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Bacterial isolates and their antibiotic susceptibility pattern from sterile body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Senay Getahun, entitled:

"**Bacterial isolates and their antibiotic susceptibility pattern from sterile body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**" and submitted in partial fulfillment of the requirements for a Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviations and acronyms

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
CRE	Carbapenem-resistant Enterobacterales
CNS	Central nervous system
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase-negative Staphylococci
CSF	Cerebrospinal fluid
DRERC	Departmental Research and Ethical Review Committee
ESBL	Extended-spectrum beta-lactamases
ETB	Ethiopian Birr
GNB	Gram-negative bacteria
MDR	Multidrug resistance/resistant
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCCLS	National Committee for Clinical Laboratory Standards
spp.	Species
SPSS	Statistical Package for Social Science
SOP	Standard operating procedure
TASH	Tikur Anbessa Specialized Hospital
WHO	World Health Organization

Abstract

Background: Sterile body fluid infection requires rapid and accurate diagnosis and treatment because its morbidity and mortality are high. Continuous laboratory surveillance is important to guide clinicians in providing effective preventive and evidence-based therapies to achieve this goal.

Objectives: The study aimed to determine bacterial isolates and antibiotic susceptibility patterns from sterile body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Methods: A hospital-based cross-sectional study was conducted from May 1 to December 30, 2023, among 205 randomly selected patients. Data were collected through interviews using a pretested questionnaire. Sterile body fluids submitted to the laboratory were processed according to standard laboratory procedures. Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method according to CLSI guidelines. ESBL & CRE detection were made using cephalosporin/ clavulanate combination discs & modified Hodge test, respectively. Data entry and analysis were performed using SPSS v26. Bivariate and multivariate logistic regressions were used to assess the association between the outcome and predictor variables. A P-value <0.05 was considered to indicate statistical significance.

Result: Approximately one-fifth (19.5%, n=40) of the processed samples were culture-positive. Most of the pathogens detected were gram-negative bacteria (GNB), 72.5%, while gram-positive bacteria accounted for 27.5%. Gram-negative isolates were 86.2% sensitive to amikacin & meropenem (76%), while gram-positive isolates were sensitive to ciprofloxacin (72.7%). Among GNB, 68.2% were extended-spectrum beta-lactamase producers, while 36.4% were carbapenemase producers. MDR, mostly associated with penicillins and third-generation cephalosporins, was detected in 67.5% of the isolates. Culture positivity was associated with rural residence (AOR: 3.86, 95% CI: 1.58-9.42, P = 0.003), inpatient status (AOR: 2.65, 95% CI: 1.17-5.99, P = 0.019), comorbidities (AOR: 5.46, 95% CI: 2.22-13.40, P = 0.001) and turbid appearance (AOR: 3.37, 95% CI: 1.17-9.70, P = 0.024).

Conclusion: In this study, significant drug resistance to commonly prescribed beta-lactam antibiotics and a high MDR were observed, necessitating the use of prudent antibiotics in hospital and community settings by all healthcare workers and policymakers.

Keywords: sterile body fluid infection, antimicrobial resistance, extended-spectrum beta-lactamase, antibiotic stewardship, Tikur Anbessa, Ethiopia

1. INTRODUCTION

1.1. Background

Sterile body sites are those in which there are no commensal bacteria or microbes when they are in a healthy state. Similarly, body fluids obtained from sterile body sites are expected to be free of pathogenic or commensal microorganisms [1]. Body fluids such as cerebrospinal fluid (CSF), pleural, peritoneal, pericardial, and synovial fluids are defined as sterile body fluids. The detection of organisms in fluids that are normally sterile indicates a significant infection that can be life-threatening. Infections caused at these sites could be due to pathological agents or skin contaminants contained in intensive care units [2].

Sterile body fluids play an important role in the transport of nutrients and waste products, the regulation of body temperature, and the evaluation of the respiration process [3]. Different types of body fluids are found in our body, such as pleural fluid, peritoneal fluid, CSF, synovial fluid, and pericardial fluids. These fluids are generally free of microorganisms, including bacteria, fungi, viruses, and parasites; Invasion of microorganisms can lead to morbidity and mortality [4].

Body fluids such as CSF, pleural, peritoneal, synovial, and pericardial fluids are frequently obtained in microbiology laboratories for culture in suspected infections. These infections are associated with considerable morbidity and mortality [2, 5]. Microbiologically, any growth in the culture of these fluids is accepted as the causative agent, except for suspicion of contamination. Microorganisms can invade sterile body sites, causing serious invasive diseases and resulting in a critical phenomenon. Bacterial infections change the physicochemical nature of body fluids, such as increasing white blood cell (WBC) count and protein concentration and decreasing glucose concentration [4]. In response to infection, fluid can accumulate in any body cavity associated with invasive diseases, such as bacteremia, sepsis, bacterial meningitis, bacterial peritonitis, and other complications [7].

Sterile body fluids represent an important source for the diagnosis of infectious diseases because they can be sampled using sterile methods that bypass the normal bacterial flora. Generally, a wide spectrum of microbiological analyses for sterile body fluids, including bacterial, fungal, viral, and mycobacterial agents, is required [8].

CSF analysis is used to identify a disease or condition that affects the central nervous system (CNS), such as infection, malignancy, or bleeding within the brain or skull. Synovial fluid analysis is used to identify the origin of joint swelling, discomfort, and/or inflammation. Pleural fluid analysis helps identify the origin of pleural inflammation (pleuritis, pleurisy), fluid accumulation in the pleural space (pleural effusion), or possibly cancer. Similarly, pericardial fluid analysis is performed to identify the origin of fluid build-up around the heart. Meningitis, pericarditis, pleural infection (complicated parapneumonic effusion or empyema), and septic arthritis are the main forms of sterile body fluid infection [9].

In general, *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas aeruginosa*, *Citrobacter spp*, and *Acinetobacter spp*. are the most frequently isolated gram-negative bacteria, while *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *enterococci* are among the gram-positive bacteria that invade and infect sterile body fluids leading to morbidity and life-threatening infections [10, 11].

The development of resistance to commonly used antibiotics is becoming a challenge worldwide and undermines treatment success in hospital-acquired infections in general and sterile body fluid infections in particular [10]. In particular, infections with extended-spectrum beta-lactamases (ESBLs) and carbapenem-resistant *Enterobacteriaceae* (CRE) are associated with increased mortality rates, prolonged hospital stays, increased hospital expenses, and reduced clinical and microbiological response rates [11, 12]. This phenomenon is more severe in developing countries such as Ethiopia, where there is over-the-counter availability and a lack of regulation and quality control of drugs.

1.2. Statement of the problem

Sterile body fluid infection is a medical emergency and requires early diagnosis and effective treatment. To achieve this goal, definitive epidemiological data are needed, as empiric and preventive treatments are mainly based on these data.

Antibiotic resistance is the result of bacteria changing in ways that reduce or eliminate antibiotic effectiveness and is estimated to contribute to more than 2 million infections and 23,000 deaths annually in the United States alone, according to the US Centers for Disease Control and Prevention [12]. This results in a direct cost of $20 billion and additional productivity losses of $35 billion [13]. A similar scenario is reflected in Europe, which registers approximately 25,000 deaths a year and costs €1.5 billion a year, all attributable to infections with antibiotic resistance [14]. Few data are available for various countries in Africa showing the morbidity and mortality burden of antibiotic-resistant infections. For example, these infections were found to contribute to increased mortality in newborns with suspected neonatal sepsis in a Tanzanian study [15].

Although few studies [7, 13, 16, 17] targeting body fluid infections have been conducted in Ethiopia, there are limited data on the rates of body fluid infections and the occurrence of multidrug-resistant bacteria. To the best of the authors' knowledge, no published article in Ethiopia has investigated the prevalence and role of ESBLs and CREs in sterile body fluid infection. Furthermore, regular monitoring of prevalent pathogenic organisms and their sensitivities is essential for better patient management and formulation of the hospital's antibiotic policy. Knowledge of the prevalent strains and their pattern of antimicrobial resistance is essential, as this knowledge will help clinicians select the appropriate antibiotic therapy.

Therefore, the present study was carried out to understand the current state of the bacterial profile and its susceptibility patterns from various body fluids collected from patients attending a tertiary care hospital in Addis Ababa.

1.3. Significance of the study

Body fluid analysis plays a critical role in the diagnosis and prognosis of a disease. A change in the concentration or composition of a particular biochemical component in body fluids is used as an indicator of a physiological or pathological condition. Therefore, a particular component of body fluid can be considered a marker for the detection of a disease. Detection of these markers early in life can lead to a prompt diagnosis, which can lead to appropriate treatment.
Knowledge of the bacteriological and antimicrobial profiles of sterile body fluids is necessary for the effective treatment of life-threatening infections and thus the prevention of antimicrobial resistance.

The findings of this study may help increase awareness of the local antimicrobial susceptibility pattern and common bacterial pathogens that cause body fluid infections. It also helps to establish an antimicrobial susceptibility surveillance system and improve current infection control programs in hospitals. Regular monitoring of prevalent pathogenic organisms and their sensitivity will help clinicians select antibiotic therapy appropriately in the absence of a culture report and prevent the development of antibiotic resistance.

This study could also serve as a baseline for other related studies that may follow in the future, as it requires periodic analysis and surveillance. Since Tikur Anbessa Specialized Hospital (TASH) serves as the main referral center for all patients from different parts of the country, it provides a good representation of the etiology of bacteria and the pattern of resistance to body fluid infections.

2. LITERATURE REVIEW

A cross-sectional study carried out in northern India from April 2018 to March 2019 revealed that 14 patients developed pleural fluid infections. The main isolated bacteria were *Staphylococcus aureus* (12.5%) followed by *E. coli* (23.86%) [18].

Similarly, a study carried out in Egypt from May 2018 to April 2019 showed that of a total of 121 collected pleural fluid samples, 57(47%) samples had positive cultures, 31(50%) of which were aerobic bacteria, and positive for Gram bacteria, 27 (43.5%) were bacteria negative for Gram bacteria, and 4 (6.5%) were anaerobic bacteria. The organism encountered most frequently was *Staph aureus* (n=15, 24.2%), followed by *Klebsiella pneumoniae* (n=10, 16.1%), *Pseudomonas aeruginosa* and *Pneumococci spp.*(n = 8, 12.9%) and *Escherichia coli* (n =6, 9.7%) [19].

In another study conducted in India from August 2019 to October 2019 involving 380 patient samples, *Escherichia coli* was among the bacteria isolated from ascitic fluid. From the pleural fluid, *Escherichia coli* and *Klebsiella pneumoniae* were isolated. *Staphylococcus aureus* was isolated from the synoial fluid and CSF. Among the 11 culture-positive samples, mainly gram-negative organisms (72.7%) were isolated and the remaining were gram-positive organisms (23.7%) [20].

