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Bacterial profile and antimicrobial resistance pattern among cancer patients who are taking Chemotherapy at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Seid Yimam, entitled:

Bacterial profile and antimicrobial resistance pattern among cancer patients who are taking Chemotherapy 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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Abbreviations

ALL…………………...........................Acute Lymphoblastic Leukemia

AML……………………….……….…Acute Myeloid Leukemia

AMR…………………..........................Antimicrobial resistance

BSI………………………..…….........Bloodstream Infections

CI……………………..........................Confidence Interval

CLSI…………………….…….……........Clinical and Laboratory Standards Institute

CONS………………………….….…....Coagulase-Negative Staphylococcus

DRERC……………………..………...Departmental Research and Ethics Review Committee

ESBL...Extended-Spectrum β-Lactamase

GNB ………………………………....Gram-Negative Bacteria

GNB……………………………….......Gram positive bacteria

MDR……………………………….......Multi-Drug Resistance

MRSA………………………..……......Methicillin-Resistant S. aureus

NCD…………………………………Non-Communicable Diseases

SOP…………………………………......Standard Operation Procedure

SPSS …………………………………...Statistical Package for social sciences

UTI……………………………………..Urinary tract infection

WHO…………..World Health Organization

Abstract

Background: Bacterial infection is one of the most common complications among cancer patients. Bacterial infection and antimicrobial resistance remains a major cause of life-threatening complications in patients receiving anticancer chemotherapy. This study was used to assess the epidemiology of bacteria in cancer patients who are taking chemotherapy in Tikur Anbessa Specialized hospital, emphasizing antibiotic resistance of the isolated organism.

Objective: To assess bacterial profile and antimicrobial resistance pattern among cancer patients active on chemotherapy at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Methods: In this cross-sectional study, 197 cancer patients active on chemotherapy participated from February to May, 2021 at Tikur Anbessa specialized Hospital in Addis Ababa. Blood and urine from each participant was taken. Using a convenient sampling technique, a total of 197 blood cultures and 197 urine cultures were performed. Early morning midstream urine (MSU) specimens were collected using lick-proof re-usable sterile plastic containers. All blood and urine samples were cultured and bacterial isolates were identified by their colony morphology, gram staining reaction, and biochemical tests. Drug susceptibility testing was done using the Kirby Bauer disc diffusion technique. Data analyses were performed using SPSS version 24.0

Result: Out of 197 blood cultures, 30/197(15.23%) were positive, and from the total 197 urine cultures, 29/197(14.72%) isolates were positive. Gram-negative bacteria 42/59(71.19%) were the predominant pathogens. *CoNS* 7(23.33%) and *S. aureus* 6(20%) were the most dominant bloodstream bacteria. *E.coli* 9(31.03%) was the predominantly isolated bacteriuria. Resistance for three classes of antibiotics was detected, 4/8(50%) of *multidrug resistance CoNS*, 5/8(62.5%) of *Methicillin-resistant Staphylococcus aureus*, 3/14(21.43%) of *MDR Escherichia coli*, 2/11(18.18%) *MDR Klebsiella pneumoniae*, 1/3(33.33%) *MDR K.oxytoca* and 1/1(100%) *MDR Enterobacter aerogenes* isolated.

Conclusion: Both Gram-positive and Gram-negative bacteria showed an increasing level of resistance for most of the antibiotics used empirical therapy is alarming. Routine determination of the microbial and drug resistance spectrum of pathogens should be an essential component especially cancer patients active on chemotherapy infection control and care in our setting.

Keywords: Bacterial pathogen, Septicemia, UTI, Antimicrobial resistance, Oncology patients

1. Introduction

1.1Background

Cancer is a group of disease that are abnormal cells grow uncontrollably that can begin in nearly any organ or tissue of the body; go beyond their usual boundaries. The metastasizing process is a major cause of death from cancer. It is the broadening of malignant tumor cells from a primary neoplasm to distant parts of the body where they multiply to create new growths, and this process is a major cause of death from cancer. Other common names for cancer are a neoplasm and malignant tumor [1]. Cancer center patients are at risk for a wide range of pathogens because they are immunosuppressed. So, a prevention program is extremely important to reduce the risks of bacterial infections [2].

Immunosuppression is the major hallmark of cancer in patients who are receiving cancer treatment. Cancer treatment modalities like radiation therapy and chemotherapy kill cancer cells, but also cells that are part of our defense mechanism against infections. In addition, the cancer itself, undernourishment, invasive procedures and surgical procedure further contribute to immune suppression rendering cancer patients highly susceptible to a wide range of infections. A bacterial infection is one of the most common complications among cancer patients. Bacterial infections, especially bloodstream infections (BSI) are among the most frequent complications in immunosuppressed patients with cancer and are associated with considerable morbidity and mortality and high economic costs [3].

The organisms commonly isolated among cancer patients with sepsis are bacterial or fungal pathogens, with the leading pathogens being S. aureus, Pseudomonas species, E. coli, and Candida species [4, 5]. Laboratory investigations in sepsis include measurement of inflammatory markers, organ function tests, and identification of infectious sources through blood culture plus any culture specimens to identify the source of infection [6, 7].

In the global report on surveillance in Antimicrobial Resistance, The World Health Organization (WHO) declared that AMR in a wide range of infectious agents has become a serious public health difficulty and an after-antibiotic era is a real option for the 21st century [8].

Antimicrobial resistance occurs when microorganisms change in ways that turn into the medications used to cure the infections they cause unsuccessful. The microorganisms that become resistant to most antimicrobials are often called "superbugs". Antimicrobial resistance is a key concern because a resistant infection may kill, can expand to others, and imposes vast costs to individuals and society. The main reason of antibiotic resistance is usage of antibiotics. When we use antibiotics, some bacteria die but resistant bacteria can stay alive and even multiply to distant parts. Excess use of antibiotics makes resistant bacteria more common [9].

1.2 Statement of the problem

Cancer is the world's second biggest cause of mortality, with an estimated 9.6 million fatalities in 2018, or one death for every six patients. The most common types of cancer in male includes lung, prostate, colorectal, stomach, and liver cancer, while breast, colorectal, lung, cervical, and thyroid cancer are the most common among female [10]. Every year, cancer is expected to kill more than 7.9 million people around the world, accounting for over a quarter of all deaths [11]. More than 3 million individuals are expected to be diagnosed with cancer each year in Asian nations such as India, Indonesia, the Philippines, Vietnam, and China [12].

In 2018, 752,000 new cancer cases and 506,000 cancer deaths were reported in Sub-Saharan Africa. Breast, cervical and prostate cancers account for the majority of cancer cases in Sub-Saharan Africa [13]. In Ethiopia, cancer is estimated to account for about 5.8% of total national mortality. Although population-based data do not exist in the country except for Addis Ababa, it is estimated that the annual occurrence of cancer is approximately 60,960 cases and the annual mortality is more than 44, 000 [14].

Cancer is one of the primary causes of bacterial infection, with cancer patients having a tenfold increased risk of infection when compared to non-cancer individuals [15]. The global epidemiology of bacterial infections in cancer patients has evolved over time, with a shift from Gram-negative bacteria to Gram-positive bacteria, and also a new return of Gram-negative bacteria in many countries [16, 17].

Antimicrobial resistance is increasing dramatically in all parts of the world including Ethiopia. Antibiotic resistance poses a threat to contemporary cancer treatment now and in the future. Ineffective antibiotic treatment for bloodstream infections caused 70% of cancer patients to die within a month, compared to less than 20% of those who received proper antibiotics. Inadequate treatment and resistant microorganisms, as well as cancer recurrence or relapse, were linked to poor outcomes [18].

Infections were responsible for over 2 million of the 12.7 million new cancer cases diagnosed in 2008[19]. Bacterial infection is thought to be contributed for around 15% of malignancies worldwide, or roughly 1.2 million cases per year [20]. Bacterial Infectious complications are a major cause of morbidity and mortality in oncology patients, particularly those with underlying hematological malignancies. Although there are fewer data on infectious mortality in patients with solid organ tumors, an infection is believed to be the primary or secondary cause of death in about half of these patients (i.e., chemotherapy reagents, immunosuppressant therapies, and antimicrobial use,) [21].

