, Ethiopia, from May2025 to March 2026 (n=422) 23

Table 2 Clinical characteristics of pediatric cancer Patients active on chemotherapy at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, from March 2025- May 2026(n=422) 24

Table 3 Distribution of ESKAPE pathogen isolates from blood and urine specimens among pediatric cancer patients who were active on chemotherapy at the Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia, from March 2025- May 2026. 26

Table 4.1 Antimicrobial Susceptibility test of Gram- positive bacteria isolated from blood culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia,2025 and 2026. 27

Table 4.2 Antimicrobial Susceptibility test of Gram- positive bacteria isolated from urine culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia,2025 and 2026. 28

Table 4.3 Antimicrobial Susceptibility test of Gram- negative bacteria isolated from blood culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia,2025 and 2026 30

Table 4.4 Antimicrobial Susceptibility test of Gram-negative bacteria isolated from urine culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, 2025 and 2026. 32

Table 5 Bivariate and Multivariate logistic Regression analysis of Risk Factors Associated with ESKAPE infection among pediatric cancer patients 34

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List of Abbreviations

AMR.....Antimicrobial resistance

BSI.....Blood stream infection

CDC.....Center for Disease Control

CON.....Coagulase Negative *Staphylococcus*

ESKAPE.....*Enterococcus faecium, Staphylococcus aureus*
Klebsiella pneumoniae, Acinetobacter
Pseudomonas aeruginosa, and Enterobacter

ETB.....Ethiopian birr

GN.....Gram-negative

GP.....Gram positive

HAIs..... healthcare-associated infections

MSU..... midstream urine

MDR.....Multidrug resistance

ST.....Solid Tumor

TASH.....Tikur anbessa specialized hospital

UTI..... Urinary tract infection

WHO..... World health organization

Abstract

Background: *ESKAPE (E: Enterococcus. Faeciem S: Staphylococcus aures, K: Klebsiella aeruginosa, A: Acinetobacter baumanni, P: Pseudomonas aeruginosa, E: Enterobacter spp)* is the group of pathogens capable of 'escaping' the biocidal action of antibiotics. These pathogens rank among the most prevalent causes of life-threatening infections, and their ability to acquire and disseminate antimicrobial resistance is well known. *ESKAPE* pathogens are usually resistant to standard empirical treatment and in severely immunocompromised patients may easily lead to a life-threatening infection or even death. Following the chemotherapeutic and radiation-related neutropenia in cancer patients, bacterial infections are serious complications encountered by cancer patients.

Objective: This study aims to determine the prevalence and antimicrobial resistance pattern of *ESKAPE* pathogens among pediatric cancer patient suspected to blood stream infection, urinary tract infection and associated risk factors at Tikur Anbesa Specialized Hospital Addis Ababa Ethiopia.

Methods: Hospital based cross-sectional study was be conducted at Tikutr Anbesa Specialized Hospital from 422 pediatric (birth to 18 years) cancer patients who are taking chemotherapy and suspected of blood stream infection (BSI) and urinary (UTI). Participants were included using a convenient sampling technique from April 2025 to May 2026. Conventional bacterial culture with subsequent biochemical tests were used to isolate the bacteria. SPSS version 25.0 was entered and analyze the data employing both descriptive and analytical statistic.

Result: Out of 232 blood culture, 67/232(28.88%) were positive and from the total of 190 urine samples cultured, 53/190(27.89%) were positive. Among 120 positive bacterial urine and blood isolates, Gram-negative *ESKAPE* pathogen detected in 76/120(63.33%) and *K. pneumonia* 45/120(37.5%) was the most frequent gram negative *ESKAPE* pathogen from both blood and urine culture isolates followed by *Acinetobacter spp, P. aeruginosa, Enterobacter. spp,* were *8.83%, 10%, 7.5%*. respectively. From gram positive pathogens, Enterococcus spp 26(21.67%) was most predominant followed by *S. aures* 19(15.83%). Resistance for more than three class was detected: in 3/14(21.42%), from blood and urine 4/12(36.38) were isolated. The prevalence of Vancomycin

resistant *Enterococcus was* 4/11 (36.36%) and 3/7 (42.86%) from blood urine sample respectively. Among the bacterial isolates, multidrug resistance (MDR) was observed in *24*/24(100%) MDR *K. pneumonia,* 6/9(66.67%) MDR *Acinetobacter spp,* 4/4(100%) MDR *P. aeruginosa*, 4/6(66.67%) MDR *Enterobacter spp* isolated. The factors associated with ESKAPE pathogen infection in cancer patient were age, stage of cancer, length of hospital stay urinary catheter, and neutropenia.

Conclusion:

Gram negative *ESKAPE* pathogen were predominant among pediatric cancer patients active on chemotherapy. *K. pneumonia* and *Enterococcus spp* being the most frequent isolate *ESKAPE* pathogens. A significant number of bacterial isolates showed MDR. Regular surveillance to assess bacteria resistance pattern must be conducted to modify antibiotic stewardship in the hospital.

Keywords: Prevalence**,** Antimicrobial susceptibility, cancer*, ESKAPE,* MDR

Introduction

1.1 Background

The "*ESKAPE*" acronym was developed in 2008, by Rice to describe the organisms that can escape the biocidal action of antibiotics, *including E: Enterococcus faecium, S: Staphylococcus aureus, K: Klebsiella pneumoniae, A: Acinetobacter baumannii P: Pseudomonas aeruginosa, and E: Enterobacter spp*. Considering their economic impact and overall mortality, deserve significant attention at both clinical and research and development levels. Infections caused by these pathogens are among the most common causes of death, and they can acquire and spread antibiotic resistance. They are responsible for more than half of all health-care-associated infections and have been linked to high rates of antimicrobial resistance (AMR)(1).

ESKAPE pathogen are considered opportunistic pathogens, which have the capacity to affect critically ill patients and immune-compromised patients. In recent years, these pathogens have shown resistance to multiple antibiotic families. The presence of high-risk clones within this group of bacteria contributes to the spread of resistance mechanisms worldwide. Resistance mechanisms identified in *ESKAPE* group bacteria are structural modification of the target site, enzymatic modification of antimicrobials, and changes in membrane permeability are the main mechanisms of antimicrobial resistance detected in the *ESKAPE* group. They have the ability to persist in the hospital environment, aided by various mechanisms, such as biofilm formation, well described in the case of *P. aeruginosa* or the ability to resist stress conditions, such as the presence of disinfectants, as described in *A. baumannii* is well known. The presence of these microorganisms in the hospital environment contributes to coinfections (2).

ESKAPE also stems from the word "escape" because these pathogens escape antibiotic treatments. *ESKAPE* pathogens are listed under critical and high-priority groups in the "WHO Priority Pathogens List" because of their ability to cause life-threatening conditions. The treatment of patients with *ESKAPE* infection can be challenging for healthcare providers because of the limitations of antimicrobial agents, reduced treatment options, and increased disease burden. AMR cases cannot be treated with first-line agents; thus, multiple antimicrobial agents are used, leading to toxicity and increased healthcare costs(3).

Infections are one of the most frequent complications seen in patients with cancer, and a patient with cancer has a 3 times greater risk of dying from a fatal infection than a patient without cancer. Infections are thought to play a primary or associated role in the cause of death in approximately 50% of patients with hematological malignancies or solid tumors, even if drug-resistant infections are rarely recorded as the official cause of death-on-death certificates. The most common cause of infections in patients with cancer are bacteria. Disruption of anatomic barriers, surgery, chemotherapy-related and radiation related neutropenia, and stem cell transplantation are the most known risks of developing an infection. Patients with cancer Under neutropenic conditions are subjected to prolonged treatment of antibiotics prophylactically and empirically. The use of widespread and prolonged antibiotics to reduce mortality and morbidity from infections in patients with cancer are likely contributors to the emergence of resistance. In addition, patients with cancer are vulnerable to healthcare-acquired infections as a major source of antibiotic- resistant organisms(4).

ESKAPE pathogens resistant to antibiotics present the most significant threat to patients who develop bloodstream infections (BSIs) due to a lack of suitable treatment options and effective management. Some challenges that health-care providers encounter include a limited understanding of the invading species, restricted antimicrobial options, and an uncontrolled host reaction to an infection called sepsis. Consequently, patients with BSIs caused by *ESKAPE* pathogens require immediate and efficacious antibiotic treatment to improve their chances of recovery. Understanding the epidemiology and effectiveness of empirical antibiotic treatment and developing appropriate measures to control the emergence and spread of *ESKAPE* pathogens is essential to understanding the epidemiology of BSIs caused by these pathogens(5).

Bacterial infection is the most common complications during cancer treatment and it is a considerable cause of infection-related mortality in cancer patients. Cancer patients are at a high risk of bacterial infection due to surgical complications, chemotherapy and radiotherapy-related neutropenia, and the use of immunosuppressive drugs during cancer treatment. *ESKAPE* pathogens are at the forefront of bacterial infection and antibiotic resistance during cancer treatment. The *ESKAPE* pathogens were significantly associated with more prolonged infections, longer duration of infection, and higher mortality. Antibiotic resistance in cancer patients often correlates with

increased susceptibility to infections that eventually reduce the patient's survival time; it also poses a significant threat to the accomplishments achieved in cancer treatment(6)

1.2 Statement of the problem

Following the chemotherapeutic and radiation-related neutropenia in cancer patients, bacterial infections are serious complications encountered by cancer patients. Some infections caused by Multi-Drug Resistant (MDR) *ESKAPE* are difficult to treat and broad-spectrum antibiotics used against them further impart toxicological manifestations on cancer-affected children(7).

Antibiotic resistance has become a public health problem, with high morbidity and mortality rates affecting mainly countries with emerging economies. The World Health Organization (WHO) considers that in 2050, infections associated with (AMR) due to *ESKAPE* will be responsible for 10 million deaths per year. The Centers for Disease Control and Prevention (CDC) estimates that in the United States of America (USA), infections related to antibiotic-resistant microorganisms are responsible for at least 23,000 deaths per year. This group of microorganisms has highly extensive antibiotic resistance, as well as being capable of acquiring multiple genes that confer them multidrug resistance. Also, they are considered the cause of most healthcare-associated infections (HAIs), especially for severely ill and immunocompromised patients (8).

ESKAPE have developed alarming levels of antibiotic resistance, rendering conventional treatments increasingly ineffective have demonstrated the ability to "escape" the antimicrobial effects through various mechanisms, including the acquisition of resistance genes and the formation of biofilms (9).

Cancer is the main cause of death worldwide. According to WHO data, every year, 400,000 children suffer from cancer, and almost 100,000 dies. Infections are a frequent cause of morbidity and mortality in hematological and oncological children due to a compromised immune system. Moreover, they are one of the most frequent complications reported in children with cancer, and a patient with cancer has a three-times greater risk of dying from a fatal infection than a patient without. In addition, to a compromised immune system, important risk factors include the following: hospitalization, broad-spectrum antibiotic therapy, invasive procedures, and biomaterials (e.g., catheters, tubes, drains)(10).

Some report indicates that 2 million AMR infections with a death toll of 29,000 occur in the United States per annum, at an attributable healthcare cost of more than $4.7 billion. In Europe, over 33,000 deaths and 874,000 disability-adjusted life years are attributed to hospital-acquired (HA)

and community-acquired (CA) AMR infections each year, accounting for $1.5 billion in direct and indirect costs due to *ESKAPE* (11).

ESKAPE pathogens are usually resistant to standard empirical treatment and easily lead to a life-threatening infection or even death in severely immunocompromised patients treated with intensive chemotherapy, in cancer patients(12). CDC announced the *ESKAPE* bacteria as life-threatening microorganisms worldwide and all these antibiotic-resistant bacteria are in the WHO priority group for which new antibiotics are urgently needed(13). Globally, the prevalence of *ESKAPE* pathogens causing BSI has been reported, with an isolation rate of >40% among *ESKAPE* pathogens(14). According to statistics, in 2019, 1.27 million deaths were directly caused by bacterial resistance to antibiotics due to *ESKAPE*, and 4.95 million deaths were associated with this bacterial resistance (15). Ten million extra deaths are predicted by 2050 due to drug-resistant *ESKAPE* pathogen infections. These pathogens can severely limit treatment options, and increase the risk of treatment failure and patient mortality(16).

ESKAPE causes severe infections, morbidity, and mortality in which 75.7–78.9% of total bacterial isolates from hospitals in Italy cause bloodstream infections. Meanwhile, in the United States, the Centre for Disease Control and Prevention (CDC) projected that MDR pathogens cause almost 2 million diseases and 23,000 deaths/year(17).

Underdeveloped countries reported an incidence of 57%, which represents 8 million new cases, and 65% mortality, which represents 5.3 million deaths annually (48% of these happened during the first 5 years after the diagnosis). According to the National Cancer Institute of the United States (part of the National Institutes of Health (NIH)), more than 60% of new cancer cases globally are found in Asia, Africa, and South and Central America, and 70% of all cancer deaths are concentrated in these regions. According to the International Agency for Research on Cancer, between 2001 and 2010, approximately 300,000 cases of cancer were diagnosed in children and teenagers every year. About half of them die each year due to the severe complications of cancer. (18).

