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Patterns of Ascites fluid infections, etiology and antimicrobial profile among adult Cirrhosis patients attending at St. Paul Specialized Hospital Millennium and Yekatit 12 Hospital Medical Colleges, Addis Ababa, Ethiopia

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This is to certify that the Thesis prepared by Abubeker Shemsu, entitled:

Patterns of Ascites fluid infections, etiology and antimicrobial profile among adult Cirrhosis patients attending at St. Paul Specialized Hospital Millennium and Yekatit 12 Hospital Medical Colleges /AAU-CHS, A.A Ethiopia from February 2019-march 2021 G.C and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AST	Antimicrobial sensitivity test
BA	Bacteria ascites
CNNA	Culture Negative Neutrocytic Ascites
GI	Gastrointestinal
MNB	Monomicrobial non-Neutrocytic Bacterascite
MOH	Ministry of Health
PMN	Polymorphonuclear
SAFI	Spontaneous Ascitec Fluid Infection
SBP	Spontaneous Bacterial Peritonitis
SPSHMMC	St. Paul specialized Hospital Millennium Medical college

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Abstract

Background: Ascites is one of the most common complications in patients with cirrhosis. Ascites fluid infections are common and potentially life-threatening infections frequently encountered in patients with cirrhotic ascites. The diagnosis of ascetic fluid infections is based on the number of polymorph nuclear neutrophil (PMN)) cells and the result of ascetic fluid culture.

Objective: The main objective of this study was to evaluate the pattern, clinical and microbiological profile of Spontaneous ascites fluid infection (SAFI), in patients with liver cirrhosis attending at selected public hospital from March 2020 to March 2021.

Methods: Hospital based cross sectional study was conducted from March 2020 to March 2021. Saint Paul Specialized Hospital and Yekatit 12 hospital medical colleges were chosen by purposive sampling method. Cirrhosis patients with ascites who presented to the hospitals were conveniently enrolled and consecutively recruited. Structured questionnaire was used to collect demographic data, clinical diagnosis and laboratory investigation. Culture and drug resistant pattern studied following standard methods. Data was analyzed using statistical software IBM (SPSS 23) and multinomial regression analysis was done to assess association between outcome and explanatory variables.

Results: 51 out of 218 patients (23.34%) were found to have SAFI, out of which 19 (37.3%) were female and 32 (62.7) were male. 40 (78.43%) patients had Culture Negative Neutrocytic ascites (CNNA), 11 (21.57 %) had Classic SBP (Spontaneous bacterial peritonitis) and 1 (2.0%) had Bacterascites. Gram negative (mainly *Escherichia coli* n=4 (36.36%), and *Klebsiella pnuemoniae* n=3 (27.27%)), and *Staphyloccus aureus* n=1 (9.09%), Streptococcus viridans n=1 (9.09) and *CoNs* (*coagulase negative staphs*) n=2 (18.27) . Most identified *E.coli* were resistant to *Ceftriaxone.* Abdominal distension (62%), pedal oedema (60%), abdominal pain (62%) and jaundice (52) were the main clinical features in those with SAFI. History of jaundice, fever, low serum Albumin, high ALT, and low Ascites fluid albumin were among independent predictors.

Conclusion: Spontaneous Ascites fluid infection (SAFI) was common among cirrhotic patients with ascites attended at SPSHMMC and Yekatit 12 hospital medical college. Jaundice, low arterial blood pressure, low platelet, low serum albumin, and low Ascites fluid albumin were among highly indicative SAFI and diagnostic paracentesis should be done instantly on admission to confirm the diagnosis preferably before starting empiric antibiotic therapy to enhance culture positive rate.

Key words: Cirrhosis patients, spontaneous bacterial peritonitis, Drug susceptibility, Addis Ababa.

1 Introduction

1.1 Background

Cirrhosis is a complication of many liver diseases characterized by abnormal structure and function of the liver. Hepatitis and chronic alcohol abuse are frequent causes of liver cirrhosis. Ascites is the abnormal buildup of fluid in the abdomen mostly caused by cirrhosis (1, 2, 3, 4).

Spontaneous ascites fluid infections (SAFI) and or spontaneous bacterial peritonitis (SBP) are a well-known infection in patients with cirrhosis and ascites. SAFI is associated with significant morbidity and mortality**.** The current available evidence suggests that the abnormal buildup of fluid in the abdomen, weakened immune status of the patient and subsequent overgrowth of a specific organism in the intestine, translocation of that microbe from the intestine to mesenteric lymph nodes, and resulting spontaneous bacteremia and subsequent colonization of susceptible ascites fluid are the basis for SBP (5, 6, 7).

Clinical presentation of 87% of patients with SBP is symptomatic at the time the infection diagnosed, the symptoms and signs of infection are often indirect, such as a slight change in mental status. Without prompt paracentesis, the diagnosis and treatment of infected ascites may be delayed, often resulting in the death of the patient. The commonly encountered signs and symptoms of spontaneous ascetic fluid infections are fever, abdominal pain, abdominal tenderness and altered mentation (8, 9, 10).

Timely diagnosis of ascites fluid infection requires a high index of suspicion and a low threshold for performing a paracentesis. Clinical deterioration, especially fever or abdominal pain, in a patient with ascites should raise the suspicion of infection and prompt a paracentesis. Even though, peritoneal carcinomatosis, pancreatitis, hemorrhage into ascites, and tuberculosis (TB) can lead to an elevated Ascitec fluid poly-morpho nuclear neutrophil (PMN) count, most cases of neutrocytic ascites are caused by bacterial infection. If the Ascitec fluid PMN count is elevated, the working diagnosis is Ascitec fluid infection until proved otherwise (9, 10, 11).

SBP is diagnosed by cells particularly aPMN count in ascetic fluid equal to or greater than 250/ mm^3, nearly 40% of SBP episodes are culture positive**.** Many older studies reported that Gram-negative enteric bacteria were involved in the majority of SBP episodes **(**3, 12, and 13).

Third-generation cephalosporin is recommended by international guidelines as empirical treatment for SBP and quinolones as secondary prophylaxis (14, 15)**.** However, in recent study Gram-positive bacteria and antibiotic-resistant bacteria have been increasingly found to cause SBP (3, 16-18). This change in antimicrobial susceptibility has been due to prolonged and common quinolone use and increased prevalence of hospital and intensive care unit admissions. These findings have raised doubts about the currently recommended antibiotic strategy in SBP. The prevalence of antibiotic-resistant pathogens was significantly geographical (19). Antibiotic consumption has been identified as the main cause for increasing rates of antibiotic resistance. Some country has known for a restrictive antibiotic policy and has had the lowest antibiotic use for years (19, 20, 21).

1.2 Statement of the Problem

Spontaneous ascetic fluid infections are the most common infection of ascetic fluid that occurs in about 10%-30% of patients with ascites (14, 18). Studies in the past have shown mortality rates due to SBP to be as high as 80%-90%, but this can be decreased to 10%-40% with early diagnosis and effective therapy with broad-spectrum antibiotics. Studies have shown that patients with upper gastrointestinal tract (GIT) bleeding are at higher risk of developing SBP. An estimated 22% of these patients have already been infected prior to admission and 30%-40% may develop SBP during hospitalization (9- 11).

Despite the known fact that these infections carry a significant mortality and morbidity and even with the presence of recognizable health care cost of investigating and managing these patients (4, 9, 11), no formal data exists to help manage these infections and no standardized local guidelines formulated based on local study. The current shift in the etiology of SAFI and or SBP the widespread use of antibiotics noted in clinical practice, a shift in the causative agents of these infections and a change in the resistance pattern of the pathogens are clearly expected (12).

Consequently, microbiological study results in SAFI in our country could differ from those observed in other countries like Spain, Greece, Germany, the United States, Ghana, and Nigeria over time (1, 22, 30, 31). This would mean that international guidelines for prophylaxis and treatment of SBP would need to differentiate between countries based on antibiotic resistance rates. These are some of the facts that why doing this research as it would shade some light on some of the questions we have about and possibly flag the way for further studies to be conducted on related topics in the future. Therefore, this study investigated causative microorganisms in patients with cirrhosis and ascites in cross sectional manner who were hospitalized with the last 1-year in selected public health hospital in Ethiopia. In addition, the study aimed to identify the patients most at risk for SAFI and or SBP and to evaluate the associated factors.

1.3 Significance of the Study

As ascites fluid infection is a common infection and spontaneous bacterial peritonitis (SBP) is a serious complication and common cause of death in patients with liver cirrhosis, this study provided up to date information that which bacterial pathogenswere responsible for SAFI in cirrhosis patients. Identifying and typing of organism whether it is gram negative or gram positive and studying their respective drug sensitivity pattern would provide important information for clinicians for better management of cirrhotic patients. This study may also be utilized for formal data construction and possibly an input to treatment guideline in Ethiopia context for the care of cirrhotic patients.

2 Literature review

A study conducted in USA in December 2000 by Evans LK and *etal* on asymptomatic outpatients of cirrhotic patients with ascites shows the patterns of Ascites fluid infection in the population of 427 cirrhotic outpatients as defined by neutrocytic ascites (absolute neutrophil count >250 cells/mm3) was 3.5%. Of this 1.4% were culture positive (2.1%), 1.9% others had bacteria ascites. The organisms cultured from ascetic fluid in these asymptomatic patients with culture positive neutrocytic ascites and bacterascites (Non neutrocytic bacteriascites) predominantly gram positive (22).