Patients receiving chemotherapy, organ transplants and other advanced therapies are particularly susceptible to bacterial infection. The treatment of bacterial infections in patients with cancer who are taking chemotherapy should often rely on the use of established guidelines, along with concern of the local epidemiology and antibiotic resistant patterns of the potential etiologic agents. However, in Tikur Anbessa Specialized Hospital identification and antibiotics resistance testing is not routinely performed, so patient management is based on empirical treatment. The actual burden of urinary tract infection, blood stream infection, and antimicrobial resistance pattern of bacterial isolates on cancer patients active on chemotherapy are not known. Thus, the present study provides baseline information on the spectrum of bacterial isolates and antimicrobial resistance patterns in cancer patients who are taking chemotherapy attending at Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia.

1.3 Significance of the study

This study would be helpful to determine bacterial profile and its antimicrobial resistance pattern among cancer patients and also which type of bacteria is more prevalent in cancer patients and which type of bacteria more resistance and for what type of antimicrobials resistant for each of bacteria.

This study would help us to design strategies that involve about health services, which provides invaluable support for cancer patient in order to achieve successful cancer treatment goals and promoting health environments regarding bacterial infection for those patients.

The findings of this study would help in strengthening the information available so far and will help policy makers to design effective strategies to combat UTI and BSI among cancer patients.

This study would provide the current bacterial infection and its antimicrobial resistance pattern among cancer patient who are taking chemotherapy and used to plan intervention activities in the future.

The study also will serve as base line data for the upcoming researchers in this area

2. Literature review

2.1 Bacterial profile and antimicrobial resistance pattern

According to cross-sectional a study done by Khodashahi R *et al*., 2019 in Iran, A total of 50 patients were enrolled in the study. This study had a positive blood culture; Gram-negative (30%) bacteria were three, and Gram-positive bacteria were five (50%) [22].Study conducted in the same area by Aminasnafi A *et al.,* 2017, 310 patients had a recorded culture result (438 cultures in total). *P. aeruginosa* was the most common isolated pathogen (34.1 %). Only 63 positive cultures containing *pseudomonas* were tested for antimicrobial resistance, and 55.5% were resistant and 42.8% were sensitive to ceftazidime. From nine isolated *E.coli* cultures, 66.6% were resistant and 33.3% were sensitive to ceftazidime. For *Acinetobacter* and *Staphylococcus epidermidis* species, only one positive culture (out of 33 and 34 respectively) was tested, and in both cases was resistant to ceftazidime [23].

In Iran a retrospective descriptive cross-sectional study conducted by Amanati A *et al.*, 2021, a total of 414 of the 2393 blood cultures examined were positive. Gram-negative bacteria were isolated in greater numbers (63.3%, 262) than Gram-positive bacteria (36.7%, 152). *Escherichia coli* (123/262, 47 %), *Pseudomonas spp.* (82/262, 31%), and *Klebsiella pneumoniae* (38/262, 14.5%) were the most common GNB. *CONS* was the most often isolated gram-positive bacteria (83/152, or 54.6 percent). *Acinetobacter spp., Pseudomonas spp., Enterobacter spp., E. coli, and K. pneumoniae* were found to be the most carbapenem-resistant bacteria (77.8, 70.7, 33.3, 24.4, and 13.2 percent, respectively [24].

A retrospective study was conducted by Saravanan, M ,2020 at a tertiary care cancer centre in Northern part of Kerala, from the total of 3169 urine samples studied ,705 (22%) patients were significant bacteriuria. From those, Gram negative bacilli accounted for 79%, Gram-positive cocci for 16% and *Candida species* for 5%, the most prevalent organisms from Gram-negative pathogens isolated were *Escherichia coli* (43%) followed by *Klebsiella pneumoniae* (28.5%). In Gram-positive isolates, *Enterococcus spp.* (60%); *Enterococcus faecalis* = 39% and *E. faecium*= 17%) was the predominant species followed by *Staphylococcus spp.* [28.5%; *(CoNS)* = 16% and *S. aureus* = 12.5%]. Resistance was found to be higher to the aminoglycosides (47%), cephalosporins (65%) and fluoroquinolones (70%). Out of the total 14 *S. aureus* isolates, 57% were *MRSA* [25].

In India a retrospective study conducted by Kokkayil *et al.,* 2018, 56 isolates from 53 patients were isolated of which the majority were gram-negative bacilli (GNB; n = 52 or 93%). *Klebsiella pneumonia* (43%, n = 24) was the most frequently isolated bacteria followed by *Enterobacter sp* (20%, n = 11) and

Escherichia coli (12%, n = 7) [26]. A retrospective analysis of cancer patients was conducted by Parikh and Bhat, 2015 in same area, of the 497 samples, 100 were positive for bacterial growth. Overall, *E.coli* (40%) was the predominant isolate followed by *Klebsiella pneumoniae* (25%), *Pseudomonas aeruginosa* (11%), *Enterococcus spp* (11%) and *Proteus mirabilis* (5%). Resistance was found to be higher to the aminoglycosides (46%), cephalosporins (67%) and fluoroquinolones (90%) [27].

According to a retrospective study conducted in India by Sawargaonkar M *et al.*, 2019, of which 242 blood culture samples, Out of 97(40%), positive cultures, Gram positive were 60 (62%) and Gram negative were 37 (38%). The most common Gram-positive isolate was *MRSA* (38%) and Gram-negative isolate was *Escherichia coli* (35%). High degree of resistance was found to cephalosporins and piperacillin + tazobactam [28]. Another study in the same area conducted by Bhat S. *et al.*, 2021, Out of 638 cancer patients, 140 patients had positive cultures, representing 272 specimens and 306 isolates. 214 (69.9%) were gram-negative bacilli, and 92 (30.1%) were gram-positive cocci. The most wide spread detected were *Klebsiella spp.* (18.30%), *Pseudomonas spp.* (17.65%), *Escherichia coli* (14.71%) and *Staphylococcus aureus* (13.72%). Among the GNB, the antibiotic resistance rates reported to fluoroquinolones, aminoglycosides, and third-generation cephalosporins were 45.13%, 39.20%, and 48.58%, respectively [29].

Based on cross sectional study conducted by Shrestha, G *et al.*, 2021 in Nepal, from the total 308 patients, 73 (24%) samples had bacterial growth. The most common bacteria isolated were *E. coli, Staphylococcus* and *Klebsiella* (58%, 11% and 10%) respectively. Nitrofurantoin (54/66, 82%) and Amikacin (30/51, 59%) were the most common from limited antibiotic pattern. Multidrug resistance was 89% of the positive culture [30].

Based on A retrospective study a study was conducted by Atikur AR, *et al.*,2013 in Bangladesh, 45 patients who had 58 febrile neutropenic episodes were included. No patient presented with localizing signs. The urinalysis was negative in 53 episodes and positive in 5 episodes. Four patients had 5 UTIs. The frequency of UTI was 8.6%. Four patients had bacteremia, none of whom had a UTI [31].

Based on A retrospective study a study was conducted by GOLLI L A *et al.,* 2019 in Romania, a total of 157 bacterial isolates were obtained, of which *Staphylococcus aureus* accounted (37) (23.56%), followed by *Streptococcus pneumonia* (23)- 14.64%), *Klebsiella spp.* and *Escherichia coli* (22 - 14, 01%). High rates of MDR were found for *E. coli* (63.63%), *MRSA* (61, 11%), and *Klebsiella spp.* (54, 54%), while one-third of the isolated strains of *Pseudomonas aeruginosa*, *Acinetobacter spp.* and *Proteus spp.* were MDR [32].

In Quantitative research Study conducted by Póvoa, H.C *et al.,* 2018 in Brazil, from a total of 8 patients evaluated with Gram-negative microorganisms, half of the patients were female and half were male. From 18 patients evaluated were Gram-positive bacteria 66% were female and 34% were male. Gram-positive bacteria are around 60% of documented bacteremia. This study was concluded that Excess use of antimicrobials leads to the suppression of drug-sensitive microorganisms from the intestinal flora and promotes the persistence and growth of resistant bacteria [33].Another prospective study was conducted by Velasco E *et al.*, 2004 in the same area showed from the total, 1039 microorganisms were isolated blood, of which Gram-negative bacilli accounted for 56%. High rates of ceftazidime resistance were identified among *Acinetobacter spp.* (40%) and *Enterobacter spp.* (51.2%). *E. coli* and *K. pneumoniae* were isolated frequently from hematology patients, and *Enterobacter spp*.Oxacillin resistance was detected in 18.7% of *Staphylococcus aureus* isolates [34].

A retrospective cross-sectional study was conducted by Al-Mulla, N *et al.*, 2014 in Qatar, A total of 70 patients (38%) were included; 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 haemolyticus* (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) [35].