Pediatric patients undergoing chemotherapy, organ transplants, and advanced therapies are particularly susceptible to bacterial infections. Effectively managing these infections in cancer patients requires strict adherence to established clinical guidelines, as well as consideration of local epidemiological data and patterns of antibiotic resistance among potential pathogens. However, at

Tikur Anbessa Specialized Hospital, there is a notable lack of routine identification and antibiotic resistance testing before initiating antibiotic therapy. This deficiency has resulted in reliance on empirical treatment strategies for patient care. Consequently, the prevalence of bloodstream infections and antimicrobial resistance among bacterial isolates in pediatric cancer patients receiving chemotherapy remains largely unknown.

There are a limited number of studies that comprehensively evaluate the prevalence and AMR of *ESKAPE* pathogens in pediatric cancer patients in Ethiopia. Therefore, this study aimed to assess the status of these pathogens and their antibiotic resistance profiles, among pediatric cancer patients. This study also provides foundational data on the AMR profile of the *ESKAPE* pathogen among pediatric cancer patients undergoing chemotherapy at Tikur Anbessa Specialized Hospital in Addis Ababa, Ethiopia.

1.3 Significance of the study

This study provides the current bacterial infection and its antimicrobial Susceptibility pattern of *ESKAPE* among pediatric cancer patients are taking chemotherapy. Understanding these aspects can lead to improved treatment strategies, better infection control measures, and ultimately enhanced outcomes for patients facing the challenges of cancer and related infections.

The information gathered from this study can assist clinicians in making informed treatment decisions, optimizing patient care, and customizing interventions to effectively address infections in this vulnerable population. Additionally, the data generated can provide valuable insights for policymakers, aiding in the planning and implementation of targeted interventions, efficient resource allocation, and the development of guidelines aimed at enhancing infection management in pediatric oncology settings.

This study also benefits patients by reduces the risk of side effects and antibiotic toxicity by avoiding unnecessary or ineffective treatments using antibiotics in a way that is specific to the individual patient and the bacteria causing the infection. Early and appropriate treatment for infections like BSI and UTI significantly lowers the risk of severe complications and prolonged illness

The findings of this study significantly contribute to improving the care and outcomes of pediatric cancer patients, while also serving as a foundational resource for future research in this area. By

establishing baseline data on bacterial infections and antimicrobial resistance patterns, the study paves the way for further investigations and innovations that aim to enhance our understanding and management of infectious complications in pediatric oncology.

Literature review

A Retrospective Survey Conducted Benko R et al., at Tertiary Care Teaching Hospital in Hungary. After the removal of duplicates, n = 6887 individual bacterial isolates were recovered, out of which n = 4974 (72.22%) were *ESKAPE* isolates. *E. coli* was the most frequent isolate (2193, 44.1%), followed by the *Klebsiella* genus (664; 13.4%). The third most frequent isolate was *S. aureus* (561, 11.3%)(19)

The retrospective multicenter nationwide study was conducted by Jaremeke K et al., between 2012 and 2021 in Poland A total of 9121 newly diagnosed children were treated for malignancy and 1697 pediatric patients underwent hematopoietic cell transplantation. *K. pneumoniae* infections were diagnosed in 527 patients. Their overall incidence was 4.86% in pediatric hematology and oncology patients and 4.95% in patients who underwent hematopoietic cell transplantation. The incidence of infection was higher in patients with acute leukemia than with solid tumors (7.8% vs. 4.1%; OR = 2.0; 95% CI = 1.6–2.4; $p < 0.0001$). The most frequent source of infection was in the urinary tract at 55.2%. More than 57% of *K. pneumoniae* strains were extended-spectrum β-lactamase-positive and almost 34% were multidrug-resistant. Infections with *K. pneumoniae* contributed to death in 3.22% of patients.(10)

A retrospective study was conducted by Kara T et al., Between January 2010 and November 2015 Ankara hospital. Turkey. 71 pediatric patients with 111 BSIs were included in the study. All blood samples were cultured with a BACTEC 9240 system (Becton Dickson, Sparks, MD, USA). Antimicrobial susceptibility patterns of microorganisms were detected with the Phoenix 100 ID/AST system. In total, 71 patients who had 111 bloodstream infection episodes were included. About 35.1% gram-positive bacteria, 60.5% gram-negative bacteria, and 4.4% were fungi. *Escherichia coli* was the most commonly detected gram-negative pathogen (16.7%) and *CONS* was the most commonly isolated gram-positive microorganism (16.7%)(20).

A cross-sectional study was conducted by Mohammed AK et al., at the Central Teaching Hospital of Pediatric in Baghdad from the period of Sept. 2019 till Aug. 2020 and 18 (22.5 %) gram-positive and 62 (77.5 %) gram-negative bacteria were isolated from the total sample of 80 (100.0 %). The percentage of males was 45 more than females which represents 35 in most age groups between age (1 day-3 year) and 33 (100.0 %). In most distribution of organisms according to the type of sample among gram-positive *Staphylococcus aureus* accounted 13 (16.2 %) and among gram-

negative bacteria, *Escherichia coli* 21 (26.3 %), *Klebsiella pneumoniae* 13 (16.2 %), and, *Pseudomonas aeruginosa* 11 (13.8 %) were detected (21).

A retrospective observational study was carried by Sherbin GM et al., out at the departments of pediatric hematology-oncology and microbiology of a tertiary care cancer center in North India in over a period of 24 months (October 2012 to September 2014). A total of 1747 culture samples were sent of which 1240 (71%), 271 (15.5%) and 236 (13.5%) were from blood, urine, and other sites, respectively. Of these, 238/1747 (13.6%) isolates grew bacteria. Gram-positive isolates accounted for 26.4% of all positive cultures and S. aureus was the most common amongst these (77%). Gram-positive organisms were most frequently isolated from the bloodstream and other sites and only occasionally from urine ($P < 0.001$). Gram-negative infections accounted for 74% of all positive isolates, with *E. coli* (71/176) and *Klebsiella* (70/176) were the most common (74%) pathogens identified, and *E. coli* and *Klebsiella* comprised 80% of them. A high incidence of extended spectrum beta-lactamase producing organisms (84%), beta-lactam beta-lactamase inhibitors (78%), and carbapenem resistance was observed (29%). Bloodstream infection with multi-drug resistant *Klebsiella* was associated with high mortality. The gram-positive bacteria isolated were predominantly *staphylococcus aureus* and were antibiotic sensitive. (22).

Across sectional study was conducted by Haddaishagaris S et al., at the National Cancer Institute (NCI), Cairo, Egypt a total of 175 Gram-negative bacterial isolates were isolated from different samples collected from cancer patients admitted to the National Cancer Institute (NCI), Cairo, Egypt, and processed by standard microbiological methods. 143 bacterial isolates were recovered from adult patients, and 32 were recovered from children. *Escherichia coli* showed the highest frequency (36%), followed by *Klebsiella pneumonia* (30.85%), *Acinetobacter baummannii* (14.28%), and *Pseudomonas sp*. (9.14%). Antibiotic profiles revealed that bacterial isolates are highly resistant to the most commonly available antibiotics. Amikacin and gentamicin were the most effective antibiotics against isolated Gram-negative bacteria. Moreover, most bacterial stains produce virulence factors, including EsβLs, biofilm, and siderophores. *E. coli isolates* produced ESβLs with rates of 25.28%, *Klebsiella pneumonia* (11.0%), and *Pseudomonas sp*. (25.0%). Among these collected bacterial isolates, 132 (75.4%) can form a biofilm to different degrees. Also, most bacteria isolate generated siderophores, with 133 (75.94%) (23).

A cross-sectional study was conducted Shwk S et al., at the National Cancer Institute; Cairo University was analyzed. Identification and antimicrobial susceptibility of isolates were done using Microscan Walk Away 96. Of 116 patients, the *ESKAPE* pathogens isolated were *Enterobacter spp*., methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterococci* in 12%, 23%, 37%, 10%, 9%, and 9% of episodes, respectively. Health-care acquired infections constituted 75% of *ESKAPE* infections. Duration of episodes and overall mortality were significantly higher in *ESKAPE* BSIs when compared to *CoNS* (14.5 ± 7.6 versus 09.9 ± 6.9), and (26% versus 4%); respectively, p value <0.001.(24).

A hospital-based cross-sectional study was conducted by Restane D et al., at the University of Gondar Hospital, cancer treatment center from February to April, 2017. A total of 216 cancer patients were included in the study. Among the 42 culture positive cases, 6% (6/100) were from the urine of asymptomatic patients. The majority of the isolates were from urine (n=32) and wound swab (n=7), with a low frequency of the isolates from blood (n=3) and ear discharge (n=1) samples. GPB species (25, 58.1%) were higher than GNB species (18, 41.9%). GPB accounted for 41.9% (18/43) of the UTI, 9.3% (4/43) of wound infections, and 7% (3/43) of blood stream infections while GNB attributed to 32.6% (14/43) of UTI and 7% (3/43) of wound infections. The overall predominant bacterial isolates were *S. aureus* (12, 28.6%), followed by *CONS* (11, 26.2%) and *E. coli* (9, 21.4%). The most common antimicrobial isolates identified in UTI were *E. coli* and *CONS* (9, 28.1%) each and nalidixic acid (6, 66.7%). Moreover, fluoroquinolone (ciprofloxacin and/or norfloxacin) resistance was observed in 33.3% of *E. coli*(25).

A hospital-based, cross-sectional study was conducted by Worku Met al., on cancer patients at University of Gondar comprehensive specialized hospital from March to July 2021. In this study, out of 200 cancer patients included and 67.5% (135/200) of them were males. The majorities of study participants, 56% (113/200) of cancer patients were pediatrics and 26.5% (53/200) of them belong under five years of age. Out of 200 patient samples that had undergone culture, 27% (54/200) samples had bacterial growth. Gram-positive bacteria isolates were predominant, 61.1%, and *S. aureus* was the predominant Gram-positive iso-late, (51.5.6%), followed by coagulase-negative *staphylococci* (48.5%). Moreover, *K. pneumoniae* (47%) and *P. aeruginosa* (29.5%) were the most common Gram7-negative bacterial isolates. Among patients who had BSIs, the highest

prevalence of BSIs was observed among males (66.7%), and in pediatric cancer patients (44.2%) (26).

A case-control study was performed by Olive P et al., at the National Cancer Institute (Instituto Nacional do Cancer [INCA]) PICU, Rio de Janeiro, Brazil. Data were collected from all infection episodes related to GNB that occurred between January 1, 2009, and December 31, 2012, in PICU patients, aged between 0 months and 18 years, who were hospitalized for more than 24 h. There were 765 admissions to the PICU between January 1, 2009, and December 31, 2012. There were 101 episodes of GNB infection in 76 patients in 86 admissions, and 47 (46.5%) of these episodes were related to MDR-GNB infection. The most frequently occurring pathogens among the MDR-GNB were as follows: *A. baumannii*, seven (17%); *S. melophilia*(27), seven (15%); *Enterobacter* spp., seven (15%); and *Klebsiella pneumonia*, seven (15%). The most frequently occurring pathogens among the non-MDR-GNB were as follows: *P. aeruginosa*, 22 (41%); *A. baumannii*, 14 (26%); and *K. pneumoniae*, eight (15%). Polymicrobial infection was present in one MDR-GNB patient (2.0%, n = 1/47) and two non-MDR-GNB patients (3.8%, n = 2/54)(27).

A retrospective study conducted by Star M et al., at the Royal Children's Hospital, Melbourne, on GN bacteremia oncology patients from 2003 to 2010 were retrospectively identified from the hospital microbiology laboratory database. Blood cultures were processed using the Bact/alert system. Identification and susceptibility testing by using disk diffusion method. 280 positive blood culture episodes of GN bacteremia in 210 patients were identified. Of these, 42 episodes in 35 patients were caused by an AR GN organism Overall, *Escherichia coli* was the most common bacterium (33.2%), followed *by Klebsiella spp*. (21.8%) and *Enterobacter spp*. (13.6%). Of the non-lactose fermenters, *Pseudomonas aeruginosa* (11.1%) was the most frequently isolated (28)

A retrospective chart review study was conducted by Castagola E et al., in centers located in Australia (n = 1), Brazil (n = 1), Canada (n = 1), Chile (n = 1), Germany (n = 3), Italy (n = 4), Russian Federation (n = 1) and Switzerland (n = 3). between 1 January 2015 and 31 December 2017 in patients aged ≤ 18 years, with a disease treated with chemotherapy. A total of 1291 BSIs with 1379 strains were reported in 1031 patients. Among Gram-negatives, more than 20% were resistant to ceftazidime, cefepime, piperacillin-tazobactam, and ciprofloxacin while 9% were resistant to meropenem. Methicillin resistance was observed in 17% of *S. aureus* and vancomycin resistance in 40% of *E. faecium*. Previous exposure to antibiotics, especially to carbapenems, was

significantly associated with resistant Gram-negative BSI while previous colonization with methicillin-resistant *S. aureus* was associated with BSI due to this pathogen(29).

cross-sectional study was conducted by Yusuf K et al., at the Institute of Child Health, Lahore, Pakistan by utilizing a non-probability sampling technique (convenient sampling) for 6 months. 300 pediatric patients who received prescriptions with at least one antibiotic suffering from cancer and were treated with at least one antibiotic were included. The study findings depicted that children aged between 4 to 7 years were affected by various cancers and lymphoblastic leukemia (47%) was the most frequently occurring cancer. Urine, blood, and mucosal swabs were used for sampling, but most samples were blood (29%) samples used to detect AMR. The study population was observed to be more susceptible to gram-negative than gram-positive bacteria. The incidence of AMR was higher in gram-negative bacteria than in gram-positive bacteria. Among the collected samples the bacteria showed sensitivity for ciprofloxacin (23%), amikacin (21%), piperacillin (24%), and ceftazidime (23%). Considering the resistance patterns among the tested antibiotics that were prescribed to the cancer patients various penicillin antibiotics and ceftazidime showed a higher incidence of the AMR(7).

