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Bacterial Profile of Sterile Body Fluids and their Antibiotic Susceptibility Patterns Among Patients Attending Minilik Ii Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

Kaleab Getahun*

Wollo Univeristy College of Medicine and Health Sciences Department of Medical Laboratory Science, Ethiopia

***Corresponding Author:**
Kaleab Getahun, Wollo Univeristy College of Medicine and Health Sciences Department of Medical Laboratory Science, Ethiopia.

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Medical Laboratory Education and Services Directorate

On behalf of Wollo University, medical laboratory education and services directorate, college of medicine and health science, we the advisors of this research entitled "Bacterial profile in sterile body fluids and their antibiotic susceptibility patterns among patients at Minilik II comprehensive specialized hospital and Yekatit 12 hospital medical college, Addis Ababa, Ethiopia." read and confirm as MSc. Thesis for the student and recommend that it can be submitted as fulfilling the thesis requirements.

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Abstract

Background: Sterile body fluids (SBF) are fluids that, under typical human body conditions, do not contain any microorganisms which are important in transporting nutrients as well as waste products, regulating body temperature and assessing respiration process. The SBF infections are serious and urgent condition that requires immediate treatment because untreated infections at SBF sites can lead to severe potentially life-threatening infection throughout the body.

Objective: This study is designed and aimed to assess the bacterial profile of sterile body fluid (SBF) infection and their antibiotic susceptibility patterns from patients attending at Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College, Addis Ababa.

Method: A hospital based cross-sectional study was conducted from February 2024 to June 2024 at Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College in Addis Ababa. A total of 186 study participants were recruited using convenient sampling technique. Any consenting patients submitting SBF specimens (CSF, pleural, synovial and ascetic fluid) for testing at clinical laboratory were included and analyzed using conventional culture methods and biochemical tests. The antimicrobial susceptibility test (AST) was determined using the disk diffusion method and interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines. Data was entered into IBM SPSS version 27.0 and analyzed. Bivariate logistic regressions analysis was used.

Result: A total of 186 SBF including CSF, pleural, synovial and ascetic fluid. Pleural and CSF fluids respectively were the most dominant samples in the study. This study revealed 9.1% (17/186) prevalence of bacterial SBF infection. From the overall 17 isolated bacteria, 70.6 % (12/17) were gram negative bacteria. From the isolated bacteria Pneumonia and E. coli were the frequently isolated bacteria. The highest bacteria isolates were found, <10 years old participants and CSF samples. In the present study, from the isolated bacteria, 70.6 % (12/17) shown MDR pattern for the antibiotic drugs tested.

Conclusion: The prevalence of bacterial isolates in this study from SBF was remarkable and, Klebsiella pneumonae was found as the most dominant etiologic agent for SBF infection. Higher resistance of bacteria for 3rd-generation cephalosporin was recorded. Culture and AST practice with continuous surveillance should be an integral part of the laboratory investigation for better outcome and management of patients' national concern.

Keywords: Sterile Body Fluids, Bacterial Profile, MDR, Addis Ababa and Ethiopia

Introduction

Background

Sterile body fluids (SBF) are fluids that, under typical human body conditions, do not contain any microorganisms. The purpose of these fluids is to reduce inter-organ friction by bathing the organs and membranes. According to human physiology, these bodily fluids play a crucial role in the transportation of waste materials and nutrients, the regulation of body temperature, and the evaluation of the respiratory process. Our bodies include a variety of bodily fluids, including blood, pericardial, synovial, pleural, peritoneal, and cerebrospinal fluid (CSF). These fluids are usually free of microorganism's including bacteria. Critical phenomena can occur when germs infiltrate sterile bodily areas and cause severe invasive illnesses. Fluids may build up in any bodily cavity as a result of infection and are linked to invasive disorders like sepsis, bacterial meningitis, bacterial peritonitis, bacteremia, and other complications. Infections of the SBF, when occurs typically have greater clinical urgency and these infections could be life threatening [1-3].

Low inoculum of pathogenic bacteria and early administration of empirical antibiotics there were fewer chances of retrieving positive cultures the morbidity and ability to cause life threatening infections has categorized the cases as a medical emergency that demands early diagnosis and suitable treatment thereby, it will assist the clinician to start more targeted treatment earlier and shortens the patients' hospital stays [4,5].

The SBF infections can have a variety of conditions, such as burn injuries, trauma, and surgery could expose the typically sterile areas to infectious angent. Infection of each sterile site or SBF is separately defined and may describe the anatomical site, organ involved, etiology, clinical parameters/presentation and pathophysiology such as bacteremia, sepsis, bacterial meningitis and bacterial peritonitis [6].

Globally, infections brought on by resistant pathogens continue to be a major source of serious infections, with rising rates of morbidity and mortality. SBF infections caused by antimicrobial-resistant (AMR) bacteria can even be more dangerous and lead to longer hospital stays, higher medical costs, and increased mortality [3].

AMR and bacterial infections are major public health issues in poor countries. Understanding the local antimicrobial sensitivity pattern and the causing bacteria is crucial for empirical treatment. Moreover, for better management of patients and framing the antibiotic policy, the knowledge of likely prevalent strains along with their AMR pattern is essential [4].

The pathologic and noticeable accumulation of fluid in the peritoneal cavity is referred to as "ascites" or peritoneal effusions. Ascites is the most common complication of cirrhosis and 60% of patients with cirrhosis develop ascites within 10 years during the course of their disease. The pathophysiology of spontaneous bacterial peritonitis (SBP) is thought to be the movement of bacteria across the intestinal-mucosal barrier from the intestinal lumen to mesenteric lymph nodes or other extra-intestinal locations.

Likely, the CSF, which is created by ultra-filtration or secretion, travels through the ventricles and the spinal cord, providing brain cells with nourishment and acting as a cushion. A severe headache, fever, muscle rigidity, seizures, elevated intracranial pressure and stroke are all signs of meningitis, an inflammation of the meninges that line the central nervous system (CNS). Bacterial meningitis is a medical emergency that require urgent rational antibiotics therapy. An estimated 200,000 deaths occur worldwide each year, with mortality that rates ranging from 16% to 32% in

underdeveloped nations, particularly Sub-Saharan Africa. Similarly, synovial fluid is a viscous substance that lubricates the joints and many bacteria which are responsible for the occurrence of arthritis due to presence of bacterial cell wall fragments and the bacterial DNA as indicated from experiments. Numerous microbes could be implicated for causing of arthritis, including S. aureus, S. epidermidis, S. pyogenes and N. gonorrhoeae. Pleural effusions are caused by an excessive buildup of fluid in the pleural space. The fluid appearance possibly can be clear, straw-colored, odorless and non-viscous fluids [2,4,7].

In Ethiopia context, scarcity of data about the bacterial profile and AMR of bacteria causing infection of various body fluids is obstacle for the health service. Data on AST of the organisms isolated from SBF over a period of time can be used to create a local SBF infections antibiogram. Knowledge on common causative organisms in various sterile body sites and their antimicrobial susceptibility pattern can help in starting appropriate empirical antibiotics [8,9].

Statement of the Problem

Critical SBF infections necessitate prompt identification and the start of the right antibiotic treatment. SBF infections are rising globally as a result of contemporary medicine's sophisticated and intrusive treatment. One of the main causes of morbidity and death for people with liver cirrhosis is bacterial infections that are due to colonization of the peritoneal organs and fluid. This account for 25%–46% of hospital admission due to acute decompensating events in these patients [6,10].

A diverse species profile of bacterial have been observed causing SBF infections including gram-negatives, such as Pseudomonas spp., E. coli., Acinetobacter spp., Klebsiella spp., as well as gram-positive bacteria like N. meningitidis, Streptococci spp., Enterobacter spp., Staphylococci spp. Based on recent epidemiological studies on the burden of SBF infections, pleural bacterial infections have increased from 7.6% to 14.9% worldwide, with a 20% fatality rate. The mortality risk by bacterial pleural infection on elderly or chronically ill individuals ranges from 25% to 75%. Similarly, the collection of pus in the pleural space, known as empyema thoracis (ET), continues to be a major condition of death and morbidity in children which is caused by S. aureus and S. pneumoniae. Bacterial pneumonia is also the most typical condition for pleural effusion among those who are living with AIDS, and S. pneumoniae accounts for more than 50 percent of parapneumonic effusions [2-4,11].