Based on prospective observational cohort study conducted by Kanafani, Z. A., *et al.*, 2006 in Lebanon, We included 177 episodes of neutropenic fever. The most common underlying malignancy was lymphoma (42.4%).Gram-negative organisms were account for 78.8% (26/33) of bloodstream infections compared to 33.3% (11/33) with Gram-positive organisms [36].

A retrospective cross-sectional study was conducted by Mvalo, T *et al.*, 2018 in Cape Town, South Africa showed: 343 positive cultures were identified, for 150 BSI episodes among 89 patients;-positive isolates. From gram-negative isolated *Escherichia coli* and *Klebsiella species*; gram-positive bacteria were 49.1% of the culture isolates, 41.6% were Gram-negative bacteria, and 9.3% were fungal. *CoNS* and *viridian's group Streptococcus* were the most known Gram-positive bacteria. The majority of BSI episodes occurred in patients with hematological malignancies (74%), in the presence of severe neutropenia (76.4%), and was associated with chemotherapy (88%). Complications occurred in 14% of

BSI. This study was concluded that BSI mainly caused by Gram-positive bacteria and was associated with a low case-fatality rate [37].

A descriptive cross-sectional study conducted by Rybojad, P., *et al* 2011in Zimbabwe, a total of 142 participants were included; 50 (35.2%) had positive blood cultures, from positive blood culture 56.0% were Gram-positive, and 42.0% were Gram-negative bacteria isolated. Common species isolated included *CoNS* (22.0%), *E. coli* (16.0%), *K. pneumonia* (14.0%), *E. faecalis* (14.0%) and *S. aureus* (8.0%). Gram-negative isolates exhibited high resistance to 61.9% of gentamicin and 71.4% of ceftriaxone. Amikacin and meropenem Appeared 85.7 and 95.2% activity against all Gram-negative isolates respectively. Vancomycin and linezolid were successful against all Gram-positive isolates 96.2 and 100.0% of respectively. Ten (66.7%) of the *Staphylococcus spp.* were methicillin-resistant. This study was concluded that *S. aureus* were the major microbial and methicillin resistance carriage [38].

In a prospective single-center cohort study conducted by Amer W. *et al.*, 2017 in Egypt, 67 bloodstream infections were detected in 46 patients. The major risk factors for bloodstream infections were Neutropenia (69.7%) and Hematological malignancies (67.4%). Gram-positive bacteria represented were (53.7%) including primarily *CoNS* (38.9%) and *Streptococci* (30.6%). Methicillin resistance was detected in all S. aureus, 71.4% of *Coagulase-negative Staphylococci* that were sensitive to ciprofloxacin (85.7%, 100%), gentamicin (85.7%, 100%), and Clindamycin (71.4%) respectively. Gram-negative bacteria represent (46.3%) mainly *Klebsiella pneumonia* (38.7%). ciprofloxacin (100%), Amikacin (100%), piperacillin/ tazobactam (69.2%) and sulbactam/ cefoperazone (53.8%) were sensitive to *ESBL* in Enterobacteriacaea was (81.3%). This study concluded that Carbapenem resistance was alarming and mandating more evaluation of β-lactamase inhibitors in treatment of ESBL [39].

Based on A hospital-based cross-sectional study was conducted by Fentie A. *et al*, 2018 at the University of Gondar Hospital, Northwest Ethiopia, The overall prevalence of bacterial infection on cancer patients was 19.4%. The predominant bacterial isolates were *S.aureus* (28.6%), followed by *CoNS* (26.2%) and *Escherichia coli* (21.4%). Multidrug resistance was detected in 46.5% bacterial isolates. Methicillin resistance was detected in 25% of *S. aureus* and 45.5% of *Coagulase-negative staphylococci*. Fluoroquinolone resistance was detected in 33.3% of *E. coli* isolates [40].

A hospital-based comparative cross-sectional study was conducted by Tigabu A, *et al.*, 2020, at Gondar; the overall occurrence of asymptomatic bacteriuria in cancer patients was 23.3% while 6.7% in apparently healthy blood donors. The predominant uropathogenic bacteria was *E. coli* (32.1%)

,*Klebsiella species* (25.0%), *S. aureus* (21.4%), *Enterococcus species* (10.7%), *Serratia species* (7.1%), and *Enterobacter aerogenes* (3.6%). Most Gram-negative bacteria were more sensitive to ceftazidime, cefoxitin, nalidixic acid, nitrofurantoin, norfloxacin, ciprofloxacin, and tobramycin, whereas extremely resistant to ampicillin, penicillin, tetracycline, and ceftazidime. Nitrofurantoin was 100% susceptible for *S. aureus* [41].

A hospital-based cross-sectional study was conducted by Tolera M. *et al.*, 2018 at Hiwot Fana Specialized University Hospital, Eastern Ethiopia, the prevalence of culture-confirmed bacterial nosocomial infection was 6.9%. *S.aureus* (18.5%) was the most frequent isolate followed by *Escherichia coli* (16.7%). Chloramphenicol and erythromycin showed 80% resistance to *S. aureus*, and cephalexin and tetracycline were 70% resistant to *S. aureus*. MDRS made up 88.9% of all *S. aureus* isolates. *P.aeruginosa* were 83.7% resistance to each of ceftazidime and cephalexin, and 66.7% to chloramphenicol. Both *P. aeruginosa* (30.4%) and *S. aureus* (21.7%) were the most common multidrug-resistant isolates. This study was concluded that *S. aureus, E. coli*, and *S. pneumoniae* were the most common causes of nosocomial infections [42].

The cross-sectional study was conducted by Arega B *et al.*, 2018 at Tikur Anbessa in Addis Ababa. From 112 blood culture tests of the 76 patients, 82 pathogens were isolated: 71 (86.6%) were bacteria and 11 (13.4%) were fungi. Gram-positive bacteria were the preponderance (60.5%) of the detected, where *S. aureus* was leading (72%) and (68%) of *S. aureus* were resistant to ceftriaxone and oxacillin. Gram-negative bacteria were 39.5% of the isolates. *Stenotrophomonas maltophilia* (17.9%) was the most frequent in Gram-negative isolate. In Gram-negative bacteria, the maximum rates of resistance were identified in amoxicillin-clavulanic acid (80%), followed by ceftriaxone (73.3%) and trimethoprim-sulfamethoxazole (73.3%).Multidrug resistance were seen on ceftriaxone, tetracycline, and trimethoprim-sulfamethoxazole. MDR was identified in 26.3% of GPB and 40% of GNB[43].

Hospital based prospective cross-sectional study was conducted by Sime WT *et al* , 2020 at Tikur Anbessa hospital from the overall of 292 urine samples tested, the positivity rate were 18(6.3%), *Escherichia coli* (44.4%) was the predominant followed by *K. pneumoniae* (22.2%) and *Citrobacter diversus* (16.7%). The antibiotic susceptibility pattern showed meropenem and nitrofurantoin as the most successful treatment for *E. coli*, *K. pneumoniae*, and *Citrobacter diversus* isolated and also against MDR isolates. The rate of multidrug resistant was 33.3% (6/18) [44].

3. Objectives

3.1 General objective

The general objective of this study was to assess the bacterial profile and their antimicrobial resistance pattern among cancer patients who were taking chemotherapy at Tikur Anbessa Hospital, Addis Ababa, Ethiopia from February 2021-May 2021

3.2 Specific objectives

- To investigate septicemia among oncology patients who are taking chemotherapy attending Tikur Anbessa specialized hospital
- To identify bacterial uropathogens among oncology patients who are taking chemotherapy
- To determine the antimicrobial resistance patterns of bacterial isolates among oncology patients who are taking chemotherapy
- To explore the potential risk factors associated with bacterial infection in oncology patients

4. Materials and methods

4.1Study area

This study was conducted at Tikur Anbessa 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 open 24 hours for emergency services. The hospital is administered by Addis Ababa University and the largest teaching hospital among all in Ethiopia providing teaching for about 300 medical students and 350 Residents every year. The hospital has 929 academic staff, 825 nurse professionals, 55 laboratory technologist, 74 pharmacist, 69 midwife professional, 39 anesthesia professional, 14 physiotherapist, 37 radiology technology, 15 biomedical professional, 6 environmental health, 5 medical doctors, 15 others and 891 administrative staff. It offers diagnosis and treatment for approximately 370,000- 400,000 patients a year. The hospital has 800 beds, with 130 specialists, 50 non-teaching doctors. The emergency department sees around 80,000 patients a year. It is the only cancer referral treatment center and providing service for over 60,000 cancer patients annually in Ethiopia.