A cross-sectional study conducted in Delhi, India, from January 2015 to December 2015 analyzed 405 samples. Among the 405 samples, 122 fluid samples showed culture growth with an isolation rate of 30%. The isolates from different fluids were *E. coli*, *Acinetobacter spp*., *Klebsiella spp*., *S. aureus*, *Enterococcus spp., Pseudomonas spp.,* and *Citrobacter spp*. Gram-negative isolates were mostly sensitive to carbapenems, colistin and polymyxin B (100%), and gram-positive isolates were highly sensitive to vancomycin (100%) linezolid (100%), and ciprofloxacin (70%). *Acinetobacter* is the most resistant pathogen to many antibiotics. In this study, the predominant organisms were *E. coli* (28.6%) and *Acinetobacter spp*. (27%), followed by *Klebsiella spp*. (19.6%), *S. aureus* (10.6%), *Enterococcus spp*. (7.3%), *Pseudomonas spp*. (4.9%) and *Citrobacter spp*. (1.6%). In these studies, *Acinetobacter spp*. and *E. coli* were the most common organisms isolated from pleural effusion samples [21].

A study carried out in Iran from 2003 to 2013 showed that of the 107 CSF samples collected, the commonly isolated bacteria were *Streptococcus pneumoniae* (34.5%), *Haemophilus influenzae*

type b (23.36%), *Neisseria meningitidis* (6.54%), Serratia spp. (6.54%) and Klebsiella pneumoniae (5.6%) [35]. A similar study was conducted in Iran between 1998 and 2008, with a total of 11269 CSF samples collected from suspected cases, among which 329 (2.9%) were culture-positive for coagulase-negative *Staphylococci* (40.1%), *S. pneumoniae* (9.1%), *S. aureus* (7.6%), *Klebsiella spp*. (6.1%), *P. aeruginosa* (6.1%), *Haemophilus spp*. (5.8%), *E. coli* (5.3%), *Acinetobacter spp*. (4.6%), hemolytic *streptococci* (except *S. pneumoniae*) (4.6%), *N. meningitides* (2.7%). In terms *staphylococci* are resistant to tooxacillin and vancomycin, *S. pneumonia* is resistant to penicillin, and gram-negative bacteria are resistant to ampicillin [22].

In another study conducted in Delhi, a review of the patient's microbiology laboratory records of bacterial isolates from pus and body fluid samples from January to December 2012 was performed.The laboratory records of body fluid and pus samples from 5593 patients were analyzed. The age participants in the study group group ranged from 1 month to 72 years, with a mean age of 25 years. Among the study participants, 63.6% were men, while 36.4% were women. *Staphylococcus aureus* was the most frequent isolate (22.3%) followed by *Pseudomonas spp*. (22.0%), and *Klebsiella spp*. (18%). Gram-positive isolates were more susceptible to levofloxacin, vancomycin, linezolid, and teicoplanin. Most gram-negative isolates were susceptible to imipenem, meropenem, piperacillin, tazobactam, polymyxin B, and colistin. Only 7.3% of the isolates were susceptible to all antibiotics tested, while 81.8% of the isolates were resistant to two or more classes of antibiotics [23].

Similary, a study conducted in India from January 2017 to December 2017 involving the recruiting of 1800 study samples showed that 15% (n=270/1800) of body fluids exhibited bacterial growth. Most bacteria were isolated from cerebrospinal fluid (CSF) 56.4% and pleural fluid 34.3%. The most frequent bacterial isolate was *K. pneumoniae* 66.7% followed by alpha *Streptococcus* 30% (n=8/54) and *E. coli*. Gram-negative and gram-positive bacteria exhibited the greatest resistance to gentamycin (76%) and erythromycin (59%), respectively. The MDR 62.9% [24].

Similarly, in a study conducted in Ataturk city, clinical samples of sterile body fluid (cerebrospinal, pleural, peritoneal, pericardial, and synovial fluids) were obtained from January 2017 to December 2020. The identification bacterial and antibiotic susceptibility was performed using conventional and automated systems. A total of 221 (9.74%) organisms were detected among the 2269 samples. Particularly common gram-negative bacterial agents were listed at the top of the list (*Escherichia*

coli, *Pseudomonas spp. Klebsiella spp*., *Acinetobacter baumannii*, *Acinetobacter calcoaceticus* complex). Staphylococcus aureus was the most common Gram-positive strain, followed by enterococci. Most *A. baumannii* isolates were resistant to multiple drugs, and more than 20% of Pseudomonas spp. were resistant to ceftazidime, cefepime, and piperacillin-tazobactam. All the enterococci were susceptible to vancomycin and a strain of S. *aureus* was resistant to methicillin. All isolates of the *Mycobacterium tuberculosis* complex were found to be susceptible to first-line antituberculosis drugs [25].

A study conducted in Arba Minch from January 1 to November 30, 2020, showed that 152 hospitalized patients were enrolled in this study. The general incidence of bacterial pleural infection was 27.6%. The predominant bacterial isolates were *Staphylococcus aureus* at 34.9%, followed by *Escherichia coli* at 11.6%. Multidrug-resistant (MDR) isolates represent approximately 48.8% of all isolates, and gram-positive bacterial isolates represented 71.4%. Methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (CONS) were found in 26.7% and 33.3% of the isolated *Staphylococcus aureus* (MRSA) strains, respectively. An isolate of *Enterococcus spp*. was found to be resistant to vancomycin. All isolated *Streptococcus pneumoniae* and *Streptococcus pyogenes* strains were susceptible to penicillin, erythromycin, cotrimoxazole, and vancomycin [26].

In another study conducted in Ethiopia, Ayder Comprehensive Specialized Hospital, Mekelle, northern Ethiopia, a total of 218 patients were investigated, of which 146 (67%) were men. The age of the study subjects ranged from 2 days to 80 years, with 96(44%) 15 years and older age group. The total bacterial number of bacterial infections was 44 (20.2 %) of which gram-positive bacteria were prevalent, with 23 (52.3%) and gram-negative bacteria were prevalent with 21 (47.7%). The predominant bacteria isolated were *S. pneumonia*, followed by *K. pneumoniae*, *S. aureus*, and *E coli*. Multidrug resistance was observed in 12 (100%) isolated gram-positive bacteria and 6 (75%) isolated gram-negative bacteria [27].

A study carried out in Bahir Dar from 01 April to 30 July 2018 revealed 176 study participants, 112 (63.6%) of whom were men and 70 (39.8%) of whom were infants. The mean age of the study participants was 14.3 years. Eight (4.5%) CSF samples were found to be positive for bacteriological culture. Of these, five (62.5%) were gram-negative, including three with *E. coli* and a case of *K. pneumoniae* and one with *P. aeruginosa*. The remaining three isolates were *S.*

aureus. In this study, the overall rate of multidrug resistance (MDR) was 75%. Gentamicin and ciprofloxacin were found to be effective against *S. aureus*. Similarly, gram-negative isolates were found to be sensitive to ceftazidime and ceftriaxone [28].

Another cross-sectional study conducted at Dilla University Referral Hospital from February 1, 2019, to March 30, 2020, revealed causative bacteria in 38 (13.2%) out of 287 cultured CSF samples. Among the culture positive patients, the most frequent isolate was *Streptococcus pneumoniae* 13 (34.2%) followed by *Staphylococcus aureus* 7 (18.4%), *Neisseria meningitidis* 6 (16%), and *Escherichia coli* 6 (16%). *Haemophilus influenzae* b was isolated from 4 (10.5%) children with meningitis. The other cause of meningitis was *Streptococcus agalactiae* (approximately 10.5%). *Cryptococcus neoformans* was detected in 4 (1.9%) patients with meningitis. Approximately 42.1% (16/38) of the bacterial isolates were multidrug resistant. Approximately 38.5% of *S. pneumoniae* strains were multidrug-resistant, while approximately 33.3% of *N. meningitis* strains, 50% of *H. influenzae* strains, 57.1% of *S. aureus* strains, and 40% of *E. coli* strains exhibited multidrug resistance. One study revealed a high incidence of bacterial meningitis and a high rate of drug resistance [29].

Another cross-sectional study conducted in Ethiopia from April 1, 2014, to August 30, 2018, showed that a total of 654 body fluids of patients were analyzed for isolation, identification, and antimicrobial susceptibility tests, of which 75 (11.6%) were culture-positive. Forty-nine (7.5%) culture-positive samples were obtained from female patients, while 26 (4%) were from male patients. Among the total positive culture samples, 56 (74.6%) and 19 (25.4%) were gram-negative and gram-positive, respectively. The predominant isolated bacterial species were *Escherichia coli*, 13 (17.3%), and *Acinetobacter species*. 12 (16%), *Klebsiella pneumoniae* 10 (13.3%), *S aureus* 10 (10.3%), *Neisseria meningitidis* 9 (9.3%), and *Pseudomonas species* 5 (6.3%) [30].

3. OBJECTIVES

3.1. General objective

- Determine bacterial isolates and antibiotic susceptibility patterns from sterile body fluids at TASH.

3.2. Specific objectives

- To isolate and identify aerobic bacterial pathogens from sterile body fluid samples in TASH.
- Determine the antimicrobial susceptibility pattern of bacterial isolates from sterile body fluid samples in TASH.
- Determine the prevalence of extended-spectrum beta-lactamases (ESBLs) and Carbapenem-resistant *Enterobacteriacea* (CRE).
- Determine the underlying associated factors of sterile body fluid infection.

4. MATERIALS AND METHODS

4.1. Study setting

The study was carried out at the Tikur Anbessa Specialized Hospital, which is the largest general public hospital in the country. The faculty is the oldest and largest among the health training institutions in the country and is staffed with the most senior specialists. The hospital provides tertiary-level referral treatment and is open 24 hours for emergency services. TASH has more than 800 beds and offers diagnosis and treatment for approximately 470,000 to 500,000 patients a year. The emergency department sees approximately 80,000 patients a year.

4.2. Study design and period

A hospital-based prospective cross-sectional study was conducted at the TASH Laboratory. The study was carried out from May 1 to December 30, 2023.

4.3. Population

4.3.1. Source population

All patients who visited TASH and were suspected of having sterile body fluid infections.

4.3.2. Study population

Patients suspected of having sterile body fluid infections during the study period and those who provided consent were included in this study.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

- All patients suspected of having sterile body fluid infection at Tikur Anbessa Specialized Hospital were included, regardless of age or sex.

4.4.2. Exclusion criteria

- Patients with a history of antibiotic therapy in the previous two weeks and blood samples were excluded from the study.

4.5. Study variables

4.5.1. Dependent variables

- Magnitude of bacterial isolates from sterile body fluid
- Antibiotic susceptibility pattern of isolated bacterial species

4.5.2. Independent variables

- Sociodemographic factors: age, sex, outpatient status, inpatient status, educational status, residence, monthly income, marital status, occupation, etc.

4.6. Sample size determination and sampling method

4.6.1. Sample size determination

The sample size was determined using a single population proportion formula considering a 14.1% proportion (p) of bacterial infection as reported in an earlier study [31], with a confidence interval (CI) of 95% and a marginal error of 5% (d).

$$n = \frac{(Z\propto_{/2})^2 \times pq}{d^2}$$

Where:

- n = sample size
- Z $_{(a/2)}$ = Z-score at a 95% confidence interval = 1.96
- p = 0.141 (proportion of bacterial infection)
- q = 1 - p
- d = 0.05 (5% error margin)

Therefore, n becomes:

$$n = \frac{(1.96)^2 \times 0.141 * 0.859}{(0.05)^2} = 186$$

Assuming a 10% non-response rate: **(0.1) * (186) ≈ 19**

186 + 19 = 205

4.6.2. Sampling method

Participants were recruited using a simple random sampling technique after completing the physician's office visit session before specimen collection. Subsequently, patients were approached and invited to participate in the study until the required sample size was achieved.