When bacteria infiltrate the CSF, bacterial meningitis ensues. If no medical managements taken, between 5 to 10% of patients pass away within 24 hours of the onset of symptoms, and between 10% and 20% of those who survive have serious neurological aftereffects such as, learning difficulties and hearing loss [12].

Sub-Saharan Africa has the highest rate of meningococcal meningitis. For decades, Meningococcal meningitis epidemics are particularly prevalent in Africa, with an area called "African meningitis belt" that extends from Sudan and certain areas of Ethiopia to the whole west coast. The mortality rate recognized as high burden that is around 13%. From the epidemic occurred, there were 149,166 cases of meningitis and 15,750 deaths in over 22 countries of the African meningitis belt were particularly affected.

The mortality rates for meningitis and meningococcemia are 16% and 85%, respectively, according to studies on the epidemiology of meningococcal meningitis in adult Ethiopians [13,14]. The magnitude of bacterial infection in the sterile area of the body in Ethiopia varies with different locations. In Ethiopia, Mekelle and Addis Ababa, burden of SBF infections, stated as 20% and 11% respectively according to studies [8,15].

Infections induced by AMR bacteria have become a public health concern and antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality. To a great extent, it is more common in developing nations with insufficient access to health care. Ethiopia also faces similar challenges due to a habit of using self-medication of antibiotics, lack of microbiology facilities and diagnostic capacity, and poor personal hygiene and infection control which leads to AMR. Therefore, to minimize future complications an early diagnosis and proper antibiotic treatment are required [16-20].

Studies that look at the bacterial profiles of different body fluids are needed in Ethiopia because they can yield accurate and representative data that compiles findings from earlier research. In order to empirically treat infections as soon as feasible and lower morbidity and mortality, it is important to regularly examine the pattern of bacterial susceptibility in a given area. In order to assess the prevalence, organism profile, and pattern of antibiotic susceptibility of isolates derived from bodily fluid infections in Addis Ababa town, Yekatit 12, and Menilik Specialized II Hospital, the current study was created, which are serving a wide range of population that can represent a broad range community of the country [21].

Significance of the Study

For the fact that SBF analysis is not widely applicable in the health services setup and as it leads to misdiagnosis and AMR, this study is potential to provide the current information on bacteria etiology that is associated with SBF infection on body sites for better patient care, diagnosis. The primary beneficiaries from this study are infected patients. Through culture and sensitivity test results that will reduce patient`s length of stay in the hospital, avoid misuse of treatments, and reduce complications as a result of the infection.

Most importantly, in accordance to AMR pattern is raising concerns over time in Ethiopia, which majorly can be associated with empirical treatment practices for SBF infections, this study is capable to indicate the current picture of AMR patterns of the bacterial isolates of SBF infections to draw wide national status at community level.

Also, the findings of this study will assist physicians in developing safe and efficient treatments, creating logical prescription regimens, deciding on policies, and ultimately evaluating the effectiveness. In addition, this will help policy makers to amend or develop new infection control programs in local situations. It could also be used as input data for drug selection and purchase, and monitoring of microbial resistance. Furthermore, the results of this study will also help for future researchers and all people who are interested to know more about this study.

Literature Review

Epidemiology of Sub Bacterial Isolates

A study conducted in USA, Michigan, analyzed a total of 1,157 specimens, 19.6% (227/1157) yielded culture-positive and 60.9% (138/227) were gram-positive bacteria. Dominant isolates were S. aureus (51/138, 37%), CoNS (50/138, 36.2%), Enterococcus spp. (18/138, 13%) and Streptococci spp. (19/138, 13.8%). The remaining were gram-negative bacteria (54/227, 23.8%) and anaerobes (9/227, 4%). From gram-negative bacilli, E. coli was the dominant bacterial isolated counting 22.2% (12/54) followed by Serratia marcescens (5/54, 9%), Klebsiella spp. (4/54, 7.4%), and P. mirabilis (4/54, 7.4%) [22].

Another study conveyed from Turkey on 412 specimens summarized that 103 (25%) were culture positive. The most frequently isolated microorganisms were gram-positive cocci 61.7% and remaining 38.3% were gram-negative bacilli. From gram-positive isolates, CoNS (31.8%) were predominant isolates, followed by S. aureus and S. pneumoniae (9.5% each) likewise, E. coli (25%), K. pneumoniae (7.5%), and other organisms were detected [23].

In a study conducted in Bangladesh, out of 1660 samples 34% growth was obtained from total samples. From the isolated organisms, Pseudomonas spp took 29.1% followed by Acinetobacter spp. 27.5%, E. coli 10.3% and Klebsiella spp. 9.7%. Additionally, Sauers, Enterobacter spp, Citrobacter sp, Enterococcus spp, Providencia spp and Serratia spp altogether accounted 10.6% of the isolates [24].

From study in Brazil, eighty-two body fluids samples were analysed and the dominant isolated bacteria were Pseudomonas aeruginosa, E. coli and S. aureus. Also, S. pneumoniae, S. anginosus, S. viridans, Enterococcus faecalis, CONs, Acitinobactor baumannii were identified [25].

In a study conducted in India from 333 body fluid samples were collected and gram-negative isolates were 21.3% and the common isolates were Pseudomonas (20.7%), Acinetobacter (11.6%), Citrobacter (10.7%) and E. coli (10.7%) [26]. Another Similar study from India showed a result from 122 samples, 30% of total showed growth and 28.6% of isolates were E. coli and 27% Acinetobacter species [27].

In other study document from India indicates that from the screened 380 body fluid samples E. coli and K. pneumoniae were frequently isolated from pleural fluid; synovial fluid and CSF showed growth of S. aureus. From eleven [11]. culture positive samples 72.7% were Gram negative organisms the remaining were Gram positive organisms [28].

Another studies from India, Hubli city and Mumbai respectively, revealed that from a total of 635 samples studied which were 233 (36.6%) pleural fluids, 222 (34.9%) ascetic fluids, 174 (27.4%) cerebrospinal fluids and 06 (0.9%) pericardial fluids. The predominant organisms isolated were E. coli (23.23%) and non-fermenting Gram-negative bacteria (19.01%) followed by Pseudomonas (14.08%), Klebsiella spp. (13.38%), S. aureus (10.56%) and Citrobacter spp. (7.04%) [29]. The Mumbai study involved 100 patients having cirrhosis with ascites, which 58% were Spontaneous Bacterial Peritonitis (SBP) cases and 42% non-SBP cases showed that amongst the 58 SBP cases, 53.45% cases were of classic spontaneous bacterial peritonitis. E. coli were the commonest bacteria isolated, followed by Pseudomonas aeruginosa and Acinetobacter species [30].

A cross-sectional descriptive study conducted in Namibia showed out of 7,267 CSF samples submitted,701 (9.6%) showed growth of microorganisms of these 503(71.8%) grew bacteria. The most frequent gram-positive organisms isolated were Streptococcus species (n = 206, 40.9%), Staphylococcus (n=36, 7.2%), and Enterococcus species (n=9, 1.8%) sequence [31].

From study in North-west Ethiopia Debremarkos city, it was reported that the overall magnitude of SBF infection among study participants was 7.5% (14/187). The majority of the isolates were Gram-negative bacteria and the predominant species were, Enterobacter cloacae accounting for 28.57% (4/14). Also, it was found that 78.57% (11/14) of them were multidrug-resistant isolates [32].

Studies from Southern Ethiopia, Arbaminch general hospital focused on peritoneal and pleural infection showed that, from 147 patients having peritoneal effusions screened and 19.05% of ascetic fluids collected had bacterial growth, which 76.6% of isolated pathogen was Gram-negatives. The frequently isolated bacteria were E. coli, 36.67% (11/30),

followed by Klebsiella spp 20%, S. aureus 13.33%, and P. aeruginosa 13.33%. Likewise, another study conducted on total of 152 hospitalized patients with pleural infection, result conveyed that bacterial magnitude as 27.6%. Predominantly isolated bacteria were S. aureus with 34.9%, followed by E. coli with 11.6% magnitude [11,33].

In a study from Eastern Ethiopia, Harar, Hiwot fana hospital, (HFSH), 204 specimens were collected for the study, and 34 of total samples were culture-positive. The highest frequency and distribution were gram-negative bacteria (24/34, 70.6%). The prevalence of bacteria for SBF infection was 16.7%. The gram-negative bacteria were dominant that hold 70.6% of all isolates. The most isolated bacteria were K. pneumoniae (26.5%) and E. coli (20.6%) [34].