A cross-sectional study was conducted by Toor KM et al., at Department of Pediatric Foundation University School of Health Sciences, Islamabad between January 2023 and January 2024 on Pediatric cancer patients aged 1 to 18 years with febrile neutropenia (FN) during or after chemotherapy were included. The study included 135 pediatric cancer patients with febrile neutropenia. The study cohort consisted of 47% children aged 6-10 years, with a male predominance (56%). Leukemia was the most common cancer type (47.4%). All patients presented with fever; other symptoms included fatigue (63.7%), cough (36.3%), and abdominal pain (24.4%). Of the 78 positive blood cultures, *Staphylococcus aureus* (28.2%) was the most common gram-positive bacterium, exhibiting high resistance to penicillin (95%) and methicillin (60%). Among gram- negative bacteria, *Escherichia coli* (25.6%) showed significant resistance to cephalosporins (90%) and aminoglycosides (35%). *Klebsiella pneumoniae* (11.5%) and *Pseudomonas aeruginosa* (9.0%) also demonstrated notable resistance patterns(30).

Prospective cohort study was conducted by Batashi A et al., between January 2015 and October 2017 of pediatric oncology patients at the Royal Hospital in Oman. A total of 74 episodes of positive bacterial blood cultures were detected in 38 oncology patients (positive blood culture rate

of 51%). Fifty-seven percent were positive for gram-negative organisms with *Klebsiella* (21%) being the most common gram-negative organism cultured, and the most common gram-positive organism was Staphylococcus (coagulase-negative *Staphylococcus* (CONs) and *S. Aureus*) (30%). The majority of patients had gastrointestinal complaints (74%), and almost half (51%) had prolonged periods of neutropenia (>7 days). One-third of gram-negative organisms were resistant to four or more antibiotics with a major resistance of 31% to piperacillin-tazobactam. Of the gram-positive organisms, 38% were resistant to at least four antibiotics and 30% were pan-resistant (except for vancomycin) (1).

A cross-sectional study was conducted by Shresm G et al., at B.P. Koirala Memorial Cancer Hospital (BPKMCH), Bharatpur, Chitwan, Nepal between July 2018–June 2019. Out of 308 patients who had undergone culture, 73 (24%) of the samples had bacterial growth. The most common organisms isolated were *E. coli* (58%), *Staphylococcus* (11%), and *Klebsiella* (10%). These bacteria had undergone susceptibility testing to 27 different antibiotics in various proportions. Of the limited antibiotic testing levels, nitrofurantoin (54/66, 82%) and amikacin (30/51, 59%) were the most common. Among those tested, there were high levels of resistance to antibiotics in the "Access" and "Watch" groups of antibiotics (2019 WHO classification). In the "Reserve" group, both antibiotics showed resistance (polymyxin 15%, tigecycline 8%). Multidrug resistance was seen among 89% of the positive culture samples.(31)

A retrospective study was conducted by Kapoor G et al., in the pediatric hematology-oncology department of a tertiary care cancer center in north India over 24 months (2012-2014). During the study period, a total of 1747 culture samples were sent of which 1240 (71%), 271 (15.5%), and 236 (13.5%) were from blood, urine, and other sites, respectively. Of these, 238/1747 (13.6%) isolates grew bacteria. Gram-positive isolates accounted for 26.4% of all positive cultures and S. aureus was the most common (77%). Gram-positive organisms were most frequently isolated from the bloodstream and other sites and only occasionally from urine ($P < 0.001$). Gram-negative infections accounted for 74% of all positive isolates, with *E. coli* (71/176) and *Klebsiella* (70/176) being the most frequently identified organisms followed by *Pseudomonas* (29/176), *Acinetobact*er (4), *Enterobacter* (1), and *Proteus* (1). Among Gram-negative isolates, *E. coli* was most commonly isolated from urinary specimens (61%), while *Klebsiella* accounted for most (50%) of the bloodstream organisms—probability (32).

A prospective observational analysis study was conducted by Haddaishagari S et al., at a tertiary care center for pediatrics at India in 2013. Of the cumulative 207 cultures sent (83 blood and 124 urine) in both hematological and solid tumors, 89 cultures (33 blood and 56 urine) were positives. Out of 89 positive cultures 76 were gram-negative isolates. The most common gram-negative bacterial isolates were Escherichia coli 33 (37%), followed by *Pseudomonas* 21 (23.5%). *Acinetobacter* grew in 2 patients (2.2%). Extended-spectrum beta-lactamases (ESBLs), carbapenem-resistant and pan-resistant organisms were seen in 28 (31.4%), 5 (5.6%), and 2 cases (2.3%) respectively. Overall, Gram-positive organisms were 13/89 (12.3%). *Staphylococcus* was the most common Gram-positive organism and methicillin-resistant *Staphylococcus aureus was* seen in 5 each.(33).

A retrospective surveillance study was conducted by Bhat Set al., in the Department of Oncology of Kasturba Medical College Hospital (KMCHA) during the period from 1st January 2015 to 31st December 2016. Blood culture was done by BacT/ALERT system (BioMerieux, USA). Out of 638 cancer patients diagnosed with infections in the 2 years, 140 patients had positive cultures, representing 272 specimens and 306 isolates. Common specimens sent for culture were blood sputum, urine, and pus. 214 isolates (69.9%) were gram-negative bacilli, and 92 (30.1%) were gram-positive cocci. The most common isolates were *Klebsiella spp*. (18.30%), *Pseudomonas spp*. (17.65%), and *Escherichia coli* (14.71%) followed by *Staphylococcus aureus* (13.72%). Among the gram-negative organisms, the antibiotic resistance rates reported to fluoroquinolones, aminoglycosides, and third-generation cephalosporins were 45.13%, 39.20%, and 48.58%, respectively. 26.92% of the organisms are resistant to all three antibiotics. 50.4% of *Klebsiell*a *spp*. and *Escherichia coli* were ESBL producers. Gram-negative organisms showed 11.63% resistance to β-lactam/ β-lactamase inhibitor combination, and 22.22% of gram-negative organisms are resistant to carbapenems. 50% of the *Staph- ylococcus spp*. were methicillin resistant, but all were sensitive to vancomycin. Out of 638 cancer patients diagnosed with infections in the 2-year period, 140 patients had positive cultures, representing 272 specimens and 306 isolates. Common specimens sent for culture were blood sputum, urine, and pus. 214 isolates (69.9%) were gram-negative bacilli, and 92 (30.1%) were gram-positive cocci. The most common isolates were *Klebsiella spp*. (18.30%), *Pseudomonas spp*. (17.65%), and *Escherichia coli* (14.71%) followed by *Staphylococcus aureus* (13.72%). Among the gram-negative organisms, the antibiotic resistance rates reported to fluoroquinolones, aminoglycosides, and third-generation

cephalosporins were 45.13%, 39.20%, and 48.58%, respectively. 26.92% of the organisms are resistant to all three antibiotics. 50.4% of *Klebsiella spp*. and *Escherichia coli* were ESBL producers. Gram-negative organisms showed 11.63% resistance to β-lactam/ β-lactamase inhibitor combination, and 22.22% of gram-negative organisms are resistant to carbapenems. 50% of the *Staphylococcus spp*. were methicillin resistant, but all were sensitive to vancomycin(34).

Prospective cohort study of pediatric oncology patients was conducted by Battashi A et al., between January 2015 and October 2017 at the Royal Hospital in Oman. Patient demographics, clinical data, laboratory parameters, microbial etiology and susceptibility, and outcomes were retrieved and analyzed. A total of 74 episodes of positive bacterial blood cultures were detected in 38 oncology patients (positive blood culture rate of 51%). Fifty-seven percent were positive for gram-negative organisms with *Klebsiella (*21%) being the most common gram-negative organism cultured, and the most common gram-positive organism was *Staphylococcus* (*coagulase negative Staphylococcus* (CONs) and *S.aureus*) (30%). The majority of patients had gastrointestinal complaints (74%), and almost half (51%) had prolonged periods of neutropenia (>7 days). One third of gram-negative organisms were resistant to four or more antibiotics with a major resistance of 31% to piperacillin-tazobactam. Of the gram-positive organisms, 38% were resistant to at least four antibiotics and 30% were pan-resistant (except for vancomycin)(35).

Retrospective study conducted by All mulla NA et al., during the period of 2004–2011; a total of 70 (38%) patients were diagnosed with bloodstream infections. Bacterial etiology was determined, along with their susceptibility patterns. A total of 70 patients (38%) were diagnosed with acute leukemias, lymphomas, solid tumors, or brain tumors; those patients experienced 111 episodes of bacteremia. The most common Gram-positive (n=64 [55%]) isolates were Staphylococcus epidermidis (n=26), *Staphylococcus hominis* (n=9), and *Staphylococcus hemolytic* (n=7), and the common Gram- negative (n=52 [45%]) isolates were *Klebsiella pneumoniae* (n=14), *Pseudomonas aeruginosa* (n=10), and *Escherichia coli* (n=7)(36).

A Prospective observational study conducted by Hassoune D et al., at a 200-bed university referral cancer center for adults in Barcelona, Spain. From January 1, 2006 to December 31, 2011, all episodes of bacteremia occurring in hospitalized patients with cancer were included. Of 1,148 episodes of bacteremia, 392 (34 %) were caused by *ESKAPE* pathogens. Fifty-four episodes (4.7 %) were due to r*ESKAPE* strains (vancomycin-resistant *Enterococcus faecium* 0, methicillin-

resistant Staphylococcus aureus (MRSA) 13, extended-spectrum beta-lactamase (ESLB)-producing *Klebsiella pneumoniae* 7, carbapenem- resistant *Acinetobacter baumannii* 4, carbapenem- and quinolone-resistant *Pseudomonas aeruginosa* 18 and derepress ion chromosomic ß-lactam and ESBL-producing *Enterobacter species* 12. Risk factors independently associated with r*ESKAPE* bacteremia were comorbidities, prior antibiotic therapy, urinary catheter and urinary tract source. Inappropriate empirical antibiotic therapy was more frequent in patients with r*ESKAPE* bacteremia than in the other cases (55.6 % vs. 21.5 %, p <0.001). Persistence of bacteremia (25 % vs. 9.7 %), septic metastasis (8 % vs. 4 %) and early case-fatality rate (23 % vs. 11 %) were more frequent in patients with r*ESKAPE* bacteremia than in patients with other etiologies (p <0.05)(37).

A prospective observational study conducted by Bodro M et al., at a 200-bed university referral cancer center for adults in Barcelona, Spain. From January to December. Of 1,148 episodes of bacteremia, 392 (34 %) were caused by *ESKAPE* pathogens. Fifty-four episodes (4.7 %) were due to r*ESKAPE* strains (vancomycin-resistant *Enterococcus faecium* 0, methicillin-resistant *Staphylococcus aureus* (MRSA) 13, extended-spectrum beta-lactamase (ESLB)-producing *Klebsiella pneumoniae* 7, carbapenem resistant *Acinetobacter baumannii* 4, carbapenem- and quinolone-resistant Pseudomonas aeruginosa 18 and derepress ion chromosomic ß-lactam and ESBL-producing *Enterobacter species* 12. Risk factors independently associated with r*ESKAPE* bacteremia were comorbidities, prior antibiotic therapy, urinary catheter and urinary tract source. Inappropriate empirical antibiotic therapy was more frequent in patients with r*ESKAPE* bacteremia than in the other cases (55.6 % vs. 21.5 %, p <0.001). Persistence of bacteremia (25 % vs. 9.7 %), septic metastasis (8 % vs. 4 %) and early case-fatality rate (23 % vs. 11 %) were more frequent in patients with r*ESKAPE* bacteremia than in patients with other etiologies (p <0.05)(38).