4.7. Data collection procedure

4.7.1. Data and sample collection

Data were collected after informed consent was obtained from each study participant. Information on sociodemographic and clinical characteristics, such as patient setting (inpatient/outpatient),

type of ward, comorbidities, instrumentation, hospital admission, etc. was recorded through face-to-face interviews using structured and pre-tested questionnaires. Body fluid samples were drawn by experienced physicians using proper aseptic precautions with a sterile syringe and needle and sent to the Department of Microbiology within 2 hours of collection at room temperature.

4.8. Sample Processing, Culture and Antimicrobial susceptibility testing

4.8.1. Sample processing

Microbiological analysis was performed using universal safety precautions and standard laboratory procedures to isolate and identify bacterial infection from sterile body fluids. Samples greater than 1 ml were centrifuged at a rate of 2500 revolutions per minute (rpm) for 10 minutes to concentrate any organisms, after which the sediment was seeded for culture. However, samples less than 1 ml and purulent samples were inoculated directly into the culture medium.

4.8.2. Culture

The collected samples were cultured on blood agar for gram-positive bacteria, MacConkey agar for gram-negative bacteria, and chocolate agar for fastidious organisms (Oxoid, UK). Blood agar and MacConkey agar were inoculated and aerobically incubated at 37°C for 48 hours, and chocolate agar was microaerobically incubated (in a candle jar to provide a concentration of 5-10% CO_2 to provide a chance for the growth of fastidious microaerophilic bacteria). Culture plates were initially examined after 24 h and finally, after 48 h to determine the appearance of growth. Cultures were declared sterile if there was no growth in the plates after 48 hours of incubation according to the 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines. Bacterial pathogens grown in culture media were identified by Gram staining and colony morphology, and further by biochemical tests. The morphological features of the colonies, such as size, shape, pigmentation, and opacity, were noted and recorded. Gram staining was performed to distinguish gram-positive bacteria from gram-negative bacteria by their shape and arrangement. The biochemical characteristics of Gram-positive bacteria were determined by performing the catalase test, coagulase test, bile esculin test, optochin sensitivity test, novobiocin sensitivity test, bacitracin sensitivity test, pyrrolidonyl arylamidase (PYR) test, mannitol test, DNase test and hemolytic activity on blood agar. However, indole production test, citrate utilization test, lactose and glucose fermentation test, urease test, oxidase test, lysine decarboxylase (LDC) test, methyl red/voges

proskauer test (MR / VP), motility test, and hydrogen sulfide gas production test were performed for the identification of gram-negative bacteria.

Enterobacteriaceae were classified to species level using triple sugar iron, indole, citrate, urea, lysine decarboxylase, and motility. After identification, each *Enterobacteriaceae* strain was subjected to detection of ESBL and carbapenemase according to the CLSI 2020 guidelines.

4.8.3. Antimicrobial susceptibility testing

The susceptibility of isolates to antimicrobial agents of different classes was evaluated using the Kirby-Bauer disk diffusion method according to a commercially prepared antibiotic disc of known concentration according to the CLSI 2020 guidelines on Mueller-Hinton agar standard media. Using a sterile wire loop, 3-5 well-isolated pure colonies from an overnight culture were picked and emulsified in 5 ml of normal saline (0.85% NaCl) to make an inoculum suspension with a turbidity equivalent to a 0.5 McFarland standard. The inoculum suspension was evenly spread across the entire surface of Mueller-Hinton agar plates (HiMedia, Mumbai, India) by swabbing in three directions with a sterile cotton swab. The appropriate disks impregnated with antimicrobial agents were then applied within 15 minutes after inoculation. The agar plates were inverted (to prevent the accumulation of moisture on the agar surface) and the disks were secured on the agar surface. Finally, plates were incubated within 15 minutes after disk application at 37°C for 18-24 hours. Enterococcal isolates were inoculated on Muller-Hinton agar supplemented with 5% defibrinated sheep blood. After incubation, the diameter of the inhibition zone was measured to the nearest millimeter and isolates were classified as sensitive, intermediate, and resistant according to the CLSI guidelines [32]. The susceptibility of *Staphylococcus aureus* to oxacillin (methicillin) to *Staphylococcus aureus* was interpreted using 30 µg of cefoxitin as a surrogate test for Methicillin-resistant *Staphylococcus aureus* (MRSA) [32].

According to the CLSI guideline, the bacterial isolates were tested for Amikacin (30µg), Amoxycillin (2µg), Amoxycillin/clavulanic acid (3µg), Ampicillin (10µg), Cefepime (30µg), Cefotaxime (30µg), Cefotaxime-clavulanate (30 µg/10 µg), Cefoxitin (30µg), Ceftazidime (30µg), Ceftazidime-clavulanate (30 µg/10 µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Clindamycin (2µg), Erythromycin (15µg), Gentamicin (10µg), Meropenem (10µg), Oxacillin (5µg), Penicillin G (10µg), Trimethoprim/sulphamethoxazole (25µg) and Vancomycin (30µg) (Oxoid, UK). Among gram-negative bacteria, extended-spectrum beta-lactamase (ESBL) was detected by phenotypic methods using cephalosporin/ clavulanate combination discs (30 µg of cefotaxime and

30 μg of ceftazidime with and without 10 μg of clavulanate. The modified Hodge test was used for the phenotypic detection of carbapenemase production.

4.8.3. ESBL detection

The initial screening of *Enterobacteriaceae* for ESBL was performed based on the diameters of the zones of inhibition produced by ceftazidime (30 μg), ceftriaxone (30 μg), and cefotaxime (30 μg) according to the CLSI screening criteria. The breakpoints indicative of suspicion for ESBL production were ≤22 mm for ceftazidime, ≤25 mm for ceftriaxone, and ≤27 mm for cefotaxime. The combined disk method was used as a confirmatory phenotypic method for the detection of ESBL according to the CLSI. Ceftazidime (30 μg) and cefotaxime (30 μg) disks alone and in combination with clavulanic acid (30 μg/10 μg) were used for phenotypic confirmation of the presence of ESBLs. A ≥5 mm increase in zone diameter was interpreted for cephalosporin disks or their respective cephalosporin/clavulanate disks as producers of ESBL.

4.8.4. Carbapenemase detection

Carbapenem (meropenem) resistant or carbapenem-intermediate *Enterobacteriaceae* were checked for the production of carbapenemase using a modified Hodge test (MHT; cloverleaf test) according to the CLSI. When the *Escherichia coli* American Type Culture Collection (ATCC) 25922 grew around the streaked organism and showed indentation, the isolate was recorded as a carbapenemase producer, while no growth of the ATCC *E. coli* 25922 along the streaked organism indicated a negative test and the isolate was not a carbapenemase producer.

4.9. Data quality assurance

The quality of the data was ensured by properly designing the tool, and the questionnaire was pretested in 5% of randomly selected patients at St. Paul's Hospital Millennium Medical College before actual data collection, and some minor modifications were made accordingly. The completeness and consistency of the collected data were checked daily.

To maintain data quality, standard operating procedures were strictly followed, verifying that the media and AST discs met the expiration date and quality control parameters according to the CLSI guidelines. Visual inspections were performed for cracks in medium or plastic Petri dishes, thin or uneven fill, hemolysis, unusual medium color, evidence of freezing, desiccation, and contamination according to standard guidelines [30]. As an internal quality control, the

uninoculated medium (negative control) was incubated and must remain clear to check sterility. Reference strains from the American Type Culture Collection (ATCC), such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and/or *Staphylococcus aureus* (ATCC 25923), were used as positive controls (for culture and monitoring of reagent quality). Known strains of the Oneworld Accuracy External Quality Assessment (EQA) scheme based in Vancouver, Canada: *E. coli* and *S. aureus* were also used for drug susceptibility tests. The inoculum density of the bacterial suspension was adjusted to the 0.5 McFarland standard to perform antibiotic susceptibility tests.

4.10. Data analysis and interpretation

Data entry and analysis were performed using the Statistical Package for Social Sciences (SPSS) version 26. Descriptive statistics, including frequencies, percentages, and medians, were used to summarize baseline sociodemographic data from patients and assess the distribution of responses. Logistic regression analysis was performed to identify any associations between predictors and outcome variables. Factors with a P-value < 0.25 in the bivariate analysis were exported to the multivariate logistic regression analysis. Multivariate analysis was performed using logistic regression to control for the effect of potential confounding variables. Consequently, statistically significant associations were determined based on the adjusted odds ratio (AOR) with its 95% CI and the value of P <0.05.

4.11. Ethical clearance

Ethical approval was obtained from the Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences of the College of Health Sciences of Addis Ababa University (DRERC/722/23/MLS). A permission letter was also issued from TASH. Furthermore, prior to data collection, each patient was informed about the purpose of the study, the selection procedures, the need to collect small amount of body fluid samples and access their medical records, and the assurance of confidentiality by not using names to improve anonymity. Subsequently, the patient's willingness to participate in the study was confirmed by obtaining written consent.

5. RESULTS

5.1. Sociodemographic characteristics of the study participants

A total of 205 study participants were recruited and enrolled in this study. Among them, men comprised the majority of respondents, 117 (57.1%). The median age of the participants was 25 years (IQR 7-48). More than half of the respondents (54.6%) resided in rural areas. Almost a quarter of the participants (24.4%) attended their secondary education and 46.3% were employed. Approximately half of the patients earned a monthly salary of less than 1500 ETB a month. (Table 1).

Table 1. Sociodemographic characteristics of the study participants in TASH, Addis Ababa, Ethiopia, 2023.

Variables	Category	Frequency (%)
Age, median (IQR)		25 years (7-48)
Age group (yrs.)	≤20	93 (45.4)
	21 – 40	46 (22.4)
	41 – 60	49 (23.9)
	≥ 61	17 (8.3)
Gender	Male	117 (57.1)
	Female	88 (42.9)
Marital status	Under age*	83 (40.5)
	Single	31 (15.1)
	Married	84 (41)
	Divorced	4 (2)
	Widowed	3 (1.5)
Residence	Urban	93 (45.4)
	Rural	112 (54.6)
Educational level	Pre-school	66 (32.2)
	No formal education	14 (6.8)
	Primary ed. (grade 1 - 8)	38 (18.5)
	Secondary ed. (grade 9 - 12)	50 (24.4)
	College and above	37 (18)
Occupation	Unemployed	51 (24.9)
	Student	46 (22.4)
	Gov't/Private Employee	78 (38)
	Self-employed	17 (8.3)
	Homemaker	5 (2.4)
	Retired/Pension	8 (3.9)
Monthly income (ETB)	< 1500	102 (49.8)
	1500 - 5000	62 (30.2)
	> 5000	41 (20)

IQR, interquartile range; **ETB**, Ethiopian birr.

*Non-candidates for marital status.

5.2. Clinical and sample-related characteristics of the study participants

A total of 205 body fluid samples were collected and investigated. Approximately half (48.3%) of the processed samples were CSF, followed by pleural fluid (31.2%), peritoneal fluid (15.6%), synovial fluid (3.4%) and pericardial fluid (1.5%). Most of the samples collected (34.6%) were from patients in the outpatient department (OPD). Among the 40 (19.5%) positive patients, isolates were identified predominantly from CSF samples (58%), from patients admitted to the hospital for 7 days or more (80%), and from patients with a type of instrumentation or medical device (80%) (Table 2).