Another study from Gondar University Teaching Hospital executed on 390 CSF fluids of participant patients. From culture study, bacterial pathogens were isolated from 22 patients, 5.6% prevalence of bacterial infection. The most dominant grown bacteria were N. meningitidis 10 (45.5%) and S. pneumoniae 7 (31.8%) from all isolates [7].

Similar studies were conducted in Addis Ababa. The study from Tikur Anbesa Specialized hospital, CSF samples accounted 57.4% following pleural fluids 33.3%. Culture positive findings showed that 40.7% gram positive and 59.3% Gram-negative. From the bacteria isolated, the frequent were Pneumonia e 16.7% followed by CoNS which accounted 15.0%. Correspondingly, a retrospective study from EPHI, in spite of total recorded 654 cases; out of culture-positive results, 11.5% were gram-positive bacteria and 74.6% gram-negative. The predominant isolates were Escherichia coli (n=13; 17.3%), followed by Acinetobacter spp. (n=12; 16%), K. pneumoniae (n=10; 13.3%), S. aureus (n=10; 13.3%) Neisseria meningitidis (n=9; 9.3%) and Pseudomonas spp. (n=5; 6.3%) of total isolated bacterial [2,4].

Antimicrobial Susceptibility Pattern of Isolates

In a study conducted in India, Gram negative isolates observed sensitive to carbepenems, colistin and polymyxin B (100%) while, gram positive isolates were highly sensitive to vancomycin (100%). In advance, Acinetobacter was the most resistant pathogens to many antibiotics also 38.5% of S. aureus isolates were MRSA. Another similar study from India showed that overall susceptibility of bacteria to aminoglycosides, quinolones and Cephalosporins were 92.31%, 79.17% and 58.33% respectively [27,30].

Another study conducted in Nepal showed that from 10.7 % (n=196) bacterial isolates, 30% of organisms showed MDR and 10% of the isolates were XDR. Moreover, 35% of E. coli and K. pneumonia were extended spectrum β-lactamase (ESBL) producers. From S. aureus isolates 30% were MRSA and also 90% of S. aureus was resistant to penicillin [35].

Study from India, Ballard, founds that most gram-positive cocci were 100% sensitive to vancomycin., S. aureus and Streptococcus spp. were sensitive to gentamicin (100%) and cotrimoxazole (80-100%) however, 28.57% of S. aureus were MRSA. Accordingly, similar study found Gentamicin (47.5%), Piperacillin Tazobactam (51.6%), Amikacin (56.7%), and Cefoperazone-Sulbactam (65.3%) were the most effective antibiotics against gram negative pathogens. Ciprofloxacin (48%) was the most effective antibiotic against gram positive isolates, which accounted for 9% of the samples, followed by Cotrimoxazole (40%) and Erythromycin (28.6%) [26,36].

A study was conducted with a retrospective observational study at tertiary care Hospital in India. From the findings of this study, the AST pattern stated as the Gram-negative isolates were 100% sensitive to Imipenem followed by Amikacin (78%), gentamicin (74%), Cefepime (69%) and gram-positive isolates were 100% sensitive to vancomycin and linezolid followed by gentamicin (96%), cefepime (94%), and amikacin (92%). The Pseudomonas spp. was sensitive to imipenem (96%) and, higher resistance rate observed to Ceftazidime, Cefoperazone and Levofloxacin. Also, another descriptive cross-sectional study from Namibia also showed that Streptococcus species, H. Influenzae, Staphylococcus, N. Meningitidis, and E. coli were isolated and all were resistance for Cephalosporin and Streptococcus (34.3%) was resistance to penicillin [29,31].

In the study from southern Ethiopia, Arbaminch hospital, more than half of gram-negative bacilli found resistant to cefepime, ceftriaxone, cefuroxime, ciprofloxacin, and ampicillin while, gentamicin (69.6%) and meropenem (65.2%) showed better responses. The predominant isolate E. coli showed high resistance to ampicillin (72.7%), ceftriaxone (81.8%) and ciprofloxacin (81.8%). Similarly, 75% of P. aeruginosa isolates were resistance for cefepime and ciprofloxacin. While, 75% of P. aeruginosa isolates were sensitive for piperacillin and meropene [33].

According to a study conducted in Tikur Anbesa specialized hospital, 75.9% of the bacterial isolates showed MDR. In which, 90% (n=8/9) of K. pneumoniae and (100%, 8/8) of CoNS of the isolates were MDR. Additionally, both Gram-negative and Gram-positive bacteria were resistant to gentamicin (65.6%), ampicillin (62.5%), ciprofloxacin (53.1%), ceftriaxone (50%), and tobramycin (50%) [2].

Another local study from Gondar indicates, among Gram positive organisms S. pneumoniae showed a high level of drug resistance against chloramphenicol (57%), from Gram negative bacteria, N. meningitidis was found resistant to co-trimoxazole (50%), chloramphenicol (30%), gentamicin (30%) and ampicillin (20%), while related study from Arbaminch hospital, MDR isolates account 48.8% of total isolates. Gram-positive bacterial isolates accounted 71.4% of all MDR isolates. Methicillin-resistant was observed as 26.7% and 33.3% of S. aureus and Cons respectively [7,11].

A study was conducted at EPHI found, Gram-negative isolates, 36.6% Enterobacterales, 16.6% non-Enterobacterale and, 28.9% of Gram-positive isolates were found as MDR. K. pneumoniae and E. coli 100% were resistant to ampicillin. From isolated Gram-positive bacteria, Enterococcus spp. showed 100% resistance to penicillin. S. aureus also showed 36.7% resistance to vancomycin and oxacillin 36.4%. Proteus spp. were 100% sensitive to chloramphenicol, ceftriaxone, gentamicin and meropenem [37].

According to different study summaries, the increasing trend of resistance in both Gram-negative and Gram-positive isolates, which warrants regular surveillance studies. Careful use of antibiotics, awareness development on community along with strict adherence to hospital infection control may result in a significant decline in morbidity and mortality among patients [38].

Conceptual Framework

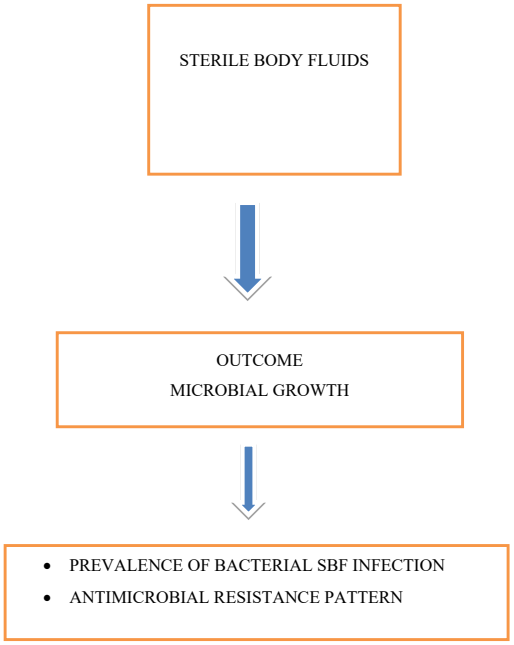

Figure 1: Conceptual Frame Work Ilustration

Objective

General Objective

The general objective of this study is to assess bacterial profiles and their antibiotic susceptibility pattern among patients attending Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College from February – June, 2024.

Specific Objective

- To determine the prevalence of bacterial isolates in sterile body site fluids.
- To assess the drug susceptibility patterns of the bacterial isolates.

Methodology

Study Area

The study was conducted at Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College, which are located in the capital city of Ethiopia, Addis Ababa. The hospitals provide service for wide scale of population from the city and for those who are from all corner regions with diverse socio-demographic background resident society. Minilik II Comprehensive Specialized Hospital has various professionals that included more than 85 subs specialized and medical physicians, 203 nurses, 123 other health professionals, and 250 administrative staff, making nearly 700 staffs.

Yekatit 12 Hospital Medical College was build and starts to serve for the last 100 years which makes the hospital one of the few oldest institutes in Ethiopia. It is supposed to serve more than 5 million patients who are from the city and away from the catchment area. It has six major departments. In the hospital estimated 200 to 250 patients are being served per day through six units of the OPD.