A hospital based cross-sectional study conducted in India in 2018 by Leuica K *etal* on 200 patients with chronic liver disease and ascites showed 42 patients (21%) were found to have SBP, out of these female accounts 2 (5%) and male 40 (95%). Thirty-five (83.33%)) patients had Culture Negative Neutrocytic ascites (CNNA), 6 (14.28%) had Classical SBP and 1 (2.38%) had Bacterascites. Most of them were gram negative, mainly *Escherichia coli n=5* (71.42%), *Klebsiella pnuemoniae n=1 (14.28%)* and *Staphyloccus aureus n=1 (14.28%).* Most of microbial isolates were susceptible to *ceftriaxone*. The most common presenting feature in SBP patients was fever (88.09%), abdominal pain (85.71%), jaundice (71.42%), hepatic encephalopathy (69.04%) and UGI bleed (28.57%). So, the prevalence of Spontaneous bacterial peritonitis was 21% with *Escherichia coli* being the commonest organism in this setting. History of alcohol consumption, abdominal pain, fever, low Ascitec fluid total protein, high indirect bilirubin and low serum protein were found to be predictors of spontaneous bacterial peritonitis (23).

Another study conducted in Pakistan in December 2007 by Zaman A *etal* on 50 patients showed 28 (56%) were diagnosed to have spontaneous bacterial peritonitis or its variants. Classic spontaneous bacterial peritonitis was present in 11 patients (39.28%), 16 (57.14%) patients were found to have culture negative neutrocytic ascites and one patient (3.57%) had bacterascites. Among 28 cases of spontaneous bacterial peritonitis 12 samples of Ascitec fluid showed positive culture reports. *E. coli* was the most frequently cultured organism isolated in 8 (66.66%) cases, *Streptococcus pneumonae*in 2 patients (16.66%), *Staphylococcus aurus* and *Klebsiella* each in 1 case (8.33%). This study concluded that SBP and its variants is a common complication of liver cirrhosis with ascites. *E. coli* is the most frequent offending organism in these cases (25).

A retrospective study conducted in Spain by Llovet JM et al, in 1997 included two hundred and twenty-nine consecutive episodes of spontaneous bacterial peritonitis, (193 in patients without

(Group A) and 36 in patients with *norfloxacin* prophylaxis (Group B)), were analyzed. In 100 episodes (86 and 14, respectively), the responsible organism was isolated in Ascitec fluid. There were marked differences ($p<0.001$) between group A and B in the frequency of peritonitis caused by gram-negative (67.4% vs. 14.3%) and gram-positive (30.2% vs. 78.6%) bacteria. The study found three polymicrobial episodes. Bacteria resistant to *cefotaxime* and gram-negative bacilli resistant to quinolones were isolated in Ascitec fluid in nine (seven in Group A and two in Group B) and three episodes (all in Group A), respectively. No differences in the course of infection and patient survival were observed between groups. This study concluded that SPB in patients with and without prophylaxis with norfloxacin are not different in clinical features, response to treatment and prognosis. SBP caused by gram-negative organisms resistant to quinolones is extremely uncommon in patients with cirrhosis receiving prophylactic *norfloxacin* (26).

In a prospective study conducted by Borzio M and etal in 2001 showed 12% prevalence of bacterial peritonitis in a total of 405 in patients with cirrhosis at the time of admission to the hospital or during the hospitalization. Enteric flora accounted for 62% of infections, *Escherichia Coli* being the most frequent pathogen. Low Ascitec fluid total protein concentrations, as well as the phagocytic (both motile and stationary) dysfunction associated with cirrhosis, are risk factors for bacterial infection. GI hemorrhage is an under-recognized risk factor for the development of spontaneous bacteremia and SBP. The cumulative probability of infection during a single hospitalization for bleeding is approximately 40% (27).

A study conducted in Romania by Tudorașcu DR *et al* in 2016 on 64 patients suffering from liver cirrhosis, with an episode of SBP, who were admitted to the IInd Medical Clinic of the County Hospital of Craiova, which included control group of 61 patients with liver cirrhosis with an episode of decompensation of liver disease showed the most frequent etiology of SBP is represented in 67% of the cases by Gram negative germs, and thus, the antibiotic therapy orientated against this etiological segment. In this study most of the germs were sensitive to third generation cephalosporins, quinolones, *carbapenems* and *vancomycin* (28).

A retrospective double-cohort study conducted in Netherland by Oey *et al*.which encompasses all Ascitec cultures from patients with cirrhosis obtained 2003–2005 and 2013–2014included a total of 312 patients, 125 patients in the first and 187 patients in the second cohort. SBP was diagnosed in 132 of 840 analyzed Ascitec fluid samples; 62 samples were culture positive. An

increase of Gram-positive bacterial isolates was noted from 26% to 46% between cohorts (p ¼ 0.122). In this study the prevalence of multidrug-antibiotic–resistant pathogens increased from 25% to 32% (p ¼ 0.350. The Authors concluded in this single-center study in the Netherlands found a modest but non-significant increase in the proportion of patients with SBP caused by Gram-positive bacteria and multidrug-antibiotic–resistant bacteria over a 10-year period. The findings of this study differ from reported data in other countries and suggest empiric antibiotic prophylaxis and treatment of SBP should be based on national and regional microbiological findings and resistance pattern (29).

There was a cross-sectional study conducted in Ghana by Duah A *et al* involving 140 patients with ascites irrespective of the underlying cause from 25th March 2016 to 25th November 2016. In this study demographic information and clinical data were collected using a standardized questionnaire. The study used Ascitec fluid culture as the gold standard for SBP diagnosis and Ascitec fluid cell count was also done. Positive Ascitec fluid culture and/ or Ascitec polymorpho nuclear leukocyte ≥250cells/mm^3 were considered a diagnostic for SBP. The determined prevalence of SBP was 21.43% (30/140). Majority, (41.7%) of the bacteria isolated from Ascitec fluid with SBP was Escherichia coli. History of jaundice, low arterial blood pressure on admission and encephalopathy were found to be independent predictors of SBP. The study concluded SBP is common among patients with ascites admitted at the Hospital. Jaundice, encephalopathy and low blood pressure are highly suggestive of SBP (30).

Retrospective study conducted in Nigeria by Oladimeji AA *et al* on thirty-one patients with liver cirrhosis and ascites who were admitted into the medical ward of a hospital from August 2009 to July 2010 were involved. All the study subjects underwent abdominal paracentesis which was done within 48 hours of admission under aseptic condition. The mean age of the studied subjects was 62±9 years (age range 43-78 years). Of the 21 that developed SPB, culture positive SBP was present in 66.7% (14/21) while CNNA was found in 33.3% (7/21). The prevalence of patients with positive cultures on Ascitec fluid but without neutrocytic ascites were classified as having *mono-bacterial bacterascites* (MNB) was 26% (8/31) in this study. Of those with SBP, 93% had monomicrobial infection with aerobic Gram negative bacilli being responsible in 66.7% of the cases with *E.coli (70%)* being the predominant organism followed by *Klebsiella species*. Gram positive organisms accounted for 33.3% with *Streptococcal species (60%)* being the predominant

organism followed by *Staphylococcus aureus (40%).* Patients with SBP had significantly lower platelet count when compared with those without SBP, $p < 0.05$. Also, international normalization ratio (INR) was significantly higher in those patients with SBP compared with those without SBP, $p < 0.05$. The poor prognostic indicators found in this study were; low Ascitec protein, hepatic encephalopathy, coagulopathy, renal dysfunction (creatinine>2mg/dl) and leukocytosis ($p < 0.05$). Th**e** study concluded that it is imperative to do diagnostic abdominal paracentesis for cell count and culture in any patient with onset of ascites or cirrhotic patients with ascites and suggestive symptoms compatible or suggestive of SBP (31).

There was no study conducted on this specific topic in Ethiopia, but there was a study conducted at Gonder University by Muhie OA in 2018 which aimed assessing the causes and clinical profile of ascites. This study included a total of 52 patients from November1, 2018 to March 30, 2019. Thirty (57.7%) of them were males and the majority (77%) of the participants were fifty years old or younger. The mean age was 43.8 ± (14). Thirty-eight (73%) patients take alcohol occasionally while 11(21.2%) patients take alcohol frequently or massively. Chronic liver disease (CLD) was the major cause of ascites in this study in 24 (46.2%) patients. The other main causes of ascites were heart failure from various causes (19.2%), tuberculosis and hepatosplenic *Schistosomiasis* contributing to 11.5% each and chronic kidney disease (5.8%). Five (20.8%) CLD patients had spontaneous bacterial peritonitis as a complication. Five (20.8%) and 4 (16.7%) CLD patients had hepatocellular carcinoma and hepatic encephalopathy as complications, respectively. Nine (17.3%) patients had variceal bleeding; six of the patients were diagnosed to have CLD while the remaining patients were having hepatosplenic schistosomiasis. The study concluded that liver cirrhosis is the major cause of ascites in Gondar, Ethiopia, while chronic viral hepatitis infections (hepatitis B (HBV) and C (HCV) viruses) are the main causes of liver cirrhosis. The other major causes included heart failure, tuberculosis, and hepatosplenic *Schistosomiasis*. The author suggested that it is wise to consider and give priority to these diseases whenever one is evaluating a patient with ascites (32).

3 Objectives

3.1 General Objective

- To assess patterns of ascites fluid infections, etiology, antimicrobial sensitivity and associated factors among cirrhosis patients attending at SPSMMC and Yekatit 12 Hospital medical College from March 2020 – January 2021, AA, Ethiopia..

3.2 Specific Objectives

- To assess the type of Ascites fluid infection in cirrhotic patients
- To isolate bacterial pathogen in ascetic fluids of cirrhosis patients
- To assess the antibiotic sensitivity pattern of ascites fluid culture isolates
- Assess associated risk factors for SAFI among cirrhosis patients.
- Assess the prevalence of SAFI and classic SBP

4 Material and Methods.

4.1 Study area

This study was conducted at St. Paul's Hospital Millennium medical college (SPHMMC) and Yekatit 12 hospital medical college. These hospitals are located in Addis Ababa and chosen by purposive sampling method.

