ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

BACTERIAL PROFILES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS FROM BODY FLUIDS AT TIKUR ANBESSA SPECIALIZED HOSPITAL, ADDIS ABABA, ETHIOPIA.

BY: **FREHIWOT TEKLRHAYMANOT**

A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY SPECIFITY

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This is to testify that the thesis is prepared by Frehiwot Teklehaymanot, which is entitled with Bacterial isolate and Antimicrobial susceptibility pattern from body fluids at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, and submitted in partial fulfillment of the requirements for the degree of Master of Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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LIST OF ABBREVIATIONS AND ACRONYMS

AST----------Antimicrobial Susceptibility Test

APD----------Ambulatory peritoneal dialysis

CDC----------Communicable Disease Control and Prevention

CSF----------Synovial fluid

CLSI----------Clinical and Laboratory Standards Institute

EMA----------Ethiopian Medical Association

EMLA ----------Ethiopian Medical Laboratory Association

EPHA ----------Ethiopian Public Health Association

Hib----------Homophiles influenza type b

HRS----------Hepatorenal syndrome

LPL ----------Lumbar puncture

PMN----------Polymorpho-nuclear leukocytes

RA ----------Rheumatoid arthritis

SAH ----------Subarachnoid hemorrhage

SBP----------Spontaneous bacterial peritonitis

ABSTRACT

Background: Body fluids serve as a medium for carrying nutrients and waste products. These fluids are usually sterile, even if a single colony of bacteria may be significant to invade and infect to body fluids and leads to morbidity and mortality. Assessing to the prevalence of bacteria and testing their antibiotic susceptibility helps to provide effective therapies, develop rational prescription writing and make policy decisions.

Objective: The aim of this study was determining bacterial profiles and their antimicrobial susceptibility patterns from body fluids at Tikur Anbessa Specialized Hospital, laboratory Addis Ababa, Ethiopia.

Method: A cross sectional study was conducted from july 2015 to September 2015 and 384 study participants were required. CSF, acsites, pleural and synovial fluid were collected and cultured on Blood agar, MacConkey agar and chocolate agar and incubated according to standard conditions. All specimens were subjected to Gram Stain, WBC count and AFB immediately following culturing. All culture positives were identified by gram stain and biochemical tests using the standard procedure and antimicrobial susceptibility test was performed using Kirby-Bauer method. Data was entered and analyzed using SPSS version 22.

Result: In this study 54/384(14. 0%) of bacteria were isolated from different body fluids such as; Cerebrospinal fluids 264(68.8%), Pleural fluid 76(19.8%), Peritoneal fluid 34(8.9%), and Synovial fluid 10 (2.6%) and using gram stains 41/384 (10.7%) of bacteria were seen from the total of body fluids. Among these majority of body fluids 173(44.1%) had abnormal white blood cell count (WBC) above 05 cells per mm^3, out of them 91(21.1%) had polymorphic feature. *K. pneumonia* 16.7 %(9/32) were the most common isolated bacterial following by *coagulase negative Staphylococcus* 15.0 %(8/32). Among all body fluid in CSF (31(57.4%) were the highest bacterial isolated. Out of 54 isolates, 41(75.9%) of bacteria were showing multi drug resistance. Gentamycin (76%) and erythromycin (59%) were the highest drug resistanc for gram-negative and gram-positive respectively.

Conclusion: The prevalence of bacterial isolates in this study was 14.0%. Frequency of single as well as multiple drug resistance was high. Over all 41/54, (75.9%) isolates had shown Multiple-drug-resistance of (MDR≥2) drugs.

1. INTRODUCTION

1.1 Back ground

Body fluids are important in transporting nutrients and waste products, regulating body temperature, and assessing respiration process. Different types of body fluids found in our body such as Pleural fluid, peritoneal fluid, Cerebrospinal fluid, Synovial, and Pericardial fluids. These fluids are usually free of microorganism's including bacteria, fungi, virus and parasites, if there is invasion of microorganism can lead to morbidity and mortality [1, 2].

Cerebrospinal fluid (CSF) is produced by ultra filtration or secretion, and circulates through the ventricles and spinal cord, when compared to with plasma has less protein concentration. Bacterial, fungal and viral infection could results meningitis. Among these microorganisms', bacterial meningitis is the leading cause of meningitis which often presents acutely in medical emergency [3].

Meningitis refers to the inflammation of the meninges, which line the central nervous system (CNS), which characterized by severe headache, fever, intolerance to light and sound, rigidity of muscles, seizures, raised intracranial pressure, and stroke. As the result of these, microbiological examination of CSF is the "gold standard" for diagnosis of bacterial meningitis and it is equally important to obtain the antimicrobial susceptibility of the causative bacteria to rationalized treatment. The most common bacteria that cause meningitis are *Streptococcus pneumonia*, *Neisseria meningitides; Streptococci group B, Listeria monocytogenes, and Haemophilus influenza*. In children, *Haemophilus influenzae type b* (Hib), and pneumococcal infections are the most common causes [4]. In patents with meningitis CSF, appearance could be cloudy, depending on the presence of significant concentrations of WBCs, RBCs, bacteria, and/or protein. In the present of bacterial infection in CSF, the WBC count is elevated between the range of 1000– 5000 cells/mm3 leading to a neutrophil between 80% and 95% the glucose concentration becomes decreasing around 40 mg/dL and elevated the concentration of protein [5].

Peritoneal effusions are the term of 'ascites' refers to the detectable and pathologic collection of fluid in the peritoneal cavity [6, 7]. 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. Ascites occurs, when portal hypertension developed and disables to excrete an adequate amount of sodium into urine [8]. Ascites differentiate into transudative or exudative effusions, the concept of exudative indicates the present of inflammatory or tumor with high protein concentrations, where as transudation as result of systemic conditions such as cirrhosis, heart failure or nephrotic syndrome with low protein concentrations [9, 10]. All ascitic fluid screened for the development of spontaneous bacterial peritonitis (SBP) using the gold standard procedures of polymorph nuclear leukocytes (PMN) leukocyte count in ascitic fluid $\geq 250 \times 10^6$ / L. [11, 12]. The passage of bacteria from the intestinal lumen to mesenteric lymph nodes or other extraintestinal sites across the intestinal- mucosal barrier consider as pathogenesis of SBS like non-enteric *Streptococcus sp* and Gram-negative aerobic enterobacteria *Escherichia coli* (present in approximately 70% of cases) and *Klebsiella* sp are the most commonly involve [13, 14, 15].

Pleural effusions a result of excessive fluid accumulation in the pleural space the appearance is clear, straw-colored, odorless and non-viscous fluids. These fluid classfied into transudative or exudative, the transudative caused by systemic non-inflammatory conditions such as heart failure and cirrhosis. The exudative caused by a malignant or an inflammatory, like pneumonia, tuberculosis. In general the exudateve pleural fluids elevate the WBC $> 500 \times 10^6$ / L leukocytes, with differential of neutrophil [10, 16]. It reflects the stage of the inflammatory response and narrows the diagnostic possibilities. Neutrophil predominance indicates an acute inflammatory process affecting the pleura that may occur in the case of parapneumonia, pulmonary embolism, viral infections, gastrointestinal diseases and tuberculous pleuritis. Predominance of mononuclear cells indicates a subacute or chronic process [17]. Empyema thoracis (ET) is accumulation of pus in the pleural space remains a very significant cause of childhood mortality and morbidity in children caused by *Staphylococcus aureus*, *S. pneumoniae,* group A *streptococci* or *Haemophilus influenza* [18]. Bacterial pneumonia is the most common cause of pleural effusion in AIDS, *Streptococcus pneumoniae* accounts for more than 50 percent of these parapneumonic effusions[19].While less frequent caused by *Haemophilus influenzae, Mycoplasma pneumonia* result in small pleural effusions[20,21].

Joint fluid is called synovial fluid. It is a viscous substance lubricates to most joints, an indicatives of inflammation of joints. Various microorganisms may be involved in arthritis like, viruses, fungi, bacteria such as; *Mycoplasma, Staphyllococcus aureus, Staphylococcus epidermidis, streptococcus pyogenes, Neisseria gonorrhoeae, gram negative bacilli,* and *Mycotuberculosis* [22]. Rheumatoid arthritis (RA) is an autoimmune systemic disorder, characterized by synovial hyperplasia and chronic inflammation. Experimental evidence has suggested the presence of bacterial cell wall fragments and bacterial DNA from *Neisseria*, *Acinetobacter*, *Shigella, Salmonella, Pseudomonas* and *Mycoplasma spp* identified in inflamed joints of patients with various arthritis groups [23, 24].