Study Designs and Period

A hospital based cross-sectional study was conducted from February to June, 2024.

Population

Source Population

All patients who were attended Minilik II Comprehensive Specialized Hospital and Yekatit 12 Medical College Hospital.

Study populations

All patients who were attended Minilik II Comprehensive Specialized Hospital and Yekatit 12 Medical College Hospital for diagnosis of SBF infection and ordered SBF analysis for microbiology laboratory study during the study period.

Inclusion and Exclusion Criteria

Inclusion Criteria

All suspected patients for bacterial SBF infection that were cooperative to provide the clinical specimen and other required information.

Exclusion Criteria

Patients who were on antibiotic treatment in the last two weeks prior to the data collection period and those patients who had follow up at the same time at Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College during the study period.

Sample Size Determination and Sampling Techniques

The sample size was calculated based on single population proportion formula as described below. The value of p is taken as 14.1% (0.14) referring from a study conducted at Tikur Anbessa Specialized hospital [2].

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

Where, n = sample size,
Z = Z-score, confidence interval (95% confidence interval, 1.96) P = 0.141 (Prevalence from previous study)
P = expected prevalence or proportion (P = 0.5)
d = precision (d = 0.05) and

$$\frac{(1.96)^2 \text{ X } 0.141(1\text{-}0.14)}{0.05^2} = 185.011 \sim 186$$

Therefore, a total of 186 study participants were recruited using convenient sampling technique with equal proportion of participants from Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College.

Study Variables

Dependent Variable

- Prevalence of bacterial pathogens.
- Antibiotic susceptibility patterns.

Independent Variable

Socio-demographic characteristics such as, age, gender, residence, educational background, occupational status, marital status, monthly income, and clinical related data such as, specimen type, previous SBF infection history, ward unit, other known medical disease, and appearance of specimen.

Data Collection and Laboratory Methods

Data Collection

Clinical and socio-demographic data were collected by assigned nurses at the study site hospital using a pretested structured questionnaire. The pretest was done at Kebena Health Centre, Arada Kifle Ketema, Addis Abeba.

All specimens, such as pleural fluid, peritoneal fluid, CSF, synovial, and pericardial fluids were collected and transported to the microbiology laboratory and processed within two (02), hours of collection. Simple and précised checklist was designed to collect participant's information like participants code number, age, sex, date, medical record number, inpatient, and outpatient.

Specimens Collection and Transport

Following a signed agreement, a doctor used a needle and syringe to collect samples aseptically. After being collected, the samples were sent to a lab for gram staining, culture, and WBC count. Every collected fluid's physical characteristics including whether it is clear, bloody or traumatized, turbid, or straw-like was recorded.

Culture and Bacteria Identification

Microbiological Investigation

Microbiology analysis was performed at Yekatit 12 Hospital Medical College laboratory, microbiology department for the isolation and determination of bacteria. The collected specimens were prepared in accordance with established procedures for gram staining, culture and biochemical testing followed to inoculating on blood agar, chocolate agar and MacConkey agar plates. Inoculated medium was aerobically incubated for 24 to 72 hours at 35 to 37°C. In order to

create a microareophilic state for picky bacteria, the chocolate agar plates were specially incubated in a candle jar with a CO2 concentration of 5–10%.

Plates were investigated for the growth of bacterial colonies and those which had culture growths were taken for gram stain and biochemical tests based on the standards of the laboratory procedures. Identification of isolates was applied based on standard bacteriological techniques, such as colony morphology, gram staining and biochemical test.

Biochemical characteristics of gram-positive bacteria were determined by carrying out catalase test, coagulase test, optochin sensitivity test, bacitracin sensitivity test, mannitol test, and hemolytic activity on blood agar. While indole production test, citrate utilization test, kliger's iron test, urease test, oxidase test, lysine decarboxylase (LDC) test, methyl red/voges proskauer (MR/VP) test, motility test, and hydrogen sulfide gas production test were carried out for the identification of gram-negative bacteria.

Drug Susceptibility Testing

Susceptibility of bacterial isolate to antimicrobial agents of different classes were assessed by the Kirby-Bauer disk diffusion method in compliance with a commercially prepared antibiotic discs of known concentration on Mueller–Hinton agar(MHA) standard media per Clinical Laboratory Standard Institute (CLSI) guidelines [39].

The MHA were applied with inoculums prepared with 0.5 McFarland turbidity standard following antimicrobial discs were applied on to the plate. Antimicrobial discs such as: Amikacin (30 ug Oxoid), Ceftazidime (30ug Oxoid), Ceftriaxone (30ug BD), Gentamycin (10ug Oxoid), TMP-SXT (1.25+23.75ug BD), Clindamycin (2µg BD), cefoxitin (10 ug BD), Erythromycin (15ug Oxoid), Ciprofloxacillin (5ug BD), Vancomycin (30ug BD), and amoxa-clavulanic acid (10ug Oxoid) were used.

Quality Control

The QC was performed to check the quality of the medium. Each new lot of stock reagents which were used for preparation of media was passed through quality control panel before used by testing with known standard strains. To observe the potency of performance of reagents, methods and techniques, QC was applied on staining reagents with known organisms.

Standard Operating Procedures (SOPs) were strictly applied, and verified if the media met an expiration date and quality control parameters per CLSI. Data collectors were trained before the data collection procedure and 5% of total prepared questionnaires were pre-tested before the data collection.

The Quality control of culture medias was assured by concurrent testing with the American Type Culture Collection (ATCC) strains including E. coli (ATCC 25922), P. aeruginosa (ATCC 27852), and S. aureus (ATCC 25923). Also, visual inspections of cracks in media or plastic petri-dishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed.

Data Management and Analysis

The data entry was performed by EPI INFO version 3.1 statistics software and the entered data was double-checked before the analysis. The descriptive statistics (means, percentages or frequency) and the bi-variant logistic regression analysis were performed. The final results are presented in different forms of descriptive charts, graphs and tables.

Dissemination of Results

Laboratory results were reported for respective physician/nurses using a cell phone and written report for better patient management. The findings of the study were re-reported to the hospital`s research director office. The study abstract submitted to Addis Ababa health bureau in order to use the findings as a baseline for further related studies and build general conceptual understanding about the issue also, to assist the responsible bodies to practice and thereby maximize the benefits of the patients and health services. The final report is submitted and presented to Wollo University, Department of Medical Laboratory Science

Ethical Clearance

The study was approved by the Department of Medical Laboratory Science, Wollo University. The proposal document was re-evaluated and represented to the Addis Ababa Health Bureau research directorate office and acknowledged the topic to accomplish the study at Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College. The final permission letter was received from the hospital research and social service office with letter of assistance to the researcher after presentation and explanation of the purpose and procedures of the study. All information of patients remained as confidential as national protocol suggests. The necessary information about the purpose and procedures of the study was explained to the study participants and attendants and for professionals who has participated in the data collecting. Each laboratory result of study participants was reported to the requesting physicians on time.

Operational Definition

- **Sterile Body Fluids:** biological fluids found in human body which do not contain any microorganisms at normal

state such as, cerebrospinal fluid, pleural fluid, pericardial fluid, peritoneal or ascitic fluid, synovial fluid [9].

- **Sterile Body Fluids Infection:** microbial invasion and physiochemical change in the SBF site [12].
- **Gross Appearance:** naked eye observable physical status of sample if it`s clear, turbid, bloody, yellowish, or purulent.
- **Types of Specimens:** referencing the site of the sterile fluid sample collected to classify as cerebrospinal fluid, pleural fluid, pericardial fluid, peritoneal or ascitic fluid, synovial fluid etc.
- **Multidrug Resistance (MDR):** resistant at least one agent in three or more antimicrobial categories [40].

Results

Socio-Demographic Characteristics

In this study, 186 SBF samples were collected from patients attending at Minilik II Comprehensive Specialized Hospital and Yekatit 12 Medical College Hospital from February - June, 2024. From total participants, 110 (59.1%) were female and 54 (29.0%) were over 50 years old. Correspondingly, government employees took 48 (25.7%) while, based on participant`s monthly income, 69 (36.9%) had in a range 10001-20000 ETB. Similarly, 120 (64.5%) were above college level in their education status (Table 1).