Table 2. Clinical and sample-related characteristics of the study participants in TASH, Addis Ababa, Ethiopia, 2023.

Variables	**Category**	**Culture result**		**Total** N (%)
		Growth N (%)	**No Growth** N (%)	
Patient setting	Inpatient	20 (31.7)	43 (68.3)	63 (30.7)
	Outpatient	20 (14.1)	122 (85.9)	142 (69.3)
	Total	40 (19.5)	165 (80.5)	205 (100)
Ward type	ICU	11 (37.9)	18 (62.1)	29 (14.1)
	Pediatrics	10 (28.6)	25 (71.4)	35 (17.1)
	OPD	4 (5.6)	67 (94.4)	71 (34.6)
	Surgical	3 (16.7)	15 (83.3)	18 (8.8)
	Medical	8 (38.1)	13 (61.9)	21 (10.2)
	Others*	4 (12.9)	27 (87.1)	31 (15.1)
	Total	40 (19.5)	165 (80.5)	205 (100)
Hospital admission	≤7 days admitted	8 (5.2)	146 (94.8)	154 (75.1)
	>7 days admitted	32 (62.7)	19 (37.3)	51 (24.9)
	Total	40 (19.5)	165 (80.5)	205 (100)
Comorbidity	Present	22 (37.3)	37 (62.7)	59 (28.8)
	Absent	18 (12.3)	128 (87.7)	146 (71.2)
	Total	40 (19.5)	165 (80.5)	205 (100)
Type of comorbidity	Hypertension	8 (33.3)	16 (66.7)	24 (11.7)
	Diabetes	3 (25)	9 (75)	12 (5.9)
	HIV	3 (60)	2 (40)	5 (2.4)
	Malignancy	3 (50)	3 (50)	6 (2.9)
	Others**	5 (41.7)	7 (58.3)	12 (5.9)
	None	18 (12.3)	128 (87.7)	146 (71.2)

	Total	40 (19.5)	165 (80.5)	205 (100)
Use of instrumentation (Indwelling medical devices)	Yes	32 (59.3)	22 (40.7)	54 (26.3)
	No	8 (5.3)	143 (94.7)	151 (73.7)
	Total	40 (19.5)	165 (80.5)	205 (100)
Instrumentation used	Intravenous line	25 (78.1)	7 (21.9)	32 (15.6)
	Urinary catheter	2 (20)	8 (80)	10 (4.9)
	Nasogastric tube	1 (50)	1 (50)	2 (1)
	Endotracheal tube	2 (50)	2 (50)	4 (2)
	Central venous catheter	1 (20)	4 (80)	5 (2.4)
	Hemodialysis catheter	1 (50)	1 (50)	2 (1)
	None	8 (5.3)	142 (94.7)	150 (73.2)
	Total	40 (19.5)	165 (80.5)	205 (100)
Specimen type	CSF	23 (23.2)	76 (76.8)	99 (48.3)
	Pleural fluid	11 (17.2)	53 (82.8)	64 (31.2)
	Peritoneal fluid	4 (12.5)	28 (87.5)	32 (15.6)
	Synovial fluid	1 (14.3)	6 (85.7)	7 (3.4)
	Pericardial fluid	1 (33.3)	2 (66.7)	3 (1.5)
	Total	40 (19.5)	165 (80.5)	205 (100)
Specimen appearance	Turbid	11 (36.7)	19 (63.3)	30 (14.6)
	Clear	15 (14.9)	86 (85.1)	101 (49.3)
	Clear-straw	13 (18.6)	57 (81.4)	70 (34.1)
	Trauma	1 (25)	3 (75)	4 (2)
	Total	40 (19.5)	165 (80.5)	205 (100)

*Emergency and Orthopedics

** Renal & congestive heart failure

5.3. Prevalence of Bacterial Isolates

Of the 205 samples analyzed, 40 were culture-positive. which comprised an overall prevalence of 19.5%. Gram-negative bacteria accounted for a greater proportion of positive cases (29/40, 72.5%). Among these, *K. pneumoniae* (10/29, 34.4%) and *E. coli* (7/29, 24.1%) were the most commonly isolated major gram-negative bacteria, while the remaining 11 cases were filled with gram-positive bacteria, with *S. aureus* and *Enterococcus* spp. being the main bacteria (Figure 1).

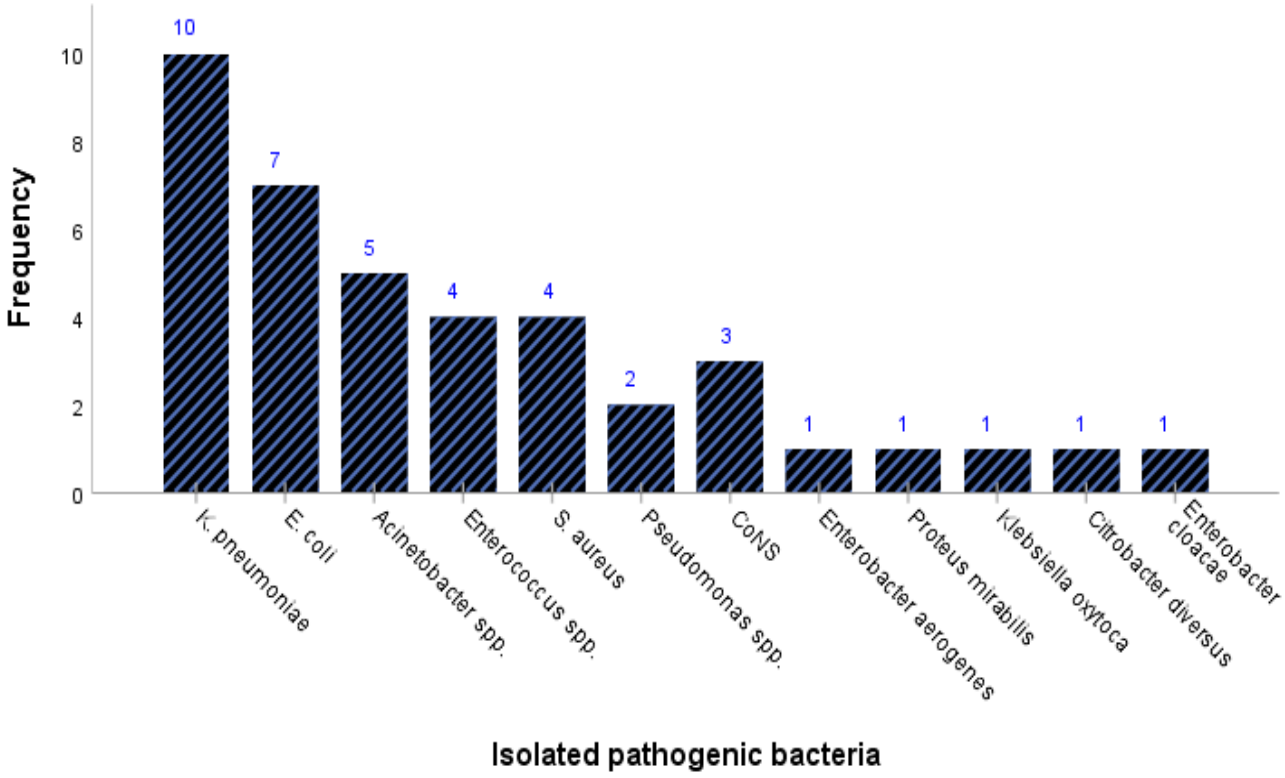

Figure 1. Bacterial isolates from patients diagnosed with sterile body fluid infection in TASH, Addis Ababa, Ethiopia, 2023.

5.4. Factors associated with positive culture

According to the bivariate logistic regression: age, residence, patient setting, comorbidities, and sample characteristics were significantly associated with p-value <0.25; these variables were selected for further analysis and exported to the multivariate logistic regression model. The results of the multivariate logistic regression analysis showed that rural residence, inpatient status, comorbidities, and turbid appearance of the samples were significantly associated with culture positivity rates at p-values <0.05 after adjustment for confounders.

Those living in rural areas (AOR: 3.86, 95% CI: 1.58-9.42, P = 0.003) were found to be more likely to be infected than their urban counterparts. In the patient setting, inpatients tended to be (AOR: 2.65, 95% CI: 1.17-5.99, P = 0.019) more vulnerable than outpatients. Patients with comorbidities were found to have a much greater chance of having a culture-positive result (AOR: 5.46, 95% CI: 2.22-13.40, P = 0.001) than those without comorbidities. The chance of being culture positive was 3.37 times higher (AOR: 3.37, 95% CI: 1.17-9.70, P = 0.024) among patients who had a turbid sample than among their counterparts (Table 3).

Table 3. Bivariate and multivariate analysis of factors associated with bacterial infection of sterile body fluids in TASH, Addis Ababa, Ethiopia, 2023.

Variables	Category	Culture Growth N (%)		Bivariate Analysis		Multivariate Analysis	
		Yes n = 40	**No** n = 165	COR (95% CI)	P-value	AOR (95% CI)	P-value
Age group (yrs.)	≤20	19 (20.4)	74 (79.6)	1.71 (0.63-4.63)	0.290	1.40 (0.46-4.27)	0.548
	41-60	13 (26.5)	36 (73.5)	2.40 (0.82-6.99)	0.107*	1.16 (0.33-4.02)	0.805
	≥61	2 (11.8)	15 (88.2)	0.88 (0.16-4.89)	0.892	0.33 (0.04-2.26)	0.261
	21-40 ®	6 (13)	40 (87)				
Gender	Male	20 (17.1)	97 (82.9)	0.70 (0.35-1.40)	0.315		
	Female ®	20 (22.7)	68 (77.3)				
Residence	Rural	31 (27.7)	81 (72.3)	3.57 (1.60-7.96)	0.002*	3.86 (1.58-9.42)	**0.003***
	Urban ®	9 (9.7)	84 (90.3)				
Patient setting	Inpatient	20 (31.7)	43 (68.3)	2.83 (1.39-5.77)	0.004*	2.65 (1.17-5.99)	**0.019***
	Outpatient ®	20 (14.1)	122 (85.9)				
Comorbidity	Present	21 (36.8)	36 (63.2)	3.96 (1.92-8.15)	0.001*	5.46 (2.22-13.40)	**0.001***
	Absent ®	19 (12.8)	129 (87.2)				
Specimen appearance	Turbid	11 (36.7)	19 (63.3)	3.31 (1.31-8.35)	0.011*	3.37 (1.17-9.70)	**0.024***
	Clear-straw	13 (18.6)	57 (81.4)	1.30 (0.57-2.95)	0.519	1.48 (0.58-3.73)	0.405
	Trauma	1 (25)	3 (75)	1.91 (0.18-19.6)	0.586	3.54 (0.26-47.8)	0.340
	Clear ®	15 (14.9)	86 (85.1)				

*Statistically significant

COR, crude odds ratio; **AOR**, adjusted odds ratio; **CI**, confidence interval; ®, reference category

5.5. Antimicrobial Susceptibility Testing

A drug sensitivity test was performed for all 40 bacterial isolates. Isolates have shown various resistance and susceptibility patterns to the different antibiotics tested. Among the antimicrobial profiles of gram-negative bacteria, amikacin (86.2%) and meropenem (76%) were the most effective drugs against gram-negative isolates. Furthermore, they showed good sensitivity to cefepime (62.1%) and gentamicin (62%). However, weak performance and resistance to penicillins, mainly ampicillin (79.3%), and third-generation cephalosporins such as ceftriaxone (69%) and ceftazidime (58.6%) were observed among gram-negative isolates.