Body fluid are naturally sterile, bacteria may be invade and infect the body fluids and cause sever deases; however in the present of treatment bacteria may develop several mechanism of drug resistance against various antibiotics. The infections caused by these resistant bacteria do not respond to treatment, leading to morbidity and mortality. Now-a-days, bacterial drug resistance is an important problem, and due to wide variation in bacterial drug resistance, results of various studies and reports in one region or in one period are not necessarily true for other region or periods [25].

1.2. Statement of the problem

Bacterial meningitis is a medical emergency that require urgent rational antibiotics therapy. Bacterial meningitis is an estimated 200,000 deaths worldwide per year, and the cases fatality rates reported between 2% and 30% with the mental retardation rate of 10%–20% cases [26, 27]. Despite of the effective antibiotics, the mortality rate is higher in developing countries, ranging from 16-32% especially in sub-Saharan Africa an estimated 300--million-population annually, and the epidemiology study in Ethiopians shows the fatality rates for meningitis and meningococcemia to be 16% and 18% respectively [28]. Bacterial meningitis are majorly caused by, which is about 80% of all cases of, *N. meningitidis, S.pneumoniae, and H. influenza* [29].

Pleural effusion is an abnormal collection of fluid in the pleural space due to malignancy or infectious cause with mortality rate of 6.4% of the patients. Pleural infusions in developed countries are caused due to malignancy while in developing countries it is mainly caused due to bacterial infections [30]. Parapneumonic effusion, defined as pleural effusions associate with lung infection, occurs up to 50% of the case due to bacterial pneumonia. Among these bacterial pneumonia 70% cases due to the bacterias of *Haemophilus influenzae*, *Mycobacterium tuberculosis*, Streptococcus *pneumoniae* and *Staphylococcus aureus* [30].

Peritonitis (SBP) is a frequent, and severe complication in cirrhotic patients with ascites, study described that the risk of SBP increased in hospitalized patients with prevalence of 10% to 30%, although the mortality rate was reported upto 90% [31]. Since *Enterobacteriaceae* and streptococci cause the majority of spontaneous infections in cirrhosis, while the drug of β-lactams and quinolones have been used widely in their treatment and the bacterial character changes through time and increase the multidrug resistant (MDR) of bacteria [32].

Though few studies have done in Ethiopia, they are insufficient to assess the rates of body fluid infections and occurrence of multidrug resistant bacterias. In addition, these studies showed that the increasing prevalence of body fluid infections and occurans of multidrug

resistance patterns of bacteria isolates suggesting that further studies to be conducted in different parts of Ethiopia. More over antibiotic resistance is changing day by day [25] Hence, the aim of this study was to determine the prevalence of bacterial isolate and their susceptibility pattern from body fluids at Tikur Anbessa Specialized Hospital laboratory, Addis Ababa, Ethiopia.

1.3. Significance of the study

This study provides current information on bacteria ethiology that cause body fluid infections. Most importantly, this study indicates the current picture of antimicrobial resistance patterns of bacterial isolates of body fluid infections as antibiotic resistance is changing over time. This will help clinicians to provide safe and effective empirical therapies, develop rational prescription programs, make policy decisions and finally assess the effectiveness of all. In addition, this will help policy makers to amend or develop new infection control programs in local situations.

2. Literature reviews

A study conducted in Pakistan from January 1996 to December 2010 showed that 112 patients had developed pleural fluid. The most isolated bacterial were *Staphylococcus aureus* (n=13) and *Streptococcus pneumoniae* (n=5) [33].

A study conducted in Iran from September 2003 to March 2006 showed that twealve cases, having pleural fluids with clinical of empyema thoracis among them [seven girls and five boys] with a median age of 5.2 years (range, 6 month to 16 years) with ascetic fluid. All cases were culture positive, and gram stain was positive in five (38.5%) of them. The most isolated bacterial were *S. pneumoniae* in 5 (38.5%), *E. coli* in 2 (15.3%), *S. viridans* in 2 (15.3%), and *K. pneumoniae*, *H. influenza*, *Enterococci*, and nontypable Streptococcus each in one (7.7%).All of them except *Enterococci* were sensitive to ciprofloxacin and ceftriaxone [34].

Another study was conducted in Iran form 2003 to 2013 showed that in 107 CSF the commonly isolated bacterias were *Streptococcus pneumoniae* (34.5%), *Haemophilus influenzae* type b (23.36%), *Neisseria meningitidis* (6.54%), *Serratia* spp. (6.54%), and *Klebsiella pneumoniae* (5.6%) [35].

A similar study conducted in Iran from 1998 to 2008 a total of 11269 CSF collected from suspected cases among them 329 (2.9%) were culture positive for *coagulase negative Staphylococci(40.1%), S.pneumoniae (9.1%), S. aureus (7.6%), Klebsiella spp. (6.1%), P. aeruginosa (6.1%), Haemophilus spp. (5.8%), E. coli (5.3%), Acinetobacter spp.(4.6%), hemolytic streptococci (except of S.pn eumoniae) (4.6%), N. meningitides (2.7%.* According to antimicrobial susceptibility, oxacillin and vancomycin were resistance for *staphylococci*, penicillin was resistant for *S. pneumonia*, and ampicillin was resistant to gram-negative bacteria [29].

A study conducted in India from 1993 to 2008 showed that a total of 449 sterile body fluids like cerebrospinal fluid, ascitic fluid, and pleural fluid with the clinical condition of Meningitis (34.3%) and pneumonia (33.9%). The frequent isolated bacteria were S pneumonia (43.3%) and majority of them had a minimum inhibitory concentration for penicillin, chloramphenicol, co-trimoxazole, erythromycin, and cefotaxime [36].

Another study conducted in India from January 2009 to December 2010 a total of 100 patients suspected cirrhosis with ascites among them SBP (58%) and non-SBP 42 %. The most common type of bacteria's isolated from SBP was *Escherichi*a *coli, Pseudomonas aeruginosa* and *Acinetobacter* species. The susceptibility pattern for aminoglycosides, quinolones and cephalosporins were 92.31%, 79.17% and 58.33% respectively with mean polymorph nuclear count (PMN) 755.19 ± 788.04 / mm3 [37].

A similar study conducted in India from December 2011 to November 2012 showed that atotal of 32 ascetic fluids collected from patients with suspected of SBP. The bacteria isolation rate were 31 %, and the most common of isolated bacteris were *E. coli* 17 (54.9%), *Klebsiella spp.* 5 (16.2%), *Staphylococcus aureus* 6 (19.3%) and *Pseudomonas aeruginosa* 3 (9.6%) [31].

A cross-sectional study conducted in Israel from July 1992 to December 1996 showed that 144 Synovial fluids obtained from patients with arthritis. The most common isolated bacteria were *Staphylococcus aureus* from 12 patients, *Streptococcus pneumonia* and *Kingella kingae* from 4 patients each, *group G streptococci* were isolated from 3 patients, *Staphylococcus epidermis*, and members of the family *Enterobacteriaceae* from 2 patients, and *Streptococcus mitis* and *Peptostreptococcus prevotii* from 1 patient each [38].

A prospective study conducted in England from January 1990 to December 1993 a total case of 1158 septic arthritis. The infection was more common in children; among them in males were higher prevalence than females. The most common isolated bacteria were *staphylococcal* 40.6% (470), and *streptococcal species* 40.6% (470) [*Streptococcus pneumonia* and *Lancefield group A B C* and *G*], and *Homophiles influenza* 23.3% [39].

A study conducted in Nepal from March 2003 to February 2004 a total case of 81 patients with cirrhosis, UGI bleeding and symptoms of SBP. The prevalence of culture positive was 35% (n=7) from peritoneal fluid. The most common isolated bacteria's were *Escherichia coli* (n=4), and S*treptococcus pneumonia (*n=3) [40].