A prospective, single-center, observational, cohort study was conducte by Faizan M et al., or clinical audit. Eighty-three patients, from age 1 month to 16 years, who were admitted to the Pediatric Oncology department at the Children's Hospital Lahore, Pakistan. The total number of hospitals acquired infection was 101 while the total number of infected patients was 83. The number of infections per 1000 patient days was 6.66. The most common HAI was bloodstream infection (BSI). Sixty-nine percent of HAIs were gram- negative while 31% were gram-positive. *Klebsiella* species was the most common gram-negative and *Staphylococcal Aureus* was the most

common gram-positive organism. Pan-resistance was encountered in 25.74%, Carbapenem resistant *Enterobacteriaceae* in 16%, Vancomycin-resistant *Enterococc*i in 3%, and Methicillin-resistant *Staphylococcus Aureus* in 2%. Mean hospital stay was 20.22 days, 60.25% of patients were discharged, and 39.75% expired(39).

A cross-sectional study was conducted by Paul M et al., at a tertiary care teaching hospital located in Rishikesh, Uttarakhand, for a time period of 7 months from January to July 2019. Mean age ± SD of the study population was 32.39 ± 10.56 years with a male to female ratio of 1.55:1. 74.18% of the blood samples were received from patients suffering from hematological malignancies. Microbiologically confirmed BSIs were observed in 27.1% patients. Gram-negative bacilli were predominantly isolated in culture with *Klebsiella spp*. being the most common. Percentage resistance values of gram-negative bacilli to aminoglycosides, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones, cephalosporins, carbapenems, chloramphenicol, ampicillin, co-trimoxazole, and doxycycline were 26.6 to 91.7%, 8.3 to 86.6%, 10 to 66.7%, 13.3 to 73.3%, 8.3 to 73.3%, 80 to 93.3%, 13.3 to 20%, 16.7 to 66.6%, and 13.3 to 16.7%, respectively(40).

A prospective study, conducted by Moghim Met al., at Shahid Sadoughi hospital in from 2012 to 2015. Out of 212 participants, 62.3% (132/212) were suffering from hematologic malignancies, and 37.7% (80/212) had solid tumors. Gram-negative bacteria were the predominant microorganisms (84.9%). *E. coli* was the most frequently isolated pathogen (38.68 %), followed by *Klebsiella* (14.15%) and *Acinetobacter species* (11.32%). In addition, *Staphylococcus epidermidis* was the most common isolated Gram-positive bacteria (8.5%). Gram-negative bacteria were susceptible to ciprofloxacin with a response range of 53.7% to 100%. The majority of *E. coli* isolates were sensitive to ceftazidime (87.8%) and were resistance to Co-trimoxazole (15.8%). *Klebsiella* isolates were 100% susceptible to cephalosporins, meropenem and imipenem(41).

An institutional-based prospective cross-sectional study was conducted by Amsale G et al., on 370 pediatric (age birth-15 years) patients suspected of sepsis at the University of Gondar Comprehensive Specialized hospital from December 2020 to November 2021. Out of the total 370 study subjects, 21.6% (80/370) of them were culture positive. Of these, 43 (53.8%) and 37 (46.3%) were Gram-positive and Gram-negative bacterial pathogens, respectively. The most prevalent Gram- positive bacterial isolate was *Staphylococcus aureus* (n = 24; 30%) and *coagulase negative*

staphylococci (n = 7; 8.8%). Among the Gram-negative bacterial isolates, the leading bacteria was *Klebsiella pneumoniae* (n = 20; 25%) followed by *Escherichia* coli (n = 7; 8.8%). Clindamycin, Chloramphenicol, Gentamicin and Ciprofloxacin were the most effective antibiotics against Gram-positive bacterial isolates while Amikacin, Meropenem and Chloramphenicol were effective against Gram-negative pathogens. Methicillin resistance was detected in 45.8% of Staphylococcus aureus. Multi-drug resistance (MDR) antimicrobial susceptibility pattern was observed in 76% of the bacterial isolates(42).

Article review conducted by Rabi K et al., at Technological University Dublin in 2024.The current healthcare environment is at risk due to the facilitated transmission and empowerment of the *ESKAPE* pathogens, comprising of *Enterococcus faecium, Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*. These pathogens have posed significant challenges to global public health and the threat has only amplified over time. These multidrug-resistant bacteria have become adept at escaping the effects of conventional antibiotics utilized, leading to severe healthcare-associated infections and compromising immunocompromised patient outcomes to a greater extent(43).

A retrospective study, analyzing by Shawky S et al., the surveillance results of blood stream infections in collaboration with the Pediatric Oncology department at National Cancer Institute, Cairo University from June to December 2012. In a 6-month period, *ESKAPE* pathogens were isolated from non-duplicate blood cultures in 81 episodes of 72 cases of pediatric cancer patients, while CONS were isolated from 135 blood cultures of 116 patients. The *ESKAPE* pathogens isolated were *Enterobacter spp*., methicillin- resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterococci* in 12%, 23%, 37%, 10%, 9%, and 9% of episodes, respectively. Health-care acquired infections constituted 75% of *ESKAPE* infections. Duration of episodes and overall mortality were significantly higher in *ESKAPE* BSIs when compared to CONS (1.5 ± 7.6 versus 09.9 ± 6.9), and (26% versus 4%); respectively, p value <0.001 (44).

Objective

3.1 General Objective

To investigate the prevalence and antibiotic resistance pattern of *ESKAPE* pathogens among pediatric cancer patients suspected (BSI) and (UTI) and associated risk factors at Tikur Anbessa Specialized Hospital.

3.2 Specific Objectives

- To determine prevalence of *ESKAPE* pathogens among pediatric cancer patient with BSI and UTI.
- To assess antibiotic resistance profile of *ESKAPE* pathogens isolated from blood and urine cultures of pediatric cancer patients with BSI and UTI.
- To identify the associated risk factors for *ESKAPE* infection among pediatric cancer patient with BSI and UTI.

4. Materials and methods

4.1 Study area

This study was conducted at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. It is one of Ethiopia's largest general public hospitals in the country. It was established in 1964 and now the main teaching center for both clinical and preclinical training of most disciplines. The hospital provides a tertiary level referral treatment and is open 24 hours for emergency services. The hospital is administered by Addis Ababa University and is the largest teaching hospital in Ethiopia. It is the only cancer referral treatment center that provides service for over 60,000 cancer patients annually in Ethiopia. In the pediatric oncology department, there are 25 beds at D7 ward, 10 beds at, C7 ward, and, at pediatric emergency.

4.2 Study design and period

A cross-sectional study design was employed to identify the prevalence and antimicrobial susceptibility pattern of *ESKAPE* pathogen and associated risk factors among pediatric cancer patients who will be taking chemotherapy at TASH from March 2025 to June 2026.

Population

Source population

All pediatric cancer patients attending the TASH oncology department during the study period.

4.3.2 Study population

All pediatric oncologic patients who were be taking chemotherapy for pathologically confirmed cancer and who fulfill the inclusion criteria were be our study group at TASH during the study period.

All pediatric cancer patients during chemotherapy with feasible symptom of BSI and UTI.Who are willing to participate in the study and able to provide either consent or assent from April 2025 to May 2025. Mothers/guardians of pediatrics who will not volunteer for written consents will be excluded Patients who are too ill to participate in the study.

4.5 Study Variables

4.5.1 Dependent Variables

- ESKAPE pathogen prevalence
- Antimicrobial resistance pattern

4.5.2 Independent Variables

- Age
- Sex
- Type of malignancy
- Stage of cancer
- Type of chemotherapy taken
- Round of chemotherapy
- Use of catheter
- Imaging test
- Radio therapy
- Being neutropenic
- Surgery
- Antibiotic taken

4.6 Sample size calculation and sampling method

4.6.1 Sample size calculation

The sample size calculation is based on single sample size estimation formula. The value of P is taken as 0.5 because there is no previous study. Considering 95% confidence interval, 5% margin of error and using single sample size estimation formula the sample size is calculated as follows

$sample\ size\ n = z2 * p(1-p)/\ d2$

Where n = Sample size

α = level of significance

z = at 95% confidence interval Z value =>Z α/2=1.96

p = Estimated proportion of the population (0.5 for unknown)

d = Margin of error at (5%) (0.05)

n = (1.96^2 * 0.5 * (1-0.5)) / 0.05^2 = 384.16

n≈ 384

The initial sample size was be estimated as 384 participants, and finally, by computing a 10% (38 subjects) nonresponse rate, the final sample size was be consolidated as **422**.

4.6.2 Sampling method

A convenient sampling technique was be used to recruit study participants to the study.

4.7 Measurement and Data Collection

4.7.1 Sample and Data Collection Procedure

For blood sampl,1 ml, 2 ml, 3–5, and 5–10 ml of blood was collected from children birth- 1, 2–5, 6–12, and 13–18 years old respectively in two bottles by nurses and clinicians under strict aseptic conditions using 70% alcohol followed by povidone-iodine, and also bottles were labeled with the patient's identification number and date of collection. Blood was transferred to a blood culture bottle containing a 1:5 blood-to-broth dilution was made. Two blood culture bottles were processed for each patient. Daily checks of culture bottles were done for up to 6 days. For urine sample, early morning 5 ml midstream urine (MSU) specimens were collected using lick proof sterile plastic containers. Structured and pretested questionnaires were used to record all relevant socio-demographic and clinical data of participants. The required information was obtained via interview and from patient medical records.

4.7.2 Laboratory Analysis

4.7.2.1 Culture and Identification

4.7.2.1.1 Blood culture identification

Using the standard procedure, the blood culture was processed in the microbiology laboratory by the conventional method. The blood culture bottle was incubated at 37˚C for 24hr then sub-cultured. Bottles that showed signs of growth based on turbidity or hemolysis were sub-cultured on blood agar and McConkey agar. The blood agar and McConkey agar was incubated aerobically at 37°C for 24 to 48 hrs. Blood culture bottles that show no sign of growth was further incubated

at 37˚C then was checked the growth inspection daily for up to 7 days. Blood culture broths with no microbial growth after 6 days were sub-cultured for 48 hours before being reported as a negative result. Colony morphology, gram staining reaction from the growth colony, and biochemical tests were used for the identification of bacteria.

4.7.2.1.2 Urine culture identification

Urine sample from each patient was inoculated onto cysteine-lactose-electrolyte deficient agar CLED/ (Oxoid, Basingstoke, Hampshire, England) plates using a calibrated inoculating loop with a capacity of 0.001 ml. The inoculated plates were be incubated for 24–48 h at 37 °C aerobically. Growths of a colony count of $\geq 10^5$ cfu/ml were be considered significant bacteriuria. Then sub-cultured to MacConkey agar.

4.7.2.2.1 Drug Resistance Patterns

Drug susceptibility patterns of bacterial isolates were performed based on CLSI 2025 guidelines. The suspension was be prepared using a sterile wire loop; from a pure culture 3-5 pure colonies of bacteria were picked and emulsified in nutrient broth standard inoculums were adjust to 0.5 McFarland and swabbed onto Muller Hinton agar. The antibiotic discs were included: ampicillin (10µg), ceftriaxone (30µg), cefepime(30µg), ceftazidime(30µg), amoxicillin(25µg), gentamicin (10 µg), trimethoprim + sulfamethoxazole (1.25 + 23.75 µg), ciprofloxacin (5 µg), for gram-negative isolated bacteria were used. For gram positive bacteria isolates, vancomycin ampicillin (10 µg), amoxicillin (25 µg), penicillin G (10 units), gentamicin (10 µg), trimethoprim + sulfamethoxazole (1.25 + 23.75 µg), erythromycin (15 µg), were used. Using the Kirby Bauer disk diffusion method, antimicrobial susceptibility testing was performed for each pure isolated bacterium and incubated at 37 °C for 18-24 hours. The zones of inhibition were measured to the nearest millimeter and isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI 2025.

4.8 Quality Assurance

The quality of the study was be assured by preparing SOPs. Samples were be collected in a sterile container and following SOPs. The microbiological evaluation of samples was be carried out following the protocol in the clinical and laboratory standards institute. Culture results were be recorded before entry to the statistical tool. Before the analysis, the data were be checked for consistency and completeness, and incomplete data were be excluded before proceeding to data analysis. The quality of the study was be also assured by implementing quality control measures

that include sterility and performance checks throughout the whole process of the laboratory work. *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923, *K. pneumonia ATCC* 700603, *Enterococcus spp* ATCC 29212 control strains were be used.

4.9 Data Analysis and Interpretation

Collected data were processed, edited, and analyzed using SPSS version 25.0 (Statistical Package for Social Sciences, SPSS) statistical. The descriptive data like frequency, percentage, mean, standard deviation, prevalence of BSI and UTI was calculated. Bi-variate and multivariate logistic regression analyses using forward conditional method were used to assess the possible risk factors of surgical site infection. The level of significance P value <0.25 in bi-variate analysis were included in multivariate logistic regression(17). Odds ratio with 95% confidence interval was calculated to determine degree of association between dependent and independent variables. A P-value below 0.05 was considered statistically significant for all cases

Operational definition

Multi-drug Resistance is defined as non-suability to at least one agent in three or more antimicrobial categories

4.10 Ethical Consideration

The proposal of this study was submitted to the ethical review board of Addis Ababa University, College of Health Sciences, and the Department of Laboratory Sciences review board. Before data collection ethical clearance letter was received from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, and the Department of Laboratory Sciences protocol number DRERC/603/21/MLS. A permission letter was also obtained from the study site. The objective of this study was explained to the study participants' families. The purpose of this study and the benefit of this research was explained in detail and laboratory samples were taken voluntarily.