Among the antibiograms of gram-positive bacteria, which comprised (n=11/40) of the total isolated bacteria, they were resistant to penicillin g (9/11, 81.8%), oxacillin (8/11, 72.7%), ampicillin (7/11, 63.6%) and trimethoprim-sulfamethoxazole (7/11, 63.6%). Moreover, ciprofloxacin, gentamicin, and amoxicillin-clavulanic acid exhibited high to moderate activity (72.7%, 57.1% & 54.5%), respectively, against gram-positive isolates. *S. aureus* isolates (3/4, 75%) were resistant to oxacillin and cefoxitin, indicating the presence of methicillin-resistant *S. aureus* (MRSA). The resistance of Staphylococcus spp. to methicillin was tested with a cefoxitin disc (30 μg) as described by the Clinical and Laboratory Standard Institute. (Diffusion of the cefoxitin disc ≤21 mm for *S. aureus*). Positive culture reports were analyzed and percentages and proportions were calculated using appropriate statistical analysis. Furthermore, isolates of *Enterococcus* species were susceptible to vancomycin; however, they were resistant to penicillin g, ampicillin, and trimethoprim-sulfamethoxazole. The antibiotic susceptibility patterns of the recovered gram-positive and ram-negative isolates are shown in Tables 4 and 5, respectively.

Table 4. Antimicrobial susceptibility patterns of gram-negative bacteria isolated from sterile body fluid samples in TASH, Addis Ababa, Ethiopia, 2023.

Antimicrobial Classes													
N = 29		Penicillins			Cephalosporins				Carbapenem	Aminoglycosides		FLQ	Sulfonamide
Isolated Organism	Pattern	AMC	AMP	AMX	CTX	CRO	CAZ	FEP	MEM	GEN	AMK	CIP	SXT
K. pneumoniae (10)	S I R	6 1 3	1 - 9	6 - 4	3 2 5	4 - 6	5 - 5	5 1 4	7 1 2	6 1 3	9 - 1	4 - 6	4 - 6
E. coli (7)	S I R	5 - 2	1 1 5	3 1 3	4 - 3	1 - 6	2 - 5	5 - 2	6 - 1	4 - 3	6 - 1	2 1 4	2 - 5
Acinetobacter spp. (5)	S I R	3 1 1	2 - 3	2 - 3	3 - 2	1 - 4	- 1 4	3 - 2	4 - 1	3 - 2	4 1 -	2 - 3	2 1 2
Pseudomonas spp. (2)	S I R	- 1 1	1 - 1	1 - 1	1 - 1	1 - 1	1 - 1	1 - 1	1 1 -	1 1 -	2 - -	1 - 1	- 1 1
P. mirabilis (1)	S I R	- 1 -	- - 1	1 - -	1 - -	1 - -	1 - -	1 - -	1 - -	1 - -	1 - -	1 - -	- 1 -
K. oxytoca (1)	S I R	- - 1	- - 1	- - 1	- - 1	- - 1	- - 1	- - 1	- - 1	1 - -	1 - -	1 - -	1 - -
Enterobacter aerogenes (1)	S I R	- - 1	- - 1	1 - -	- 1 -	- - 1	1 - -	1 - -	1 - -	1 - -	1 - -	- - 1	- - 1
Citrobacter diversus (1)	S I R	1 - -	- - 1	1 - -	- - 1	1 - -	- - 1	1 - -	1 - -	1 - -	- - 1	1 - -	- - 1
Enterobacter cloacae (1)	S I R	- - 1	- - 1	- - 1	- - 1	- - 1	1 - -	1 - -	1 - -	- - 1	1 - -	- 1 -	1 - -
N (%)	S I R	15(51.7) 4(13.8) 10(34.4)	5(17.2) 1(3.4) 23 (79.3)	15(51.7) 1(3.4) 13(44.8)	12(41.4) 3(10.3) 14(48.3)	9(31) - 20(69)	11(37.9) 1(3.4) 17(58.6)	18(62.1) 1(3.4) 10(34.4)	22(76) 2(6.9) 6(20.7)	18(62) 2(6.9) 9(31)	25(86.2) 1(3.4) 3(10.3)	12(41.4) 2(6.9) 15(51.7)	10(34.4) 3(10.3) 16(55.1

Abbreviations: FLQ, fluoroquinolone; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; AMX, amoxicillin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; MEM, meropenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; S, sensitive; I, intermediate; R, resistant.

Table 5. Antimicrobial susceptibility patterns of gram-positive bacteria isolated from sterile body fluid samples in TASH, Addis Ababa, Ethiopia, 2023.

Antimicrobial Classes													
N = 11		Penicillins				Cephalosporins		Macrolide	Glycopeptide	Lincosamide	AMG	FLQ	Sulfonamide
Isolated Organism	Pattern	PENG	OXA	AMC	AMP	FOX	CRO	ERY	Vancomycin	Clindamycin	Gentamicin	CIP	SXT
S. aureus (4)	S I R	- - 4	1 - 3	2 - 2	- 1 3	- 1 3	2 - 2	2 - 2	NA NA NA	2 - 2	3 - 1	4 - -	2 - 2
Enterococcus spp. (4)	S I R	1 - 3	2 - 2	2 1 1	1 - 3	NA NA NA	1 - 3	1 1 2	3 - 1	1 2 1	NA NA NA	2 2 -	- 1 3
CoNS (3)	S I R	1 - 2	- - 3	2 - 1	1 1 1	- 1 2	1 - 2	1 - 2	NA NA NA	2 - 1	1 - 2	2 - 1	1 - 2
N (%)	S I R	2(18) - 9(81.8)	3(27.2) - 8(72.7)	6(54.5) 1(9) 4(36.3)	2(18.1) 2(18.1) 7(63.6)	- 2(29) 5(71.4)	4(36.3) - 7(63.6)	4(36.3) 1(9) 6(54.5)	3(75) - 1(25)	5(45.4) 2(18.1) 4(36.3)	4(57.1) - 3(42.9)	8(72.7) 2(18.1) 1(9)	3(42.9) 1(9) 7(63.6)

Abbreviations: AMG, aminoglycoside; FLQ, fluoroquinolone; PENG, penicillin G; OXA, oxacillin; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; FOX, cefoxitin; CRO, ceftriaxone; ERY, erythromycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CONS, *coagulase-negative staphylococci*; S, sensitive; I, intermediate; R, resistant; NA, not applicable.

5.6. Bacterial resistance and clinical profiles of patients

Drug resistance was detected in 95% (38/40) of the isolated bacteria. MDR (resistance to at least one agent in ≥3 classes of antimicrobial agents) was identified in 67.5% (27/40) of the isolates. Compared with that in gram-negative isolates, the multidrug resistance pattern in gram-positive isolates was not as common. Among the MDR isolates, 23 (85.2%) were gram-negative bacteria, while only 4 (14.8%) were gram-positive. Furthermore, 22.5% (9/40) of the isolates were possibly extensively drug resistant, while 10% (4/40) were possibly pandrug resistant. The general results of the AST indicated that penicillin, oxacillin, ampicillin, ceftriaxone, ceftazidime, and trimethoprim-sulfamethoxazole had relatively high resistance rates. More than half of the resistant bacteria in all resistance categories (DR, MDR, XDR and PDR) were isolated from patients with comorbidities, hospital admission for 7 days or more, and indwelling devices (instrumentation) during treatment. The proportions of resistant bacteria that were isolated from patients with

characteristics relevant to antimicrobial resistance categories (presence of comorbidities, hospital admission, and instrumentation) are listed in Table 6 & 7.

Table 6. Multidrug resistance patterns for bacterial isolates from body fluids samples at TASH

Bacterial isolates	Level of antibiotic resistance						Total MDR isolates (≥R3) n (%)
	R0	R1	R2	R3	R4	R>4	
Gram Negative							
K. pneumoniae (10)	0	0	0	0	3	7	10 (100)
E. coli (7)	0	0	1	0	2	4	6 (85.7)
Acinetobacter spp. (5)	0	1	1	0	0	3	3 (60)
Pseudomonas spp. (2)	0	0	0	2	0	0	2 (100)
P. mirabilis (1)	1	0	0	0	0	0	0 (0)
K. oxytoca (1)	0	0	0	1	0	0	1 (100)
Enterobacter aerogenes (1)	0	0	0	1	0	0	1 (100)
Citrobacter diversus (1)	0	0	1	0	0	0	0 (0)
Enterobacter cloacae (1)	0	0	1	0	0	0	0 (0)
Total (29)	1	1	4	4	5	14	23 (79.3)
Gram Positive							
S. aureus (4)	0	0	2	0	0	2	2 (50)
Enterococcus spp. (4)	1	2	0	1	0	0	1 (25)
CoNS (3)	0	0	3	1	0	0	2 (50)
Total (11)	1	2	5	2	0	0	4 (36.3)

Note: R0: resistance to no antibiotics, R1-R4: resistant to 1, 2, 3, and 4 classes of antibiotics, respectively, ≥R3: resistance to 3 or more antibiotics from different classes (nonsusceptibility to at least one agent in three different antimicrobial classes). MDR: multidrug-resistant.

Table 7. Association of isolate resistance profiles with clinical characteristics of the patients in TASH, Addis Ababa, Ethiopia, 2023.

Isolate characteristic	Resistance profile	Total N (%)	Bacteria type		Patient characteristic							
					Sex		Comorbidity		Hospital admission		Instrumentation	
			G+ve N (%)	G-ve N (%)	Male N (%)	Female N (%)	Present N (%)	Absent N (%)	>7 days N (%)	≤7 days N (%)	Yes N (%)	No N (%)
	Drug-resistant (DR)	38(95)	10(26.3)	28(73.7)	17(45.9)	20(54.1)	21(56.8)	16(43.2)	29(78.4)	8(21.6)	29(78.4)	8(21.6)
	Multidrug-resistant (MDR)	27(67.5)	4(14.8)	23(85.2)	11(40.7)	16(59.3)	16(59.3)	11(40.7)	20(74.1)	7(25.9)	22(81.5)	5(18.5)
	Extensively drug-resistant (XDR)	9(22.5)	1(11.1)	8(88.9)	5(55.6)	4(44.4)	6(66.7)	3(33.3)	7(77.8)	2(22.2)	7(77.8)	2(22.2)
	Pandrug-resistant (PDR)	4(10)	0(0)	4(100)	2(50)	2(50)	3(75)	1(25)	3(75)	1(25)	3(75)	1(25)

5.7. ESBL-positive *Enterobacteriaceae*

The overall prevalence of ESBL-producing *Enterobacteriaceae* was 68.2% (n=15/22). Among the *Enterobacteriaceae* strains tested for ESBL, *K. pneumoniae* (60%, n=6/10), *E. coli* (71.4%, n=5/7), *K. oxytoca* (100%, n=1/1), *Enterobacter aerogenes* (100%, n=1/1), *Enterobacter cloacae* (100%, n=1/1), and *Citrobacter diversus* (100%, n=1/1) were positive (Table 8).