	Category	Frequency	%
Age	<10	26	14.0
	11-20	21	11.3
	21-30	8	4.3
	31-40	35	18.8
	41-50	42	22.6
	>50	54	29.0
Sex	Male	76	40.9
	Female	110	59.1
Educational Status	Pre-school	10	5.4
	Unable to read or write	1	0.5
	Primary	33	17.7
	Secondary/High school	22	11.8
	College and above	120	64.5
Occupation	Government employee	48	25.8
	Labor worker	26	14
	Student	46	24.7
	Farmer	14	7.5
	Housewife/househusband	9	4.8
	Merchant	17	9.1
	Other	26	14.0
Monthly-income	No income	50	26.9
	100-5000	16	8.6
	5001-10000	26	14
	10001-20000	69	37.1
	≥20000	25	13.4

Table 1: Socio-Demographic Character of Participants in SBF Infection Suspects at Yekatit 12 Hospital and Minilik II Hospital, February-June 2024

Clinical and Sample-Related Characteristics

Sterile samples were collected aseptically from four (04) different sites secreting SBFs. In this study; CSF, pleural, synovial and peritoneal fluids were collected from participants. From these collected samples, Pleural fluid 55 (29.6%), Cerebrospinal fluids 52 (28.0%), Ascitic/peritoneal fluid 43 (23.1%) and synovial fluid 36 (19.4%) were encountered in the study. Among these samples' types collected and investigated for bacterial growth on culture, CSF found with the highest burden among the collected SBF sample followed by pleural fluid, 6/17 (35.3%) and 5/17 (29.4%) respectively (Figure 2).

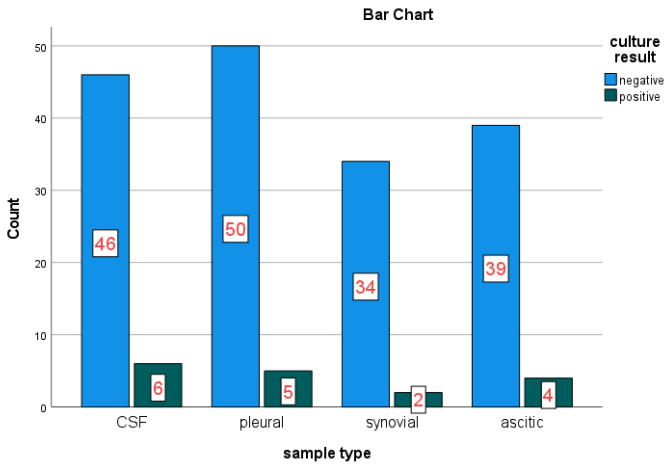

Figure 2: The SBF Sample Type Proportion Causing SBF Infection at Yekatit 12 Hospital and Minilik II Hospital, February-June 2024

Prevalence of Bacterial Isolates

According to the present study, the overall prevalence of SBF bacterial infection among participants is 17 /186 (9.1%) (CI=95%, 3.8-14.5). Regarding to the gram stain characteristics of the isolated bacteria, 12/17 (70.6%) were gram-negative bacteria. From the culture growth isolated bacteria, K. pneumoniae was found as the most predominant one 29.4% (5/17) and followed by E. coli attaining 23.5% (4/17) for SBF infection (Figure 3).

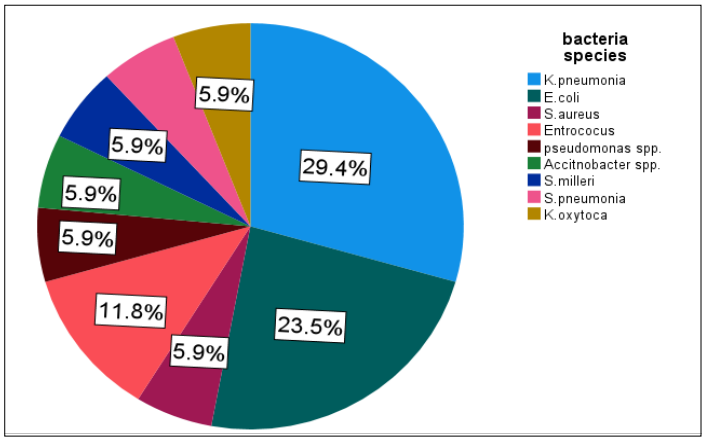

Figure 3: Bacterial Isolates on Patients with SBF Infections at Yekatit 12 Hospital and Minilik II Hospital, February-June 2024

In this study, the incidence of bacterial SBF infection was higher in females when compared to males. From the isolated bacteria, 11/17 (64.7%) were from female study participants. Similarly, the highest prevalence was recorded from under ten (<10) years study participants with burden of 6/17 (35.5%). (Figure 4).

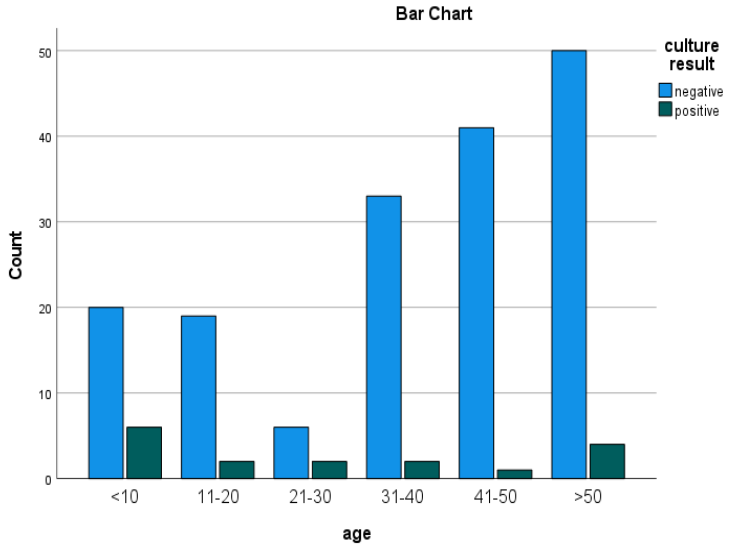

Figure 4: Prevalence of SBF Bacterial Infection Amidst to Patient's Age Category at Yekatit 12 Hospital and Minilik II Hospital, February-June 2024

Antimicrobial Susceptibility Patterns

A drug sensitivity test was performed for all (17) bacterial isolates. These isolates had shown various resistance and susceptibility patterns against different antibiotics that were tested on MHA. The AST pattern of gram-negative bacteria was observed that antibiotics drugs of Meropenem and Amikacin had showed the most effectiveness against gram-negative isolates both with 83.3% effective action on the isolates consequently also, Amoxa-clavlanic acid at 75% effect over the isolated bacteria. However, highest resistance rate was observed on third-generation cephalosporin, Ceftazidime and Ceftraxone with 58.3% weak effect on gram-negative isolates also; Trimethoprim-Sulfamethoxazole had expelled 41.7% resistance to isolated gram negatives.

Most effective antibiotics against K. pneumoniae isolates (n=5) on AST were Amikacin, Meropenem and Amoxa-clavlanic acid which showed 80% effectiveness. In contrast, the highest resistance was observed for Ceftraxone and Ceftazidime each with 60% résistance rate. All the E. coli isolates (n=4) were susceptible to Amikacin, Meropium and Amoxa-clavulanic acid 75% for each isolate. But they were 50% resistant to Gentamycin, Trimethoprim-Sulfamethoxazole and third-generation cephalosporins such as Ceftazidime and Ceftriaxone.

The overall resistance rate of Gram-positive bacteria had showed that, Trimptoprin-sulfamethoxazole 60% (3/5), ceftriaxone 60% (3/5) and ciprofloxacilin 40% (2/5). However, the gram-positive isolates showed no resistance for Cefoxtin and Vancomycin that isolates were 100% sensitive and Erythromycin showed 80% effective action on isolates (Table 2).