Another study conducted in Nepal from May 2012 to April showed that 252 CSF was collected from patients <15 years old having clinical features of meningitis. The most common isolated bacteria's were *Group B streptococcus* 3 (16.7), *S. pneumoniae* 2 (11.1), *H. influenza* 2(11.1), *N. meningitides* 7(38.9), *E. coli* 3(16.7) isolated. Among isolated *S. pneumonia, H. influenza N. meningitides* were resistance for Ampicillin, and rifampicin was resistance to *N.meningitidis* [41].

A study conducted in Namibia from 2009 to 2012 showed that 100 CSFshowed that *Streptococcus species, N.Meningitidis, H. Influenzae, Staphylococcus, and Escherichia coli* were isolated and all were resistance for Cephalosporin and *Streptococcus* (34.3%) was resistance to penicillin [4].

A study conducted in turkey during 2013 showed that 49 year olds, who had unstrangulated umbilical hernia, and diagnosed his peritoneal fluid by Gram stain, and culture, the result was no microorganism seen for gram stains and the isolated bacteria's were *E. coli* and *Enterococcus spp.* both of the isolated were sensitive for ciprofloxacin [15].

A study conducted in Korea from May 2000 to February 2008 showed that 111 patient suspected for peritonitis, the prevalence of bacteria growth for gram-positive and gram-negative were 56.3% and 21.4% respectively. The most frequently isolates were *Coagulase-negative staphylococcus* (26.8%), *Streptococcus sp* (17.0%), *Staphylococcus aureus* (17%), *Corynebacterium species (4.5%), Escherichia coli* (4.5%), *Acinetobacter species, and Enterobacter sp* (2.7%) [42].

A study conducted in the Rama Medical College from October 2013 to September 2014 showed that in atotal of 100 different body fluids with the prevalence of 31%. The frequently isolates bacterias were *E. coli, S. aureus, S. pneumonia, Klebsiella spp*. and *Pseudomonas sp* [43].

A study conducted in Brazil from November 2002 to April 2004 in eighty-two body fluids among these the isolates bacterias were *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus, Streptococcus pneumonia*, *Streptococcus anginosus*, *Streptococcus viridans*, *Enterococcus faecalis*, coagulase-negative *Staphylococcus*, *Acitinobactor baumannii* [44].

Another study conducted in Brazil from November 2001 to November 2006 in atotal of 691 ascetic fluids *Escherichia coli* (31.7%), *Streptococcus pneumonia* (7.9%), *Staphylococcus aureus* (7.9%), and *Klebsiella pneumoniae* (7.9%) were isolated [45].

A similar study conducted in Brazil from 1984 to 1990 showed that in1083 CSF. The commonly isolated bacterias were *mycobacterium tuberculosis* (4.3%), *pseudomonas spp* (0.5%), *Entrobacter spp* (0.4%), *and staphylococcus areuse* (0.3%) [46].

A study conducted in Angola from 2003 to 2012 showed that atotal of 23,134 pleural fluid and CSF collected from children, then examined for the gram-stain, and culture. The most commony identified bacteria's were *S. pneumonia* (40%), *Staphylococcus aureus* (26%), *H. influenza*, *Escherichia coli* (4%), *Streptococci pneumococcal* (3%), *Proteus* spp. (3%), *Klebsiella* spp. (2%), and other gramnegative bacteria (4%), however there was no detection of *Mycobacterium tuberculosis* [47].

A study conducted in Egypt from May 2008 to January 2009 showed that Synovial fluid were collected from 100 patients. The most frequently isolated bacteria's were *Staphylococcus aureus* 11(55%), *Staphylococcus epidermis* 5(25%) and *Escherichia coli* 4(20) [48].

Study conducted in Sudan during 2011 within 10 months, showed that in 31 cases presenting with symptoms and signs of peritonitis (52.8% men, 47.2% women) were on continuous ambulatory peritoneal dialysis (CAPD). The mean total white cell count was 2497 cells/mm^3 (range: 100 – 14 500 cells/mm^3). The gram-positive infection was higher than that of gram-negative infection (53.8% vs. 46.2%). *Staphylococcus aureus* was the most common gram-positive organism (24.2%), and *Escherichia coli* was the most common gram-negative organism (12.1%)[49].

A study conducted in Gondar from September 2002 to August 2003 showed that in 22 CSF with the prevalence of 5.6%. commonly isolated bacteria's were *Neisseria meningitidis* 10(45.5%) *and Streptococcus pneumonia* 7(31.8%), and their drug resistance for *S.pneumoniae* showed against chloramphenicol 4(57%), tetracycline 3(43%), co-trimoxazole 3(43%), ampicillin

3(43%), and gentamicin 1(14%), and also *N. meningitidis* was resistant to co-trimoxazole 5(50%), chloramphenicol 3(30%), gentamicin 3(30%) and ampicillin 2(20%)[25].

Hypothesis

The overall prevalence of bacterial profiles from body fluids and their antimicrobial susceptibility pattern from body fluid.

3. Objective

3.1. General Objective

- To determine the bacterial profiles and their antimicrobial susceptibility pattern from body fluid in patients attending in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

3.2. Specific Objectives

- To determine the bacterial profiles from body fluid.
- To determine antimicrobial susceptibility pattern on bacterial isolates from body fluid.

4. Materials and Methods

4.1. Study area

The study was done at Tikur Anbessa Specialized Hospital that is found in Lideta Sub-city, Addis Ababa Ethiopia. In 1998, it handed by Addis Ababa University by the Ministry of Health as a teaching hospital for the medical faculty. The faculty is the largest and the oldest among health training institutions in the country, staffed with the most senior specialists. It is now the main teaching hospital for both clinical and preclinical trainings of most disciplines and it is where specialized services rendered. The Hospital provides tertiary level referral treatment and open 24 hours for emergency services. The hospital is diagnosis and treating for approximately 370,000-400,000 patients yearly with 800 beds, 130 specialists and 50 non-teaching doctors. The emergency department sees around 80,000 patients per year.

4.2. Study Design and Period

- A hospital based cross-sectional study conducted from July 2015 to September 2015

4.3. Population

4.3.1. Source Population

- All patients visited at Tikur Anbessa Specialized Hospital and suspected for infection.

4.3.2. Study Population

- Patients visited at Tikur Anbessa Specialized Hospital and have body fluid analysis at the microbiology laboratory.

4.4. Eligibility

4.4.1. Inclusion Criteria

- All patients have visited at Tikur Anbessa Specialized Hospital and suspected for body fluid pathogens during the study period.

4.4.2. Exclusion Criteria

- Contamination, incorrect lablling, dalay of sample(>2hrs) and who started antibiotics before fourteen days

4.5. Study Variables

4.5.1. Dependent Variable

- Bacterial isolates from body fluid.
- Antibiotic susceptibility pattern of the isolated bacterial species.

4.5.2. Independent Variable

- Age, sex, outpatient, inpatient, WBC, differential count, gram stains and AFB stains.

4.6. Sample Size and sampling technique

4.6.1. Sample Size

The sample size calculated based on single sample size estimation as shown below. The value of p was taken as 50 %(0.5).

$$n= Z\ 2\ P\ (1- P)\ /d2$$

Where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion (P = 0.5), and d = precision (d = 0.05), Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

(1.96) 2 X 0.5(1-0.5) = 384.16.

Therefore, the sample size was 384 Sampling techniques. A total of 384 study participants were recruited using consecutive convenient sampling techniques.

4.7. **Data collection and processing**

4.7.1. Data and specimen collection

Simple checklist was prepared to collect information like sex, age, inpatient, and outpatient.

All body fluids were collected by physicians using aseptic procedure then send in to laboratories for culture, granstains, AFB stains, WBC, and differential count. The appearance of fluids is clear, bloody/traumatic, cloudy and clear straw was recorded. For cloudy and less volume of fluids (<1 ml) was done gram stain, and AFB stain with out centrifugation, if the volume of fluids 2-3 ml centrifuged for 15 min at 2500-3000 rpm and had done gram stain, and AFB stain form cediments.

4.7.2. Transport of specimens

Body fluid collected aseptically with a needle and syringe, inorder to maintain anaerobic bacteria close the syringe by cap, the air will be expelled from the syringe and needle or the specimen injecte into an anaerobic transport tube, oxygen will be diffuse slowly through plastic syringe. Cerebrospinal fluid (CSF) processed in a microbiology laboratory within 1 hour after collection, if not processing within 1 hour stored at 370C incubator or inoculated into Trans-Isolate (T-I) medium to transport from one laboratory to another laboratory .