4.2 Study design and period

A cross-sectional study design was employed to identify the bacterial profile and antimicrobial resistance pattern among cancer patients who are taking chemotherapy at Tikur Anbessa specialized hospital from February to May, 2021.

4.3 Population

4.3.1 Source population

All patients attending Tikur Anbessa specialized hospital oncology department during the study period

4.3.2 Study population

All oncologic patients who were taking chemotherapy for pathologically confirmed cancer at Tikur Anbessa specialized hospital during the study period.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- All cancer patients under chemotherapy suspected of bloodstream infection and UTI.
- Who are willing to participate in the study and able to provide either consent or assent.

4.4.2 Exclusion criteria

- Those patients who are not able provide appropriate specimens.
- Patients who have taken antibiotic agents within 10 days.
- Who are too ill to provide specimen were excluded from the study.

4.5 Study Variables

4.5.1 Dependent Variables

- Bacterial profile
- Antimicrobial resistance pattern

4.5.2 Independent Variables

- Age
- Sex
- Marital status
- Educational status
- Occupational status
- Type of malignancy
- Stage of cancer
- Grade of cancer
- Tobacco and smoke user

4.6 Sample size calculation and sampling method

4.6.1 Sample size calculation

The amount of sample size that infers the target population in this study was calculated for estimating a single population proportion at 95% confidence interval (CI) ($Z\alpha/2$ = 1.96), 5% margin of error, and 10% non-response rates.

Using the formula $n=Z_{\alpha/2}^{2} *p*(1-p) / d^{2}$

Where q= 1-p, z=1.96, p=0.866, q=0.134, d=0.05, n=178

The prevalence was taken from the previous study was conducted between December 2011 and June 2012 at Tikur Anbessa Hospital in Addis Ababa [43] and then the total sample size was 197 including a 10% non-response rate.

4.6.2 Sampling method

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

4.7 Measurement and Data collection

4.7.1 Sample and Data collection Procedure

Ten and five ml milliliter of blood were collected from adults and children, respectively by nurses and clinicians under strict aseptic conditions using 70% alcohol followed by povidone-iodine, and also bottles were labeled with patient's identification number and date of collection. Blood was transferred to a blood culture bottle containing 90ml of brain heart infusion broth for adults and 45 ml for children. Daily check of culture bottles was done for up to 7 days [45]. In addition, early morning 10 ml midstream urine (MSU) specimens were collected using lick proof re-usable 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 record.

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 process in the microbiology laboratory by the conventional method. Blood culture bottle was incubated aerobically 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, chocolate agar, and macConkey agar. The blood agar and macConkey agar were incubated anaerobically. Whereas chocolate agar was incubated in a carbon dioxide atmosphere using a candle jar at 37°C for 24 to 48 hrs. Blood culture bottle which shows no sign of growth were further be incubated at 37˚C then checked the growth inspection daily up to 7 days. Blood culture broths with no microbial growth after 7 days were sub-cultured for 48hrs 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 [46]. The identification of true *Coagulase-negative Staphylococcus (CoNS)* was considered when there was growth in the two blood bottle drawn from separate sites.

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 incubated for 24–48 h at 37 °C aerobically. If the growths of a colony count of ≥105 cfu/ml were considered significant bacteriuria. Then sub-cultured to macConkey agar (Oxoid, Basingstoke, Hampshire, England) and blood agar (Oxoid, Basingstoke, Hampshire, England)[47].

4.7.2.2 Drug resistance patterns

Drug resistance patterns of bacterial isolates were performed based on CLSI guidelines. The suspension was prepared using a sterile wire loop; from a pure culture 3-5 pure colonies of bacteria were pick and emulsify in nutrient broth standard inoculums were adjust to 0.5 McFarland and swab onto Muller Hinton agar. The tested antibiotic discs include: ampicillin(10 µg), augmentin(30µg), ceftriaxone(30µg), cefepime(30µg), ceftazidime(30µg), amoxicillin(25µg),gentamicin(10 µg), trimethoprim + sulfamethoxazole(1.25 + 23.75 µg), chloramphenicol(30 µg), ciprofloxacin(5 µg), for gram negative isolated bacteria were used. For gram positive bacteria isolates, ampicillin(10 µg), augmentin(30 µg), ceftriaxone(30 µg), cefepime (30 µg), ceftazidime(30 µg), cefoxitin(30 µg), amoxicillin(25 µg), penicillin G (10 units),gentamicin(10 µg), trimethoprim + sulfamethoxazole(1.25 + 23.75 µg), chloramphenicol(30 µg), ciprofloxacin(5 µg), erythromycine(15 µg), tetracycline(30 µg) were used. Using the kirby bauer disk diffusion method, antimicrobial susceptibility testing was performed for each pure isolated bacterium and incubating 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.

4.8 Quality Assurance

The quality of the study was assured by the training data collector, preparing SOPs. Samples were collected in a sterile container and following SOPs. The microbiological evaluation of samples was carried out following the protocol in clinical and laboratory standards institute. Culture results were recorded before entry to the statistical tool. Before the analysis, the data were checked for consistency and completeness and incomplete data were excluded before proceeding to data analysis. The quality of the study was also assured by implementing quality control measures that include sterility and performance checks throughout the whole process of the laboratory work. *E. coli ATCC 25922*, *P. aeruginosa ATCC 27853*, and *S. aureus ATCC 25923* control strains were used.

4.9 Data Analysis and interpretation

Collected data was process, edit, and analyze using SPSS version 24.0 (Statistical Package for social sciences, SPSS) statistical. Variables with bivariant and variables with p-value <0.25 in the bivariant analysis were included in the multivariate logistic regression to determine the association of potential factors with BSIs and UTIs. P≤0.05 were considered statistically significant.

4.10 Operational Definition

Multidrug resistance: - Bacterial isolates which is resistant for three or more antibiotics that belong to different classes.

Septicemia: - the occurrence of bacteria in the blood/bacterial bloodstream infection.

Urinary tract infection: the presence of pathogenic microorganisms within the urinary tract in a significant quantity ($\geq 10^5$ cfu/ml)

4.11 Ethical Consideration

Ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, and the Department of Laboratory Sciences before starting the thesis work; protocol number DRERC/603/21/MLS. A permission letter was also obtained from the study site. The objective of the study was explained to the study participants and written informed consent was obtained before obtaining sample and personal information from each participant. All the information which was obtained from the study participants were kept confidential. For each confirmed infection case, the responsible clinician of the participant was informed and treatment was started as per the culture result and drug susceptibility pattern.

5. Result

Socio demographic characteristics of cancer patients active on chemotherapy

A total of 197 cancer patients actively taking chemotherapy at Tikur Anbessa specialized Hospital was enrolled in the present study. For each patient, blood and urine samples were obtained. Out of these, 99(50.3%) were males and 98(49.7%) were females. The mean age of study participants was 29.72±17.855 years, with maximum age of participants 80 years and minimum age 1 year. The majority of the participants were single 92(46.7%), married 84(42.6%), housewives 47(23.9%), no formal education 96 (48.7%) (Table 1).

Table 1. 1 Socio-demographic characteristics of cancer patients active on chemotherapy at Tikur Anbessa Hospital, Addis Ababa, Ethiopia, 2021 (n=197)

Characteristics		Frequency, n=197	Percent (%)
Sex	Male	99	50.3
	Female	98	49.7
Age	< 15 years	41	20.8
	15- 29 years	67	34.0
	30 - 44 years	43	21.8
	45- 59 years	30	15.2
	≥ 60 years	16	8.1
Marital status	Single	92	46.7
	Married	84	42.6
	Divorced	2	1.0
	Widowed	8	4.1
	Others	11	5.6
Occupational status	Civil servant	19	9.6
	Merchant	8	4.1
	House wife	47	23.9
	Farmer	33	16.8
	Others	90	45.7
Educational status	No formal education	96	48.7
	Primary	51	25.9
	Secondary	29	14.7
	Tertiary	21	10.7

Clinical data of study participants during study period

Out of 197 cancer patients active on chemotherapy, 152 (77.16%) had hematologic malignancy and 45 (22.84%) had solid tumors. Among the total, acute myeloid leukaemia 58(29.44%) was the highest, followed by acute lymphoblastic leukaemia 57(28.93%). From solid tumors; breast cancer, and nasopharyngeal carcinoma accounts for 9(4.57%) and 7(3.6%) respectively. Among the recorded grading of cancer patients, Non-grading 140(71.1%) were the highest. Regarding stage of cancer, 86(43.65%) of them were early stage, 111(56.35%) cancer patients were advanced stage (Table 2).