GRAM POSTIVE ISOLATES									
		AMC	**CLN**	**FOX**	**VA**	**SXT**	**CRO**	**CPR**	**ERY**
S.aureus (N=1)	S	1	1	1	1		1	1	1
	R					1			
Enterococcus (N=2)	S	1	1	2	2	1	1	1	2
	R	1	1			1	1	1	
S.milleri (N=1)	S	1		1	1	1		1	1
	R		1				1		
S. pneumoniae (N=1)	S		1	1	1				1
	R	1				1	1	1	
N (%)	S	3(60)	3(60)	5(100)	5(100)	2 (40)	2(40)	3(60)	4 (80)
N (%)	R	2(40)	2(40)	0(0)	0(0)	3 (60)	3(60)	2 (40)	1(20)
GRAM NEGATIVE ISOLATES									
		CPR	**MER**	**CRO**	**AMC**	**SXT**	**GEN**	CTZ	AMK
K.Pneumonia (N=5)	S	3	4	2	4	3	3	2	4
	R	2	1	3	1	2	2	3	1
E.coli (N=4)	S	3	3	2	3	2	2	2	3
	R	1	1	2	1	2	2	2	1
Pseudomonas (N=1)	S	1	1	1	1	1	1	1	1
	R								
Acitnobacter (N=1)	S		1		1	1	1		1
	R	1		1				1	
K.oxytoca (N=1)	S	1	1				1		1
	R			1	1	1		1	
N (%)	S	8(66.7)	10(83.3)	5(41.7)	9(75)	7(58.3)	8(66.7)	5(41.7)	10(83.3)
N (%)	R	4(33.3)	2 (16.7)	7(58.3)	3(25)	5(41.7)	4(33.3)	7(58.3)	2 (16.7)

Table 2: Antibiotic Resistance Pattern of Gram Positive and Gram-Negative Bacteria Isolates from Suspected Patients Who Had Body Fluids Infections at Yektit 12 Hospital and Minilik II Hospital, Addis Ababa, 2024

Note: SXT: Trimethoprim-sulfamethoxazole; CRO: Ceftriaxone; AMC: Amoxicillin-Clavulanic acid; GN: Gentamicin; VA: Vancomycin; CPR: Ciprofloxacin; FOX: cefoxitin; ERY: Erythromycin; CLN: Clindamycin; CTZ: Ceftazidime: AMK, Amikacin: S: sensitive; R: resistant

Multi-Drug Resistance

According to the present study, 12 (70.5%) bacteria isolated were MDR. From these, 66.7% (8/12) MDR isolates were gram-negative isolates and 80% (4/5) of isolates of Gram-positive isolates. From the frequently isolated bacteria, 75% (3/4) of *E. coli* and 2/5 (40%) of K. Pneumoniae isolates were found as MDR (Table 3).

Bacterial isolates	Antibiotic-resistant						
Gram-negative	**R0**	**R1**	**R2**	**R3**	**R4**	**≥R5**	**MDR**
K. pneumoniae (n=5)	-	2	-	2	-	1	3
E. coli (n=4)	-	1	-	1	2	-	3
Pseudomonas (n=1)	-	1	-	-	-	-	0
Acitnobacter (n=1)	-	-	-	1	-	-	1
K.oxytoca (n=1)	-	-	-	-	1	-	1
Total (n=12, %)	**0(0%)**	**4(33.3)**	**0((0)**	**4(25)**	**3(25)**	**1(8.3)**	**8 (66.7%)**
Gram-positive bacteria							
S. aureus (n=1)	-	-	-	-	1	-	1
Enterococcus spp. (n=2)	-	1	-	1	-	-	1
S. milleri (n=1)	-	-	-	1	-	-	1
S. pneumoniae (n=1)	-	-	-	-	-	1	1
Total n=5, (%)	**0**	**1(20)**	**0(0)**	**2(40)**	**1(20)**	**1(20)**	**4(80%)**

Table 3: Multidrug Resistance Pattern of Bacterial Isolates

Note: R0: Sensitive for all, R1: resistance to one drug, R2: resistance to two drugs, R3: resistance to three drugs, R4: resistance to four drugs, ≥R5: resistance to five and above.

Discussion

Biological fluids collected from sterile bodily areas are anticipated to be free from both pathogenic and commensal bacteria. Any pathological agents or environmental skin pollutants could be the source of infection in these SBFs. Although SBFs, such as pleural fluid, ascitic fluid, and CSF, are typically sterile, they may become contaminated by various microorganisms and develop potentially fatal diseases. Microbial invasion of normally sterile parts of the body can cause systemic illness. Meningitis, pericarditis, pleural infection either complicated parapneumonic effusion or empyema and septic arthritis cover major forms of SBFs infections [41-45].

In the present study, the overall prevalence of bacterial SBF infection was 9.1% (17/186). This prevalence finding in some extent is close with most previous studies, while offset with few studies. Accordingly, the prevalence of bacterial SBF infection in this study found similar to the study conducted in Turkey in 2021, which was 9.7%, and Nepal in 2019, 10.7% [35,42]. In advance, studies from Addis Ababa, Ethiopia, at EPHI and TASH, reported a prevalence of bacterial SBF infection as 11.5% and 14.1%, respectively [2,4]. These results tell that the prevalence of bacteria causing SBF infection in studies done in Addis Ababa in some extent is similar. Although, for the least contradictions observed, study population socio-economic background, study methods and sample sizes are noted as contributing factors.

In contrast, the prevalence of this study is found higher than study from Debremarkos, North-western Ethiopia, (7.5%), and lower to the study from Mekele, Ethiopia, (20.2%) [21,32]. For the variations of the prevalence's among studies can be attributed to differences in sample processing/laboratory technique, environmental status of the study area and the practice of infection control. Accordingly, the appropriate management of patient, early detection and identification of organism with the results of AST is crucial. Positive cultures are usually low because of a smaller number of pathogens and prior administration of empirical antibiotics in patients [42-45].

In the present study, the most frequently isolated bacteria are gram-negative bacteria (70.6%) that`s identical with the study conducted at HFSH, Harar, Ethiopia. Similarly, study from India, gram negative isolates were 71% likely, from EPHI, Addis Ababa, Ethiopia, 74.6% of total isolates were gram negative [4,34,46].

In contrast, clashing with this study, studies conducted in Mekelle, Ethiopia and India, gram positive bacteria were the dominant isolates as 52.3% and 85.2% of total isolates respectively [21,47]. This obverse finding on the bacterial characteristics in these studies, can possibly be due to differences on sample sizes, study areas, hospital-acquired infections, and different epidemiological factors also standard infection control precautions applied on the sites [48].

In general, from isolated bacteria in this study, K. pneumoniae (5/17, 29.4%), E. coli (4/17, 23.5%), and S. aureus (2/17, 11.8%) were the most frequently isolates causing SBF infection. This data record is similar with earlier studies done in Ethiopia (HFSH and TASH), Turkey, India, and Kenya. This uniform data pattern observed could be explained by their distinctive structure of gram-negative bacteria, are their increasingly resistant presentation to most available antibiotics or opportunistic nature of organisms [9,34,42,49].

In the present study, antibiotics, Meropenem (83.3%) and Amikacin (83.3%) found with the most effective action against gram-negative isolates. Whereas, cefraxone and cefepime found as the least effective antibiotics against gram -negatives in which 58.3% of each antibiotic were resisted by isolates. This result is complementary with the studies

carried out from HFSH, Harar, tertiary care hospital, India, Accra, Gana, and with Debremarkos, North west Ethiopia [9,32,34,50].

In contrast to the Gram positives bacteria isolates of this study, 100% of the isolates were noted as sensitive to Cefoxin and vancomycin, and 80% of the isolates were found sensitive to Erytromycin. Contrarily, 60% of gram positives executed resistance to ceftriaxone and Sulfamethoxazole-trimethoprim. In advance, S. pneumoniae and Entrococcus were found resistant for most classes of antibiotics such as Sulfamethoxazole-trimethoprim, ciprofloxacilin and ceftriaxone. This record of data is homogenous with study from Turkey that showed both isolates, S. pneumoniae and Entrococcus were the most frequent isolate with the most problematic resistance patterns [42].

From this study, the increased rate of phenomenon of beta-lactam including third generation cephalosporins drug resistance may be linked to various factors, including but not limited to the frequent use of antibiotics, their easy availability, and the practice of self-medication, the scarcity of diagnostic facilities, the inappropriate use of antibiotics and the tradition of manipulation of prescriptions without susceptibility data. Also, a survey in Sub-Saharan Africa and Asia reveal that affordable first line agents such as ampicillin and gentamicin are unlikely to be clinically efficacious in a substantial proportion of infections. This results in increasing reliance on the third generation cephalosporins for empirical treatment of serious infections. However, the spread of extended-spectrum beta-lactamase producing strains into the community, probably accelerated by this increased consumption, is eroding the usefulness of these drugs. Alternative agents for treating multi-resistant coliform infections, such as the carbapenems, are unaffordable for treatment of community-acquired infections in low-income countries. In addition, these organisms have a range of mechanisms to prevent the action of many antimicrobials used in clinical medicine such as efflux pumps, alteration of the drug binding site and membrane permeability, degradation enzymes [51,52].