5. Dissemination of Results

The findings of this research were disseminated to different bodies. One copy was submitted to Addis Ababa University, the College of Health Sciences, and the Department of Laboratory Sciences. The findings of this study were presented to internal and external examiners as per the

requirements of the department. The study was submitted for consideration in peer-reviewed journals.

5. Results

5.1 Socio demographic characteristics of pediatric cancer patients active on chemotherapy

A total of 422 patients involved who fulfilled the inclusion criteria, pediatric cancer patients actively taking chemotherapy at Tikur Anbessa specialized Hospital between March 2025 to May 2026 were included to the study. Out of these 273 (64.7%) were male and 149 (35.3%) were female. The mean age of the participants were 2.94 ± 0.93 years with maximum age of participants 18 years and minimum age 5 months. Addis Majority of participants, 257 (60.9%) were from Addis Ababa (Table 1).

Table 1 Socio-demographic characteristics of pediatric cancer patients active on chemotherapy at Tikur Anbessa Hospital, Addis Ababa, Ethiopia, from March 2025 to May 2026 (n=422)

Variable	Category	Frequency, n	Percent (%)
Age group	0-1	21	5
	2-5	130	30.8
	6-12	123	29.1
	13-18	148	35.1
Gender	Male	273	64.7
	Female	149	35.3
Residence	Addis Ababa	257	60.9
	Out of Adis Ababa	165	39.1

5.2 Clinical data of study participants

Out of 422 pediatric cancer patients, 346(82%) had hematologic malignancy and 76(18%) had solid tumors. Among the total, acute lymphoblastic leukemia 238(69.2%) was the highest, followed by acute myeloid leukemia 38(%). From solid tumors; rhabdomyosarcoma, wilms tumor and brain tumor accounts for 36(46.2%), 16(20.5%) and 11(14.1%) respectively. More than half of 238(56.4%) patients were on the second cycle of chemotherapy and 176(41.7%) of participants had advanced stage cancer, most patients 298(70.6%) acquire infection after 72 hours of admission. Longer duration of hospital stay observed among 131(31.5%) patients. X-ray and ultrasound were the most frequently 262(62.1%) utilized imaging tests observed in 262 (62.1%) participants. Only 37(8.8%) of patient undergone radiotherapy. 194(46.0%) of participant had urinary catheter insertion, considerable proportion 173(41.0%) of patient were neutropenic and 125(29.6%) had surgery (Table 2).

Table 2 Clinical characteristics of pediatric cancer Patients active on chemotherapy at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, from March 2025- May 2026(n=422)

Variable		Frequency (422)	Percent (%)
Type of malignancy	Hematologic	346	82
	Solid	76	18
Hematologic malignancy	ALL	238	69.2
	AML	38	11.0
	CLL	3	0.9
	MS	1	0.3
	NHL	21	6.1
	BL	35	10.2
	Lymphoma	8	2.3
Solid malignancy	Brain tumor	11	14.1
	Wilms tumor	16	20.5
	RMS	5	6.4
	Medulloblastoma	3	3.8

	Neuroblastoma	5	6.4
	Hepatoblastoma	5	6.4
	Erwin sarcoma	2	2.6
Round of chemotherapy	First	184	43.6
	Second	238	56.4
Stage of cancer	Early	246	58.3
	Advanced	176	41.7
Length of hospital stay	1 Month	289	68.5
	2 Month	133	31.5
Onset	Before 72 hours of admission	124	29.4
	After 72 hours of admission	298	70.6
Do you taken Antibiotics	Yes	142	33.5
	No	280	66.4
Types of imaging test	X-ray	39	9.2
	Ultrasound	38	.09
	CT-scan	2	5
	X-ray	262	62.1
	X-ray, Ultrasound and CT scan	57	13.5
	CT-scan and Ultrasound	24	5.7
MRI	-	-	-
Radio therapy	Yes	37	8.8
	No	385	91.2
Catheter	Yes	194	46.0
	No	228	54
Types of catheters	Foley	176	41.7

	Canula	246	58.3
Neutropenia	Yes	173	41.0
	No	29	59.0
Surgery	Yes	125	29.6
	No	297	70.4

5.3 Prevalence of ESKAPE isolates from blood and urine sample

Out of 422 pediatric cancer patients who are taking chemotherapy and investigated for blood stream infections and urinary tract infections, 120(28.4%) ESKAPE pathogens were isolated. From 120 ESKAPE pathogens, 67/120(55.83%) were isolated from blood culture while 53/120(44.17%) were identified from urine culture. From the total ESKAPE pathogen, 76/120(63.33%) were gram negative ESKAPE pathogen while, 44/120(36.67%) were gram positive bacteria. From a total bacterial pathogen isolated from blood K. *pneumonia24* were dominant accounting 24(35.8%) for blood and, 21 for urine. (Table 3)

Table 3 Distribution of ESKAPE pathogen isolates from blood and urine specimens among pediatric cancer patients who were active on chemotherapy at the Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia, from March 2025- May 2026.

Bacterial isolates	Blood, = 232		Urine, n = 190	
	Frequency (67)	Percent (%)	Frequency (53)	Percent (%)
Enterococcus. spp	13	20.9	13	22.6
S. aures	11	16.4	7	13.2
K. pneumonia	24	35.8	21	39.6
Acinetobacter spp	9	13.4	1	1.9
P. aeruginosa	4	5.9	8	15.1
Enterobacter spp	6	8.9	3	5.7

5.4 Antimicrobial Susceptibility pattern of bacterial isolate

Regarding antimicrobial resistance pattern for gram negative ESKAPE pathogen isolated from blood and urine culture, high resistance rates were observed to ceftriaxone (92.10%), ceftazidime (85.53%) ciprofloxacin (81.58%), amikacin (78.95%), trimethoprim-sulfamethoxazole (77.63%), cefepime (75%), pip-tazobactam (72.37%), gentamicin (69.74%), meropenem (64.47%),

nitrofurantoin (61.84%) In contrast, low resistance rates were detected to tobramycin (6.98%). (Table 4.1). For gram positive ESKAPE isolated from blood and urine culture, high resistance rates were detected bacteria isolated from urine sample on nitrofurantoin (78.95%), ampicillin (57.89%), ciprofloxacin (57.89%), vancomycin (57.89%), (73.80%). On the other hand, a low resistance rate was detected for oxacillin (5.26%) and (Table 4.2).

Table 4.1 Antimicrobial Susceptibility test of Gram- positive bacteria isolated from blood culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, from March 2025 to May 2026.

Antibiotics	**AST**	**GP ESKAPE Isolates from blood**		
		***Enterococcus spp* (14)**	***S. aureus (11*)**	**TOTAL (%)**
V	S	4(28.57)	ND	8.89
	I	0(0)	ND	0
	R	10(71.43)	ND	22.22
PG	S	4(28.57)	3(27.27)	28
	I	0(0)	0(0)	0
	R	10(71.43)	8(72.73)	40
CPR	S	4(28.57)	8(72.73)	36.67
	I	3(21.43)	0(0)	6.67
	R	7(50)	3(27.27)	40
CLI	S	ND	5(45.45)	20
	I	ND	0(0)	0
	R	ND	6(54.54)	24

ERY	S	ND	4(36.36)	16
	I	ND	0(0)	0
	R	ND	7(63.63)	28
GEN	S	ND	7(63.63)	28
	I	ND	0(0)	0
	R	ND	4(36.36)	16
OXA	S	ND	5(45.45)	20
	I	ND	0(0)	0
	R	ND	6(54.54)	24
TRI	S	ND	7(63.63)	28
	I	ND	0(0)	0
	R	ND	4(36.36)	16

CLI-clindamycin, CIP-Cipro floxacillin, ERY-Erythromycin, GN-Gentamicin, PG Penicillin G, OXA- oxacillin, TRI-Trimethoprim-Sulfamethoxazole, V-vancomycin, ND-not done

Table 4.2 Antimicrobial Susceptibility test of Gram- positive bacteria isolated from urine culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, from March 2025 to May 2026.

Antibiotics	**AST**	**GP ESKAPE Isolated from urine**		
		***Enterococcus spp* (12)**	***S. aureus (7)***	**TOTAL (%)**
V	S	1(8.33)	ND	5.26
	I	0(0)	ND	0
	R	11(91.67)	ND	57.89

CPR	S	2(16.67)	6(85.71)	42.11
	I	0(0)	0(0)	0
	R	10(83.33)	1(14.28)	57.89
AMP	S	1(8.33)	ND	5.26
	I	0(0)	ND	0
	R	11(91.67)	ND	57.89
NF	S	2(16.67)	2(28.57)	21.05
	I	0(0)	0(0)	0
	R	10(83.33)	5(71.43)	78.95
GN	S	ND	7(100)	36.84
	I	ND	0(0)	0
	R	ND	0(0)	0
OXC	S	ND	6(85.71)	31.58
	I	ND	0(0)	0
	R	ND	1(14.29)	5.26
PG	S	ND	2(28.57)	10.53
	I	ND	0(0)	0
	R	ND	6(85.71)	31.58
TSX	S	ND	0(0)	0
	I	ND	0(0)	0
	R	ND	7(100)	36.84

Table 4.3 Antimicrobial Susceptibility test of Gram- negative bacteria isolated from blood culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, from March 2025 to May 2026.

Antibiotics	AST	GN ESKAPE Isolated from blood (43)				
		***K. pneumoniae* (24)**	***A.baumani(9)***	***P. arigenosa (4)***	***E. bacter spp(6)***	***TOTAL (%)***
CFT	**S**	2(8.33)	0(00)	ND	1(16.67)	6.98
	I	0(0)	0(0)	ND	0(0)	0
	R	22(91.67)	9(100)	ND	5(83.33)	83.72
CPR	S	2(8.33)	3(33.33)	1(25)	3(50)	20.93
	I	0(0)	0(0)	1(25)	0(0)	2.33
	R	22(91.67)	6(66.67)	2(50)	3(50)	76.74
GT	S	2(8.33)	1(11.11)	ND	ND	6.98
	I	1(4.17)	0(0)	ND	ND	2.33
	R	21(87.5)	8(88.87)	ND	ND	67.44
ME	S	3(12.5)	1(11.11)	3(75)	5(83.33)	27.91
	I	0(0)	0(0)	0(0)	0(0)	0
	R	21(87.5)	8(88.87)	1(25)	1(16.67)	72.09
CFP	S	1(4.17)	1(11.11)	2(50)	1(16.67)	11.68
	I	1(4.17)	0(0)	0(0)	1(16.67)	4.65
	R	22(91.67)	8(88.87)	2(50)	4(66.67)	83.72

CFZ	S	1(4.17)	1(11.11)	1(25)	1(16.67)	9.30
	I	0(0)	0(0)	1(25)	0(0)	2.32
	R	23(95.83)	8(88.87)	2(50)	5(83.33)	81.39
PIP	S	3(95.83)	0(0)	1(25)	2(33.33)	13.95
	I	0(0)	1(11.11)	0(0)	0(0)	2.32
	R	21(87.5)	8(88.87)	3(75)	4(66.67)	83.72
AM	S	2(8.33)	3(33.33)	ND	5(83.33)	23.25
	I	0(0)	0(0)	ND	0(0)	0
	R	22(91.67)	6(66.67)	ND	1(16.67)	67.44
ERT	S	3(12.5)	ND	ND	5(83.33)	18.60
	I	0(0)	ND	ND	0(0)	0
	R	21(87.5)	ND	ND	1(16.67)	56.16
TRA	S	2(8.33)	1(11.11)	ND	1(16.67)	9.30
	I	0(0)	0(0)	ND	0(0)	0
	R	22(91.67)	8(88.89)	ND	5(83.33)	81.39
NIT	S	ND	ND	ND	0(0)	0
	I	ND	ND	ND	0(0)	0
	R	ND	ND	ND	ND	0
TB	S	ND	ND	1(25)	ND	0
	I	ND	ND	0(0)	ND	0
	R	ND	ND	3(75)	ND	6.98

CFT-ceftriaxone, CPR-ciprofloxacin, GT-gentamycin, ME-meropenem, CFP-cefepime, CFZ-ceftriaxone, PIP-pip tazobactam, AM-amikacin, NIT-nitrofurantoin, TB-tobramycin

Table 4.4 Antimicrobial Susceptibility test of Gram-negative bacteria isolated from urine culture among pediatric cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, from March 2025 to May 2026.