St Paul's Millennium Medical College, as it is known today, was established through a decree of the Council of Ministers in 2010, although the medical school opened in 2007 and the hospital was established in 1968 by the late Emperor Haile Selassie. It is governed by a board under the Federal Ministry of Health. The College initiated Ethiopia's first integrated modular and hybrid problem-based curriculum for its undergraduate medical education, and is currently expanding to postgraduate programs and diversifying its undergraduate program offerings. The college is in the process of building its capacity quickly in a short period of time, growing from 3 to 250 faculty members in the last six years, and expanding teaching facilities. Collectively it has more than 2800 clinical, academic and administrative and support staffs that provide medical specialty services to patients who are referred from all over the country, teaching medicine and nursing students and doing basic and applied researches. While the inpatient capacity is more than 700 beds, The College sees an average of 1200 emergency and outpatient clients daily. These study was done at the internal medicine department particularly gastroenterology and Hepatology sub unit both on inpatient and outpatients.

Yekatit12 Hospital was established in 1923 as one of modern medical service delivery centers in the country. After many decades of medical service delivery, in 2011, it became a medical College by a decision of the City Government of Addis Ababa. The City Government, recognizing the long-aged service that the Hospital has been rendering to the residents of Addis Ababa and taking in to account its present statues, decided to reestablish it as center for training medical professional combining with medical service delivery. The establishment proclamation (proc.no.31/2011), issued by the Council of the City Government of Addis Ababa, renames the Hospital as Yekatit 12 Hospital Medical College.

The college is devoted for training medium and higher level of health professionals in amalgamation with health service delivery through applying new method of training that combines theoretical training with practical application. The training method stipulated in the establishment proclamation and instituted in this legislation is newly introduced to the country

under the auspices of applying practice oriented medical training in higher magnitude. The Hospital was redesigned to be used as a research center for the college in addition to its medical service that it renders to the public being a college hospital. This research was conducted at internal medicine department on patients who were at outpatient and inpatient service.

4.2 Study design and Period

Hospital based cross-sectional study was conducted from March 2020 to March, 2021

4.3 Population

4.4 Source population

All Patients who visited internal medicine department who attended as outpatient and inpatient of the selected public health hospitals for chronic liver disease with ascites is the source population of the study.

4.5 Study population

Patients with cirrhosis who visited the internal medicine department of SPSHMMC and Yekatit 12 Hospital Medical College and meeting the inclusion criteria.

4.6 Inclusion and Exclusion criteria

4.7 Inclusion criteria

- Patients who have evidence of cirrhosis and ascites based on clinical and ultrasound evaluation
- Age> 18 years

4.8 Exclusion criteria

- Who have evidence of secondary peritonitis

4.9 Study Variables

4.10 Dependent variables

- Prevalence of SAFI and classic SBP
- Bacterial Isolates from Ascites fluid Culture
- Antimicrobial sensitivity pattern of bacterial isolates

4.11 Independent variables

- The independent variables of the study are categorized into three which are demographic information, clinical feature and laboratory features. The demographic information are age and sex. The clinical features include Arterial BP, BMI, body temperature, history of upper GI bleeding, history of jaundice, encephalopathy, previous episodes of spontaneous bacterial peritonitis, abdominal distension, abdominal pain, weight loss, pedal oedema,

ascites, fever, chills. Laboratory features include LFT, CBC, and PT/INR and hepatitis serology.

4.12 Measurement and Data collection

4.12.1 Sample size determination

As far as my literature review, no any research was done on prevalence SBP among adult cirrhosis patients in Ethiopia. Therefore, Patterns of Ascites fluid infection among adult patients with ascites attending Korle-Bu Teaching Hospital in Ghana which is 21.4% is used to calculate sample size.

Z=Standard score corresponding to 95% confidence level

d = the margin of error (precision) = 5%

n = the required sample size

$$n = (Z\alpha/2)^2 \, P\,(1-P)/d^2$$

$$n = ((1.96)2x0.214(1-0.214))/(0.05)^2 = 258$$

About 85% of the determined sample size incorporated on this research

4.12.2 Sampling method

The study sites were selected by purposive sampling method. Convenient sampling method used to select each study subjects. Cirrhosis patients with ascites whether symptomatic or not for SBP who presented to the internal medicine department, particularly Gastroenterology and Hepatology unit of selected public health hospitals was collected consecutively recruited.

4.12.3 Data collection procedure

Patients' medical records were reviewed to find out relevant history including alcohol use, physical characteristics including clinical features of liver cirrhosis (ascites, hepatomegaly, splenomegaly, and abdominal pain, presence of collaterals cvain). Diagnosis of ascites was made based on criteria of abdominal distention, presence of shifting dullness, positive fluid thrill and were confirmed by diagnostic paracentesis or abdominal ultra sound scan (34).

After thoroughly explaining the study to patients, those who give informed consent was recruited and a questionnaire administered (to obtain socio-demographic data and clinical history of the patients. The questionnaire addressed socio demographic information, clinical sign and

symptoms, laboratory investigations, ultrasound result and other necessary information were collected related to the study.

4.13 Principles of each Laboratory analysis

4.13.1 Ascites fluid Sample collection

Abdominal paracentesis was performed using an aseptic technique at the right or left iliac fossa, 3cm above and 3cm medial to the anterior superior iliac spine. Exactly 20mls of Ascites fluid were collected using a sterile syringe by senior gastroenterologist or resident doctor and 10mls inoculated into blood culture bottle at the bed side. Additionally, Ascites fluid analysis were performed as part of clinical utility and cell count, differential, Ascites fluid albumin, and total protein were collected from the patient card.

4.13.2 Isolation bacterial pathogen

After the sample was inoculated on blood culture bottle (broth) (BHI and TSB), the culture medium were incubated at $37^{o}C$ for 24 hours using incubator. After 24 hours the culture medium were observed for possible microbial growth. For those who show microbial growth, a portion of the sample were transferred to blood agar plate, chocolate agar plate, and MacConckey Agar. Mannitol salt agar also was used to isolate staphylococcus species.

4.13.3 Identification of bacterial pathogen

Bacterial identification was made using biochemical tests, namely indole, citrate, oxidase, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, mannitol fermentation from the pre collected and stored samples.

4.13.4 Antimicrobial sensitivity testing (AST)

If growth were detected on the culture medium, antimicrobial sensitivity testing was done based on the identified bacterial pathogen for antibiotic disc choice. Antimicrobial sensitivity of the bacterial isolates was done by the Kirby-Bauer disc diffusion method. In the procedure fresh sub-cultures of bacterial isolates were used after overnight growth on Muller Hinton Agar. The

inoculums were prepared by suspending several of the colonies in sterile phosphate buffered saline (pH 7.2) to achieve a turbidity of 0.5 McFarland standards. This resulted in a suspension containing approximately1-2 x 10^8 CFU/ml. A sterile cotton swab was dipped into the bacterial suspension, elevated above the liquid and rotated several times against the inside wall of the tube to remove excess of the inoculum. The swabs then were streaked evenly in three different directions onto the Muller Hinton Agar. Susceptibility Testing were done by discs of choice using the Kirby-Baur disk diffusion method and the interpretation of results were made by CLSI guideline, January 2020 (30^{th} Edition) for Sensitive, Intermediate and Resistance Zones. All the results were collected using appropriate data collection sheet.

The following antimicrobial discs with respective concentration were used for gram negative enterobacteriacea were: Amikacin (30 µg), Ampicillin (10µg), Amoxilin/k clavulinate (20µg),Cefazolin (30µg), Cefepime (30µg), Cefotetan (30µg), Cefotaxime (30 µg), Cefoxitin(30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Ciprofloxacin (5µg), Gentamycin (10µg), Meropenem (10µg), Levofloxacin (5µg), Tobramycin (10µg), and Trimethoprim/Sulfomethoxazole (1.25/23.35µg). Whereas for gram positives were: Ampicillin (10µg), Cefepime (30µg), Cefotaxime (30 µg), Ceftriaxone (30µg) Azithromycin (15µg), Clindamycin (2µg), Doxycycline (30µg), Erythromycin (15µg), Gentamycin (10µg), Oxacilin (30µg), Tobramycin (10µg), Penicilin (1 unit), Tetracycline (30µg), Trimethoprim/Sulfomethoxazole (1.25/23.35µg), and vancomycin (30µg) discs were selected based on prescription pattern and recommendations from CLSI. Zone of inhibition diameters were interpreted as sensitive, intermediate and resistant according to the principles established by CLSI 30^{th} edition.

4.13.5 Ultra sound Scan

All patients underwent an abdominal ultrasound scan after overnight fasting and the following details were obtained from patients card: maximum vertical span of the liver; nodularity of liver surface; spleen size (length of its longest axis); presence of collateral vessels, portal vein dimension and presence of ascites.

4.13.6 Other Investigations

Normally all cirrhosis patients were undergoing laboratory investigation for hemoglobin (HB), white blood cell count (WBC), platelet (PLT) count, international normalized ratio (INR), and serum concentrations of total protein (TP) and direct bilirubin (DB), total bilirubin (TB), serum total protein, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Alkaline phosphatase (ALP), Serum sodium (Na+), potassium (K+), urea and Creatinine testing Hepatitis B surface antigen (HBsAg) and anti-bodies to hepatitis C virus (anti HCV-Ab) as part of clinical utility and Such data were collected from patient's card.

4.14 Quality assurance

4.14.1 Pre-analytical

Ascites fluid were collected by assigned physicians (mostly by resident doctor). Sterility check were performed for each batch of media. The questionnaire was pre-tested to check its appropriateness.

4.14.2 Analytical

Quality control for the culture medium and reagents were done using ATTC. The Ascites fluid were inoculated at bedside into blood culture bottle (Broth) to minimize contamination. The quality of the antibiotic disc were done using recommended antibiotic disc control procedure (CLSI guideline 30^{th} edition, 2020).

4.14.3 Post- analytical

Results were collected on using result collection sheets that were part of standard questionnaire.