5.8. Carbapenem-resistant *Enterobacteriaceae*

Regardless of their ESBL results, all *Enterobacteriaceae* strains were tested for carbapenemase production. Among the 22 isolated *Enterobacteriaceae* strains, 81.8% (n=18/22) showed intermediate or high resistance to meropenem and carbapenemase production was suspected. The overall prevalence of carbapenem- resistant *Enterobacteriaceae* (CRE) was 36.4% (n=8/22). The carbapenemase-producing organisms in this study were *K. pneumoniae* (40%, n=4/10), *E. coli* (42.9%, n=3/7), and *K. oxytoca* (100%, n=1/1) (Table 8).

Table 8. Extended-spectrum beta-lactamase and Carbapenemase-Producing Enterobacterales

Isolated organism	ESBL screening		Carbapenemase screening	
	Positive	Negative	Positive	Negative
K. pneumoniae (n=10)	6	4	4	6
Escherichia coli (n=7)	5	2	3	4
K. oxytoca (1)	1	0	1	0
P. mirabilis (1)	0	1	0	1
Enterobacter aerogenes (1)	1	0	0	1
Enterobacter cloacae (1)	1	0	0	1
Citrobacter diversus (1)	1	0	0	1
Total (n=22)	15	7	8	14

ESBL, Extended-spectrum beta-lactamase

6. DISCUSSION

Microbial pathogens, as well as their antibiotic resistance patterns, may change from time to time and from place to place. The emergence of antibiotic-resistant organisms, the increase in the frequency of nosocomial infections, and the increasing number of immune-compromised patients have combined to keep body fluid infections from normally sterile sites common [34].

The present study was conducted in a tertiary care hospital and consisted of 205 different samples of sterile body fluids. The fluid most frequently encountered was CSF 99 (48.3%), followed by pleural fluid 64 (31.2%), peritoneal fluid 32 (15.6%), synovial fluid 07 (3.4%), and pericardial fluid 03 (1.5%). This study is consistent with the study by Sodani and Hawaldar, in which 216 body fluids were processed; (31.02%) of which were CSF followed by pleural fluid (17.13%) [20]. Approximately one-fifth (19.5%, n=40) of the sterile body fluid samples analyzed had a culture-positive result. The prevalence of bacterial pathogens was comparable to that reported in previous studies conducted in India (22%) [35] and Mekelle, northern Ethiopia (20.2%) [10]. In contrast, it is higher than that reported in India (9.7%) [36], Turkey (9.74%) [37], and Harar, Eastern Ethiopia (16.7%) [38]. However, the current findings are lower than those of studies conducted in southern (29.9%) [21] and northern India (31.12%) [39]. The reason for this wide disparity in sterile fluid positivity rates could be attributed mainly to differences in laboratory techniques (molecular technique versus conventional methods), antibiotic use, sample size and duration of study. Other factors such as geographical location, study population, and infection control practices could also have contributed to the observed variations [40].

In this study, gram-negative bacteria (GNB) were predominant (72.5%) over gram-positive bacteria (27.5%). This predominance is consistent with the findings of a similar study conducted by Rouf and Nazir [11] that showed 70% GNB and 30% gram-positive bacteria. Harshika *et al*. [35] and Vijaya and Anuradha [41] also showed a similar predominance of GNB. This may be due to their wide prevalence in the hospital environment and frequent resistance to antibiotics may be the reason for their persistence and spread [42, 43]. Among these, *K. pneumoniae* (34.4%) and *E. coli* (24.1%) were the most commonly isolated GNBs. This is consistent with studies in Harar, eastern Ethiopia [38], and northern India [24]. In contrast, studies from Mekelle, northern Ethiopia [10], reported that gram-positive bacteria were the most common isolates. This difference could be due to different hospital-acquired infections and different standard infection control precautions [44]. In addition, different sample sizes and study areas could have contributed to the differences.

Among gram-positive bacteria, *S. aureus* and *Enterococcus* spp. were the most common, accounting for 72.7% of the total.

According to the logistic regression analysis, rural residence, hospitalization status, comorbidities, and turbid appearance of the samples were found to have significant associations ($p < 0.05$) with culture-positive outcomes. Compared with their urban counterparts, rural patients were 3.86 times more likely to develop bacterial body fluid infections ($P = 0.003$). This could be attributed to the misuse or widespread use of antibiotics by healthcare professionals, poor drug quality, high incidence of infections, unsafe conditions, and lack of antimicrobial surveillance. In the patient setting (hospitalization), inpatients tended to be 2.65 times ($P = 0.019$) more vulnerable to sterile body fluid infections than outpatients were. The higher rate of culture-positive results among hospitalized patients could be due to secondary sources of infection (cirrhosis, surgical procedures, trauma) and low immune status due to underlying medical conditions (burn, transplant surgery, and intensive care support) [45]. In addition, this was probably due to nosocomial infections acquired at the time of admission [46]. The results indicated that patients with comorbidities were 5.46 times more likely than those without comorbidities to develop a culture-positive result ($P = 0.001$). Similarly, the chances of culture positivity were 3.37 times greater among patients with a turbid sample than among their counterparts ($P = 0.024$). This finding is consistent with a previous study conducted in Ethiopia [38]. The high detection of bacteria with a turbid appearance can be partially attributed to rapid changes in patient physiology and immunology as a result of bacteria. Body fluids invaded by these bacteria are characterized by an increase in the WBC count and protein concentration, which is responsible for the turbid appearance of the sample [47].

In this study, the antimicrobial susceptibility test (AST) showed different levels of drug efficacy among various groups of antibiotics. Amikacin (86.2%) and meropenem (76%) were found to be the most effective against gram-negative isolates. Furthermore, gentamycin (62%) and the fourth-generation cephalosporin cefepime (62.1) also showed good effectiveness. On the other hand, lower performance was observed among beta-lactam antibiotics such as ampicillin (20.7%) and third-generation cephalosporins, such as ceftriaxone (31%) and ceftazidime (41.4%). This finding is comparable to those of previous studies in Ethiopia [10, 31], India [41], and Nepal [48].

Most gram-positive bacteria were sensitive to ciprofloxacin and gentamicin. The strains were the least sensitive to penicillin and erythromycin. Furthermore, three-quarters of the *S. aureus* isolates (3/4, 75%) in this study were methicillin-resistant (MRSA). This is in conjunction with the study

by Singh *et al.* from India [36], but it is higher than that in another report (38.5%) [11]. This could be due to geographic variation, differences in infection control practices, and differences in the treatments used. Higher proportions of resistant bacteria were isolated from patients with comorbidities, longer hospital admissions, and indwelling devices (instrumentation). This could be because patients with these conditions are usually in immunosuppressed states and are more susceptible to various nosocomial infections due to their underlying diseases and exposure to various invasive medical devices that make them more prone to various healthcare-associated infections, similar to what has been reported in a study in northern India [39].

As with gram-negative bacteria, resistance to commonly prescribed beta-lactam drugs were also observed in gram-positive isolates. These strains were resistant to penicillin (81.8%), oxacillin (72.7%), cefoxitin (71.4%), and ceftriaxone (63.6%). However, approximately three-fourths (72.7%) of the isolates were sensitive to ciprofloxacin, gentamicin (57.1), and amoxicillin-clavulanic acid (54.5%). Similarly, high levels of resistance to beta-lactam agents have been reported in previous studies in Ethiopia [48] and India [41]. The increased rate of resistance to beta-lactam drugs can be correlated with the frequent use of these antibiotics, the ease of availability, self-medication practices, limited diagnostic facilities, inappropriate antibiotic use, and the issuance of prescriptions without susceptibility data [49, 50].

Multidrug resistance (MDR) was detected in 67.5% of the isolates mostly to penicillins, a third-generation cephalosporin class of antibiotics, and sulfonamides, specifically trimethoprim-sulfamethoxazole. The high level of MDR in this study with respect to different drugs is in agreement with studies conducted in India (62.9%) [51], Addis Ababa, Ethiopia (75%) [46], and Mekelle, Ethiopia (90%) [10]. In this study, extensive and pan-drug-resistant bacteria accounted for 22.5% and 10%, respectively. Among the gram-negative isolates screened, 68.2% were ESBL producers while 36.4% were carbapenemase producers. The availability of drugs without prescriptions and the indiscriminate/prolonged use of common antibiotics have led to the rapid and extensive spread of antimicrobial resistance. Additionally, since TASH is a referral tertiary care hospital, critical patients already have prior exposure to antibiotics, which could have resulted in high antimicrobial resistance.

7. STRENGTH AND LIMITATION

7.1. Strength

The fact that the study was carried out in a tertiary care hospital serving as the main referral center for many, if not all, patients from different parts of the country provides an ideal representation and a good picture of the etiology of bacteria and the pattern of resistance to body fluid infections. Additionally, first-hand information was obtained through face-to-face interviews, and laboratory tests were performed on the same day as the data collection without further delays.

7.2. Limitation

The present study was conducted on limited samples; this may not reflect the entire study scenario. This highlights the need to conduct more studies with larger sample sizes. Additionally, our laboratory cannot identify anaerobic organisms. The isolation rates could be underestimated because the etiology of sterile fluid infections also includes anaerobic bacteria, which were not included in this study.

8. CONCLUSION AND RECOMMENDATION

8.1. Conclusion

In general, the study shows the spectrum of bacterial isolates observed from sterile body fluid samples collected in our laboratory and helps in the empirical treatment of patients according to antibiotic susceptibility patterns. This study also highlights the importance of adhering to antibiotic sensitivity results and infection control practices to prevent the spread of multidrug-resistant infections in the hospital environment.

8.2.Recommendation

Culture and susceptibility tests must be an integral part of routine laboratory tests for the treatment of infections in patients, and the choice of drugs must be based on the results of the sensitivity tests. The antibiotic sensitivity profile of this study suggested that amikacin and meropenem may be the drugs of choice for treating infection. However, based on microbiological evidence, beta-lactam antibiotics, such as third-generation cephalosporins, ampicillin, and penicillin, should be used. Regular monitoring of prevalent pathogenic organisms and their sensitivities is needed to help clinicians in the appropriate selection of antibiotic therapy in the absence of a culture report

and further prevent the development of antibiotic resistance. Our laboratory also needs to focus on anaerobic cultivation methods.

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10. ANNEXES

Annex I: Information sheet and consent (Amharic & English versions)

Title of the Research Project: Bacterial isolates and their antibiotic susceptibility patterns from sterile body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Principal Investigator: Senay Getahun (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 an MSc candidate, from the Addis Ababa University. Your participation is voluntary. The research teams will include one principal investigator and two advisors; from Addis Ababa University Medical Laboratory Department. Please take as much time as you need to read or listen to the information sheet.

Purpose of the research project: The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring response to treatment, epidemiological surveillance, prevention, and research. Therefore, the purpose of the present study is to evaluate bacterial profiles and antimicrobial susceptibility patterns in sterile body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. You have been chosen for this study. Therefore, we invite you to participate in this study.