Antibiotics	AST	GN ESKAPE Isolated from urine (33)				
		***K.pneumoniae* (21)**	***A baumani (1)***	***P.arigenosa* (8)**	***E.bacter spp* (3)**	***TOTAL (%)***
CFT	S	2(9.52)	ND	ND	0(0)	6.06
	I	0(0)	ND	ND	1(33.33)	3.03
	R	19(90.48)	ND	ND	2(66.66)	63.64
CPR	S	1(4.71)	0(0)	2(25)	1(33.33)	12.12
	I	2(9.52)	0(0)	0(0)	0(0)	6.06
	R	18(85.71)	1(100)	6(75)	2(66.66)	81.82
GT	S	3(14.28)	0(0)	ND	1(33.33)	12.12
	I	0(0)	0(0)	ND	0(0)	0
	R	18(85.71)	1(100)	ND	2(66.66)	12.12
NF	S	7(33.33)	0(0)	ND	0(0)	21.21
	I	0(0)	0(0)	ND	0(0)	0
	R	14(66.67)	1(100)	ND	3(100)	54.54
ME	S	8(38.09)	0(0)	4(50)	1(33.33)	39.39
	I	0(0)	0(0)	0(0)	0(0)	0
	R	13(61.90)	1(100)	4(50)	2(66.66)	60.61
CEF	S	1(4.76)	0(0)	2(25)	0(0)	9.09
	I	0(0)	0(0)	0(0)	1(33.33)	3.03

	R	20(95.24)	1(100)	6(75)	2(66.66)	87.88
CEZ	S	1(4.76)	0(0)	2(25)	0(0)	9.09
	I	0(0)	0(0)	0(0)	1(33.33)	3.03
	R	20(95.24)	1(100)	6(75)	2(66.66)	87.88
PIP	S	7(33.33)	0(0)	2(25)	1(33.33)	30.30
	I	0(0)	0(0)	1(12.5)	0(0)	3.03
	R	14(66.67)	1(100)	5(62.5)	2(66.66)	66.67
AMI	S	5(23.81)	0(0)	0(0)	1(33.33)	18.18
	I	0(0)	0(0)	0(0)	0(0)	0
	R	16(76.19)	1(100)	8(100)	2(66.66)	81.82
ER	S	5(23.81)	ND	ND	1(33.33)	18.18
	I	1(4.76)	ND	ND	0(0)	3.03
	R	15(71.43)	ND	ND	2(66.66)	51.52
TRI	S	1(4.76)	0(0)	ND	0(0)	3.03
	I	0(0)	0(0)	ND	0(0)	0
	R	20(95.24)	1(100)	ND	3(100)	72.72
TO	S	ND	ND	3(37.5)	ND	9.09
	I	ND	ND	0(0)	ND	0
	R	ND	ND	5(62.5)	ND	15.15

CET-ceftriaxone, CPR-ciprofloxacin, GT-gentamycin, NF-nitrofurantoin, ME-meropenem, CEF-cefepime, CEZ-ceftazidime, PIP-piperacillin-tazobactam, AM-amikacin, ER-ertapenem, RTI-, TO-tobramycin

5.4 Factors associated with infection of ESKAPE pathogens

In logistic regression analysis, children aged 0-1 years are 3.85times more likely to get infected by ESKAPE pathogen (AOR=3.852; 95% CI=1.212-12.241; P=.0.022) than older children. advanced stage cancer patients were 2.5 times more likely to be infected by ESKAPE pathogen (AOR=2.11 ;95%CI=1.208-3.532; P=0.004) compared with children with early stage of cancer. Children who stay longer in hospital (length of hospital stay) had 0.5 higher odd of acquiring ESKEPE pathogen (AOR=0.536 ;95%CI=0.311-0.924; P=0.025) than those of short stay. Children who use urinary catheter had 1.76 times high odd of infection with ESKAPE pathogen (AOR=1.758 ;95%CI=1.054-2.931; P=0.031) infection compared with children who did not use urinary catheter. In addition, children who are neutropenic are 8.69 times more likely to be infect with ESKAPE pathogen than non-neutropenic with cancer. Most of children acquire infection after 72 hours 'of admission.

Table 5 Bivariate and Multivariate logistic Regression analysis of Risk Factors Associated with ESKAPE infection among pediatric cancer patients.

Covariates	Category	ESKAPE		COR (95%CI)	AOR (95%CI)	P value	
		Positive	Negative				
Age	0-1	10	11	2.47(1.001-6.44)	3.65(1.18-11.28)	0.024	
	13-18	39	109	1	1	-	-
Length of hospital stay	1 month	59	230	3.13(2.011-4.892)	1.818(1.059-3.123)	0.030	
	2 moths	61	72	1	1	-	
Urine catheter	Yes	65	129	1.58(1.036-2.42	1.859(1.123-3.077)	0.016	

	No	55	173	1	1		
Neutropenia	Yes	92	81	8.965(5.472-14.68)	8.35(4.85-14.85)	0.001	
	No	28	221	1	1	-	-

Abbreviations: AOR-Adjusted odd Ratio, OR-Crude odd Ratio, CI-Confidence Interval.

Covariates	**Category**	**ESKAPE**		**COR (95%CI)**	**AOR (95%CI)**	**P value**
		Positive	**Negative**			
Age	0-1	10	11	0.976(0.568-1.678)	3.852(1.212-12.241)	0.022
	13-18	39	109	1	-	-
Gender	Male	74	199	1.201(0.775-1.861)	1.21(0.77-1.89)	0.395
	Female	46	103	1	-	-
Place of residency	AA	69	188	1.219(0.793-1.879)	1.273(0.91-1.71)	0.37
	Out of AA	51	114	1		
Type of malignancy	Hematological	100	246	1	-	-
	Solid	20	56	1.215(0.25-1.790)		
Stage of cancer	Advanced	66	110	0.516(0.336-0.791)	2.116(1.268-3.532)	0.004

	Early	54	192	1	-	-
Length of hospital stay	1 -2 month	59	230	1	-	-
	2 -4 moths	61	72	0.536(0.311-	3.14(2.01-4.89	
use catheter	1 yes	65	129	0.6(0.412-0.965)	1.758(1.054-2.931)	0.03
	2 No	55	173	1		
Neutropenia	Yes	92	81	0.112(0.68-0.18)	8.685(5.007-11.069	0.001
	No	28	221	1		
Surgery	1 Yes	47	78	0.570(0.364-0.895)	1.405(0.823-)	0.213
	2 No	73	224	1		

Abbreviations: AOR-Adjusted odd Ratio, OR-Crude odd Ratio, CI-Confidence Interval

6. Discussion

Childre with cancer highly vulnerable to infection due to change of immune system because of anti-cancer therapy

The prevalence of *ESKAPE* infections among pediatric cancer patients active on chemotherapy isolated from blood culture and urine culture in our finding were 67/232(28.9%) and 53/190(27.9%) respectively. The overall prevalence of isolated bacteria from blood and urine culture were 28.44%. This finding is comparable with the study conducted in Pediatric Oncology department of NCI, Cairo University (24.11%) (24), our finding was lower when compared with studies conducted previously in India (42.99%) (33), in this study *K. pneumonia* was the most

frequent ESKAPE pathogen from both blood and urine and from gram positive *Enterococcus. Spp* was most frequent in both blood and urine. The variation in prevalence might be explained by the fact that differences in geographical location, the characteristics of the study population, and the difference in methodology used for culture.

Among the 120 bacterial isolated from urine and blood identified in our study, most of them were Gram-negative organisms, 76/120(63.33%) while only 44/120(36.67%) were gram positive. Our finding was in line with the studies reported from in Oman in which Gram-negative were most frequent organism (57%)(35), and Gonder 66.1%(26). Similar finding conducted from other countries has also shown gram negative bacteria were predominant in India(85.39%)(33), in North India (74%)(22), in Qatar (44.8%)(36), in Spain(65.05%)(38) in Jordan(66%)(37), and in Lahore (69%)(39). In our finding gram negative bacteria were the predominant cause of bacterial infection of pediatric cancer patients active on chemotherapy as compared to gram positive bacteria. As indicated in many study this may be the lower use of indwelling medical devices, low utilization of prophylactic antibiotic regimes in cancer patients have been indicated as factors in dominance of gram negative bacteria(26). In our study among gram negative bacteria K. pneumonia was the most prevalent 45/76(59.21%) which was in line with a study conducted in Oman(1), and in university of Gonder(26). The pre dominance *of K. pneumonia* may be due to the fact that *K. pneumonia* is a normal flora that become opportunistic pathogen due to chemotherapy related neutropenia, mucosal barrier disruption prolonged hospitalization, invasive procedures these all facilitate bacteria over growth, trance location and recently, *K. pneumonia* emerged as a significant cause of hospital acquired infection.

Gram negative bacteria of blood stream and urinary tract infections detected a high resistance rate to cephalosporins (ceftriaxone 92.10%, ceftazidime 85.53%, cefepime 75%), carbapenem (meropenem 64.47%, ertapenem 56.16%), aminoglycoside (gentamicin ,69.75%, amikacin 78.95%), fluoroquinolones (ciprofloxacin 81.58%), sulfonamide (trimethoprim-sulfamethoxazole 77.63%), beta lactam (pip-tazobactam 72.37%) nitrofurans (nitrofurantoin 61.84%).This study comparable with the study conducted in Tanzania fluoroquinolones, cephalosporins, carbapenems, co-trimoxazole, and were 86.6%, 66.7%, 73.3%, 73.3%, and 66.6%, respectively resistant(40). And another study in Tanzania gentamicin (83%), ceftazidime (83%), ciprofloxacin (79%), trimethoprim-sulfamethoxazole (75%), meropenem (67%) resistant (14) and in Cairo, Egypt(23),

The study conducted in Shahid Sadeghi hospital incompatible with our study *K. pneumonia* 100% susceptible to all third generation cephalosporin and carbapenem (41), and in Gonder Ethiopia(42) Gram negative bacteria were increasingly resistant to antibiotics that were previously effective. The underlining factors for the high rate of antimicrobial resistant among gram negative bacteria may be the over use of as empirical treatment which promote the emergence of new resistant gen. This new resistant gen can be easily transferred among gram negative bacteria through mobile genetic elements(42)

In Gram positive ESKAPE pathogen detected from urine sample were high resistance rates; nitrofurantoin (78.95%), ampicillin (57.89%), ciprofloxacin (57.89%), vancomycin (57.89%). This study comparable with the study conducted in Iran (13). Gram positive bacteria isolated from blood stream infection were low resistant to 28% vancomycin, 16%trimethoprim sulfamethoxazole, 18% gentamycin, 24% oxacillin, 28% erythromycin,24% clindamycin, 28% vancomycin, 40% penicillin, and 40% ciprofloxacin. Our result supported by the study conducted in Gonder university(42).

The rise of AMR in pediatric cancer patients can be attributed to several factors including the over use and misuse of antibiotics, prolonged hospital stays, and frequent exposure to health care associated pathogen. In particular, pediatric cancer patient are often subjected to repeated cycles of chemotherapy, which recurrent episodes of febrile neutropenia leading to repeated courses of broad-spectrum antibiotics. This cyclic use of antibiotics places selective pressure on bacterial population, fostering emergence and spread of resistant strain(30).

Many factors were identified as risk factors for *ESKAPE* pathogen infection of pediatric cancer patients. In our finding, pediatric cancer patient aged 0–1-year were3.8 times more likely infected *ESKAPE* pathogen (AOR=3.8 ;95%CI=1.212-12.241; P=0.022) compared with older aged children. This may be related immaturity of immune system in infant. In our finding advanced stage cancer patients were 2.540 times more likely to infected *ESKAPE* pathogen infection (AOR=2.11 ;95%CI=1.208-3.532; P=0.004) compared with children with early stage of cancer. This is probably due to patients with advanced stage cancer prolonged hospitalization, round of chemotherapy and immune comptonization. In this study prolonged hospital stay associated with infection of *ESKAPE* pathogen. Children who stay longer in hospital 46.4% higher odd of acquiring *ESKEPE* pathogen (AOR=0.536 ;95%CI=0.311-0.924; P=0.025) than those short stay.

This may be prolonged hospitalization expose to cross-transmission with in hospital setting. In previous study in China children with BSI due to *ESKAPEE*c had longer hospital stay (45). In our finding children use urinary catheter had 1.76 times high odd of infection with *ESKAPE* pathogen (AOR=1.758 ;95%CI=1.054-2.931; P=0.031) infection compared with children who did not use urinary catheter. This may be urinary catheter can serve as a rout for microbial entry. Previous study in Spain Urinary catheter, (OR=2.5; 95 %CI, 1.2–5.2)as risk factors independently associated with *rESKAPE* bacteremia.(38). In this study children being neutropenic had 8.69 times more likely to infect with ESKAPE pathogen (AOR=8.685;95%CI=5.007-15;004; P< 0,001) infection when compared no neutropenic children with cancer. This could be children taking chemotherapy low number of neutrophils that directly related to low immunity, that lead to children easily infected by *ESKAPE* pathogen. as shown by previous study in Oman the severity and duration of neutropenia remained the most important risk factors. .

Strength and Limitation of study

The study was conducted on specific group (pediatric) cancer patient who are taking chemotherapy in order to asses both bacteremia and bacteriuria with its resistance pattern and adequate sample size

Limitation

Molecular technique not done for some species of bacteria because lack of resource.