4.15 Data analysis and interpretation

Descriptive statistics were performed for all continuous variables and data presented in appropriate graphs and tables. The prevalence of spontaneous bacteria peritonitis was

determined. Further analysis was done to determine if there were any associations between spontaneous bacterial peritonitis and the clinical or laboratory parameters. Chi square test were used to determine the level of association. Binomial and multinomial logistic regression analyses were conducted using SPSS version 23 for possible association. P value < 0.05 were taken as a significant association for clinical or laboratory.

4.16 Ethical considerations

Ethical clearances were granted from department of medical laboratory science, CHS, AAU, Saint Paul Specialized Hospital Millennium medical college and Yekatit 12 hospital medical college. Consent form were issued to each participant on the study, confidentiality were maintained during labeling and name and other unique identifier were avoided from the questionnaire and the sample. The participants were informed as he/she can withdraw from the study at any time during the study progress.

4.17 Dissemination of Results

The findings of this study presented to Addis Ababa University and disseminated to St. Paul specialized hospital and Yekatit 12 hospital medical college. In addition, it may be issued to the MOH and may be used by some policy makers in improving the current SBP management in cirrhosis patients. Publication and presentation on certain conferences also considered.

4.18 Operational definitions

Cirrhosis Patients: Patients with liver cirrhosis, diagnosis established by using clinical, biological and imagistic criteria.

Classical spontaneous bacterial peritonitis: is defined as ascites fluid polymorph nuclear count ≥250/mm3 and positive ascites fluid culture [(23).]

Culture negative neutrocytic ascites (CNNA): is defined as ascites fluid neutrophil count ≥250/mm3 with negative Ascitec fluid culture [(23)].

Non neutrocytic Bacterascites (NNBA): is defined as ascites fluid neutrophil count ≤250/mm3 with positive ascites fluid culture [(23)].

Secondary bacterial peritonitis: describes peritoneal infections secondary to intra-abdominal lesions, such as perforation of the hollow viscous, bowel necrosis, nonbacterial peritonitis, or penetrating infectious processes [(35)].

5 Results

5.1 Socio demographic characteristics

A total of 218 patients with ascites were recruited for this study with a mean age of 38.67 ±12.0 years (age range 19 to 76 years) with majority of age group between 18 and 40. One hundred forty-six (67%) patients were males with male to female ratio of 2.03:1. One hundred thirty-six (62.8%) were in the 18-40 age group and fifty-six (25.7%) participants were single (Table 1).

Table 1. Socio demographic characteristics of chronic liver disease in SPSHMMC and Yekatit 12 hospital medical college, Addis Ababa, Ethiopia March 2020 to March, 2021(n=218).

Socio-demographic data	number	Percent (%)
Age		
18 – 40	136	62.4
41 – 60	66	30.3
41 – 80	16	7.3
Sex		
Male	146	67.0
Female	72	33.0
Marital status		
Single	56	25.7
Married	158	72.5
Separated	4	1.8

5.2 Clinical presentation of CLD patients

Among 218 chronic liver disease patients 29.4% presented with Upper GI bleeding, 50.5% with abdominal pain, 49.5 with abdominal pain, 39.4% with sleeping disturbance, 41.75% with pedal edema, 42.2% with fever and 38.5% with chills (Table 2).

Table 2. Clinical presentation of Patients with chronic liver disease in SPSHMMC and Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia March 2020 to March, 2021(n=218)

Clinical presentation		Number	Percent (%)
History of upper GI bleeding	**Yes**	64	29.4
	No	154	70.6
Abdominal pain	**Yes**	110	50.5
	No	108	49.5
History of jaundice	**Yes**	69	31.7
	No	149	68.3
Sleeping disturbance/ memory impairment	**Yes**	86	39.4
	No	132	60.6
Abdominal distention	Yes	110	50.5
	No	108	49.5
Previous episode of SBP	Yes	60	27.1
	No	158	72.5
Fever	**Yes**	92	42.2
	No	126	57.8
Chills	**Yes**	84	38.5
	No	134	61.5
Pedal edema	**Yes**	91	41.7
	No	127	58.3
Systolic BP	Low (<90)	37	17.0
	High (>129)	28	12.8
	Normal (90-129)	152	69.7
Diastolic BP	Low (<60)	37	17.0
	Normal (60-90)	181	83.0
	High (>90)	0	0.0

5.3 Ascites fluid infection and Laboratory investigations

5.3.1 Organ function tests and Cell count

The organ function test for patients with chronic liver disease who have ascites fluid infection show the mean value of AST, ALT, ALP was 75.7, 74.2, and 140.9 with standard deviation of 120, 151.3 and 76.1 respectively whereas non-ascites fluid infection was 50.1, 31.5, and 129.2 with standard deviation of 43.8, 24.9 and 78.2 respectively (Table 3)

Table 3. Descriptive Statistics of continuous lab parameters for patients with ascites fluid infection vs non-ascites fluid infection at SPSHMMC and Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia March 2020 to March, 2021(n=51).

Descriptive Statistics of continuous lab parameters for patients with liver cirrhosis										
Laboratory parameters	**Ascites fluid infection**					**Non-ascites fluid infection**				
	N	Minimum	Maximum	Mean	Std. Deviation	N	Minimum	Maximum	Mean	Std. Deviation
Aspartate amino transferase (U/L)	50	13	828	75.7	120.0	166	10.6	265.7	50.1	43.8
Alanine aminotransferase (U/L)	50	8.1	1025	74.2	151.3	166	9.1	247.6	31.5	24.9
Alkaline phosphatase (U/L)	50	44.1	426	140.9	76.1	164	22.7	400.0	129.2	78.2
Total bilirubin (mg/dL)	50	0.16	33.6	3.9	7.2	164	0.1	33.6	1.5	3.8
Direct Bilirubin (mg/dL)	50	0.09	21.6	2.1	4.1	164	0.1	21.6	0.8	2.3
Creatinine (mg/dL)	49	0.36	4.28	1.1	0.9	163	0.2	10.0	1.2	1.6
Urea (mg/dL)	49	6.4	209	37.3	41.0	165	9.1	248.0	31.9	43.2
Sodium (mmol/L)	47	120	147	135.5	5.8	162	110.0	150.2	135.3	6.6
Potassium (mmol/L)	47	2.3	7.36	4.3	0.8	162	2.3	5.7	4.3	0.5
Chloride (mmol/L)	46	79	112.2	99.8	7.4	157	79.0	117.2	99.6	6.2
WBC(x 10^3cells/mm^3	50	3.0	157.0	14.0	20.9	165	1.0	35.0	6.2	4.4
Hgb (g/dL)	50	2.3	18.0	12.6	3.3	165	4.0	18.2	13.6	2.8

The organ function test of Classic-SBP patients with chronic liver disease show the mean value of AST, ALT, ALP was 76.8, 51.6, and 144.8 with standard deviation of 57.8, 31.7and 95.0 respectively (**Table 4)**

Table 4. Descriptive Statistics of continuous lab parameters for Classic-SBP patients at SPSHMMC and Yekatit 12 hospital Medical college, Addis Ababa, Ethiopia March 2020 to March, 2021(n=11).

Descriptive Statistics of lab parameters for classic SBP					
	N	Minimum	Maximum	Mean	Std. Deviation
Aspartate amino transferase (U/L)	11	14.8	187.4	76.8	57.8
Alanine aminotransferase (U/L)	11	11.1	112.0	51.6	31.7
Alkaline phosphatase (U/L)	10	32.0	368.0	144.8	95.0
Total bilirubin (mg/dL)	11	0.4	33.6	7.6	10.1
Direct Bilirubin (mg/dL)	11	0.1	21.6	4.4	6.6
Serum total Albumin (g/dL)	11	0.0	2.0	0.4	0.8
Serum Total protein(g/dL)	11	0.0	1.0	0.4	0.5
WBC ($x10^3$ cells/mm^3)	11	3.0	13.3	9.7	3.1
Hgb (g/dL)	11	2.3	16.2	11.5	4.0
Creatinine (mg/dL)	11	0.6	2.5	1.2	0.7
Urea (mg/dL)	11	10.9	110.4	44.6	32.7
Sodium (mmol/L)	11	120.0	147.0	134.1	8.7
Potassium (mmol/L)	11	2.3	4.8	4.0	0.7
Chloride (mmol/L)	10	90.2	119.0	99.9	7.6

Serum albumin and total protein level of patients with ascites fluid infection show 54.9%, and 62.7% of these patients had albumin level of between 2.1 and 2.5 g/dL respectively and one hundred fourteen (67.9%) from one hundred fifty-nine participants with non- ascites fluid infection had serum albumin level of greater than 3 g/dL. About 94.1% patient with ascites fluid infection had platelet value of less than $150x10^3$/ mm^3 and 13.7% have INR value of greater than 1.2. (**Table 5**).

Table 5. Categorized lab parameters of SAFI and non-SAFI patients at SPSHMMC and Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia March 2020 to March, 2021(n=218).

Test Parameters	Categories	Non- ascites fluid infection		Ascites fluid infection	
		Number	Percent	Number	Percent
Serum Albumin	2.1-2.5 g/dL	31	18.5	28	54.9
	2.51 - 3.0 g/dL	14	8.3	12	23.5
	> 3 g/dL	114	67.9	11	21.6
	Total	159	94.6	51	100.0
	Missing	9	5.4	0	0
Serum Total protein	< 5.1 g/dL	25	14.9	32	62.7
	5.1 - 8.3 g/dL	123	73.2	18	35.3
	> 8.3 g/dL	11	6.5	1	2.0
	Total	159	94.6	51	100.0
Missing	System	9	5.4	0	0
Total		168	100.0	51	100
PLT	Low < 150000	84	50.0	48	94.1
	High> 450000	3	1.8	3	5.9
	Normal 150000 - 450000	78	46.4	0	0.0
	Total	165	98.2	51	100.0
Missing	System	3	1.8	0	0
Total		168	100.0	51	100
INR	High > 1.2	108	64.3	7	13.7
	Normal ≤1.2	54	32.1	44	86.3
	Total	162	96.4	51	100.0
Missing	System	6	3.6	0	0
Total		168	100.0	51	100.0

Hepatitis marker test on 218 CLD patients show about one hundred thirty-three (61%) positive for hepatitis B, twenty-two (10.1%) was positive for Hepatitis C and two (1%) were positive for Hepatitis B and C (Figure 1).