Multi drug resistance (MDR) pattern at 70.6% of isolates was recorded in this study. This result in fewer extents is similar with the studies from HFSH, TASH in Addis Ababa, and Debremarkos, north-west Ethiopia with 76.4, 75% and 78.6% respectively. However, this finding had higher MDR rate with the study from Mekele, Northern Ethiopia, which is 40.1% also, higher than study in Nepal that had 30% MDR rate. There are many possible explanations for this difference and increased prevalence among the studies conducted socio-demography and clinical determinants status including: Self-medication, poor adherence to complete antibiotic regimens and low quality, often counterfeit drugs are all common. The disease burden from bacterial pathogens is greatest different bacterial strains, geographic variation, patients' awareness towards the use of the antimicrobials, the difference in infection control practice, the difference in antibiotic prescribing policies, easy availability of some drugs without a prescription, and indiscriminate/prolonged use of common antibiotics lead to rapid and extensive spread of antimicrobial resistance Also, the environmental persistence of resistance genes or drug residues. Antibiotic resistance spreads through a variety of environmental reservoirs, such as soil, water, hospitals, industries, farm waste, and other contaminated ecological niches [1,2,32,34,53].

Strength and Limitations of the Study

Limitation of the Study

The etiology of bacteria for infections of SBF being at minimal dose, detection rate is reduced as of the growing inquiry on culture media also, the avoidance of anaerobic bacteria enrichment, which were excluded from this investigation due to the seldom availability of national laboratory setup, the overall culture positivity rate of SBF samples was relatively low. Since the diagnosis of anaerobic bacteria is expensive and requires special facilities and expertise to perform this study doesn`t include anaerobic bacteria. The risk of false-negative results in agar-based culture media is high because only a small number of microorganisms may be present in the specimens.

Strength of the Study

The present study, unlike most previous studies, it is conducted at the two of the largest highest hospitals, Minilik II Comprehensive Specialized Hospital and Yekatit 12 Hospital Medical College which brought a better diverse participant population that built the study finding represents a wide and diverse population so that it assures to develop a better generalization for further hypothetical study.

Conclusion and Recommendation

Conclusion

A variety of gram-positive, as well as gram-negative organisms, were isolated from collected SBF samples analyzed in the study, which yielded 17 of 186 (9.1%). Dominantly, K. pneumoniae and E. coli were the leading causative agents for SBF infection. Amikacin and meropenem were found to be the most effective drugs, both executing 83.3% effective action against gram-negative isolates. However, the gram negatives were 58.3% resistance to third-generation cephalosporin. Also, the gram-positive isolates showed 60% resistant to trimethoprim-sulfamethoxazole and ceftriaxone. While, cefoxitin and erytromycin, were observed with effective action against gram- positive isolates 100% and 80%, respectively. The majority, (70.5%) of the isolates have shown multidrug resistance (MDR) pattern, especially to commonly in use and easily available drugs.

Recommendations

The following recommendations are made based on the findings of the present study.

For Yekatit 12 Hospital Medical College and Minilik II Comprehensive Hospital

The hospital should acknowledge that Culture and susceptibility testing has to be an integral part of routine laboratory tests for the best management of infections in patients, and the choice of drugs should be based on results of sensitivity testing.

For Addis Ababa Health Bureau

This data should be used as a baseline for development of better formulation of practical antibiotic policy and a regular surveillance of hospital-associated infections and monitoring of antibiotic susceptibility pattern based on the current study are suggested. In addition, advancement and technical support for laboratories found under AAHB on the bacteria growing and identification technique like molecular and automated machines is preferable for a better detection capability.

For Health Care Workers

The antibiotic sensitivity profile suggests that ciprofloxacin and amikacin are a choice of drugs for sterile body infections. However, Beta-lactam antibiotics such as third-generation cephalosporins, ampicillin, and penicillin should be used carefully unless tested.

The careful prescribing practice or rational use of antibiotics based on sensitivity testing result is needed to halt the trend of increasing antibiotic resistance. Besides, great care should be given to inpatients, as they are more vulnerable to bacterial infections in sterile body areas.

For future researchers

Since this study was not conducted alongside the nation with well enough data. The present study was conducted on a limited number of samples; this may not reflect the entire scenarios of the study. This highlights the need for further studies to be conducted on larger sample sizes. Further, antimicrobial resistance is also gradually increasing, which needs much attention. Thus, continuous monitoring of susceptibility profile of the clinically important pathogens is of great importance to guide effective antimicrobial therapy.

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Annexes

Annex I: Participant Information Sheet and Consent Form (English and Amharic Version)
English Version of Participant Information Sheet, Consent Form

Participant Information Sheet

Title

Bacterial isolates and their antibiotic susceptibility pattern from body fluid aspirates at Menilik II Specialized Hospital and Yekatit 12 Teaching hospital, Addis Ababa, Ethiopia

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

Nutrients and waste products are transported between and from cells via most sterile body fluids. Pleural, peritoneal, cerebrospinal, synovial, and pericardial samples are the most common kinds that are commonly observed in clinical laboratories. Septic arthritis, pleural effusions, meningitis, spontaneous bacterial peritonitis (SBP), and pericardial effusions are among the catastrophic disorders that can result from the infiltration and infection of these normally sterile body fluids by germs such bacteria, fungi, viruses, and parasites. In order to correctly detect and treat individuals whose bodily fluids were caused by bacteria, antibiotics must be administered to lower morbidity and mortality.

Aim of the Study

The concern of the study is to determine the profile of bacterial isolates and their susceptibility pattern from body fluid on patients who are attending at Menilik II Hospital and Yekatit 12 Hospital, Addis Ababa, Ethiopia.

Benefits for Participants

There will be no financial rewards or other inducements for study participants to take part. However, their doctor will receive the results for therapy or counseling. Most significantly, this study will help establish health programs for health policy makers and provide data or information for future, nationwide research.

Risks and Complication

There are no serious dangers to study volunteers, with the exception of the discomfort experienced at the puncture site during sample collection, especially CSF collection, which can be a little uncomfortable and pain.

Confidentiality

To preserve the confidentiality of the participants' information, the samples will be coded and their names will not be

shared. The freedom to withdraw from the study at any time will not be restricted. No personal information will be disclosed to third parties or used in any research publications.

Assurance of Principal Investigator

I have signed below to attest that I am now in charge of the research project's technical, scientific, and ethical behavior as well as providing progress reports to all project participants.

Kaleab Getahun (PI): Signature: _____ Date: _____
Note: Feel free to ask any questions you may have regarding this study at any point during its duration by getting in touch with:

PI Address: Kaleab Getahun: Department of Medical Laboratory Sciences, Collage of medicine and health sciences, Wollo university, Dessie, Ethiopia
E-mail: kaleabgetahun6@gmail.com; Tel.: +251914603080

Informed Consent

I, participant in the study, properly informed on the basic aim of the study, that is the prevalence bacterial sterile body fluid infection and the antibiotic resistance pattern of isolated bacteria in patients attending at Menilik II Specialized Hospital and Yekatit 12 Teaching Hospital, Addis Ababa, Ethiopia.

The goal and application of the study were briefly explained to me. I also understand that I have the freedom to decline information, to choose to participate, and to leave the study at any moment, and that none of these choices will affect my overall health care in any way.

I freely gave the researcher my informed consent for the aforementioned study after thoroughly comprehending the circumstances. I concurred that a microbiological test was performed on the materials. I was given the chance to ask questions on the project, and I got satisfactory responses in a language I could understand. I was also informed that the results of the analysis of body fluid isolates of any organism would be sent to the medical facility, and I could ask for the information if I wanted it.