8. Conclusion and recommendation

Conclusion

In general burden of *ESKAPE* pathogen infection among pediatric cancer patient were significantly high. Gram negative *ESKAPE* pathogen were the most prevalent pathogen isolated from blood stream and urinary tract of pediatric oncology patients active with chemotherapy. K. pneumonia was the most frequent bacterium from both blood and urine. Gram negative *ESKAPE* pathogen of blood stream and urinary tract infection detected high resistant rate to ceftriaxone, ceftazidime, cefepime, amikacin, gentamicin, meropenem, ertapenem, ciprofloxacin, cot rim, pip-tazobactam. From gram positive bacteria isolate; high resistant rate was detected vancomycin, ciprofloxacin, ampicillin and penicillin G. Multidrug resistances for three or more drugs were observed for both Gram positive and Gram-negative bacteria. Gram-positive and Gram-negative bacteria showed high level of resistance for the majority antibiotics. Therefore, routine determination of the

microbial and drug resistance spectrum of pathogens should be an essential component of cancer-related infection control especially active on chemotherapy and care in our setting.

Recommendation

- ✓ There is a need for continuous surveillance of antibiotic to the currently used antibiotics in management of urinary tract and blood stream infection among cancer patients
- ✓ Health professionals should implement education to patients in order to adhere to the treatment and thereby reducing drug resistance.
- ✓ Maximum care has to be given for pediatric oncology patients to minimize ESKAPE pathogens.

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Annex I: **Blood sample collection format**

Lab. code	Age	Sex	Time of collection	Date of collection	Name and signature

Annex II: Information sheet in English Version

Title of the Research Project: prevalence and Antimicrobial Resistance Profile of ESKAPE and Associated Risk Factors in pediatric cancer patients in Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia

Principal Investigator:

Meselech Moges (BSc, MSc candidate)

Name of the Organization:

Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences,

Introduction

You are invited to participate as a study subject in research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntary. The research teams will include one principal investigator and two advisors; from the Addis Ababa University diagnostic and public health microbiology department. Please take as much time as you need to read or listen to the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess the prevalence and antimicrobial profile of *ESKAPE* among pediatric cancer patients

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Identification and drug resistance testing are not routinely performed for those

patients in Ethiopia. Therefore, the present study will provide a baseline and update information on the prevalence and antimicrobial profile of *ESKAPE* among pediatric cancer patients

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also a specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staff as it is needed. The required clinical sample will be collected by residents of study participants. Then, you are requested to give your consent to the sample collector. After consent, a blood sample will be taken from a vein puncture. Moreover, there will be a face-to-face interview for additional questions.

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 15 minutes. We will also collect 5ml venous blood (about from you by sterile-disposable vacutainer tube and needle. We will conduct a laboratory examination to determine different bacterial profiles.

Potential risks and Discomforts

There will be minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory professionals.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked about related to your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated because of that. Also, the result of the

study will be beneficial for the diagnosis and management of bacterial infection among oncology patients. Hence, you are indirectly benefiting other patients and society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study, you can contact the following principal investigators and advisors for further information.

Name: Meselech Moges Phone: 0910009435

E-mail: meselechmoges55@gmail.com

Annex II: Information sheet in Amharic Version

የ ተሳታፊዎችፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲጤና ኮሌጅየ ሕክምና ላቦራቶሪ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅየ ሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት

ላይ እዲሳተፉ ተጋብዘዋል፡ እባክዎበዚህ ጥናትለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎየሚገ ኘዉንምንባብበጥሞናያንብቡና ግልጽያልሆነ ልዎትን ማንኛዉምሃሳብይጠይቁ፡

መግቢያ

ጥናቱ ርዕስ “prevalence and antibiotics resistance pattern of *ESKAPE* pathogen and associated risk factors among pediatric cancer patients at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia” **የ እርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነ ት ላይ የ ተመሰረተ ነ ዉ፡ ፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በጎላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቆረትም፡፡ ፡ በጥናቱ ለመሳተፍ የሚስማሙከሆነ የ ስምምነ ት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል**

የ ጥናቱተሳታፊ ለመሆን የሚጠበቅበዎትምንድን ነ ው?

በዚህ ጥናት ለመሳተፍ የሚስማሙከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡ ፡ ከተወሰደዉናሙና ላይ የሚገ ኙ መረጃዎች ከዚህ ሆስፒታል ዉጭለሚገ ኙና ለስራዉ አግባብነ ት ላላቸዉ ሰዎች ቢነ ገ ር የ ማይቃወሙመሆኑን መስማማት ይጠበቅብዎታል፡ ፡ ይሁን እንጅ ይህ አይነ ቱ መረጃ የ ርስዎን ማንነ ት የሚገ ልጡመረጃዎችን ማለትምስም፤ አድራሻና የ ስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡ ፡ ይልቁንምለዚህ አገ ልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡ ፡ በተጨማ ስለ ርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡአንዳንድተጨማሪ ጥያቄዎችመልስ መስጠትይኖርብዎትአል

በዚህ ትናት መሳተፍ የሚያመታዉ ችግር ምንድናቸዉ?

ናሙናዉ በሚሰበሰብበት ወቅት ምንም አይነት ችግር አያጋትምም ሆኖም ግን ናሙናውን ለመሰብሰብ ልምድ ያለው ባለሙያ ስለሚመደብና አስፈላጊዉ ትንቃቀ ስለሚወሰድ የህመም ስመት አይኖረዉም

የ ህክምና መረጃበሚስጥር ተጠብቆ መቆየ ትየሚችለዉእንዴትነ ዉ?

ስለራስዎ የ ሰጡት ማንኛዉምመረጃና ከተወሰደዉናሙና ላይ የ ተገ ኘዉየ ላቦራቶሪ ዉጤት የሚዉለዉለጥናቱ አላማብቻ ነ ዉ፡ ፡ ይህን ማህደር ሊያገ ኙ የሚችሉት የ ተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡ ፡ ከዚያምበላይ ስለእርስዎ ያለውን ማንኛውንምመረጃ የ ተለየ የ ይለፍቃልባለውየ ኮምፒውተር የመረጃ ማህደር ውስጥእንዲቀመጥይደረጋል፡ ፡

በዚህ ጥናት መሳተፍየሚያስገ ኛቸውጥቅሞችምንድን ናቸው

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናትበመካፈልዎ በገ ንዘብየሚያገ ኙትጥቅምባይኖርም ከጥናቱ በሚገ ኝውውጤት ግን ተጠቃሚነ ዎት፡ ፡ የ እርሶዎ ተሳትፎ የ እርስዎንና የወገ ንዎትን በካንሰር ታማሚወች ላይ ያሉባከቲሪያ ለማወቅና ለማከታተልከ ፍተኛ ጥቅምይኖረዋል

በዚህ ጥናትተሳታፊ የመሆንዎ መብቶችምንድን ናቸው?

በዚህ ጥናት መሳተፍ ሙሉበሙሉበእርስዎ ፈቃደኝነ ት የ ተመሰረተ በመሆኑ በማንኛውምሰዓትናቢታየማቋረጥሙሉመብት የ ተጠበቀ ከመሆኑምበላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንምአይነ ት የሆስፒታል አገ ልግሎት አይኖርም፡ ፡ ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነ ት ጥያቄ የመጠየ ቅና ገ ለጻ የ ማግኘት መብት አለብዎት፡ ፡ የ ላብራቶሪ ምርመራ ውጤቱንምበነ ጻ ማግኘት ይችላሉ፡ ፡ ነ ገ ር ግን እርስዎ በሚሰጡን መረጃ የ ችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድበታላቅ አክብሮት እንጠይቃለን፡ ፡

ጥያቄ ካለኝ ወይምችግር ቢያጋጥመኝ ምን ማድረግ ይገ ባል?

ይህንን ጥናትበተመለከተወይምከዚህ ጥናትጋር በተዛመደ መልኩስለሚያጋጥሙድንገ ተኛ አደጋዎችወይምጥያቄ ካለዎት በሚመለከተውአድራሻ ይጠቀሙ፡ ፡

ስም፡ መሰለች ሞገስ

ሞባይል፡ +251-9 10 00 94 35

ኢሜል፡ meselechmoges55@gmail.com

Annex III: Informed consent form in English version

Card no…...……

I will be informed that the objective of this study is to determine the prevalence and antibiotic resistance pattern of *ESKAPE* pathogens and associated risk factors among pediatric cancer patients suspected to BSI and UTI at Tikur Anbessa Specialized Hospital. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines in Ethiopia. I will also inform you about the confidentiality of this study. The principal investigator requested me to participate in the study which would require my willingness to provide the required data including blood and urine samples, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent to provide the requested information and specimens as the doctors find best for me. Signature: _____ Date_____

Annex III: Informed consent form in Amharic version

የ ተሳታፊዎችስምምነት ማረጋገጫ

የሚስጥር ቁጥር----------የ ተሳታፊዉስም----------

እኔ ስሜ ከላይ የ ተጠቀሰውተሳታፊ “prevalence and antibiotics resistance pattern of *ESKAPE* pathogen and associated risk factors among pediatric cancer patients suspected to BSI and UTI at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia ” ጥናት ላይ በቂ ገ ለጻ ተደርጎልኛል፡ ፡ ለጥናቱም ናሙና እንደሚያስፈልግ ተገ ልጾልኛል፡ ፡ የጥናቱንም አላማዎችንምተ ረድቻለሁ፡ ፡ በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውን ምመረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የ ማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡ ፡ ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪምጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡ ፡ የ ዚህ ጥናትተሳታፊ በመሆኔ የማገኘውጥቅምየ ሁሉንም ምርመራውጤትበነ ጻ ማግኘት እንደሆነ ተረድቻለሁ፡ ፡ በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የ ተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡ ፡ ስለዚህ በዚህ ጥናትለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡ ፡ ፊርማ---------- ቀን ----/---/----------የስምምነ ትቅጹን ማንበብ ለማይችሉ ተሳታፊዎች) የአማካሪ ነርስ ስም---------- ---------- ፊርማ----------ቀን----------

Annex IV: Assent form for less than 18 years old

The objective and the application of the study were briefly explained to me. I am also informed that all information contained within the laboratory request is to be kept confidential. 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 assent form voluntarily to the researcher to give my specimen for the mentioned study and agreed to use the sample for further study in my signature.

Guardians 'name_____ Signature/fingerprint_____date _____

Participant name_____ Signature: _____ date_____

Witness's name_____Signature: _____date _____

Investigator's name_____ Signature: _____

Annex IV: Amharic versions of ascent form for participants less than 18 years of age

ይህ ገ ጽ ማለትም18 ኣመት በታች ለሆናቸው ተሳታፊዎች ስምምነት የመጠየቂያ ቅጽ ነው፡ ፡ ናሙና የምንወስድበት መሳሪያ ንጽህናው ሙሉ በሙሉ አስተማማኝና ከዚህ በፊት ጥቅም ላይ ያልዋለ ነ ው፡ ፡ ናሙና በምንወስድበት ጊዜ የሚኖር ህመም የሚፈጥር ስሜትም ሆነ አደጋ የሚያስከትል ሂደትየ ለውም፡ ፡ ለጥናቱ የሚወሰደው ናሙና ለጥናቱ አላማ ብቻ ይውላል፡ ፡ የ ናሙናው ውጤት ምስጥራዊነት የ ተጠበቀ ሲሆን በናሙናው ውስጥየ በሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈላጊውን ህክምና ያገኛሉ፡ ፡ በጥናቱ ላይ በመሳተፍዎ ምንም የገንዘብ ክፍያ አያገኙም፡ ፡ በጥናቱ ለመሳተፍ የመፍቀድም ሆነ ያለመፍቀድ እንዱሁም በማንኛዉም ጊዜ የ ማቋረጥ መብት አለዎት፡ ፡ (ስም) በጥናቱ እንዲሳተፍ/ እንድትሳተፍይፈቅዳሉ? ፈቃደኛ ከሆኑ፤

የ ተሳታፊ ፊርማ፣---------- የፈቀደውግለሰብፊርማ፣---------- ---------- አድራሻ፣----------ቀን፣---------በስምምነ ቱ ወቅት የነበሩ ምስክሮች 1. _____2. _____ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎትድንገ ተኛ ችግር በሚከተለዉአድራሻ ይጠቀሙ ሞባይል፡ +251910009435.

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Annex VI: Questionnaire

Addis Ababa University College of Health Sciences, Department of Medical Laboratory Science. A questionnaire for the demographic characteristics and Prevalence and Antimicrobial Resistance Pattern of *ESKAPE* Pathogens and associated factors among pediatric cancer patients at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia. This information will be kept confidential and only used for the study.

Circle or write the space provided.