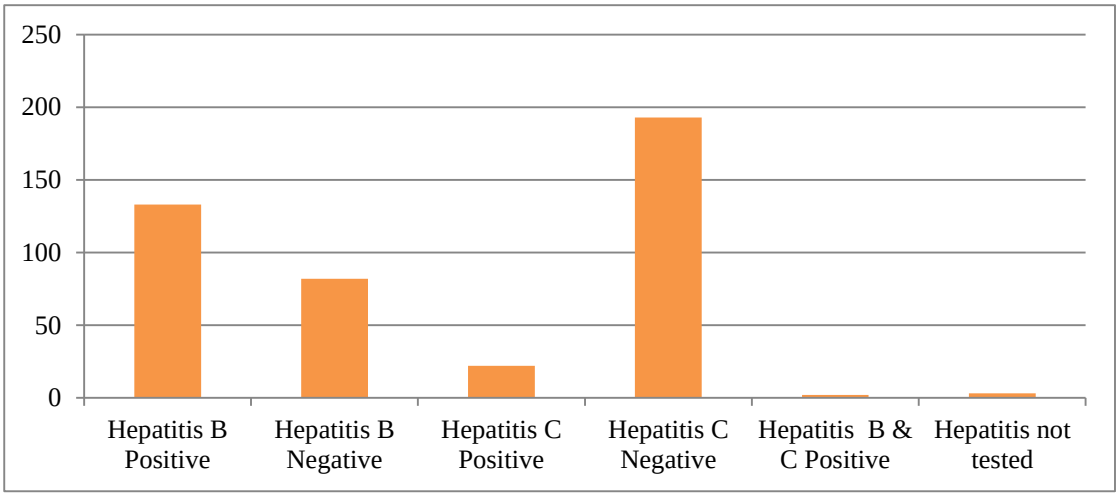

Figure 1. Hepatitis B and C distribution

5.3.2 Fluid analysis cirrhosis patient

The fluid analysis for chronic liver disease patients (n=218) demonstrated that fifty (98.0%) from fifty-one patients with ascites fluid had ascites fluid neutrophil count greater than 250 and forty nine (96.1%) from fifty one patients with SAFI had ascites fluid albumin level less than 0.5 g/dL (Table 6).

Table 6. Fluid analysis report summary of SAFI and Non-SAFI participants at SPSHMMC and Yekatit 12 hospital Medical college, Addis Ababa, Ethiopia March 2020 to March, 2021(n=218).

Test parameters	Categories	Non- SAFI		SAFI	
		Number	Percent	Number	Percent
Ascites Fluid Neutrophil Count	Neutrocytic (>250) cells/mm3)	0	0	50	98.0
	Non-neutrocytic (<250cells/mm3)	166	96.4	1	2.0
	Total	166	98.8	51	100.0
Missing		2	1.2	0	0
Total		168	100.0	51	100
Ascites Fluid ALB	≤0.5 g/dL	14	8.3	49	96.1
	0.51 - 0.75 g/dL	128	76.2	0	0
	0.76 - 1.0 g/dL	18	10.7	51	100.0
	≥ 1.1	7	4.2	2	3.9
	Total	167	99.4	51	100
Missing		1	.6	0	0
Total		168	100.0	51	100
Ascites Fluid TP	≤1.5 g/dL	88	52.4	33	64.7
	> 1.5 g/dL	78	46.4	18	35.3
	Total	166	98.8	51	100.0
Missing		2	1.2	0	0
Total		168	100.0	51	100

5.3.3 Culture pattern of spontaneous bacterial peritonitis in cirrhosis liver

SBP was present in 51 (23.39%) patients. Of the 51 patients that developed SBP, culture positive SBP was present in 22% (11/50) and CNNA was found in 78.4 % (40/50). The prevalence of MNB was 1.96% (1/51) in this study. Among patients with the culture positive SBP, 4 (36.36%) positive cultures were due to E coli, 3 (27.27%) Klebsiella spp. 1 (9.09%) *Staphylococcus aureus*, *Streptococcus viridan* accounted for 1 (9.09%) and 2 (18.18 %) CoNs (coagulase negative staphylococcus species) of positive cultures (**Figure 2 and 3).**

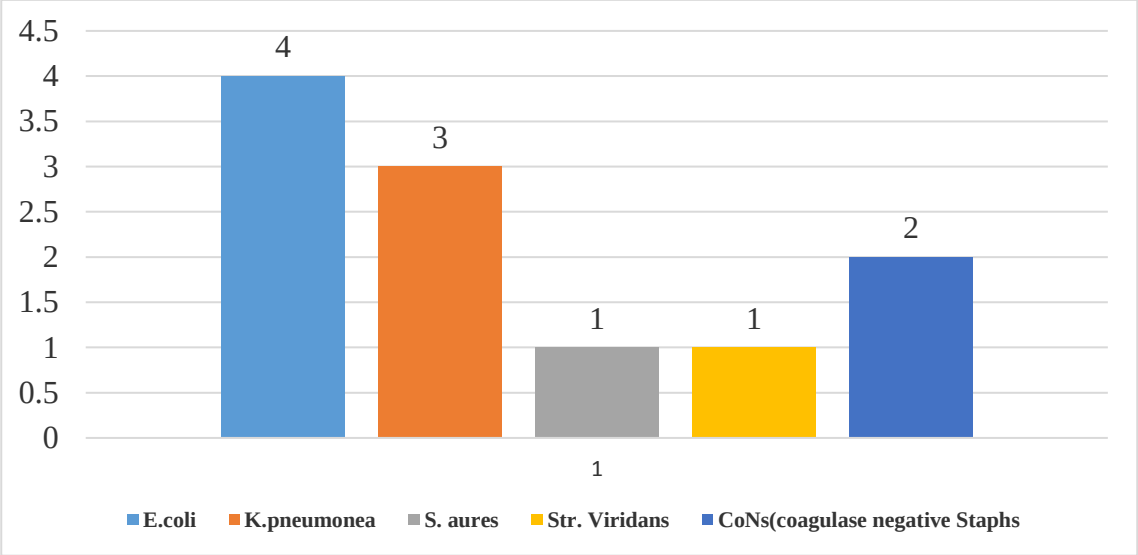

Figure 2. Microbial Isolates

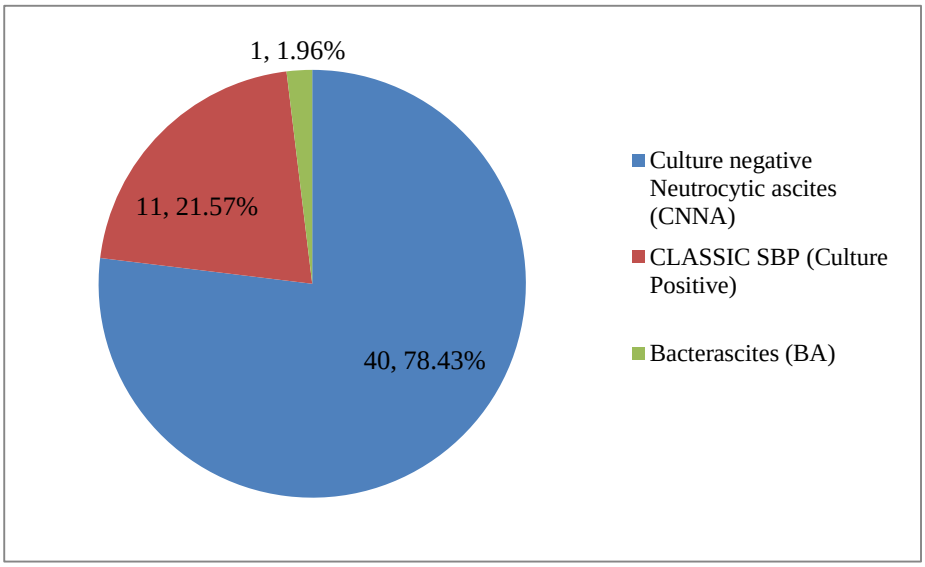

Figure 3. Variants of SBP

5.3.4 Antimicrobial Susceptibility test

Antibiotic disks used for gram-negative and gram-positive strains were Amikacin, Ampicilin, Augmentin, Cefazolin, Cefepime, Cefotaxime, Cefotetan, Cefoxitin, Ceftazidime, Ceftriaxone, Cefuroxime, Ciprofloxacin, Gentamycin, Meropenem, Levofloxacin, Tobramycin, Azithromycin, clindamycin, Doxycycline, Erythromycin, Oxacilin, Penicilin, tetracycline, Trimethoprim/Sulfomethoxazole and Vancomycin. Antibiotic disks were obtained from facilities.