4.8. Specimen processing, culture and identification

4.8.1. Microbiological investigation

All body fluids [CSF, pleural, peritoneal, synovial] were processed for WBC, gram stain, AFB, cultures according the standard of microbiological procedures by inoculating on blood agar, chocolate agar, MacConkey agar plates [(Oxoid Ltd, Basingstoke, Hampshire, UK), and nutirate broth to enrich the minimum number of organism, and incubated at 35- 37℃ aerobically for 24-72 hrs. The chocolate agar plate incubated inside candle jar to provide 5-10% CO2 concentration or to create microareophilic condition for fastidious bacteria. Each of plates were examined for the growth of bacterial colonies and all positive culture were examined using gram stain and biochemical tests according to the standard of laboratory procedures.

4.9. Drug susceptibility Patterns

The disk diffusion method was performed then after 16-18 hours of incubation at 37℃ a zone of inhibition measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI) version 1.3. Using a sterile wire loop, 3-5 pure colonies picked up from blood agar, macConkey agar and chocolate agar. Standard inoculums adjusted to 0.5 McFarland swabbed onto Muller-Hinton agar (dispensed on 150mm plate). Antimicrobial like: (Ampicillin (10µgBD), Amikacin(30ugOxoid),Ceftazidime(30ugOxoid), Chloramphenicol (30ug Oxoid), Tobramycin (10 Oxoid),Ceftriaxone (30ug BD), Rifampin(BD 5ug), Gentamycin (10ug Oxoid), TMP-SXT(1.25+23.75ug BD), Cefotaxime (30ug BD), Cefuroxime(30ug BD), Clindamycin(2µg BD),Penicillin(10 ug BD), Erythromycin(15ug Oxoid), Ciprofloxacillin (5ug BD), Vancomycin(30ug BD), and Oxacillin (1ug Oxoid) were used.

4.10. Quality Control

Standard Operating Procedures (SOP) were strictly followed, and verify that the media meet an expiration date and quality control parameters per CLSI. Visual inspections of cracks in media or plastic petri-dishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. QC performed to check the quality of medium. Each new lots had done quality controlled before use by testing the *E. coli* ATCC 25922 and/or *Staphylococcus aurous* ATCC 25923 standard strains.

4.10.1. Gram Stain and AFB staining Quality Control (QC)

To maintain the performance of reagents, methods, and techniques to verify both Gram stain and AFB stain using slide control with known organisms

Gram stains

Positive control: *Staphylococcus aureus* (stain blue to dark purple/gram-positive cocci)

Negative control: *Escherichia coli* (should stain pink to red/gram-negative rods

AFB stain

Positive control: mycobacterium tuberculosis (stains red, blue or green background stain)

Negative control: non-acid fast organisms stain blue or green

4.11. Data management and quality assurance

4.11.1. Data processing and analysis

The data entered in to EPI INFO and double-checked before analysis. Then the data exported to SPSS version 22 for analysis. The descriptive statistics (means, percentages or frequency) were calculated & the bi-variant logistic regression analysis used to see the relation between dependent variable and independent variables. Variables that were showing a significant association selected for further analysis using multiple logistic regression models with a p-value < 0.05 as statistically significant. The strength of the association interpreted using an odds ratio in a 95% confidence interval. Finally, the results presented on charts, graphs and tables.

4.12. Dissemination of results

The results of the study submitted to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, and Addis Ababa University. In addition, the results submitted to the study site. Abstract will be submitted (like EMA, EPHA and EMLA) and

other international associations to present the results during continuous medical education events or conference organized by these associations. The summary of the thesis will be submitted to international or national peer reviewed journal for publication.

4.13. Ethical clearance

The study was approved by "Department Research and Ethical Review Committee" of the Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. Permission was obtained from the study site. The purpose and procedures of the study was explained to the study participants. All information on patients were remained confidential. Laboratory results were communicated to the requesting physicians on time.

4.14. Operational definition

- **Infected body fluids:** turbid appearance, leukocytosis, identified organism by Gram stain, and culture.
- **Multidrug Resistance (MDR)**: bacterias those are resistant to antimicrobials for a minimal of two or more of drug disk.

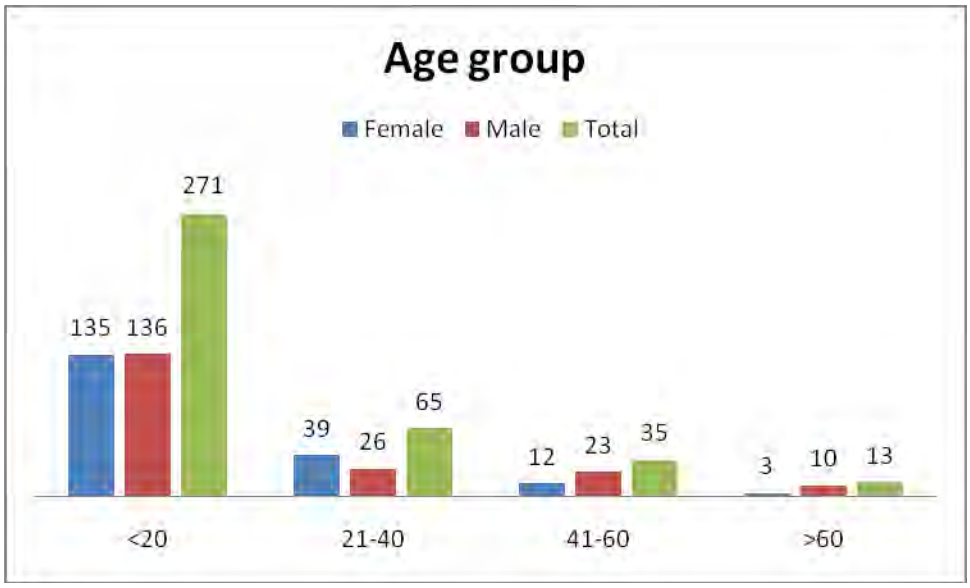
5. RESULTS

5.1. Socio-demographic characteristics

A total 384 different body fluid from 384 patients at Tikur Anbessa Specialized Hospital Laboratory from July to September 2015. Those of body fluids such as; Cerebrospinal fluids 264(68.8%), Pleural fluid 76(19.8%), Peritoneal fluid 34(8.9%), and Synovial fluid10 (2.6%).

About 195(50.8%) of body fluids were collected from out patients departments, most of body fluids 189(49.2%) were collected from male patients.

The ages range were between 24 days – 86 years, and categorized in to four age groups according the reference of [58] as showing in figure (5.1) 271(70.2%) <20 years , 65 (16.9%) between 21-40 years, 35 (9.1%) between 41-60 years ,13 (3.2%) were 61 years and above with mean (standard deviation) of 15.1(19.2).



Figer 5.1 The age grouping involved in study at Tikur Anbessa specialized Hospital laboratory from July,2o15 to September, 2015.

5.2. Bacterial isolates and cell count from body fluids

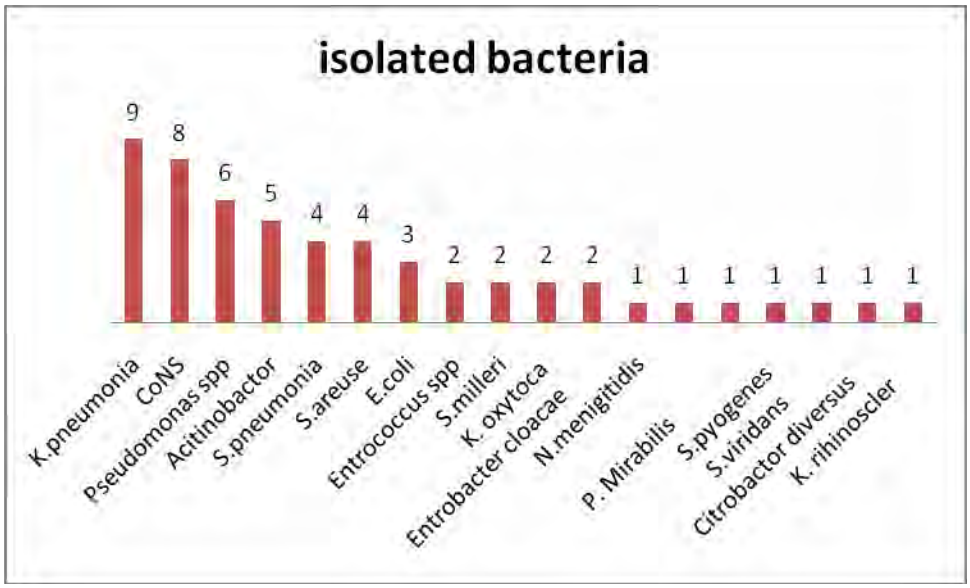
In this study, atotal of 54 (14.1%) bacteria were isolated from 384 body fluids. Among these, 32(59.3%) were gram-negative bacteria, while the reaming 22(40.7%) were gram-positive bacteria [fig.5.2].