Facility name ____Year ___Participant code _____Participants address (Sub City) _____ Telephone_____signature_____Data_collector name_____ date_____ signature _____

I. **Socio-demographic information**	
1. Age	Month..... Years.....
2. Gender	A) Male B) Female
3. Place of residence	A) Addis Ababa B) out of Addis Ababa
II Clinical information	
4. Date of admission	.....
5. type of malignancy	A, hematologic B, solid
6. If hematological what type of hematological malignancy?	A) Acute lymphoblastic leukemia B) Acute myeloid leukemia C) Chronic lymphocytic leukemia D) Chronic myeloid leukemia E) Myelodysplastic syndrome E) Ather
7. If solid what type of solid malignancy?	.....
8. Round of chemotherapy	.....
9. What Type of chemotherapy do you take?	.....
10. Stage of cancer	A) Advanced B) Early
11. Grade of cancer	Grade1 Grade2 Grade 3Grad 4 unknowns

12. Date of onset	A) Within 72 hours of admission B) After 72 hours of admission
13. Length of hospital stay	.....
14. Does the patient take any antibiotic	A, Yes B, NO
15. If yes, what types of antibiotics do you take	.....
16. Have you undergone imaging tests?	A) Yes, B) No
17. If yes which type of imaging tests?	A) X-rays, B) CT scans, C) MRI scans, D) Ultrasounds
18. Have you undergone radiotherapy	A) Yes, B) No
19. have you used a central line catheter	A) Yes, B), No
20. If yes which type of central line catheter inserted	.....
21. Have you developed neutropenia?	A) Yes B) No
22. What type of surgery did you undergo?	.....
III. Laboratory Data	
23. type of specimen	A) blood B) urine
24 Date of specimen collection	.....
25. Gram stain result	.....
26. Organisms isolated	.....
27. Drug susceptibility patter	A. Sensitive B. Intermediate C. Resistance
28. Name of principal investigator	
Signature	Date.....

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅየ ሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት በ “prevalence and antibiotics resistance profile of *ESKAPE* pathogen and associated risk factors among pediatric cancer patients at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia” ላ ይመጠየቂያ ቅጽ መጠይቅ መመሪያ አንብበው/አዬምጠውመልሱን ከተሰጡትአማራጮችእንደንያክቡወይም በ ክፍትቦታውላይይሙሉ።

ተ.ቁ	የሶሺዮ-ስነ-ሕዝብ መረጃ	ምርጫ
1	ዕድሜ	.....
2	ፆታ	1 ዎንድ 2 ሰት
3	3. የመኖሪያ ቦታ ሀ) አዲስ አበባ ለ) ከአዲስ አበባ ወጣ	ሀ) አዲስ አበባ ለ) ከአዲስ አበባ ወጣ
II	**ክሊኒካዊ መረጃ**	
4	የመግቢያ ቀን	.....
5.	የ ካንሰሩ አይነት	1 ሂማቶሎጅካል 2 ሶሊድ
6	ሂማቶሎጅካል ከሆነ ምን ዓይነት የደም ችግር?	ሀ) አጣዳፊ ሊምፎብላስቲክ ሉኪሚያ ለ) አጣዳፊ ማይሎይድ ሉኪሚያ ሐ) ሥር የሰደደ ሊምፎይቲክ ሉኪሚያ መ) ሥር የሰደደ ማይሎይድ ሉኪሚያ መ) ማይሎዳይስፕላስቲክ ሲንድሮም መ) ለላ
7	2 ሶሊድ ከሆነ የትኛው አይነት	
8	8. ምን ዓይነት ኬሞቴራፒ ነው የሚወስዱት?	....
9	የ ካንሰሩ ደረጃ	1. ከፍተኛ 2. መጀመሪያ ላይ
ነ0		
ነነ	የጀመረበት ቀን	ሀ) በ48 ሰኣታት ውስጥ ለ) ከ 48 ሰኣታት መግቢያ በኋላ

12	የሆስፒታል ቆይታ ርዝመት	.....
13	በሽተኛው ማንኛውንም አንቲባዮቲክ ይወስዳል?	ሀ, አዎ ለ, አይ
14	አዎ ከሆነ፣ ምን አይነት አንቲባዮቲኮችን ይትወስዳል?	
15	የምስል ሙከራዎችን አድርገዋል?	ሀ) አዎ ፣ ለ) አይ
16	አዎ ከሆነ የትኛው ዓይነት የምስል ሙከራዎች?	ሀ) ኤክስሬይ፣ ለ) ሲቲ ስካን፣ ሐ) MRI ስካን፣ መ) አልትራሳውንድ
17	የራዲዮቴራፒ ሕክምና ወስደዋል ?	ሀ) አዎ፣ ለ) አይ
18	ካተተር ተደርጎላቸዉ ያዉቃል	ሀ) አዎ፣ ለ) አይ
19	ምን ዓይነት ቀዶ ጥገና አድርገዋል?	
20	አዎ ከሆነ የትናዉ	.....
	III. የላብራቶሪ ውሂብ	
21	ናሙናዉ የሚሰበሰብበት ቀን	
22	የግራም እስትይን ውጤት	.....
23	ባዮኬሚካላዊ ምርመራ	.....
24	የተገኑ ባክተሪዎች	.....
25	ለመድሃኒቱ የሚሰተዉ ምላሽ	ሀ. ስሜታዊ ለ. መካከለኛ ሐ. መቋቋ

Annex V: Procedure for specimen collection, processing, and result

Standard Laboratory procedures

1. sample collection

1.1 blood collection

Aseptic blood collection procedure is important in order to avoid contamination of the blood by normal skin flora during the sample collection which can make difficult to differentiate between false and true infection.

Procedure

Cleanse the vein puncture site with 70% isopropyl alcohol, starting at the middle of the site, swab with 10% povidones-iodine solution and allow the site to air dry wipe the top of the bottle using an ethanol-ether swab.

Using a sterile syringe and needle 5 ml from a pediatric patient.

- ✓ Dispense the 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.
- ✓ Label each bottle with number of the patient, and the date of collection.
- ✓ Incubate at 35–37 °C for up to 7 days, examining and sub-culturing Blood agar, Chocolate agar, and MacConkey agar.

2. Gram stain Principle

Following staining with a crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram-negative bacteria but not from the less permeable cell wall of Gram-positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye Procedure

Prepare smear on clean slide then air-dry 50

- ❖ Flood slide with crystal violet; leave for 1 minute

- ❖ Rinse slide in clean running water
- ❖ Flood slide with Gram's iodine; leave for 1 minute
- ❖ Rinse slide in clean running water
- ❖ Apply acetone and rinse immediately under running water (exposure to acetone 5 seconds)
- ❖ Counter-stain with carbolfuchsin/safranine for 1minute
- ❖ Rinse in clean running water then dry with blotting paper
- ❖ Place a drop of immersion oil on the slide and view with 100x oil-immersion objective.

Culture media preparation and inoculation

General protocol of Culture media preparation

1. Weighing and dissolving of culture media

2. Sterilization

3. Addition of heat sensitive ingredients

4. PH testing of culture media

5. Dispensing of the culture media

6. Sterility testing

7. Quality assurance of culture media

8. Storage of culture media

Prepare media made from dehydrated products in as damp-free an environment as possible. To prevent the risk of inhaling fine particles of dehydrated media, wear a dust mask while handling dehydrated media, powder or use granulated media.

- ❖ Wash the hands immediately after preparing media.
- ❖ Once the ingredients are weighed, follow exactly the manufacturer's instructions.
- ❖ Use completely clean glassware, plastic or stainless-steel equipment that has been rinsed in pure water. The container in which the medium is prepared should have a capacity of at least twice the volume of the medium being prepared.

- Use distilled water from a glass still. Deionized water can also be used providing the exchange resins do not contain substances inhibitory to bacteria. Water containing chlorine, lead, copper, or detergents must not be used. Besides containing substances harmful to bacteria, impure water can alter the pH of a medium or cause a precipitate to form.
- Add the powdered or granular ingredients to the water and stir to dissolve. Do not shake a medium but mix by stirring or by rotating the container.
- When heating is required to dissolve the medium, stir while heating and control the heat to prevent boiling and foaming which can be dangerous and damage the medium, overheating a medium can alter its nutritional and gelling properties, and also its PH.
- Autoclave a medium only when the ingredients are completely dissolved. Always autoclave at the correct temperature and for the time specified.
- Dispense medium in bottles or tubes in amounts convenient for use.

3.2 Dispensing media into Petri dishes

1. Mix the medium gently by rotating the flask or bottle. Avoid forming air bubbles. Flame sterilizes the neck of the flask or bottle and pours 15–20 ml of medium into each dish (90–100 mm diameter). Air bubbles enter while pouring, and rapidly flame the surface of the medium before gelling occurs. Rotate the dish on the surface of the bench to ensure an even layer of agar.

2. When the medium has gelled and cooled, stack the plates and seal them in plastic bags to prevent loss of moisture and reduce the risk of contamination. Do not leave the plates exposed to bright light, especially sunlight.

3. Store at 2–8 o c

Inoculate culture media

Immediately before inoculating a culture medium check the medium for visual contamination or any change in its appearance which may indicate deterioration of the medium, e.g. darkening in color. When inoculating, or seeding, culture media an aseptic (sterile) technique must be used. This

will: Prevent contamination of cultures and specimens, prevent infection of the laboratory worker and the environment.

Aseptic techniques

- Flame sterilizes wire loops, straight wires, and metal forceps before and after use. Whenever possible, use a Bunsen burner with a protective tube.
- Flame the necks of specimen bottles, culture bottles, and tubes after removing and before replacing caps, bungs, or plugs.
- When inoculating, do not let the tops or caps of bottles and tubes touch an unsterile surface. This can be avoided by holding the top or cap in the hand. Always use racks to hold tubes and bottles containing specimens or culture media.
- Make slide preparations from specimens after inoculating the culture media.
- Decontaminate the workbench before starting the day's work and after finishing.
- Use a safety cabinet when working with hazardous pathogens. Wear protective clothing; wash the hands after handling infected material.

3. Biochemical tests

Catalase test

This test is used to differentiate those bacteria that produce the enzyme Catalase, such as staphylococci, from non-Catalase-producing bacteria such as streptococci. Catalase acts as a catalyst in the breakdown of hydrogen peroxide in to oxygen and water. Add 2–3 ml of hydrogen peroxide solution into a slide and using a sterile wooden stick pick colonies of the test organism and mix with hydrogen peroxide solution then look for bubbling.

Coagulase tests

The Coagulase test differentiates strains of Staphylococcus aureus from other Coagulase-negative species. S.aureus strains are capable of coagulating plasma. The Coagulase test can be performed using two different procedures Slide test and tube test. For both tests, clumping or clots of any size indicate a positive response.

Procedure Slide test method

1 Place a drop of distilled water on the slide

2 Emulsify a colony of the test organism to make a suspension.

3 Add a drop of plasma to the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds.

Test tube method

1. Take small test tubes and add plasma into the tube.

2. Add the test broth culture to the tube.

3. After mixing gently, incubate the tubes at 35–37oC. Examine for clotting after 24 hours.

Note: When looking for clotting, tilt each tube gently.

Results clotting of tube contents . . . . S. aureus

No clotting or fibrin clot . . . . Negative

Indole test

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is important in the identification of Enterobacteriaceae. A few colonies of the culture will be inoculated into peptone water and incubated at 37oC for 24 hours. A few drops of indicator (Kovac‘s reagent) will be added and the Color change will be then observed. If the layer of indicator reagent turns to red within 1 minute, it is indole positive and if it remains yellow it is indole negative

Urease test

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive bacteria from other Enterobacteriaceae. Urea agars will be inoculated heavily over the entire surfaces of the slant and then incubated at 370 c for 3-12 hours. A urease-positive culture produces an alkaline 55 the medium color becomes red and Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.

Triple Sugar Iron (TSI) Agar

Slant Triple sugar iron agar is used for the differentiation of enteric pathogens by the ability to determine carbohydrate fermentation and hydrogen sulphide production. 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 370 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.

Citrate utilization test citrate using Simmon's agar

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator. Pick a single isolated colony and lightly streak the surface of the slant, then incubated at 37o c aerobically for 18 to 48 hours. Blue color indicates a positive reaction and green color indicate negative reaction.

Motility Test (using motility agars)

This medium is used for checking the motility of organisms. Low agar concentration allows free movement of bacteria. 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 35-370 c for 24 hours. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

Lysine decarboxylase (LDC)

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 56 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.

Oxidase test

The Oxidase test is used to assist in the identification of bacteria which produce the enzyme cytochrome Oxidase. 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. When the organism is Oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple color.

Antimicrobial susceptibly tests

Disc diffusion susceptibility tests

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc

Test Inoculums Preparation

- 3 to 5 pure colonies of the same morphological type will be selected from blood agar plate. The colonies are transferred into a tube containing 4 to 5 ml of Tryptone soy broth.
- The turbidity of the broth culture will be adjusted with 0.5 McFarland standards.
- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.
- Left 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.

Application of Disks to Inoculated Agar Plates

- The predetermined series of antimicrobial disks is dispensed onto the surface of the inoculated agar plate.
- The plates are inverted and placed in an incubator at 37 degrees centigrade

Interpreting Results

After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition 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.

Annex VII- Declaration

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

M.Sc. candidate: Meselech Moges (B.Sc.)

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Date of submission: _____

This thesis has been submitted with our approval as advisors.

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