I _____ hereby give my consent for giving of the requested information and specimen for this study.
Participant code: _____Signature: _____Date: _____

Amharic Version of the Participant Information Sheet, Consent form

Annex II: Questionnaire

(English Version of Questionnaire)

Socio-Demographic Characteristics

Clinical and Sample Related Data (By Data Collectors)

- Specimen type
- Csf Synovial Pleural Pericardial Ascitic Ameutic other…….
- Patient type Inpatient Outpatient
- Area (wards or department) from which specimen is obtained
- -Gyn-Obs -Pediatric - Medical
- - Surgical - Nicu
- --Emergency - Isolation Center Other…….
- Appearances of fluids
- Clear Turbid Bloody Other…….
- Collection:
- Collection time ……. collected by ----------
- Time of delivery to micro-laboratory----------
- Sample volume----------
- Protein Level ……. Remark: - High Low Normal
- Glucose Level…… Remark: - High Low Normal
- Stain AFB-Stain……Gram stain……Culture……, If positive isolated Organism ……
- AST
- Resistant to
- ……..
- Sensitive to
- ……

Amharic Version of Questionnaire
Annex III: Laboratory procedures for biochemical reactions and drug susceptibility testing (CLIS guidelines).

Specimen Collection, Transport and Handling
Specimen Collection
From study participant patients, after a brief consulting about the study and sample collection method and concerns, 3-5 ml volume specimen collected by physician aseptically and drowns into a plane tube and prepared for transportation to laboratory.

Specimen Transport and Handling
The collected sample centrifuged at 2,500 rounds per minute for 10 minutes to concentrate any organisms present. Exceptionally, when less than 1 ml of sample aspirated, samples directly inoculated in to the media without centrifugation. Also, CSF samples stored at 37° C, to maintain at body temperature otherwise kept at room temperature and it does not need to be refrigerated as fastidious organisms may not able to survive at lowered temperatures. Samples that are cloudy not need centrifugation. The sediment, extracted after centrifugation, inoculated onto media prepared and for smear staining.

Direct Examination
- Appearance of fluids
- Gram stain and AFB

Procedure of Gram Stain
Gram stain reactions allowed bacteria to be classified as either gram positive or gram negative. The peptidoglycan layers in the cell walls of gram-positive bacteria are denser and thicker. In these bacteria, iodine enters the cell wall and changes the blue dye, preventing it from diffusing through the wall during decolonisation. Gram-negative cells are stained with a safranine counterstain because they lose the methyl/crystal violet.

Steps
- Smears prepare and fixed by heat.
- 0.5% crystal violet onto the smear for 30sec.
- Rinsing gently with water tilting the slide.
- (1%) Lugol's Iodine added onto the smear,
- Tilt and wash out the iodine with water.
- Decolorize by 95 - 100 % ethanol.
- Flood over with water to wash.
- Flood with 0.1% counterstain safranin.
- Rinse with water and dry to observe with microscope using 100x (immersion oil)

Interpretation
- Gram- Positive: stain deep blue/purple.
- Gram- Negative: stain pink/red.

Procedure of Ziehl-Neelsen Stain (for acid fast bacilli)
This staining technique is used to demonstrate the presence of acid and alcohol fasting bacilli having waxy envelopes which is difficult for stain and decolorizing. Heat is used to make the primary stain penetrate it`s waxy cell wall to stain cells.
- Flood slide with carbol fuchsin.
- Heat the underside of the slide until steam observed.
- Wait for 3-5min to moist with the stain.
- Rinse the slide and indirect stream of deionized water until no color appears in the water.
- Decolorize with a (3% v/v) acid-alcohol solution for 10-20sec then rinse well with water.
- Repeat decolorizing until the stained smear appears faintly. Pink and the water washing off the slide runs clear.
- Counter stain with (1% w/v) methylene blue for 20-30sec.
- Rinse with water and allow drying.
- Apply immersion oil and view under a transmitted light microscope.

Interpretation
- Positive Result: Acid-fast organisms appear red.
- Negative Result: Non-acid-fast organisms appear blue.

Culture Setup
Blood agar, Chocolate agar, MacConkey agar, incubated media at 37ºC for 24 -72 hours.

Culture Interpretation
Any organisms that grow, regardless of quantification, are going to be process for species identification and then to antibiotic sensitivity test.

Culture and Identification
- Suspected Growth of any Organism from Body Fluid on Blood Agar, Chocolate Agar and MacConkey agar.
- Positive/present.
- Negative/absent.

Identification Steps for Suspected Colonies
- Gram stains/AFB stains/
- Oxidase test positive □ /negative □/
- Catalase test positive □ /negative □/
- Coagulase test positive □ /negative □/
- Lactose fermentation from MacConkey agar: -Lactose fermenter- /non-lactose fermenter.

Biochemical Reactions
The identification and differentiation of bacterial isolates enrolls biochemical screening medias, Indole, Urease, Mannitol, Triple sugar iron (TSI), Citrate, Motility, Lysine Decarboxylase, Mannolet, and Oxidase tests.

Indole Test: Lower number of colonies inoculated into peptone water then incubated at 37oC for 24 hours. Few drops of indicator (Kovac's reagent) added and let to mix. Color change observed. When layer of indicator turns to red within 1 minute, it is Indole positive (positive result, if it remains yellow within 1 minute reported as indole negative.

Urease Test: The entire surface of the slants inoculated with urea agars in bijou bottles. It gets incubated for 3–12 hours at 37°C after the cap is loosened. The pinkish red color of the medium indicates an alkaline response produced by a urease-positive culture and yellow-pink color means that urease-negative organisms.

Triple Sugar Iron Agar Slant: Done by taping the slant's butt twice with a sterile inoculating loop, then streak the organism back and forth across the agar's surface. Following, incubate for 18-24 hours at 37°C. 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): sucrose and glucose not fermented. Splits or fissures with air bubbles indicate the generation of gas, whereas the presence of black precipitate (butt) suggests the development of hydrogen sulphide.

Citrate Utilization Test Using Simmon's Citrate Agar: As advised by the manufacturer, Simmon's citrate slopes are made in bijou bottles and kept between 2 and 80 C. After that, slopes will be stabbed and incubated aerobically for 48 hours at 37°C. A favourable reaction is indicated by blue, whereas a negative reaction is indicated if the Simmon's citrate agar slopes stay green.

Motility Test (using motility agars): A straight inoculating needle will be used to prepare and inoculate motility agar, making a single stab in the medium that is about 1-2 cm deep. Following a 24-hour period at 35–37°C, the motility will be assessed. Diffuse development distant from the line of inoculation, which manifests as medium colouring, will be a sign of motility.

Lysine Decarboxylase: By inoculating colonies in a medium containing the necessary amino acid, glucose, and a pH indicator called bromocresol purple, it is possible to identify the decarboxylation of lysine. The pH indicator will first turn yellow as a result of the medium's pH being lowered by the acids the bacteria make during the fermentation of glucose. The enzyme responsible for decarboxylating lysine to amines and neutralising the medium is activated by the acid pH. As a result, the colour shifts from yellow to purple once more. Lysine-decarboxylating bacteria cause the medium to turn purple. Additionally, H2S-producing bacteria manifest as black colonies.

Oxidase Test: A few drops of the oxidase reagent are soaked on a piece of filter paper. Next, a smear of the test organism colony is made on the filter paper. An oxidase reagent strip is an additional option. The phenylenediamine in the reagent will oxidise to a deep purple colour while the organism is generating oxidase.

Antibiotics Susceptibility Result for Bacteria Isolates
Inoculation of Test Plates
- Optimally, within 15 minutes after adjusting the turbidity of the inoculum's suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and press firmly on the inside wall of the tube above the fluid level.
- A dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.

The lid may be left for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Note: Extremes in inoculums density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inoculate for streaking plates.

Application of Discs to Inoculated Agar Plates

The predetermined antimicrobial discs dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. The plates are inverted and placed in an incubator set to 37c within 15 minutes after the discs are applied.

Reading Plates and Interpreting Results

Each plate is inspected 16–18 hours after incubation. The diameter of the disc is measured along with the sizes of the zones of total inhibition as determined by the naked eye. Using sliding calipers that are held on the back of the inverted plate, zones are measured to the closest whole millimeter.

Declaration

This thesis work entitled ”Bacterial Profile In Sterile Body Fluids And Their Antibiotic Susceptibility Patterns Among Patients At Minilik II Comprehensive Specialized Hospital And Yekatit 12 Hospital Medical College, Addis Ababa.” was carried out by me under the relevant comment, guidance and unreserved encouragement of Ms. Daniel Gebretsadik (MSc Assistant Professor) and Dr. Agumas Shibabaw (PhD, Assistant Professor) in the Department of Medical Laboratory Sciences, College of medicine and Health Sciences, Wollo University, for the award of MSc Degree in Medical Microbiology.

Name of student: Kaleab Getahun
Signature.....