The antibiotic resistance pattern of the isolated organisms indicated all of the gram-negative isolate were resistant to *Amoxicillin/K clavulanate, 85.71% to ampicillin, 57.14% to ceftriaxone,* 57.14% to *Cefazolin,* 42.85% to *Gentamycin/Sulfamethoxazole* 28.57 to *cefotaxime* and *Cefepime,* 14.28% to *amikacin, cefoxitin, ceftazidime* and *ciprofloxacin*. The isolated *S.aures* was resistant only to *penicillin* whereas *Str. viridans* was resistant to *ceftriaxone, cefotaxime, cefepime* and *penicillin*. However, the gram-negative organisms were 100% sensitive to *Meropenem,* and *levofloxacin*; 85.71% to *Cefotetan*, 71.43% to *Cefotaxime,* 57.14% to *Ceftazidime, Ciprofloxacin, Gentamycin, cefepime* and *Cefuroxime*; 42.85% to *Ceftriaxone, cefoxitime* and *cefazolin*, and 28.57% to *trimethoprim/Sulfamethoxazole*. The isolated *S.aures* was susceptible *Azitromycin, erythromycin, doxycycline, oxacillin, Gentamycin* and *vancomycin* whereas *Str.viridans* was susceptible only to *vancomycin (***Table 7).**

Table 7. Antimicrobial sensitivity of ascites fluid culture (n=9)

Antibiotic	**E.coli n=4 (36.365)**			**K. pneumoniae n=3 (27.27%)**			**Antibiotic**	**Streptococcus Viridans n = 1 (9.09%)**		
	R	**I**	**S**	**R**	**I**	**S**		**R**	**I**	**S**
Amikacin	1 (25%)	2 (50%)	1 (25%)		1 (33%)	2 (67%)	Ampicilin	1(100)		
Augmentin	4 (100%)			3 (100%)			Cefepime	1(100)		
Ampicillin	4 (100%)			2 (67%)	1(33%)		Cefotaxime	1(100)		
Cefazolin	4 (100%)					3 (100%)	Cefrtaxione	1 (100%)		
Cefepime	2 (50%)	1(25%)	1 (25%)			3 (100%)	Penicillin	1 (100%)		
Cefotaxime	2 (50%)		2 (50%)			3 (100%)	Vancomycin			1 (100%)
Cefotetan		1 (25%)	3(75%)			3 (100%)	**Antibiotic**	**Staphylococcus aureus n = 1 (9.09%)**		
Cefoxitin	1 (25%)	2 (50%)	1 (25%)	1 (33%)		2 (67%)		**R**	**I**	**S**
Ceftazidime	1 (25%)	1 (25%)	2 (50%)	1 (33%)		2 (67%)	Azithromycin			1 (100%)
Ceftriaxone	2 (50%)		2 (50%)	2 (67%)		1 (33%)	Clindamycin		1 (100%)	
Cefuroxime	2 (50%)	1 (25%)	1 (25%)			3 (100%)	Erythromycin			1 (100%)
Ciprofloxacin	1(25%)	1 (25%)	2 (50%)		1 (33%)	2 (67%)	Doxycycline			1| (100%)
Gentamicin	3 (75%)		1 (25%)			3 (100%)	Oxacillin			1| (100%)
Meropenem			4 (100%)			3 (100%)	Penicillin	1 (100%)		
Levofloxacin			4 (100%)			3 (100%)	Gentamycin			1| (100%)
Tobramycin	2 (50%)	1 (25%)	1 (25%)		1 (33%)	2 (67%)	Sulfamethoxazole/ trimethoprim		1 (100%)	
Trimethoprim\ sulphamethoxaz-ole	3(75%)	1 (25%)			1 (33%)	2 (67%)	Tetracycline			1 (100%)
							Tobramycin		1 (100%)	1 (100%)
							Vancomycin			1 (100%)

5.4 Prevalence of ascites fluid infection in adult cirrhosis patients in SPSHMMC and Yekatit 12 Hospital Medical College

A logistic regression was performed to discover the effect of age, gender, and marital status on the likelihood that participants have SBP. The logistic regression model was statistically insignificance for age (Chi square= 1.093; P value>0.05). The model explained 9% (Nagelkerkr R^2) of the variance in SBP and correctly classified 93.0 % of the cases (Table 8).

Table 8. Binomial logistic regression for demographic predictor variables

Independent Variables	Sig.	Exp. (B)	95% C.I. for EXP(B)	
			Lower	Upper
Age (18-40)	0.948	0.956	0.250	3.653
Gender (Male)	0.608	1.205	0.591	2.456
Marital status (Married)	0.878	0.832	0.080	8.620

Abdominal distension (62%), pedal oedema (60%), abdominal pains (62%) and jaundice (52) were the main clinical features. History of jaundice, low arterial blood pressure on admission and Fever were found to be independent predictors of spontaneous bacterial peritonitis. Ultrasound study depicted shrunken liver and enlarged spleen in size was the independent predictor of spontaneous bacterial peritonitis (Table **9).**

Table: 9. Multiple Logistic regression model of independent risk factors (clinical features) of Spontaneous Ascites fluid infection.

Independent Predictor	p-value	Adjusted odds ratio	95% CI
UGB	0.74	0.868	0.377 -1.999
Abdominal Pain	0.598	1.259	0.534 - 2.968
Jaundice	0.004*	3.465	1.479 - 8.117
Sleeping disturbance	0.327	0.669	0.300 - 1.493
Previous episode of SBP	0.197	0.553	0.225 - 1.359
Abdominal distention	0.304	0.602	0.229 - 1.585
Weight Loss	0.223	1.752	0.711 - 4.317
Fever	0.032*	2.651	1.088 - 6.453
Chills	0.092	2.083	0.888 - 4.885
pedal edema	0.498	1.383	0.541 - 3.531
Systolic blood pressure	0.0065*	4.794	1.552- 14.809
Diastolic blood pressure	0.711	0.805	0.255 - 2.538
Body Mass index	0.197	0.588	0.262 - 1.318
Maximum liver span	0.000*	7.521	2.620 – 21.590
Nodularity of liver surface (its longest axis)	0.313	1.577	0.650 - 3.822
Spleen size	0.023*	2.71	1.147 - 6.400
Collateral vessels	0.438	1.958	0.358 - 10.695
PVD	0.052	2.82	0.989 - 8.039

Laboratory parameters significantly predicting the presence of spontaneous bacterial peritonitis (SBP) were ALT (OR=1.026, p= 0.033, Chi-square = 187.172), serum Albumin (OR= 4.173, p=0.024, Chi-square= 45.393), total WBC count (OR=1.343, p < 0.001, chi-square= 173.323) platelet count (OR = 90.15, p<0.001, chi- square=32.157), INR (OR = 0.52, p<0.001, chi-square=47.049) and ascites albumin (OR=522.66, P= 0.000, Chi-square= 155.431), The overall model was also statistically significant (LR Chi2 = 211.430; p= 0.000) in predicting the presence of spontaneous bacterial peritonitis (SBP) (**Table 10**).

Table 10. Multiple Logistic regression models of independent risk factors (laboratory parameters) of Spontaneous Ascites fluid infection.

Independent risk factors	P-value	Adjusted odds ratio	95% Confidence Interval
AST (U/l)	0.094	0.983	0.963 - 1.003
ALT(U/l)	0.033*	1.026	1.002 - 1.05
ALP(U/l)	0.334	0.996	0.989 - 1.004
TB(U/l)	0.055	1.462	0.993 - 2.153
DB (mg/dL)	0.191	0.667	0.363 - 1.225
Serum Albumin (g/dL)	0.024*	4.173	1.202 - 14.486
TP Serum (g/dL)	0.076	20.29	0.728 - 565.356
Creatinine (mg/dL)	0.242	0.837	0.621 - 1.128
Urea (mg/dL)	0.137	1.007	0.998 - 1.016
Na	0.426	1.014	0.979 - 1.05
K	0.949	0.981	0.549 - 1.755
Cl	0.404	1.002	0.997 - 1.006
WBC	0.000*	1.343	1.207 - 1.493
Hgb (g/dL)	0.944	1.006	0.86 - 1.177
PLT (10^3/uL)	0.000*	90.15	7.392 - 1099.396
INR	0.000*	15.081	6.047 – 37.616
HBV	0.283	0.684	0.342 - 1.368
HCV	0.619	1.3	0.462 - 3.662
Total Protein Ascites (g/dL)	0.303	1.500	0.693 - 3.246
Alb Ascites (g/dL)	.000*	522.667	66.191 - 4127.136

Among different microorganisms isolated from Ascites fluid samples in the two hospitals gram negatives (63.36%) were the most prevalent causative microorganism isolated. As a whole, 18.18% of the culture-positive episodes of SAFI were owing to skin contamination CoNs.

6 Discussion

All cirrhotic patients with ascites can develop spontaneous ascites fluid infection. The prevalence of SBP in hospitalized patients range between 10%-30%. (22) With the early diagnosis of the disease, immediate and relevant antibiotic treatment, the in-patient mortality of an episode of SBP has been reduced to nearly 20%.(36**)** This study was conducted in the Department of internal Medicine, St. Paul millennium medical college and Yekatit 12 hospital medical college, Addis Ababa from march 2020 through march 2021. The study included a total of 218 patients with chronic liver disease who has ascites. Out of which 67% patients were males and 33% were females. All patients underwent diagnostic paracentesis after consent issued. 51 (23.4%) out of 218 patients were found to have SAFI, out of which 19 (39%) were female and 32 (61%) were male. A study by Kahit L. et al, (23) reported slightly lower prevalence of 21% in hospitalized patients. The mean age of presentation was 38.67 ±12.0 years (age range 19 to 76 years) (P= 0.408) which is remarkably lower than a study by Dinis-Ribeiro M *et al*. (37). In relation to the cause, the most common cause of cirrhosis in this study population was HBV 133 (61%), alcohol 63 (28.9%) and hepatitis C 22 (10%). This pattern was also different from other studies that observed in their reports, (23, 24 and 26) as most of the participants recruited from St. Paul specialized hospital millennium medical college who were visiting hepatology corner and or probably reflects that the increased prevalence of hepatitis B and C. Most of these patients have cirrhosis with portal hypertension as a complication of chronic hepatitis infection, and the conditions are more prevalent among men than females. (38, 39)

The prevalence of SAFI among adult patients with ascites who were attending internal medicine at SPHMMC and Yekatit 12 hospital medical college was 23.4%. Of the 51 patients that developed SAFI, classic SBP was present in 21.57% (11/51) while CNNA was found in 78.43% (40/51). The prevalence of MNB was 10% (1/51) in this study. The prevalence of SBP in this study was slightly higher compared with 21.4% and 21% reported by Duah A et al**.,** in Ghana **(30)** and Levica K et al **(23)**, in India respectively. This may be due to the entire participant in this study were patients only with Chronic liver disease.