Table 2. 1 Clinical characteristics of cancer Patients active on chemotherapy at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, 2021(n=197)

Characteristics		**Frequency(n)**	**Percent (%)**
Type of malignancy	Hematologic	152	77.16
	Solid	45	22.84
Specific type of cancer	Breast cancer	9	4.57
	Nasopharyngeal Carcinoma	7	3.6
	Colon	3	1.5
	Rectal	4	2
	Vulvar	5	2.5
	Brain	3	1.5
	Cervical cancer	6	3.05
	Anal cancer	3	1.5
	ALL	57	28.93
	AML	58	29.44
	CLL	19	9.64
	CML	11	5.58
	NHL	7	3.6
	Others	5	2.5
Experience of tobacco or smoke	Yes	3	1.5
	No	194	98.5
Stage of cancer	Early	86	43.65
	Advanced	111	56.35
Grade of cancer	grade 1	20	10.2
	grade 2	13	6.6
	grade 3	24	12.2
	not recorded	140	71.1

Bacterial isolates from blood and urine sample

Out of 197 cancer patients who are active on chemotherapy investigated for blood stream infections and urinary tract infections, 59(29.95%) bacterial pathogens were isolated. From 59 bacterial isolated pathogens, 30/59(50.85%) were positive for blood culture and 29/59(49.15%) were positive for urine culture. From the total bacterial pathogens, 42/59(71.19%) were gram negative bacteria and 17/59(28.81%) were gram positive bacteria. From the total 30/197(15.23%) blood stream bacterial pathogens, *Coagulase negative staphylococcus* 7(23.33%) and *S. aureus* 6(20%) were the most dominant bacteria followed by, *E.coli* 5(16.67%), *K. pneumonia* 5(16.67%) and *Acinetobacter species* 4(13.3%). From the total 29/197(14.7%) urinary tract infections, the predominant isolated bacteria were *E.coli* 9(31.03%), followed by *K. pneumonia* 6(20.9%).The overall prevalence of bacterial blood stream infections and urinary bacterial infections were 15.23 % (30/197) and 14.72% (29/197) respectively (Table 3).

Table 3. 1 Distribution of bacterial isolates from blood and urine specimens among cancer patients who are active on chemotherapy at the Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia, 2021

Bacterial isolates	**Blood, n=197**		**Urine, n=197**	
	Frequency(30)	Percent (%)	Frequency(29)	Percent (%)
Escherichia coli	5	16.67	9	31
CoNS	7	23.33	1	4
Citrobacter Diversus	1	3.33	3	10
Acinetobacter species	4	13.33	1	4
Klebseilla pneumoniae	5	16.67	6	21
Klebseilla Oxytoca	0	0	3	10
S. aureus	6	20	2	7
Pseudomonas sp.	0	0	1	4
Enterobacter cloacae	1	3.33	0	0
Enterobacter aerogens	1	3.33	1	3
Enterococcus sp.	0	0	1	3
Proteus vulgaris	0	0	1	3

Antimicrobial resistance pattern of bacterial isolated

Antimicrobial resistant pattern for gram positive bacteria isolated from blood and urine culture, high resistance rates were observed to Penicillin (94.12%), ampicillin (88.24%) and amoxicillin (58.82%). In contrast, low resistance rates were detected to trimethoprim-sulfamethoxazole (5.88%), and gentamicin (5.88%) (Table 4.1). For gram negative bacteria isolated from blood and urine culture, high resistance rates were detected on ampicillin (76.19%), augmentin (73.80%) and amoxicillin (71.43%).On the other hand, a low resistance rate were detected on trimethoprim-sulfamethoxazole (7.14%) and ciprofloxacillin (11.9%) (Table 4.2).

Table 4. 1Antimicrobial Susceptibility test of Gram positive bacteria isolated from blood and urine culture among cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, 2021

Antibiotics		**GPB Isolated(17)**			
		CoNS(8)	*S. aureus (8)*	*Enterococcus sp.(1)*	TOTAL (%)
AMP	R	8(100)	7(87.5)	ND	88.24
	I	0(0)	0(0)	ND	0
	S	0(0)	1(12.5)	ND	5.88
AUG	R	0(0)	3(37.5)	ND	17.65
	I	1(12.5)	1(12.5)	ND	11.76
	S	7(87.5)	4(50)	ND	64.7
CFT	R	1(12.5)	3(37.5)	0(0)	23.53
	I	0(0)	1(12.5)	0(0)	5.88
	S	7(87.5)	4(50)	1(100)	64.7
CFP	R	0(0)	3(37.5)	ND	17.65
	I	2(25)	2(25)	ND	23.53
	S	6(75)	3(37.5)	ND	52.94
CFZ	R	0(0)	3(37.5)	ND	17.65
	I	1(12.5)	1(12.5)	ND	11.76
	S	7(87.5)	4(50)	ND	64.7
CFX	R	ND	5(62.5)	ND	29.41
	I	ND	1(12.5)	ND	5.88
	S	ND	2(25)	ND	11.76
AMX	R	5(62.5)	5(62.5)	ND	58.82
	I	1(12.5)	0(0)	ND	5.88
	S	2(25)	3(37.5)	ND	29.41
P	R	8(100)	7(87.5)	1(100)	94.12

	I	0(0)	0(0)	0(0)	0(0)
	S	0(0)	1(12.5)	0(0)	5.88
GN	R	1(12.5)	0(0)	0(0)	5.88
	I	0(0)	2(25)	0(0)	11.76
	S	7(87.75)	6(75)	1(100)	82.35
TSX	R	0(0)	1(12.5)	ND	5.88
	I	2(28.57)	1(12.5)	ND	17.65
	S	6(75)	6(75)	ND	70.59
C	R	1(14.29)	2(25)	0(0)	17.65
	I	3(42.86)	2(25)	0(0)	29.41
	S	4(50)	4(50)	1(100)	52.94
CIP	R	3(42.86)	1(12.5)	0(0)	23.53
	I	0(0)	1(12.5)	0(0)	5.88
	S	5(62.5)	6(75)	1(100)	64.71
ER	R	5(62.5)	3(37.5)	0(0)	47.06
	I	1(14.29)	2(25)	1(100)	23.53
	S	2(28.57)	3(37.5)	0(0)	29.41
TTC	R	3(37.5)	2(25)	1(100)	35.29
	I	2(28.57)	1(12.5)	0(0)	17.65
	S	3(42.86)	5(62.5)	0(0)	47.06

Abbreviations: *AMP-Ampicillin, AUG-Augmentin, CFT-Ceftriaxone, CFP-Cefepime, CFZ- Ceftazidime, CFX-Cefoxitin, AMX-Amoxicillin, P-Penicillin, GN-Gentamicin, TSX- Trimethoprim-Sulfamethoxazole, C-Choloroamphinicol, CIP-Ciprofloxacillin, ER-Erythromycin, TTC-Tetracycline, ND-Not done*

Table 4. 2 Antimicrobial Susceptibility test of Gram Negative bacteria isolated from blood and urine culture among cancer patients at Tikur Anbessa specialized Hospital, Addis Ababa, Ethiopia, 2021