Among the bacterial isolates *K. pneumonia* found to be the most frequent isolates 16.7 %(9/54) followed by *coagulase negative Staphylococcus* 15.0 %(8/54), and *streptococcus pneumonia* 14.8 %(4/54) were the most dominate isolates.

Two hundred thirty seven (61.7%) of the body fluids were clear, 74(19.3%) body fluids were turbid, 69(18.0%) were clear straw, and 4(1%) body fluid were traumatic.

Out of 384 body fluids, 173(44.1%) of body fluids had abnormal white blood cell count (WBC) above 05/ mm^3. Among body fluids with abnormal WBC count, 92 of body fluids (21.1%) had polymorphic differential WBC feature, while 81 body fluids (19.2%) had monomorphic features.

CSF 31(57.4% were the highest body fluids with bacterial isolates followed by, pleural fluids 18(33.4%), ascetics fluids 3(5.6%), and synovial fluids 2 (3.7%).



Figer 5.2 Frequency and type of bacteria isolated from body fluids at Tikur Anbessa Specialized Hospital laboratory from July to September 2015.

Table 5.1 shows bacterial isolation at Tikur Anbessa specialized Hospital labratory from July 2015 to September 2015 in different body fluids

Isolation of bacteria	Type of specimen in %				
	CSF	pleural fluid	peritoneal fluid	synovial fluids	Total
No growth	233(59.9)	58(17.8)	31(9.0)	8(2.1)	230(89.8)
K.pneumoniae	8(1.9)	1(0.2)	0(0)	0(0)	9(2.1)
CoNS	8(1.9)	0(0)	0(0)	0(0)	8(1.9)
Pseudomonas spp	2(0.5)	4(.9)	0(0)	0(0)	6(1.4)
Acinetobacter	4(0.9)	1(0.2)	0(0)	0(0)	5(1.2)
S.pneumoniae	2(0.5)	1(0.2)	0(0)	1(0.2)	4(0.9)
S.areuse	1(0.2)	3(0.9)	0(0)	0(0)	4(0.9)
E.coli	0(0)	2(0.5)	1(0.2)	0(0)	3(0.7)
Entrococcus spp	1(0.25)	1(0.25)	0(0)	0(0)	2(0.5)
S.milleri	0(0)	0(0)	0(0)	2(0.5)	2(0.5)
K. oxytoca	1(0.25)	1(0.25)	0(0)	0(0)	2(0.5)
Entrobacter cloacae	1(0.25)	1(0.25)	0(0)	0(0)	2(0.5)
N.menigitidis	1(0.2)	0(0)	0(0)	0(0)	1(0.2)
Pseudomonas aeruginosa	0(0)	1(0.2)	0(0)	0(0)	1(0.2)
P. Mirabilis	0(0)	1(0.2)	0(0)	0(0)	1(0.2)
S.pyogenes	1(0.1)	0(0)	(0)	(0)	1(0.2)
S.viridans	1(0.2)	0(0)	0(0)	0(0)	1(0.2)
Citrobactor diversus	0(0)	0(0)	1(0.2)	0(0)	1(0.2)
K. rihinoscler	0(0)	1(0.2)	0(0)	0(0)	1(0.2)
Total	264(68.8)	76(19.8)	33(8.6)	11(2.9)	384

Table 5.2 Shows bacterial isolation at Tikur Anbessa specialized Hospital laboratory from July 2015 to September 2015 in different age groups.

Isolation of bacteria	Age in groups				Total %
	<20	21-40	41-60	>60	
No growth	244	48	28	10	330(89.8)
k.pneumoniae	6	2	0	1	9(2.1)
CoNS	7	1	0	0	8(1.8)
Pseudomonas spp	1	3	2	0	6(1.4)
Acinetobacter	1	2	1	1	5(1.2)
S.pneumoniae	2	1	1	0	4(0.9)
S .aureus	3	1	0	0	4(0.9)
E.coli	0	2	0	1	3(0.7)
Entrococcus spp	0	1	1	0	2(0.47)
K. oxytoca	2	0	0	0	2(0.47)
Entrobacter cloacae	1	1	0	0	2(0.47)
S.milleri	1	0	1	0	2(0.5)
N.mengitidis	1	0	0	0	1(0.2)
Pseudomonas aeruginosa	0	0	1	0	1(0.2)
P. Mirabilis	0	1	0	0	1(0.2)
S.pyogenes	1	0	0	0	1(0.2)
S.viridans	1	0	0	0	1(0.2)
Citrobactor diversus	0	1	0	0	1(0.2)
K. rihinoscler	0	1	0	0	1(0.2)
Total	271(70.6)	65(16.92)	35(9.11)	13(3.38)	384

5.3. Gram Stain and AFB finding

Revealed that, 22/384(5.7%) had gram negative rodes, 7/384 (1.9 %) had gram positive cocci in chain, 9/384(2.3%) had gram positive cocci in cluster, 2/384(0.5%) had gram positive diplococcic, 1/383(0.26%) had gram negative diplococcic and 2/384 (0.52%) were AFB positive one each from peritoneal and pleural fluid

5.4. Gram Stain, WBC count and appearance versus culture positive

54 bacteria were isolated from 384 body fluids, among these 41/384 (10.7%) were detected by gram stains, most of them were gram-negative 23/41. All culture positive of body fluids had shown WBC (>20/ mm3) with leading to polymorphic differential features 39/54(72.9%), and the majority of culture positive had an appearance of turbid 43/54(79.6%).

Table 5.3 showed that bacteria isolate from body fluids based Gram Stain, WBC count and appearance versus culture at Tikur Anbessa Specialized Hospital laboratory from July 2015 to Septe 2015.

Culture +ve	Gram stain		WBC		Appearance			
	Gram positive	Gram negative	Polymorphic	Monomorphic	turbid	clear	c/straw	traumatic
	18	23	39	15	43	6	4	1
54	41		54		54			

In femals 32 (62.2%) higher isolation than male 22(37.7%), however there were no significant association between genders and culture results (OR=0.624, 95%CI=0.348-1.119, P=0.114)

The prevalence of bacterial when categorized in age groups the highest isolation rate were detected in < 20 years olds 27(51.1% then followed in between 20-40 year old 17(25.5), in between 41-60 year old 7 (10.3), and above 61 year old 3(5.6) respectively . However there was significant association between the age of patients and culture results (OR=1.022, 95%CI= 1.009-1.036, P=.001.

In this study 28(51.1%) bacterial were isolated from inpatient, while 26(48.9%) of bacterial isolated from outpatients. However, there was significant association between departmens of

inpatient and culture results (OR=0.651, 95%CI=0.424-1.001, P=0.05) or outpatient and culture results (OR=1.839, 95%CI=1.126-3.002, P=0.015).