Therfore, the results of this study are anticipated to improve research in Ethiopia.

a. Procedures and expected participation

If you are willing to participate, you must understand the purpose of the study and give your consent. Not only this, but also the sample collected from you will be used for research purposes, and the results of your sample will be exposed to some concerned professional staff as needed.

b. Potential benefits to subjects and/or society

You will not receive compensation for your participation in this research study. However, depending on the result of the diagnosis, the patient will be treated accordingly.

c. Participation and withdrawal from the Study

Participation is voluntary, and you have the right not to participate in this study. You may withdraw at any time or place without consequences of any kind. You may also refuse to give any sample. You can ask any questions regarding this study and you have the right to receive a laboratory diagnosis free of charge.

The purpose of the study

The purpose of this study is to determine the profile of the bacterial isolate and its susceptibility pattern to body fluid at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. Benefits to participants: Study participants will not have financial incentives or other incentives to participate in this study. However, results have been given to physician for treatment or counseling. Most importantly, this study will contribute to providing information or data for future and future national studies and to developing health programs for health policymakers.

Risks and complication

There is no considerable risk to participants during their participation in the study other than pain at the puncture site during the collection of body fluids, especially CSF, which is boring and painful, as the CSF sample must be handled carefully and samples should not be discarded until all investigations are completed; if any are delayed, the sample shroud must be stored at room temperature and does not need a refrigerator.

Confidentiality

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

Assurance of Principal Investigator

I have signed my signature below to confirm that I assume responsibility for the scientific, ethical and technical conduct of the research project and the provision of progress reports to all stakeholders of the research project.

Senay Getahun (PI): Signature: _____ Date: _____

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

PI Address: Senay Getahun: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Email: senaygetahun1986@gmail.com

Tel: +251 926 950869

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርስቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ “Bacterial isolates and their antibiotic susceptibility pattern from sterile body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia”

የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ። በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኳን በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል።

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል። ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል። ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልፀ መረጃዎችን ማለትም ስም፣ አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ

የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል። በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎታል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

የህክምና መረጃ በሚሰጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው። ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት። የእርስዎ ተሳትፎ በሰጡት የፈሳሽ ናሙና ላይ ባክቴሪያ መኖር አለመኖሩን እንዲሁም ካለም አይነቱን ለመለየት እና ከመድሃኒት ጋር ያለውን ቁረኝነት ለማወቅ ይረዳል። ይህም ውጤታማ ህክምና ለማግኘት ይረዳል።

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 አልዎት። የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። ነገር ግን እርስዎ በሚሰጡን 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 በሚመለከተው አድራሻ ይጠቀሙ።

ስም: ሰናይ ጌታሁን

ሞባይል: +251 926 950867

ኢሜል: senaygetahun1986@gmail.com

Consent form:

I have read this consent form or I have received the information read to me. I had the opportunity to discuss this research study with the study investigator. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I can choose to withdraw at any time. I agree to participate in this research study. I understand that all efforts will be made to keep the information about my identity confidential. By signing this consent form, I have not given up any of the legal rights I have as a participant in a research study.

A. Consent for Adults (>18 years of age)

I_____ hereby gave my consent to provide the requested information and samples as the doctors found best for me.

Signature: _____ Date_____

B. Assent for children aged 12-17 years: I agree that the body fluid sample requested can be taken for this study, provided that my child gives his consent.

Parent signature: _____

C. Consent for parents/guardians under 12 years of age: With a complete understanding of the situation, I agree that the body fluid sample requested can be taken for this study, provided that my parents give their consent.

Signature of the participant: _____

Address of the participant: _____

Date: _____

(የተሳታፊዎች መረጃ ቅጽ፤ የፈቃደኝነት ማረጋጫ እና መጠይቅ)

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ: “Bacterial isolates and their antibiotic susceptibility pattern from sterile body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia” ጥናት ላይ በቂ ገለጻ ተደርጎልኛል። ለጥናቱም ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡ የጥናቱንም አላማዎችም ተረድቻለሁ፡፡ በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል። ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው። በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ። በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተቋሙ ስም---------- ዓ.ም. ---------- አድራሻ (ክፍለ-ከተማ)---------- -

የተሳታፊው መለያ ቁጥር----------አድራሻ (ክልል)----------ዞን----------ወረዳ---------- ----

የዳታ ሰብሳቢው ስም---------- ቀን---------- ፊርማ----------

ሀ) አዋቂዎች ፍቃድ የሚሰጡበት (ከ 18 ዓመት በላይ ለሆኑ)

ስለዚህ ከላይ የተጠቀሱትን ነጥቦች በመረዳት ናሙና ለመስጠት ተስማምቻለሁ።

የተሳታፊ ፊርማ፡_____

ቀን፡_____

ለ) እድሜአቸው ከ 12-17 ለሆኑ ልጆች ቤተሰብ ፍቃድ የሚሰጡበት

ልጄ በዚህ ጥናት ላይ ለመሳተፍ ፍቃዱን የሚሰጥ ከሆነ ከልጄ የሚወሰደው ናሙና በዚህ ጥናት እንዲውል ተስማምቼአለው።

የቤተሰብ ፊርማ_____

ሐ) እድሜአቸው ከ 12 ዓመት በታች ለሆኑ ልጆች ፍቃድ የሚሰጡበት

ቤተሰቦቼ በዚህ ጥናት ላይ እኔ እንድሳተፍ ከተስማሙ አኔ ስሆ ጥናቱ ሁኔታ በመረዳት ሆመሳተፍ ፍቃደኛ ነኝ፡፡

የተሳታፊው ፊርማ _____

የተሳታፊው አድራሻ _____ ቀን_____

Annex II : Questionnaire (Amharic & English versions)

No.	Variables	Category
1.	Hospital registration number (I-Care): _____	
2.	Age _____ Day(s)/Week(s)/Month(s)/Year(s)	
3.	Sex	Male ☐ Female ☐
4.	Marital status	Single ☐ Married ☐ Divorced/separated ☐ Widow/er ☐
5.	Educational level	No formal education ☐ Primary ed. (grades 1 - 8) ☐ Secondary ed. (grade 9 - 12) ☐ College & above ☐ Others, specify _____
6.	Residence	Urban ☐ Rural ☐
7.	Occupation	Student ☐ Unemployed ☐ Gov't/Private Employee ☐ Self-employed ☐ Housewife ☐ Retired/Pension ☐
8.	Monthly Income (ETB) _____	< 1500 ☐ 1500 - 5000 ☐ > 5000 ☐
9.	Healthcare access	Free ☐ Paid ☐ Others, specify _____

Part I: Questionnaires on the sociodemographic and health access of the study participants

Part II: Questionnaires on the clinical characteristics of the study participants

No.	Variables	Category
1.	Patient type	Inpatient ☐ Outpatient ☐
2.	If inpatient, ward location	A6 ☐ ER ☐ ICU ☐ B6 ☐ B7 ☐ B8 ☐ C6 ☐ C7 ☐ C8 ☐ D6 ☐ D7 ☐ D8 ☐ Others, specify _____
3.	Duration of hospital stay before the culture is taken (days)	_____
4.	Presence of comorbidities	Yes ☐ No ☐
5.	If yes, specify:	Diabetes ☐ Renal Failure ☐ HIV ☐ Malignancy ☐ Others, specify _____
6.	Use of empirical antibiotic	Yes ☐ No ☐
7.	If so, what type of antibiotics have you used?	Amoxicillin ☐ Tetracycline ☐ Ciprofloxacin ☐ Gentamycin ☐ Vancomycin ☐ Rifampin ☐ Others, specify _____
8.	Duration of empirical antibiotic used (day):	_____

9.	Use of instrumentation	Yes ☐ No ☐
10.	If yes, the type used:	Urinary catheter ☐ Intravenous line ☐ Nasogastric tube ☐ Central venous catheter ☐ Hemodialysis catheter ☐ Others, specify _____
11.	Sample type	CSF ☐ Pleural fluid ☐ Peritoneal fluid ☐ Synovial fluid ☐ Others, specify _____

ክፍል ነ፡ አጠቃላይ መግለጫዎች

ተ.ቁ	ጥያቄ	መልስ
ነ.	የታካሚ ካርድ ቁጥር (I-Care): _____	
2.	ዕድሜ _____ ቀን/ሳምንት/ዓመት (በቁጥር ይፃፍ)	
3.	ፆታ	ወንድ ☐ ሴት ☐
4.	የጋብቻ ሁኔታ	ያላገባ/ች ☐ ያገባ/ች ☐ የፈታ/ች ☐ የሞተበት/ባት ☐
5.	የትምህርት ደረጃ	መሠረታዊ ት/ት ያልተማረ/ች ☐ ነኛ ደረጃ ያጠናቀቀ/ች ☐ 2ኛ ደረጃ ያጠናቀቀ/ች ☐ 3ኛ ደረጃ ያጠናቀቀ/ች ☐ ሌላ ካለ ይገለጹ_____
6.	የመኖርያ ስፍራ	አዲስ አበባ ☐ ከአዲስ አበባ ውጭ ☐

7.	የስራ ሁኔታ	ስራ የሌለው/ላት ☐ የመንግስት ሰራተኛ ☐ የግል ሰራተኛ ☐ የቤት እመቤት ☐ ጡረተኛ ☐ ሌላ ካለ ይገለጹ_____
8.	ወርሃዊ ገቢ (በኢትዮጵያ ብር)	< 500 ☐ 500 - 1499 ☐ 1500 - 4999 ☐ > 5000 ☐
9.	የጤና አገልግሎት ሁኔታ	በነፃ ☐ በክፍያ ☐ ሌላ ካለ ይገለጹ_____

ክፍል 2: ህመም ነክ መግለጫዎች

ተ.ቁ	ጥያቄ	መልስ
1.	የህክምና አይነት	ተኝቶ ታካሚ ☐ ተመመላላሽ ታካሚ ☐
2.	ተኝቶ ታካሚ ከሆኑ፤ ከየት ዋርድ ነዎት	A6 ☐ ER ☐ ICU ☐ B6 ☐ B7 ☐ B8 ☐ C6 ☐ C7 ☐ C8 ☐ D6 ☐ D7 ☐ D8 ☐ ሌላ ካለ ይገለጹ_____
3.	ለካልቸር ናሙና ከመስጠቶ በፊት ለምን ያህል ጊዜ በሆስፒታሉ ተኝተው ቆዩ?	_____
4.	ተጓዳኝ የጤና እክል አለብዎት?	አዎ ☐ የለብኝም ☐
5.	የጥያቄ "4" መልስዎት አዎ ከሆነ ምን አይነት የጤና እክል እንዳለብዎት ይግለጹ?	የስኳር በሽታ ☐ የኩላሊት በሽታ ☐ ኤች አይ ቪ ኤድስ ☐ ካንሰር ☐ ሌላ ካለ ይገለጹ_____

6.	ያለ በቂ ምርመራ antibiotic መድሃኒት ወስደዋል/ይወስዳሉ?	አዎ ☐ አልወሰድኩም ☐
7.	የጥያቄ "6" መልስዎት አዎ ከሆነ የወሰዱትን መድሃኒት አይነት ይግለጹ።	Amoxicillin ☐ Tetracycline ☐ Ciprofloxacillin ☐ Gentamycin ☐ Vancomycin ☐ Rifampin ☐ ሌላ ካለ ይገለጹ_____
8.	ለምን ያህል ጊዜ መድሃኒቱን ወስደዋል?	_____
9.	በሰውነቶ ውስጥ የተተከለሎት አጋዝ መሳሪያ እንደ catheter ወይም tube አለ?	አዎ ☐ የለም ☐
10.	የጥያቄ "9" መልስዎት አዎ ከሆነ የመሳሪያውን አይነት ይግለጹ።	Urinary catheter ☐ Intravenous line ☐ Nasogastric tube ☐ Central venous catheter ☐ Haemodialysis catheter ☐ ሌላ ካለ ይገለጹ_____
11.	የሚሰጡትን የናሙና አይነት ይግለጹ።	CSF ☐ Pleural fluid ☐ Peritoneal fluid ☐ Synovial fluid ☐ ሌላ ካለ ይገለጹ_____

Annex III: Laboratory procedure for biochemical & drug susceptibility testing

A. Specimen Collection, Transport, and Handling

1. Specimen collection:

All body fluid will be collected by experienced physicians using an aseptic procedure and sent to the microbiology laboratory for Gram staining, culture and AST.