Antibiotics		GNB Isolated(42)									
		E. coli (14)	*Citrobacter diversus (4)*	*Acinetobacter spp. (5)*	*K. pneumoniae (11)*	*K. Oxytoca(3)*	*Pseudomonas sp.(1)*	*Enterobacter cloacae(1)*	*Enterobacter aerogens(2)*	*Proteus vulgaris(1)*	Total (%)
AMP	R	9(64.2)	4(100)	5(100)	11(100)	0(0)	1(100)	1(100)	1(50)	ND	76.19
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	ND	0
	S	5(35.71)	0(0)	0(0)	0(0)	3(100)	0(0)	0(0)	1(50)	ND	21.43
AUG	R	9(64.29)	4(10)	5(100)	10(90.9)	0(0)	1(100)	1(100)	1(50)	ND	73.80
	I	1(7.14)	0(0)	0(0)	1(9.09)	0(0)	0(0)	0(0)	0(0)	ND	4.76
	S	4(28.57)	0(0)	0(0)	0(0)	3(100)	0(0)	0(0)	1(50)	ND	19.05
CFT	R	4(28.57)	2(50)	5(100)	6(54.55)	0(0)	1(100)	0(0)	1(50)	0(0)	45.24
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0
	S	10(71.4)	2(50)	0(0)	5(45.45)	3(100)	0(0)	1(100)	1(50)	1(10)	54.76
CFP	R	4(28.57)	2(50)	3(60)	6(54.55)	0(0)	1(100)	0(0)	1(50)	ND	40.48
	I	1(7.14)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	ND	4.76
	S	9(64.29)	2(50)	1(20)	5(45.45)	3(100)	0(0)	1(100)	1(50)	ND	52.38
CFZ	R	4(28.57)	2(50)	5(100)	6(54.55)	0(0)	1(100)	0(0)	1(50)	0(0)	45.24
	I	1(7.14)	0(0)	0(0)	0(0)	1(33.33)	0(0)	0(0)	1(50)	0(0)	7.14
	S	9(64.29)	2(50)	0(0)	5(45.45)	2(66.6)	0(0)	1(100)	0(0)	1(10)	47.62
MRP	R	2(14.29)	1(25)	1(20)	4(36.36)	1(33.33)	1(100)	ND	1(50)	ND	26.19
	I	3(21.43)	0(0)	1(20)	2(18.18)	0(0)	0(0)	ND	0(0)	ND	14.29
	S	9(64.29)	3(75)	3(60)	5(45.45)	2(66.67)	0(0)	ND	1(50)	ND	54.76
AMX	R	9(64.29)	3(75)	4(80)	11(100)	0(0)	1(100)	1(100)	1(50)	ND	71.43
	I	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	ND	2.38
	S	5(35.71)	1(25)	0(0)	0(0)	3(100)	0(0)	0(0)	1(50)	ND	23.80
GN	R	4(28.57)	1(25)	1(20)	3(27.27)	1(33.33)	0(0)	0(0)	1(50)	0(0)	26.19
	I	2(14.29)	1(25)	0(0)	1(9.09)	0(0)	0(0)	0(0)	0(0)	0(0)	9.52
	S	8(57.14)	2(50)	4(80)	7(63.64)	2(66.67)	1(100)	1(100)	1(50)	1(100)	64.29
TB	R	2(14.29)	1(25)	0(0)	2(18.18)	1(33.33)	0(0)	ND	2(100)	ND	19.05
	I	2(14.29)	0(0)	0(0)	2(18.18)	0(0)	0(0)	ND	0(0)	ND	9.52
	S	10(71.43)	3(75)	5(100)	7(63.64)	2(66.67)	1(100)	ND	0(0)	ND	54.76
TSX	R	2(14.29)	0(0)	0(0)	1(9.09)	0(0)	ND	0(0)	0(0)	ND	7.14

	I	1(7.14)	1(25)	1(20)	0(0)	0(0)	ND	1(100)	0(0)	ND	9.52
	S	11(78.57)	3(75)	4(80)	10(90.91)	3(100)	ND	0(0)	2(100)	ND	78.57
CIP	R	2(14.29)	1(25)	0(0)	1(9.09)	0(0)	0(0)	1(100)	0(0)	0(0)	11.90
	I	0(0)	0(0)	2(40)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5
	S	12(85.71)	3(75)	3(60)	10(90.91)	3(100)	1(100)	0(0)	2(10)	1(10)	83.30

Abbreviations*: AMP-Ampicillin, AUG-Augmentin, CFT-Ceftriaxone, CFP-Cefepime, CFZ- Ceftazidime, AMX-Amoxicillin,GN-Gentamicin, TSX- Trimethoprim-Sulfamethoxazole, C-Choloroamphinicol, CIP-Ciprofloxacillin, ND-Not done*

Multidrug resistance pattern

In this study, the prevalence of multidrug resistance was 16/59(27.12%). Multidrug resistance for three or more drugs were observed for 9/17 (52.94%) of gram-positive bacteria and 7/42(16.67%) of gram-negative bacteria. Multidrug resistance (resistance for three classes of antibiotics) were detected 4/8(50%) of *Multi drug resistance CoNS*, 5/8(62.5%) of *Methicillin resistance staphylococcus aureus*, 3/14(21.43%) of *MDR Escherichia coli*, 2/11(18.18%) of *MDR klebseilla pneumonia*, 1/3(33.33%) of *MDR K.oxytoca* and 1/1(100%) of *MDR Enterobacter aerogenes* isolated

Factors associated with culture positivity

In logistic regression analysis, advanced stage of cancer patients were 2.540 times more likely to be culture positive (AOR=2.540; 95% CI=1.294-4.986; P=.007) as compared to patients with early stage cancer patients. Hematological cancer patients were 2.823 times more likely to be culture positive (AOR=2.823; 95% CI=1.179-6.758; P=.020) as compared to patients with solid tumor cancer patients. None of the socio-demographic factors had statistically significant association with culture positivity

Table 5. 1logistic Regression -Risk Factors Associated with culture positivity

Culture result				**COR (95% CI)**	AOR (95% CI)	**P-value**
Variables		Negative (%)	Positive (%)			
Sex	Male	70(71.4)	28(28.6)	1		
	Female	68(68.7)	31(31.3)	1.140(0.619-2.098)		0.675
Type of malignancy	Hematologic	100(65.8)	52(34.2)	2.77(1.104-6.096)	2.823(1.179-6.758)	0.020
	Solid	38(84.4)	7(15.6)	1	1	
Marital status	Single	67(72.8)	25(27.2)	1.005(0.247-4.092)		0.994
	Married	57(67.9)	27(32.1)	.792(.195-3.222)		0.744

	Divorced	1(50%)	1(50)	.375(.017-8.103)		0.532
	Widowed	5(62.5)	3(37.5)	.625(.089-4.401)		0.637
	Others	8(72.7)	3(27.3)	1		0.147
Age	< 15y	36(87.8)	5(12.2)	1.240(.351-4.383)		0.739
	15- 29y	30(73.2)	11(26.8)	1.148(.352-3.749)		0.819
	30 -44y	48(71.6)	19(28.4)	.848(.248-2.900)		0.793
	45-59y	28(65.1)	15(34.9)	1.061(.285-3.948)		0.93
	≥60y	21(70.0)	9(30.0)	1		0.144
Occupational status	civil servant	14(73.7)	5(26.3)	1.200(.393-3.664)		0.749
	Merchant	7(87.5)	1(12.5)	3(.352-25.581)		0.315
	house wife	31(66.0)	16(34)	.830(.391-1.763)		0.629
	Farmer	23(69.7)	10(30.3)	.986(.414-2.349)		0.974
	Others	63(70)	27(30)			
Educational status	no formal education	70(72.9)	26(27.1)	1.346(.489-3.706)		0.565
	Primary	33(64.7)	18(35.3)	.917(.313-2.683)		0.874
	Secondary	21(72.4)	8(27.6)	1.312(.388-4.442)		0.662
	Tertiary	14(66.7)	7(33.3)	1		
Stage of cancer	Early	66(80.5)	16(19.5)	1		
	Advanced	72(62.6)	43(37.4)	2.464(1.268-4.786)	2.540(1.294-4.986)	0.007
	yes	2(66.7)	1(33.3)	1		
Experience of tobacco/smoke user	No	136(70.1)	58(29,9)	1.172(.104-13.186)	1.179(0.285-4.879)	0.897
	Grade 1	16(80)	4(20)	1.71(.541-5.434)	0.58(.184-1.849)	0.36
	Grade 2	10(76.9)	3(23.1)	1.43(.374-5.455)	0.7(.183-2.673)	0.602
	Grade 3	14(58.3)	10(41.7)	0.6(.247-1.459)	1.67(.686-4.052)	0.26
Grade of cancer	Not recorded	98(70)	42(30)	1	1	

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

6. Discussion

In this study we report the isolation rate of bacterial infection from both blood and urine cultures of cancer patients active on chemotherapy and its antimicrobial resistance pattern for each positive sample. We also demonstrate high level of resistance among bacteria causing sepsis and urinary tract infection in our setting.

The prevalence of bacterial infections among cancer patients active on chemotherapy isolated from blood culture and urine culture in our finding were 30/197(15.23%) and 29/197(14.72%) respectively. The overall prevalence of isolated bacteria from blood and urine culture were 14.97%. This finding is comparable with the study conducted in Gondar Hospital, Northwest Ethiopia (19.4%)[40], South Africa (13.8%)[37], India (20.12%)[27], Iran (17.3%))[24], and Taleghani hospital Tehran Iran (20%)[22] among cancer patients. This finding was higher when compared with studies conducted previously in Tikur Anbessa hospital, Addis Ababa Ethiopia (6.3%) [44] and Bangladesh (8.6%)[31]. In contrast, the culture positivity rate was lower when compared with in the past from Gondar Hospital (23.3%)[41], Nepal (24%)[30], Zimbabwe (35.2%)[38], Qatar (38.7%))[35] and India (40%)[28].The variation in prevalence might be explained by the fact that differences in geographical location, the characteristics of the study population, the difference in methodology used for culture, and differences in the policies for bacterial infection management.