When categorized the prevalence of bacteria depending on an appearance of body fluids, in turbid body fluids were the highest isolates 43/54(79.6%), then following to clear body fluids 6/54(11.1%), and clear straw body fluid 4/54(7.4%). However, there were significant association between an appearance of body fluids and culture results (OR=1.64, 95% CI=1.903-1.378, P=0.00)

Table 5.4 Association of variables with body fluids culture results at Tikur Anbessa Specialized Hospital laboratory from July 2015 to Septe 2015

Variables	Culture results (n=38)		P-value	OR	95%CI
	Positive (N= %)	Negative (N= %)			
Gender			0.114	0.624	0.348-1.119
Male	22 (40.7)	173(45.1)			
Female	32 (59.3)	157(40.9)			
Total	54 (14.0)	330(85.9)			
Age in years			0.001	1.022	1.009-1.036
<20	27(50.0)	244(63.5)			
21-40	17(31.5)	48(12.5)			
41-60	7(13.0)	28(7.3)			
>61	3 (5.6)	10(2.3)			
Total	54 (14.0)	330(85.9)			
Inpatient	28(59.9)	155(40.4)	0.05	0.651	0.424-1.001
Outpatient	26(48.1)	175(45.6)	0.015	1.839	1.126-3.002
Total	54(14.0)	330(85.9)			
Type of body fluids			0.0289	1.208	0.852-1.71
CSF	31(57.4)	233(60.7)			
Pleurald	18(33.3)	58(15.1)			
Ascetic	3(5.6)	31(80.7)			
Synovial	2(3.7)	8(2.1)			
Total	54(14.0)	330(85.9)			
Appearances of fluids			0.000	1.903	1.378-2.628
Turbid	43(79.6)	31(8.1)			
Clear	6(11.1)	231(60.2)			
Clear-straw	4(7.4)	65(16.9)			
Trauma	1(1.9)	3(0.8)			
Total	54(14.0)	330(85.9)			

5.5. Antibiotics susceptibility pattern of bacterial isolates

The susceptibility rate of isolated bacteria from body fluids ranged from 0% - 100 %. Greater than 60% of gram-negative bacteria isolates from body fluids were resistance to Gentamycin 65%, Ampcillin 62%, Ciprofloxacin 53.1%, and Ceftriaxone (50%) and Tobramycin (50%), where as sensitive to Amikacin 68 %, and Chloramphenicol (62%) [Table 5.4].

Similar demonstration were done in gram-positive bacterias above 50% of them were resistance to Erythromycin(59%), Clindamycin(54%), and Trimethoprim--Sulphamethoxazol (50%), while sensitive to Vancomycin(90%)[Table 5.5].

Over all the isolated bacterias 41/54(75.9%) had shown Multiple-drug-resitance (MDR≥2 drugs). Among these isolated bacterial gram positive and gram negative had shown multiple drug resistance of 72% (n=16/22) and 96% (n=29/32) respectively [Table5.6].

K. pneumoniae 9/32(28.1%) were the predominant isolated among gram-negative bacteria showed that more than 90 %(n= 8 /9) isolated had multi drug resistance. Similarly Coagulase *negative Staphylococcus* 8/22(36%) were the predominant isolated among gram-positive organisms in all of isolates had shown 100% (n=8/8) multi drug resistance.

Table 5.5 Antimicrobial susceptibility pattern of gram negative bacterial (N=32) among suspected patients at Tikur Anbessa specialized Hospitals from July, 2015to Sept, 2015

Isolate l no		AMP	GN	CTX	CRO	CTZ	CRX	TOB	CPR	AMK	CHL	RA
K. pneumoniae	R	5(55)	9(100)	4(44.4)	6(66.7)	4(44.4)	4(44.4)	4(44.4)	5(55.5)	2(22.2)	4(44.4)	NA
9	S	4(44)	0(0)	5(55.5)	3(33.3)	5(55.5)	5(55.5)	5(55.5)	4(44.4)	7(77.7)	5(55.5)	NA
Acinitiobacter	R	3(60)	3(60)	2(40)	2(40)	4(80)	2(40)	3(60)	4(80)	4(80)	4(80)	NA
5	S	2(40)	2(40)	3(60)	3(60)	1(20)	3(60)	2(40)	1(20)	1(20)	1(20)	NA
E. coli	R	3(100)	1 (33.3)	1(33.3)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	2(66.7)	0(0)	0(0)	NA
3	S	0(0)	2(67)	2(66.7)	2(66.7)	1(33.3)	2(66.6)	1(33.3)	1(33.3)	3(100)	3(100)	NA
Pseudomonas	R	3(100)	3(100)	1 (33.3)	1(33.3)	3(100)	3(100)	1(33.3)	1(33.3)	1(33.3)	1(33.3)	NA
3	S	0(0)	0(0)	2(66.7)	2(66.7)	0(0)	0(0)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	NA
P.areginase	R	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	NA
1	S	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	NA
K. oxytoca	R	2(100)	1(50)	0(0)	1(50)	0(0)	1(50)	2(100)	0(0)	1(50)	1(50)	NA
2	S	0(0)	1(50)	2(100)	1(50)	2(100)	1(50)	0(0)	2(100)	1(50)	1(50)	NA
E. cleacea	R	1(50)	2(100)	0(0)	1(50)	0(0)	0(0)	2(100)	1(50)	0(0)	0(0)	NA
2	S	1(50)	0(0)	2(100)	1(50)	2(100)	2(100)	0(0)	1(50)	2(100)	2(100)	NA
C. divorce	R	1(100)	1(100)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	NA
1	S	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	NA
K. rihinosies	R	0(0)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(10)	NA
1	S	1(100)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	NA
P. Mirabel	R	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	NA
1	S	0(0)	0(0)	0(0)	0(00)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	NA
N. meningitis	R	1(100)	NA	0(0)	0(0)	NA	0(0)	NA	1(100)	NA	0(0)	1(100)
1	S	0(0)	NA	1(100)	1(100)	NA	1(100)	NA	0(0)	NA	1(100)	0(0)
G-VE=32		20(62)	21(65)	13(40)	16(50)	11(34)	11(34)	16(50)	17(53)	10(31)	12(37)	1(3)

NA --Not applicable: AMK. -- Amikacin, AMP--Ampicillin, SXT—Sulphamethoxazol-trimethoperem, CRO-Ceftriaxon, CTX--Cefotaxime, GN--Gentamycin, CTZ-- Ceftazidim, CPR—Ciprofloxacillin, TOB-Tobramycin,CHL-Chlorampenicol, RA-Rifampicin

Table 5.6. Antimicrobial susceptibility pattern of gram positive bacterial profile isolated (N=22) among suspected patients at Tikur Anbessa specialized Hospital laboratory from July, 2015to Sept, 2015

Total no		AM	P	CTX	CRO	CRX	CLN	ERY	VA	CPR	SXT	OX
S.aureus	S	2(50)	2(50)	2(50	2(50)	2(50)	2(50)	2(50)	NA	3(75)	1(25)	2(50)
4	R	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	NA	1(25)	3(75)	2(50)
CONS	S	3(38)	3(38)	5(64)	5(64)	4(50)	2(25)	2(25)	NA	4(50)	5(64)	5(64)
8	R	5(64)	5(64)	3(38)	3(38)	4(50)	6(75)	6(75)	NA	4(50)	3(38)	3(38)
S.pneumon	S	3(75)	3(75)	3(75)	3(75)	3(75)	2(50)	2(50)	3(75)	2(50)	2(50)	3(75)
4	R	1(25)	1(25)	1(75)	1(75)	1(75)	2(50)	2(50)	1(25)	2(50)	2(50)	1(75)
Entrococcus	S	1(50)	NA	NA	NA	NA	NA	NA	2(100)	NA	NA	NA
2	R	1(50)	NA	NA	NA	NA	NA	NA	0(0)	NA	NA	NA
S.viridans	S	1(50)	2(100)	1(50)	1(50)	1(50)	2(100)	1(50)	2(100)	2(100)	0(0)	1(50)
4	R	1(50)	0(0)	1(50)	1(50)	1(50)	0(0)	1(50)	0(0)	0(0)	2(100)	1(50)
S.milleri	S	1(1000	1(100)	1(100)	1(100)	NA	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)
1	R	0(0)	0(0)	0(0)	0(0)	NA	1(100)	1(100)	0(0)	1(100)	0(0)	0(0)
S.pyogenes	S	1(100)	1(100)	1(100)	1(100)	NA	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)
1	R	0(0)	0(0)	0(0)	0(0)	NA	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)
Total no G+ve=22	R	10(45)	8(31)	7(31)	7(31)	8(36)	12(54)	13(59)	2(9)	10(46)	11(50)	9(36)

NA --Not applicable: AM--Ampicillin, SXT---Trimethoprim —sulphamethoxazol, CRO-Ceftriaxone, CTX--Cefotaxime, CRX-- Cefuroxime, VA--Vancomycin, P--Penicillin,CPR—Ciprofloxacin, OX-Oxacilin, ERY-Erythromycin, CLN-Clindamycin,

Table 5.7 Lists of multi-drug resistance pattern for each bacterial isolates (N=54) samples were collected from patients suspected who had body fluids at Tikur Anbessa specialized Hospital laboratory from July, 2015to Sept, 2015