Classic SBP reported by Duah et al was 26.67% **(30)** higher than this study while CNNA reported in this study was higher than reported by the same study (63.33%) and lower than 83.33% reported by Levica K *et al* (23). The prevalence of MNB in this study was 1.96%, lower than the

10% prevalence reported by same study, and 26% by Oladimeji et al. [31] but nearly with that of 2.38% reported by Levica K et al (23). The differences in prevalence could be explained by differences in culture methods and techniques used as this study used manual culture method whereas almost all of the literature referred here used automethod culturing which has higher sensitivity than manual method. Recent use of antibiotics may also contribute to the relatively low prevalence of culture positive SBP as most of SBP suspected patients treated empirically before paracentesis procedure.

In this study E. coli (36.36%) was the most common organism isolated followed by Klebsiella species (27.27%). The rest of the organisms isolated were *Staphylococcus aureus* (9.09%), CoNs (coagulase negative staphylococcus *species* (18.18%) and *Streptococcus viridans* (9.09%). The isolation of these organisms is similar with studies done by Duah et al, (30) and Oladimeji et al, (31) both showed E. coli as the dominant bacteria cultured in patients with spontaneous bacterial peritonitis.

All of the participants with SBP in this study were associated with ascites caused by chronic liver disease. This supports the report in literature that SBP associated with causes apart from ascites caused by chronic liver disease are rare enough to be the subject of case reports. The causes of ascites in this study apart from liver disease were few and that can also account for the predominance of SBP in ascites caused by cirrhosis. The heterogeneity of the clinical and laboratory findings that is associated with the presence of SBP has been reported in various studies (23, 30, 31, 32).

This justifies the indication for diagnostic paracentesis in all patients with ascites visiting in the hospital. Evan's et al,(22) did not identify any clinical or laboratory parameters to be associated with the presence of SBP whilst Figueiredo et al,(40) identified serum albumin, complement C4 of Ascites fluid and upper gastrointestinal bleeding as independent predictors for the diagnosis of SBP. Guarner et al**,** (41) also identified only serum bilirubin and platelet count as independent correlation with the presence of SBP.

In this study low systolic blood pressure, jaundice, Fever, reduced platelet count, raised INR, shrunken liver and enlarged spleen size, elevated ALT level and low serum albumin, presented independent correlation with the development of ascites fluid infection. Low blood pressure was

found to have significant association with development of ascites fluid infection. This can be the first manifestation of ascites fluid infection or a complication of SAFI.

The presence of jaundice in cirrhotic patient reflects the underlying worsening of the liver condition or reflects bacteremia causing intravascular hemolysis. High INR, low platelet and low Ascites fluid albumin are consistent with advanced stage liver cirrhosis and SAFI is high in patients with severe liver disease (23) Fever and chills are common symptoms of infection and are also found in patients with ascites fluid infection. In this study, fever at the time of presentation predicted SAFI on multivariate analysis.

The antibiotic resistance rates could vary in different country based on the pattern of antibiotic consumption. A number of studies were conducted in different countries to assess the efficacy of the current guideline and help the clinicians to choose the most appropriate antibiotic as first-line treatment. Recent studies notice the emergence of resistance to third-generation cephalosporins (*cefotaxime* (62.5%, 85.7%), *ceftazidime* (73%, 82.1%) when the investigators tend to assess the trend of antibiotic resistance over time after they dichotomizing the samples into three-year periods (2005- 2008 and 2008-2011) (42). In this study the number of resistance organisms to third generation cephalosporins (*ceftriaxone, cefotaxime, ceftazidime, cefexitin* were 57.14%, 28.57% 14.28% and 14.28% respectively) lower than the above and other study even though the number of isolate were too small for generalization. *E. coli* was most sensitive to Meropenum, and Levofloxacin while *K. pnemoniea* sensitive to *cefazolin, Cefepime, Cefotaxime, meropenum* and *levofloxacin.*

Overall the antibiotic resistance pattern of the isolated organisms indicated 100% of the gram negative isolate were resistant to *Amoxicillin/K clavulanate*, 85.71% to *ampicillin*, 57.14% to *ceftriaxone*, 57.14% to *Cefazolin*, 42.85% to *Gentamycin* and *Sulfamethoxazole* 28.57% to *cefotaxime* and *Cefepime,* 14.28% to *amikacin, cefoxitin, ceftazidime* and *ciprofloxacin.* The isolated *S.aures* was resistant only to penicillin whereas *Strep.viridans* was resistant to *ceftriaxone, cefotaxime, cefepime* and *penicillin*. However, the gram negative organisms were 100% sensitive to *Meropenem,* and *levofloxacin*; 85.71% to *Cefotetan*, 71.43% to *Cefotaxime,* 57.14% to *Ceftazidime, Ciprofloxacin, Gentamycin, cefepime* and *Cefuroxime*; 42.85% to *Ceftriaxone, cefoxitime and cefazolin*, and 28.57% to *trimethoprim/Sulfamethoxazole*. The isolated *S.aures* was susceptible *Azitromycin, erythromycin, doxycycline, oxacillin, Gentamycin* and *vancomycin* whereas *Strep.viridans* was susceptible only to *vancomycin.*

7 Limitation of the study

This study was not without limitations. The diagnosis of cirrhosis in this study was based mainly on clinical, laboratory and radiologic examinations. This method of diagnosis without any histologic/Biopsy basis may be less accurate as other causes of ascites could have been missed. Also, the mode of sampling had the potential of introducing selection bias as most of the participants were recruited from hepatology department of SPSHMMC where hepetitis case is more prevalent and this may cause falsely decreased alcoholic liver cirrhosis; the study included both symptomatic and asymptomatic patients which may affect the positive rate negatively.

Due to the Covid-19 pandemic most study group were not visiting hospitals as they were more prone to this pandemic as result of their immune status and this may cause biased sampling method.

Some of the participants were on antibiotic therapy before the sample collected and this may cause false culture negative result.

The method used as enrichment media (broth Media) is manually prepared which may have less sensitive than auto method hence if fully auto method applied, the culture positive rate may be enhanced. AST was also performed manually using Muller Hinton agar plate which has its limitation as some microbe on the plate may not grow resulting false susceptible report.

8 Conclusion and Recommendation

8.1 Conclusion

Spontaneous Ascites fluid infection (SAFI) was common among cirrhotic patients with ascites visited and or admitted at SPSHMMC and Yekatit 12 hospital medical college. Jaundice, low blood pressure, high INR, low platelet, low serum albumin, increased WBC, low Ascites fluid albumin, shrunken liver and increased spleen in size were highly indicative of SAFI and diagnostic paracentesis should be done instantly on admission to confirm the diagnosis preferably before starting empiric antibiotic therapy to enhance culture positive rate. All gram negative microorganisms isolated were susceptible to Meropenem and Levofloxacin while all the gram positive isolate were susceptible to vancomycin.

8.2 Recommendations

This finding indicated that the gram negative enterobacteriaceas are still the major causes of spontaneous ascites fluid infection in this setting while the gram positive organisms can be the causative agents for SBP. If the research was done on more advanced method such as automated culture method instead of manual, the number of microorganism isolated increased perhaps changing, balancing or shifting the microbial profile.

Most of the isolated organisms were from symptomatic patients hence the next study may be conducted on similar topic should focus on patients who are symptomatic for SBP.

Treatment initiation for SBP suspected patients should rely on culture result unless and other wise an empiric treatment considered common gram negative and positive causative agents.

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10 Annexes

10.1 Information sheet in English Version

Title of the Research Project: Patterns of Ascites fluid infections, etiology and antimicrobial susceptibility among adult Cirrhosis patients attending at St. Paul Specialized Hospital Millennium and Yekatit 12 Hospital Medical Colleges /AAU-CLS, A.A Ethiopia from march 2020 –march 2021 G.C.

Principal Investigator: Abubeker Shemsu (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 a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, three advisors; Two from Addis Ababa University CLS department and one from selected public health hospital internal medicine department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess Patterns of Ascitec fluid infection in cirrhosis patients.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of locally produced data for indigenous population. Therefore, the purpose of this proposed study is to Establish base line data for Microbial characteristic, antimicrobial profile and the different risk factors for SBP in cirrhosis patients aged $\geq$18 years in Addis Ababa, Ethiopia. You have been chosen for this study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous data. The data are needed for providing better management of cirrhosis patients with SBP. Thus, result

from this study is anticipated to improve the health status of the adult population with cirrhosis at large in Ethiopia.

Procedures and the expected participation

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

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide peritoneal fluid which will be collected by specialized staff in gastroenterologist sub unit upon your admission. We will also collect 15 ml venous blood (about 1 table spoon) from you by sterile-disposable vacutainer tube and needle (7ml in SST, 4 ml in tube containing EDTA and 4 ml in citrated tube) as part of clinical utility. We will conduct laboratory examination to determine different hematological, serological, and clinical chemistry parameters.

Potential risks and Discomforts

There will be moderate discomfort in paracentesis procedure. However, there might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory professionals.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be

asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and managing of SBP. Hence, you are indirectly benefiting other patients and the society in this respect.

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. Establishing the data will be used in the future to improve the general health status of Ethiopians particularly cirrhosis patients.