Among the 59 bacterial uropathogens and blood stream infections identified in our study, most of them were Gram-negative organisms, 42/59(71.19%), while only 17/59(28.81%) were gram positive bacteria. Our finding is in line with the studies reported from in India in which Gram-negative were (69.9 %) and Gram-positive were (30.1 %)[29].Similar finding conducted from other countries has also shown gram negative bacteria were predominant like in Iran(63.3%)[24], other study in Iran(77.2%)[23] and Lebanon(78.8%)[36].Although the world epidemiology of infections in cancer patients has occurred over time, characterized by a shift from prevalent Gram-negative bacteria between the 1960s and 1970s to Gram-positive bacteria [16, 17],in our finding gram negative bacteria were the predominant cause of bacterial infection of cancer patients active on chemotherapy as compared to gram positive bacteria. This predominance of gram negative bacteria on this finding might be due to the relatively lower use of indwelling medical devices, low utilization of prophylactic antibiotic regimens, and chemotherapeutic regimens in cancer patients active on chemotherapy [48, 49].In the other hand, a study conducted in other sites and other countries reported that Gram positive bacteria were significantly more predominant isolates from cancer patients with several reasons including the high prevalence of neutropenia, intensive chemotherapy regimens, presence of indwelling invasive devices including mucosal barrier defects, and prophylactic [22, 37].

CoNS 7(23.33%) and *S. aureus* 6(20%) were the most dominant blood stream infection causing bacteria in this study. The GPB *S. aureus* and *CoNS* are everywhere in nature, which are commonly found on the skin and are the major cause of various infections, mainly in patients with solid tumors following indwelling devices, invasive surgical procedures, and contamination from hospital environments [50, 51].This study revealed more or less similar finding with those reported from Hiwot Fana Specialized University Hospital, Eastern Ethiopia *S. aureus* (18.5%) [42], and Zimbabwe *CoNS* (22%) [38]. From the total urinary tract infections, the predominant isolated bacteria in our finding were *E.coli* 9(31.03%). This finding was in agreement with the study conducted in Gondar (32.1%)[41]. On the other hand this finding lower as compared with study conducted at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia (44.4%)[44], India (40%)[27], and Nepal (58%)[30].This variation could be due to difference in sample size and study population.

Gram positive bacteria of blood stream and urinary tract infections detected a high resistance rate to penicillin (94.12%), ampicillin (88.24%) and amoxicillin (58.82%). Multidrug resistance were detected, 4/8(50%) of *Multi drug resistance CoNS*, and 5/8(62.5%) of *Methicillin resistance staphylococcus aureus*. This finding was comparable with the study conducted in Egypt (71.4% *CoNS* and Methicillin resistance in all isolates of *Staphylococcus aureus*) [39] and in University of Gondar Hospital, Northwest Ethiopia (25% of *MRSA* and 45.5% of *MRCoNS*) [40]. From gram negative bacteria isolated, high resistance rates were detected on ampicillin (76.19%), augmentin (73.8%) and amoxicillin (71.43%). Multidrug resistance were observed, 3/14(21.43%) of *MDR Escherichia coli*, 2/11(18.18%) *MDR klebseilla pneumonia*, 1/3(33.33%) *MDR K.oxytoca* and 1/1(100%) *MDR Enterobacter aerogenes* isolated. This study more or less in line with the study conducted in Gondar (ampicillin (88.2%)and amoxicillin/clavulonic acid (82.4% for GNB) [40], and in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia augmentin (61.1%), ampicillin (66.7%) and ceftriaxone (55.6%)[44]. This high resistance rate in our finding could be these antibiotics have already been in use for empirical therapy of patients with cancer and be inappropriate antibiotic use, inappropriate prescription for empiric therapy by physicians, and lack of appropriate infection control strategies increase rate of resistance in our site.

Many factors were assessed as risk factors for bacterial cultural positivity in cancer patients. However, no evidence was found to support the association between culture positivity with age, sex, experience of tobacco and smoke, grading of cancer, marital status, occupational status, and educational status ($P > 0.05$). Correspondingly, other studies has also showed that neither of the above factors mentioned in our study had no association with bacteriuria and bacteremia [40, 44]. In our finding, advanced stage cancer patients were 2.540 times more likely to be culture positive (AOR=2.540;95%CI=1.294-4.986; P=0.007)) as compared to early stage cancer patients. This reason could be the cancer has spread to other organs or parts of the body and patients with this stage of cancer may have a higher risk of infection because of changes in the immune system that control their body's defense systems due to cancer and cancer treatments. In our finding, hematological cancer patients were 2.823 times more likely to be culture positive (AOR=2.823; 95% CI=1.179-6.758; P=.020) as compared to patients with solid tumor cancer patients. This is probably due to Patients with hematological malignancy being at high risk of infections, because of neutropenia induced by intensive chemotherapy and its cytotoxic effect on the cells that line the gastrointestinal tract [52, 53]

7. Strength and Limitation of the study

The study was conducted on all ages of cancer patients who are active on chemotherapy in order to assess both bacteremia and bacteriuria with its resistance pattern.

As a drawback of this study, though in immunocompromised patient like oncologic patients, the fungal agents mainly Candida are the most frequent etiologic agent of urinary tract infections, we did not test for the fungal agents as the causal agent of urinary tract infection due to the restriction of consumables. Beyond this, we did not test extended spectrum beta-lactamase producing bacterial, which are the main sources of antibiotics resistances.

8. Conclusion and recommendation

Conclusion

In general the burden of bacterial infection among oncology patients is significantly high especially in patients with hematological, who had active on chemotherapy. Gram-negative bacteria were found to be the predominant pathogens isolated from blood stream and urinary tract bacteria in oncology patients active on chemotherapy. *E.coli* was a common gram-negative bacterium from bacteriuria in our study setting. *CoNS* and *S. aureus* were the most common bacteria from blood culture. Gram positive bacteria of blood stream and urinary tract infections detected high resistance rate to penicillin, ampicillin and amoxicillin. From gram negative bacteria isolated; high resistance rates were detected on ampicillin, augmentin and amoxicillin. 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 bacteria for cancer patients.
- ✓ Health professionals should implement education to patients in order to adhere to the treatment and thereby reducing drug resistance.
- ✓ We recommend prospective research studies to ascertain the prevalence of Urinary tract infections and blood stream infection, and antibiotic resistance patterns among cancer patients active on chemotherapy in a representative sample that provide cancer care in the country.

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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: Bacterial profile and antimicrobial resistance pattern among oncology patients at Tikur Anbessa Hospital, Addis Ababa, Ethiopia

Principal Investigator: Seid Yimam (BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

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, 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 bacterial profile and antimicrobial resistance pattern among oncology 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 spectrum of the bacterial profile, antimicrobial resistance patterns, and management of bacterial infections among oncology 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 10 ml venous blood (about from you by sterile-disposable vacutainers tube and needle. We will conduct a laboratory examination to determine different bacterial profile.

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: Seid Yimam   Phone: 09 79 70 01 63

E-mail: seidyimam2006@gmail.com

Annex II: Information sheet in Amharic Version

የተሳታፊዎች ፈቃደና መጠይቅ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ኮሌጅ የሕክምና ላቦራቶሪ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመሚቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡ ፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመቀማቅዎ በፊት ከዚህ ቀጥሎየሚ ኘዉንምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነ ልዎትን ማንኛዉምሃሳብይጠይቁ፡ ፡

መግቢያ

የጥናቱ ርዕስ “bacterial profile and antimicrobial resistance pattern among oncology patients who are taking chemotherapy at Tikur Anbessa Specialized hospital, Addis Ababa, Ethiopia”.የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሰረተ ነዉ፡ ፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኀላ ለማቋረጥ የሚወስኑ በሆንም እንከዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት

አይቋረጥም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጽሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሆል መስማማት ይጠበቅብዎታል፡፡ ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች በነገር የሚያቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ ይህን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፣ አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎችን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚሆል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለ ርስዎ አጠቃላይ የጤና ሁኔታ ለማቋርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎትአል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንምአይነት የከፋ ችግር አያጋጥምዎትም፡፡ ሆኖምግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚወስድ አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመምስሜቱ አይኖርም፡፡

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚቻለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚሆልዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸዉ፡፡ ከዚያም በላይ ስለእርስዎ ያለዉን ማንኛዉንም መረጃ የተለየ የ ይለፍ ቃል ባለዉ የኮምፒዉተር የመረጃ ማህደር ዉስጥ እንዲቀመጥ ይደረጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መሟያ እንደመሆኑ መጠን በዚህ ጥናት በመሳፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ዉጤት ግን ተጠቃሚ ነዎት፡፡ የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን በካንሰር ታማሚዎች ላይ ያሉ ባክቴሪያ ለማወቅና ለማከታተልከ ፍተኛ ጥቅም ይኖረዋል፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም፡፡ ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት፡፡ የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡ ነገር ግን እርስዎ በሚሰጡት መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

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

ስም፡ ሰይድ ይማም **ሞባይል፡** +251-9 79 70 01 63 **ኢሜል፡** seidyimam2006@gmail.com

Annex III: Informed consent form in English version

Card no…..………………….