Bacterial isolates	total no	Anti-microbial sensitivity pattern					
		R1	R2	R3	R4	R>4	Total
Gram Negative							
Pseudomonas spp	(6)	0	2	1	1	1	6
P.Aeruginosa	(1)	0	0	1	0	0	1
E.coli	(3)	0	0	0	0	1	1
K. pneumoniae	(9)	0	0	0	4	5	9
Acitinobactor	(5)	0	0	0	2	3	5
P. mirabilis	(1)0	0	0	0	1	1	
K. oxytoca	(2)	1	0	0	0	1	2
Citrobactor diversus	(1)	0	1	0	0	0	1
K. rihinoscler	(1)	0	0	0	0	1	1
Entrobacter cloacae	(2)	0	1	1	0	0	2
N. meningitidis	(1)	0	0	1	0	0	1
Total no=	32	1(3.1)	4(12.5)	4(12.5)	7(21.9)	13(40.6)	29(90.6)
Gram Positive							
S. aureus	(4)	1	2	0	0	0	3
S.pneumoniae	(4)	1	0	1	0	0	2
Entrococcus sp	(2)	1	0	0	0	0	1
CoNS	(8)	0	0	2	2	4	8
S.viridans	(2)	0	0	0	0	0	0
S.milleri	(1)	0	0	1	0	0	1
S.pyogenes	(1)	0	0	1	0	0	1
Total no=	22	3(13.6)	2(9)	5(22.7	2(9)	4(18.2)	16(72.7)

R1; resistance to one drug, R2: resistance to two drug, R3: resistance to three drugs, R4: resistance to four drugs, R>4: resistance to five and above drugs.

6. Discussion

6.1. Prevalence of bacterial from body fluids

Body fluids are usually sterile; such like Pleural, Peritoneal, Cerebrospinal, Synovial, and Pericardial fluids, however bacteria may be invade or infect to body fluids, these lead to morbidity or mortality [2]. So that accurate and confirmed laboratory result is essential to provide patient therapy, appropriate case management, and resolve public health problems. The data presented in this study can provide and facilitate information's for clinicians on public health importance in the study area to select antimicrobial drugs for the treatment of patients [25].

In this study, 14.0% of bacteria were isolated from a total of 384 body fluids, incontras to study conducted in brazile by Daur A V *et al.,* in Rama medical college by Sujatha R *etal*. Found that 23.5% , 31 % ,and 31.3% respectively [44,43]. The less prevalence of bacteria isolated in our study may be due to over diagnosing of meningitis, may be due to the fastidious nature of the organisms , may be prior exposure to antibiotics, and may be increasing the detection of malignancy, since the cause of body fluids either malignancy or infection.

Coagulase negative staph, and *acinetobacter spp* were the highest isolated in our study, in agreement with study conducted Iran by Rezaeizade *et al.*, in Iran by Babak A *et al*., in Korea by Yoon *et al*., in Brazil by Daur *et al*., [29, 37, 42, 44]. The detection of *Coagulase negative staph & acinetobacter* spp may be the present of an opportunistic pathogen associated with nosocomial infections, due to poor infection control such like: overcrowding, lack of standard facilities with controlled airflow, entry and exit points and poor sterilization of all gowns and equipment.

E. coli was the most bacteria isolated from peritoneal fluids in our study in agreement with study conducted in turkey by Dorgan *et al*., in brazile by Reginato *et al*., in Nepal by Syed *et al*.[15, 45, 41]. This may be due to passage of viable bacteria from the intestinal lumen to mesenteric lymph nodes or other extraintestinal sites across the intestinal-mucosal barrier Gram-negative aerobic enterobacteria like *Escherichia coli* [11].

S.pneumoniae and *N.meningitidis* were the most common isolated pathogens causing meningitis, which are in agreement with previous studies in, Iran by Abdinia *et al.*, in Gondar byMulat *et al.,*

in Iran by Rezaeizadeh *et al.*, in India by Kurien *et al*., in Nepal by Shrestha *et al*., in Namibia by Mengistu *et al.*[35, 25, 29, 36, 41, 5]. In this study the lower isolation of *Neisseria meningitides*, and *Streptococcus pneumonia* from CSF, may be due to the fastidious nature of the organisms, or prior exposure to antibiotics or some of the cases were not bacterial meningitis due to over diagnosis of meningitis, or the increase accessibility of vaccination in our country.

Study done in Angola by Peltola *et al*., in Iran by Babak *et al.*, in Nepal by Shrestha *et al*., in Namibia by Mengistu *et al*., [48,35,41,5] . *Haemophilus influenzae* was detected most frequently, unlike in our study no detection of *Haemophilus species*. Since, *Hemophilus influenzae* type b (Hib) once a common cause of meningitis has disappeared due to the effectiveness of the extended immunization program. The reason for this is not clear but may be due to the new vaccine have been giving as part of routine child immunization activities, which might have reduced the occurrence of invasive diseases due to *H.influenzae*.

Streptococcus pneumonias most frequently isolated organisms from pleural fluids study conducted in Pakistan by Faisal *et al*., in Qatar by Khan *et al*., India by Thomas *et al*., in Angola by Peltola *etal*.,[33, 34, 36, 48] unlike to in our study most detected bacteria were *pseudomonas spp* (12.2%). The reason for this is not clear but may be due to the new vaccine being given as part of routine child immunization activities, which might be have reduced the occurrence of invasive diseases due to *Streptococcus pneumonias* or the fastidious nature of bacteria. Where as high prevalence of *pseudomonas spp* detected in this study may be indicates the pore way of cleaning and sterility procedure.

Antimicrobial susceptibility patterns of bacterial isolates

Antimicrobial resistance of bacteria is a worldwide problem. However, the situation in developing countries like Ethiopia is high prevalence due to the absence of well-equipped bacteriological laboratories, no organized surveillance exists on drug resistance patterns among common bacterial isolated and limited number of published data on drug resistance [25].

Among gram-positive bacteria in our study, the Susceptibility pattern of *CoNS* were sensitive to Cefotaxime (75%)**,** inagrement to study conducted in Indian Jitendra *et al*. [45] found that

sensitive to Cefotaxime (95 %). This may be due to different or similar selection of regimens prescribed in the community during study period.

S. pneumoniae had high resistance against penicillin study done in Iran by Abdinia *et al*., in Gonder by mulu *et a*l., in Iran by Rezaeizade *et al.*, in Namibia by Mengistu *et al.,* in contrast to our study 50% sensitive to penicillin [29, 25, 36, 5]. This may be due to miss use of penicillin in the study area and the bacteria develop resistance through time.

Among gram-negative isolates, we found that K. *pneumoniae* sensitive to ampicillin (45%), in contrast to study conducted in Iran by Rezaeizadeh *etal***.** found that (87.5%) resistance to ampicillin, [45]. This might due to the difference methodology and study design used in the study period.

Even though there are geographical differences in the drug resistance pattern of bacteria, the problem is growing emergence of multidrug resistance strains. In this study, 75% of the isolates showed multiple antibiotic resistances (Table 5.7) in agreemet with a study conducted in Gondar by Mulat *et al.* [25]. This may be due to the absence of guidelines regarding the selection of drugs and social trend of inappropriate use of commonly prescribed drugs. The highest multiple drug resistance was also observed among gram negative bacteria's from which *Proteus species* showed highest multiple antibiotic resistance similar with previous studies in Iran by Rezaeizadeh *et al*. and Gondar by Mulat *et al,* [29, 25].

Gram stain and AFB stain

Gram stain and AFB stains are an easy and rapid method to diagnose an infected body fluid in microbiology laboratory. Allowing presumptive diagnosis in the majority of cases with sensitivity of greater than 90 % [41]. In this study for all of body fluids had used gram stains, AFB, WBC analysis. To enhance the detection of bacteria and used as confirmation of culture results test , in agreement with study done in Iran by Kerr *et al*., in turkey by Dogan *et al*, in Angola by Peltola *et al*., in Brazils by Langra *et al*. [34,15,47, 46].