2. Transport and handling of samples

All body fluids collected 3-5 ml, will be centrifuged at 2,500 rpm for 10 minutes to concentrate any microbial agent that may be present. If less < than 1 ml of body fluid is received, the samples will be directly inoculated into the medium. The CSF samples are stored in a 37°C incubator to maintain body temperature or remain at room temperature. CSF should never be refrigerated because fastidious organisms may not survive at lowered temperatures. Cloudy specimens will not be centrifuged. The sediment will be plated on the medium and gram staining will be performed.

Procedure for Gram Stain

Microorganisms are classified according to their Gram stain reaction

- Gram-positive and Gram-negative.

Gram-positive bacteria have thicker and denser layers of peptidoglycans on their cell walls. Iodine penetrates the cell wall in these bacteria and alters the blue dye to inhibit its diffusion through the cell wall during decolorization.

Gram-negative cells, which do not retain the methyl/crystal violet, are stained with a counterstain of safranine.

1. Take a clean, grease-free slide.
2. Prepare the suspension smear on the clean side with a loop of the sample.
3. Air dry and heal fix
4. Crystal violet was poured and kept for about 30 seconds to 1 minute and rinsed with water.
5. Flood the grams of iodine for 1 minute and wash with water
6. Then wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water
7. Add 0.1% counterstain safranin for about 1 minute
8. Wash with water
9. Air dry, Blot dry
10. Finally, observe under the microscope using immersion oil.

Interpretation

Gram-positive organisms stain deep blue/purple.

Gram-negative organisms stain pink/red.

III. Culture Setup

Blood agar, chocolate agar, MacConkey agar, nutrient broth, and incubated media at 37ºC for 24 -72 hours

IV. Interpretation of Culture

All body fluids are sterile, so any organism that grows, regardless of quantification, will be processed for identification and sensitivity.

B. Culture and identification

1. Suspected growth of any organism from body fluid on blood agar, chocolate agar, and MacConkey agar.

Positive (present) or Negative (absent)

2. Identification steps for suspected colonies
   a. Gram stains
   b. AFB stains
   c. Oxidase test positive or negative
   d. Catalase test positive or negative
   e. Coagulase test positive or negative
   f. Lactose

Fermentation from MacConkey agar

Lactose fermenter □ Late Lactose fermenter □ Non lactose fermenter

V. Biochemical reaction

Identification of bacterial isolates involves the use of biochemical screening mediums. Indole, urease, mannitol, triple sugar iron (TSI), citrate, motility, lysine decarboxylase, manolete, and oxydase tests.

1. **Indole test:** is a biochemical test performed on bacterial species to determine the organism to convert tryptophan into indole. Some colonies of the culture will be inoculated in peptone water and incubated at 37°C for 24 hours. A few drops of indicator (Kovac's reagent) will be added and gently shaken to mix well. The color change will be observed. If the indicator

reagent turns red within 1 minute, it is Indole positive. If the indicator reagent remains yellow in 1 minute, it is indole negative.

2. **Urease test**: the urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. The urea agars will be heavily inoculated over the entire surfaces of the slants in bijou bottles. The cap will be loosen and then incubated at 37ºC for 3-12 hours. A culture positive for urease produces an alkaline reaction in the medium, as evidenced by the pinkish-red color of the medium. Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.
3. **Triple sugar iron (TSI)**: microbiological test roughly named for its ability to test the ability of microorganisms to ferment sugars and produce hydrogen sulfide. Using a sterile inoculating needle, we stabbed the butt of the LIA slant twice and then streaked back and forth along the surface of the agar with the organism. Incubate at 37 °C for 18 to 24 h. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt red–yellow): glucose fermented only.

I. If alkaline slant–alkaline butt red–red): sucrose and glucose not fermented.

II. The presence of black precipitate (butt) indicates hydrogen sulfide production, and

The presence of splits or cracks with air bubbles indicates gas production.

4. **Citrate utilization test**: Simmons' citrate slopes will be prepared in bijou bottles as recommended by the manufacturer (stored at 2-8ºC). The slopes will be then stabbed and incubated at 37 ° C aerobically for 48 hours. The blue color indicates a positive reaction, and if the slopes of the Simmons citrate agar remained green, the color indicates a negative reaction.
5. **Motility test:** The motility of the bacterium is demonstrated in a semi-solid agar medium. This agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. Mobilization will be examined after 35-37ºC for 24 hours. Motility will be indicated by the presence of diffuse growth (appearing as a color of the medium) away from the line of inoculation.
6. **Lysine decarboxylase**: Lysine decarboxylation is based on the ability of some bacteria to cleave L-lysine to cadaverine under the liberation of carbon dioxide. Lysine decarboxylase can be detected by culturing bacteria in a medium containing the desired amino acid, glucose,

and a pH indicator bromocresol purple. The acids produced by glucose bacteria from the fermentation will initially lower the pH of the medium and cause the pH indicator to change from purple to yellow. The acid pH activates the enzyme that causes the decarboxylation of lysine to amines and the subsequent neutralization of the medium. This results in another color change from yellow to purple. Bacteria that decarboxylase lysine turn the medium purple. Additionally, bacteria that produce H_2S appear as black colonies.

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

2. Antibiotic susceptibility results for Bacteria isolates

No.	Drug	Sensitive	Intermediate	Resistant
1.	Ampicillin			
2.	Amoxicillin (Clavulanic Acid)			
3.	Amoxicillin			
4.	Amikacin			
5.	Beta-lactam			
6.	Chloramphenicol			
7.	Ceftazidime			
8.	Cefalotin			
9.	Ciprofloxacin			
10.	Clindamycin			
11.	Cefotaxime			
12.	Cefuroxime			
13.	Erythromycin			
14.	Cefepime			
15.	Cefoxitin			
16.	Gentamicin			
17.	Meropenem			
18.	Oxacillin			
19.	Tetracycline			
20.	Vancomycin			

Optimal inoculation of test plates, within 15 minutes after adjustment of the turbidity of the inoculum suspension, a sterile cotton swab is dipped in the adjusted suspension. The swab should rotate several times and pressed onto the inside wall of the tube above the fluid level. The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire surface

of the sterile agar. The lid can be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow excess surface moisture to absorb before applying the drug-impregnated disks.

Note that extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inoculates for streaking plates. Application of Discs to Inoculated Agar Plates The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed to ensure complete contact with the agar surface. The plates are inverted and placed in an incubator set at 37°C within 15 minutes after applying the discs.

Reading Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate will be examined. The diameters of the zones of complete inhibition are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter using sliding calipers, which are held on the back of the inverted plate.

Annex IV. Modified Hodge Test (MHT) Principle

The modified Hodge test (MHT) is a phenotypic method for detecting carbapenemase production by isolated pathogens in clinical laboratories. It is a reliable method to confirm that carbapenem resistance in isolated bacterial pathogens is associated with carbapenemase production.

Principle of the Modified Hodge Test (MHT)

It is based on the principle that the carbapenemase enzyme produced by the test organism hydrolyses the carbapenem antibiotic resulting in the enhanced growth of carbapenem-susceptible *E. coli*.

If the test organism produces any carbapenemase enzyme, it will hydrolyze the diffuse antibiotic in the vicinity of the streaked lines. This will enhance the growth of *E. coli*, which will otherwise be inhibited by the antibiotic, in the vicinity of the streak.

This growth enhancement results in the indented growth of *E. coli* and the test organism within the zone of inhibition of *E. coli* resultin in a clover leaf-like pattern.

Requirements for the Modified Hodge Test

1. Carbapenem sensitive *E. coli* (*E. coli* ATCC 25922 strain)

2. Mueller Hinton Agar (MHA) Plates
3. Test bacteria
4. Control Bacteria:

Positive Control: *Klebsiella pneumoniae* ATCC BAA – 1705 – MHT Positive

Negative Control: *Klebsiella pneumoniae* ATCC BAA – 1706 – MHT Negative

5. Carbapenem antibiotic disc (10 mcg discof etrapenem or meropenem)
6. Cotton swab or bent glass rod (Dolly rod) and inoculating loop
7. Other general requirements include like a Bunsen burner, sterile saline water (or broth), 0.5 McFarland standards, forceps, spirit, etc.

Procedure of the Modified Hodge Test

1. A 0.5 McFarland standard suspension of *E. coli* ATCC 25922 (or an *E. coli* strain sensitive to carbapenem) was prepared.
2. Dilute the suspension with sterile saline or broth at a ratio of 1:10 (0.5 mL of suspension was added to 4.5 mL of saline or broth)
3. Uniformly streak or spread the diluted *E. coli* suspension on a sterile MHA plate (as during the usual antimicrobial sensitivity test) and allowed to dry for approximately 3 – 10 minutes.
4. A disc of 10 mcg meropenem or etrapenem was placed at the center of the inoculated MHA plate.

[Note: no more than one (1) disc is recommended for a 10 cm Petri-plate; and no more than four (4) discs are recommended for a 15 cm petri-plate by the CLSI]

5. Using a sterile inoculating loop or swab, 3-5 colonies of freshly cultured test organisms were picked and streaked inside a straight line from the edge of the disc to the edge of the plate.

[Note: no more than one (1) test organism and two (2) control strains are recommended for 10 cm Petri-plates, and no more than six (6) test organisms and two (2) control strains are recommended for 15 cm Petri-plates by the CLSI]

[The length of the streak must be at least 20 – 25 mm]

6. The plate was incubated at 35(±2) ˚C for 16 – 24 hours at in ambient air.

Observation of the Modified Hodge Test

Following the incubation, the enhanced growth of the *E. coli* and the formation of clover leaf-like indentations at the intersection of the streak of the test organism (and controls, if used) and the zone of inhibition of the *E. coli* were observed.

Interpretation of the Results of the Modified Hodge Test

Enhanced Growth (formation of clover leaf-like pattern) = Positive Result (carbapenemase production)

1. No Enhanced Growth (no formation of clover leaf-like pattern) = Negative Result (No carbapenemase production)

K. pneumoniae ATCC BAA – 1705 will show enhanced growth of *E. coli,* forming a clover leaf-like pattern, while *K. pneumoniae* ATCC BAA – 1706 will not show enhanced growth of *E. coli*.

DECLARATION

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

M.Sc. candidate: **Senay Getahun (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as university-based advisors.

Advisor: **Dr. Kassu Desta (Ph.D, Associate Professor)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Mr. Dessie Abera (MSc, Ph.D Fellow)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.