Participation and Withdrawal from the Study

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

Contact information

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

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የተሳታፊዎችፈቃድናመተማመኛቅፅ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመመረቂያጥናትላይእዲሳተፉተጋብዘዋል፡፡እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበጥሞናያንብቡናግልጽያልሆነልዎትንማንኛዉምሃሳብይጠይቁ፡፡

መግቢያ

የጥናቱርዕስ “Patterns of Ascites fluid infections, etiology and antimicrobial profile among adult Cirrhosis patients attending at St. Paul Specialized Hospital Millennium and Yekatit 12 Hospital Medical Colleges /AAU-CLS, A.A Ethiopia.”.

የእርስዎበዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎፈቃደኝነትላይየተመሰረተነዉ::በዚህጥናትዉስጥላለመሳተፍወይ ምለመሳተፍከወሰኑበኃላለማቋረጥየሚወስኑቢሆንምእንኩዋበዚህሆስፒታልየሚሰጠዉማንኛዉምአገልግሎትአይቋረጥም::በጥ ናቱለመሳተፍየሚስማሙከሆነየስምምነትቅጹላይበጹሁፍወይምበጣትፊርማማስቀመጥይጠበቅዎታል::

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህጥናትለመሳተፍየሚስማሙከሆነናሙናዎለጥናቱእንዲሚዉልመስማማትይጠበቅብዎታል::ከተወሰደዉናሙናላይየሚገኙ መረጃዎችከዚህሆስፒታልዉጭለሚገኙናለስራዉአግባብነትላላቸዉሰዎችቢነገርየማይቃወሙመሆኑንመስማማትይጠበቅብዎ ታል::ይሁንእንጅይህአይነቱመረጃየርስዎንማንነትየሚገልጡመረጃዎችንማለትምስም፤አድራሻናየስልክቁጥርየመሳሰሉትንመረጃ ዎቸንአይጨምርም::ይልቁንምለዚህአገልግሎትብቻየሚዉልእርስዎንለማወቅየሚያስችልመለያቁጥርጥቅምላይእንዲዉልይደረ ጋል::በተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለሚቀርቡአንዳንድተጨማሪጥያቄዎችመልስመስጠትይኖርብዎትአል::

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም::ሆኖምግንናሙናዉንለመሰብሰብልምድያለዉባለሙያስለ ሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖርም::

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎየሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥናቱአላማብቻነዉ::ይህን ማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው::ከዚያምበላይስለእርስዎያለውንማንኛውንምመረጃየተለየይ ለፍቃልባለውየኮምፒውተርየመረጃማህደርውስጥእንዲቀመጥይደረጋል::

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞ ችምንድን ናቸው ?

ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልዎበገንዘብየሚያገኙትጥቅምባይኖርምከጥናቱበሚገ ኝውውጤትግንተጠቃሚነዎት::የእርሶዎተሳትፎየእርስዎንናየወገንዎትንየደምካንሰርለማወቅናለማከታተልከፍተኛጥቅምይኖረ ዋል::

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናበታየማቋረጥሙሉመብትየተጠበቀ ከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምከንያትየሚቀርብዎትምንምአይነትየሆስፒታልአገልግሎትአይኖርም፡፡ከዚህምበተ ጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየመጠየቅናገለጻየማግኘትመብትአለብዎት፡፡የላብራቶሪምርመራውጤቱንም በነጻማግኘትይችላሉ፡፡ነገርግንእርስዎበሚሰጡንመረጃየችግሩንስፋትለመከላከልእናለመቆጣጠርጠቃሚስለሆነለሚቀርብልዎት ጥያቄቀጥተኛመልስይሰጡንዘንድበታላቅአክብሮትእንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋችወይምጥያቄካለዎትበሚመለከ ተውአድራሻይጠቀሙ፡፡

አቡበከርሸምሱ

ሞባይል፡ +251-911916568

ኢሜል፡bakribinhalil@gmail.com

10.2 Annex 2. Informed consent form in English version

Participants ID…..……

I had been informed that the objective of this study is to assess *Patterns of Ascites fluid infections, etiology and antimicrobial susceptibility among adult Cirrhosis patients attending at St. Paul Specialized Hospital Millennium and Yekatit 12 Hospital Medical Colleges*. /AAU-CLS, A.A Ethiopia. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing and management of SBP. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood and peritoneal fluid, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

10.3 Informed consent form in Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔስሜከላይየተጠቀሰውተሳታፊ “Patterns of Ascites fluid infections, etiology and antimicrobial susceptibility among adult Cirrhosis patients attending at St. Paul Specialized Hospital Millennium and Yekatit 12 Hospital Medical Colleges/AAU-CLS, A.A Ethiopia.

’’ጥናትላይበቂገለጻተደርጎልኛል፡፡ለጥናቱምደምናየናሙናእንደሚያስፈልግተገልጾልኛል፡፡የጥናቱንምአላማዎችምተረድቻለሁ፡፡

በቃለመጠይቁላይየገለጽኳቸውመረጃዎችበሙሉበሚስጥርየተጠበቁእንደሚሆኑተነግሮኛል፡፡በጥናቱላይያለመሳተፍናማንኛውንምመረጃያለመስጠትእንዲሁምበማንኛውምጊዜከጥናቱራሴንየማግለልመብቴየተጠበቀእንደሆነተገልጾልኛል፡፡

ስለዚህለዚህጥናትመረጃናየስምምነትቃሌንየሰጠሁትበአጠቃላይሁኔታውንበመረዳትናበፍጹምፍቃደኝነትነው፡በተጨማሪምጥያቄለመጠየቅተፈቅዶልኝለማወቅየፈለኩትንያህልማብራሪያአግኝቻለሁ፡፡የዚህጥናትተሳታፊበመሆኔየማገኘውጥቅምየሁሉንምምርመራውጤትበነጻማግኘትእንደሆነተረድቻለሁ፡፡

በአጠቃላይእኔከላይበመተማመኛቅፅየተጠቀሱትንሁሉበሚገባናበተረጋጋመንፈስአንብቤዋለሁኝ፡፡ስለዚህበዚህጥናትለመሳተፍፈቃደኛመሆኔንበፊርማዬአረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም ---------- ፊርማ ----------

10.4 Annex-3 - Questioner

Participant ID: _____

Part –I: Demographic information

1. Age: _____ sex: **Male** ☐ **Female** ☐
2. Marital Status: **Single** ☐ **Married** ☐ **Separated** ☐ **Disparate** ☐

Part-II: Clinical Signs and symptoms

1. Does the participant have history of upper GI bleeding? **Yes** ☐ **No** ☐
2. Does the participant have been on long term antibiotic prophylaxis? **Yes** ☐ **No** ☐
3. Was the participant on antibiotic treatment for more than 6 hour? **Yes** ☐ **No** ☐
4. Are you feeling abdominal pain? **Yes** ☐ **No** ☐
5. Does the participant have history of jaundice? **Yes** ☐ **No** ☐
6. Does the participant have symptom of sleeping disturbance or memory impairment: **Yes** ☐ **No** ☐
7. Does the participant face previous episodes of spontaneous bacterial peritonitis?

   Yes ☐ **No** ☐
8. Does the participant have abdominal distension? **Yes** ☐ **No** ☐
9. Does the participant have fever? **Yes** ☐ **No** ☐
10. Does the participant have chills? **Yes** ☐ **No** ☐
11. Does the participant have pedal edema? **Yes** ☐ **No** ☐
12. Does the participant have chronic liver disease? **Yes** ☐ **No** ☐
13. Arterial BP; Systolic _____(L,N,H) Diastolic:_____(L,N,H)

Part-III: Ultrasound investigation

14. Maximum vertical span of the liver:_____
15. Nodularity of liver surface:_____
16. Spleen size (length of its longest axis):_____

17. Presence of collateral vessels:_____

18. Portal vein dimension and presence of ascites:_____

Participant ID: _____

Part-IV: Laboratory investigation record sheet.

19. Ascitec fluid neutrocytic count_____ >250, <250

20. Ascitec fluid albumin level:_____ (L,N,H)

21. Ascitec fluid total protein):_____(L,N,H)

22. Ascitec flood culture: on blood bottle: Growth No growth.

23. If there is growth, type of bacterial isolate(s) is_____(gram reaction positive/Negative)

24. The name of identified bacterial pathogen(s):_____
    _____
    _____

25. The Bacterial drug Sensitivity pattern

    Sensitive to: _____
    _____
    _____

    Resistant to:_____
    _____
    _____

26. LFT(AST_____,(L,N,H), ALT_____(L,N,H) ALP_____(L,N,H)

    TB_____(L,N,H) DB_____ (L,N,H)serum Albumin_____ (L,N,H) TP_____ (L,N,H)

27. Hematology: WBC_____ (L,N,H) Hgb_____ (L,N,H) PLT count_____,(L,N,H)INR_____(L,N,H)

28. RFT(Creatinine_____(L,N,H) Urea_____,(L,N,H)GGT_____(L,N,H)

29. Electrolytes (Na+_____(L,N,H), K+_____(L,N,H)

30. HBsAg: Pos. Neg.

31. HCV: Pos. Neg.

.

10.5 Annex-4: Sops and Guidelines

10.1.1. Sop for culture and sensitivity

10.1.2. CLSI Performance Standards for Antimicrobial Susceptibility Testing, January 2020, 30th Edition.

Declaration

I, the undersigned, declare that this thesis proposal is intended to be done for the fulfillment of MSc degree in Diagnostic and public health Microbiology at Addis Ababa University. This project is not done in any other university in Ethiopia and that all sources of materials may be used for the production of this thesis will be duly acknowledged by AAU.

M.Sc. candidate: **Abubeker Shemsu (BSc, MSc candidate)**

Signature: _____

Date of submission: _____

This Proposal has been submitted with our approval as advisors.

Advisor: **Kassu Desta (MSc, PhD Fellew, prof. of Medical microbiology)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Shambel Araya (Bsc, Msc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia

Advisor: **Dr.Yohannes Birhanu (MD, Gastroenterology and hepathologist)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.