I had been informed that the objective of this study is to assess bacterial profile and Antimicrobial resistance pattern among cancer patients. 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 had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood and bone marrow sample, 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 for providing the requested information and specimens as the doctors find best for me.

Signature: ___________________________________ Date_________________________

Annex III: Informed consent form in Amharic version
የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር---------------------------የተሳታፊውስም------------------------------

እኔ ስሜከላይ የተጠቀሰውተሳታፊ “bacterial profile and antimicrobial resistance pattern among oncology patients who are taking chemotherapy” ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱምናመጫ እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎችምተረድቻለሁ፡በቃለ ማጠይቁ ላይ የገለጽኳቸው ሚጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንምሚጃ ያለመስጠት እንዲሁምበማንኛውምጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡ስለዚህ ለዚህ ጥናት ሚጃና የስምምነት ቃሌን የሰጡት በአጠቃላይ ሁኔታውን በሚዳትና በፍጹምፍቃደኝነት ነው፡ በተጨሜምጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘውጥቅምየሁሉንምምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡

ፊርማ------------------- ቀን ----/---/----------የስምምነትቅጹን ማንበብለማይችሉ 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 ገጽ ማስትም 18 ኣመት በታች ለሆናቸው ተሳታፊዎች ስምምነት የመጠየቂያ ቅጽ ነው፡፡ ናሙና የምንወስደበት መሳሪያ ንጽህናው የተጠበቀ አስተማማኝና ከዚህ በፊት ጥቅም ላይ ያልዋለ ነው፡፡ ናሙና በምንወስደበት ጊዜ የሚኖር ህመም የሚፈጥር ስሜትም ሆነ አደጋ የሚያስከትል ሂደት የለውም፡፡ ለጥናቱ የሚወሰደው ናሙና ለጥናቱ አላማ ብቻ ይውላል፡፡ የናሙናው ውጤት ምስጥራዊነት የተጠበቀ ሲሆን በናሙናው ውስጥ የበሽታ አምጪ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈላጊውን ህክምና ያገኛሉ፡፡ በጥናቱ ላይ በመሳተፍዎ ምንም የገንዘብ ክፍያ አያገኙም፡፡ በጥናቱ ለመሳተፍ የመፍቀድም ሆነ ያለመፍቀድ እንዲሁም በማንኛውም ጊዜ የማቋረጥ መብት አለዎት፡፡ (ስም) በጥናቱ እንዲሳተፍ/ እንድትሳተፍ ይፈቅዳሉ? ፈቃደኛ ከሆኑ፤ የተሳታፊ ፊርማ ------------------------- የፈቀደው ግለሰብ ፊርማ ------------------------------ አድራሻ፣ ------------ቀን፣ -------በስምምነቱ ወቅት የነበሩ ምስክሮች

1.______________________________ 2.__________________ ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥምዎት ድንገተኛ ችግር በሚከተለው አድራሻ ይጠቀሙ

ሞባይል፡ +251979700163

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% povidine-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, withdraw about 1 ml of blood from neonate, 5 ml from a children and 10 ml from adult.

- ✓ 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.

1.2 urine sample collection

- ✓ the sample will be collected mid stream urine from the patient
- ✓ process immediately after specimen collection for culture

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 carbolfuschin/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, over heating 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, 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 work bench 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 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 slide

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

3. Add the test broth culture to tube.

4. After mixing gently, incubate the tubes at 35–37oC. Examine for clotting after 24 hour. 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 *Enterobacteriacaea*. Few colonies of the culture will be inoculated into peptone water and incubated at 37oC for 24 hours. Few drops of indicator (Kovac‘s reagent) will be added and the Colour 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 Enterobacteriacaea. 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 become 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 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 hour. 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.

4. 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 degree 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 VI: Questionnaire

Addis Ababa University College of Health Sciences, Department of Medical Laboratory Science. Questionnaire for the demographic characteristics and assessment bacterial profile and antimicrobial resistance pattern among cancer patients who are taking chemotherapy attending at Tikur Anbessa hospital. This information is only used for the study and the information will be kept confidentially. Circle or write on the space provided.

Facility name ____Year ___Participant code _____Participants address (Sub city) ________ Telephone ________signature_________ Data collector name____________date____________signature______

I. Characteristics of the Study participants	
1. Age	…………….years
2. Sex	A) Male B) Female
3. Marital status	A) single B) Married C) Divorced D) widowed
4. Occupational status	A)civil servant B) merchant C)House wife D) farmer E) others
5. Educational status	A) No formal education B) Primary C) Secondary D)Tertiary
6. What Type of chemotherapy do you take?	………………………………………..
7. type of malignancy	A, hematologic B, solid
8. Hospital admission	A, yes B, No
9.Ward of the patients	…………………………..
10. Duration of hospital stay	…………………………….
11. Stage of cancer	A) Advanced B) Early
12. Grading of cancer	A) grade 1 B) grade 2 C) grade 3 D) Not recorded
13. Previous antibiotic therapy	A, Yes B, NO
14. Do you have experience of tobacco or smoke?	A, yes B, No

II. Laboratory Data	
15. Date of specimen collection	……………………….
16. Gram stain result	…………………………
17. Biochemical test	…………………………
18. Organism isolated	…………………………
19. Drug susceptibility pattern	A. Sensitive to ………………………… B. Intermediate to …………………………. C. Resistance to……………………………..
Name of principal investigator	
Signature ________________	Date……………………..

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመሚቂያ ጥናት በ˝Bacterial profile and antimicrobial resistance pattern among cancer patients who attended a black lion hospital˝ ላይ ማጠየቂያ ቅጽ ማጠይቅ መሚያ አንብበው/አደምጠው መልሱን ከተሰጡት አማራጮች እንደንያከቡ ወይም በ ክፍት ቦታው ላይ ይሙሉ።

ተ.ቁ	መረጃ	ምርጫ
1	ዕድሜ	....
2	ፆታ	1.ወንድ 2. ሴት
3	የጋብቻ ሁኔታ	1.ያላገባ/ች 2. ያገባ/ች 3.የፈታች 4. በሞት ምክንያት ሚስቱን/ ባሉን ያጣች
4	የስራ ሁኔታ	1.የመንግስት ሰራተኛ 2. ነጋዴ 3.የቤት እመቤት 4.ገበሬ 5.ሌላ
5	የትምህርት ሁኔታ	1. መደበኛ ያልተማረ 2. መጀመሪያ ደረጃ 3. ሁለተኛ ደረጃ 4. ሶስተኛ ደረጃ

6	የካንሰሩ አይነት	1. ሂማቶሎጂክ 2. ሶሊድ
7	የካንሰሩ ልዩ አይነት	………………………….
8	የሆስፒታልቅበላ	1. አለ 2. የለም
9	የህክምናክፍል	…………………………….
10	የካንሰሩ ደረጃ	1. ከፍተኛ 2. መጀመሪያ ላይ
11	የካንሰሩ ግሬድ	1. ግሬድ1 2. ግሬድ2-4 3. ግሬድ5
12	ፀረ-ባዮቲክስመድሃኒትተጠቅመዋሉ	1. አወ 2. የለም
13	ሲጋራ/ ትምባሆይጠቀማሉ	1. አወ 2. የለም

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 used for the thesis have been duly acknowledged.

M.Sc. candidate: Seid Yimam (B.Sc.)

Signature: ___________________

Date of submission: ___________________

This thesis has been submitted with our approval as advisors.

Advisor: Melese Hailu (MSc, PhD)

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia.

Advisor: Meron Yohannes (MSc, PhD candidate)

Signature: ____________________

Date: ____________________

Place: Addis Ababa, Ethiopia.