WBC was done for suspected cirrhosis with ascites, and the polymorph nuclear count (PMN) > 250 / mm3 of cells counted for SBP [37, 49]. In agreement with our study 51.5% (17 /33) of

ascites fluids had polymorph nuclear count (PMN) >250/ mm3. Spontaneous Bacterial Peritonitis (SBP) is a frequently occurring infectious in patients with cirrhosis and ascites. To diagnosis SBP require such parameters of culture positive and/or the neutrophil count > 250 cells/mm3, there are three mechanisms involved in this infections pathogenesis: deficient local immune response (decline of phagocytic activity by hepatic macrophages), bacterial overgrowth in the intestinal lumen, and functional and structural alterations in the intestinal mucosal barrier [11].

7. Limitation of the study

The methodology could not identify anaerobic bacteria's using simple candle jar methods and increased the number of culture negative isolates.

8. Conclusion

Out of 384 body fluids 173(44.1%) of body fluids had abnormal white blood cell count among them 91 of body fluids had (21.1%) polymorphic differential feature. The prevalence rate of bacterial isolated in these sample were 14.0 %, the highest isolates were observed in CSF n=31/54(57.4%), it was collected from <20 year old. The most common isolated bacteria from different body fluids were *k. pneumonia* 9/32 (17.0%), *coagulase negative staph 8*/22 (15.0%). Even though the previous study assessing to body fluids were less, however a few of studies predicted that the choice of drugs in the treatment of bacterial isolates from body fluids is quite narrow. Similarly in this study both game-positive and gram-negative bacteria had shown multi drug resistance 72 %(n=16/22) and 96%(n=29/32) respectively. Most of gram-negative bacterias were highly resistance to Gentamycin 65%, Ampcillin 62%, and Ciprofloxacin 53%, while less resistance to Amikacin 31%. Similarly in gram-positive bacterias high level of resistance to Erythromycin 59%, Trimethoperem-sulphamethoxazol 50%, and Clindamicin 45%, while less resistance to vancomycin 10%.

9. Recommendations

- Identifiy the profile of bacteria from body fluid with their antimicrobial susceptibility is recommended to clinician for treating infected body fluid.
- A regular monitoring of antimicrobial resistance patterns of infected body fluid is essential to prevent further emergency and the spread of antimicrobial resistance.
- Effective antibiotic policy and infection control programs combined with good medical practices can help in controlling the cause of antibiotic resistance or multidrug resistant strains.

10. Reference

1. Alan B, Stan B, Ophela B, Cannon D, Healy M .the biochemistry of body fluid. *J. of association of clinical biochemistry in Ireland*. 2005; 2 (66): 1245-1256.
2. Anasua D, Swati M, Vaishali D, Renu B. Bacteriology of body fluids with an evaluation of enrichment technique to increase culture positivity. *J. of evolution of medical, and dental sciences*. 2014; 3(72):1523-1528.
3. Rodrigo H, Merijn B, Matthijs CB, Nabil K, Christiane MH, Ende VD ,etal. Risk score for identifying adults with CSF pleocytosis and negative CSF gram stain at low risk for an urgent treatable cause. *J.clinical. infect*. 2013 ; 67(2): 102–110
4. Assegid M, Johannes G , Gottfried U , Christophine N , Kennedy K , Lazarus I , *et al.* Antimicrobial sensitivity patterns of cerebrospinal fluid isolates in Namibia. *J. Pharmaceutical Policy and Practice* .2013; 6(9):4-7.
5. Allan RT, Barry JH, Sheldon LK, Bruce AK. , Karen LR., Scheld, Michael WS, *et al*. Clinical infectious diseases. *J. health and clinical Medici*. 2004;39:1267–1284.
6. Rita S. Ascites diagnosis and management. *J. of Indian Academy of Clinical Medicine*.2014; 5(1):197-199.
7. Glen E. Characteristics of ascitic fluid from patients with suspected spontaneous bacterial peritonitis in emergency units at a tertiary hospital. *J.Clin.Med.* 2005:4(7):204-209.
8. Baer Ek, Smith PG. Spontaneous bacterial peritonitis and hepatorenal syndrome in cirrhosis. *J. of Hepatology*. 2010; 53:397–417.
9. James SD, Anna SF, Andrew KB, Jenny EH. Diseases of the liver and biliary system. *J. of healthcare system*.2011; 5(7):78-92.
10. Lara MK, Culej J. The Pleural, peritoneal and pericardial effusions a biochemical approach. *J. of biochem Med*. 2014; 24(1): 123–137.
11. Declan GF, Ram GD. Sterile peritonitis in the peritoneal dialysis patients. *J. of Inter. Peritoneal Dialysis*. 2005; 25: 146–151.
12. Varghese T., Routine analysis of cirrhotic ascites for evidence of infection not worth the effort. *J .Gastroenterol*. 2011; 30(5):201–203.

13. Thiago JBR , Oliveira[II], Andrade JMO, César LM, Lamanna [I], Antonieta L, Acencio[IV], Leila MPA.Charestics of ascitics in patients due to peritonitis of bacteremia . *Med J.* 2011; 129(5):315-319.
14. Chang HP, Eun JY, Kyu SC, Sun JR, Jong SK. Klebsiella pneumoniae septic arthritis in a cirrhotic patient with hepatocellular carcinoma. *J .Korean Med Sci* .2004; 19: 608-610.
15. Dogan M, Kucukkartallar T, Feyzioglu B, Ozdemir M, Baykan M , Baysal B. Isolation of anaerobic bacteria from the peritoneal fluid of patient who has unstrangulated umbilical hernia. *J Med. Microb Diagn*. 2013; 2: 3-8.
16. Carlos JCI, Gonza DGT, Bosch MA , Jurado LV , Saiz CJ. Isolation of aurantimonas altamirensis from pleural effusions. *J.Medi. microbiology*.2010; 59:1126–1129.
17. Myths JP. In pleural fluid analysis. *J. Asian pacific society of respirology*. 2011; 16:44–52.
18. Donald EM, Eric SB. Pleural effusion and empyema. *J. companion to surgery in Africa.* 1987; 6(58):145-147.
19. Bhattacharya AK. Pleural effusion and aids. *Journal of lung India*. 2005; 22 : 101-104.
20. Khan YF, Yasin ASM, Ibrahim MH, Lingawi MT, Abbas RM. Etiology of pleural effusion among adults in the state of Qatar: a 1-year hospital-based study. *J.Eastern mediterranean health.* 2009; 17 (7):611-612.
21. Sunita H, Doddamani GB, Linganand LP, Prasad CVB. Pleural fluid analysis in HIV associated tuberculosis patients. *J. Clinical Practice*. 2012; 23(1):45-49.
22. Shehab AL, Omaima TAM, Waleed IAO. Bacteriological, and serological study on synovial fluid in septic arthritis patients. *J.Acad.biolog. Sci*. 2010; 2(2): 27- 35.
23. Moen K, Johan G, Eribe B, Emenike RK , Olsen I, Jonsson R. Oral bacterial DNAs in synovial fluids of arthritis patients. *J.Microbial Ecology in health and disease*. 2005; 17: 2-8.
24. Mardh PA, Nisson FJ, Bjellea N. Mycoplasmas and bacteria in synovial fluid from patients with arthritis. *J. reheumatoid disease*.1973; 32:319.

25. Mulu A , Kassu A , Tessema B. Bacterial isolates from cerebrospinal fluids and their antibiotic susceptibility patterns in Gondar University Teaching Hospital, Northwest Ethiopia .Ethiop. *J.Health*. 2005; 19(2):160-164.
26. Scott B. *Diagnostic microbiology*. 2002; 11(323):1678-1682.
27. Zlenitsky S, Franzuk C. Antibiotic tolerance of peritoneal bacterial isolates in dialysis fluids. *J. Antimicrob. Chemother*.2002; 49(5), 863-6.
28. Nwadioha SI. Bacteriological isolates from Cerebrospinal Fluid of suspected acute meningitis in Nigerian Children. *J. Inter. Infec. Disease*. 2013; 1(8):79.
29. Babak A, Ahangarzadeh RM, Abdoli OS. Etiology and antimicrobial resistance patterns of acute bacterial meningitis in children. *J. Medi. Iran red crescent*. 2014 ; 16(7): 17616.
30. CDC. Pneumococcal Disease. *Epide and prevention of vaccine-preventable diseases.* 2012; 580: 16.
31. Bankar S, Baveja SDA. Study of ascetic fluid for diagnosis of spontaneous bacterial peritonitis (SBP) in adult patients with cirrhosis. *J.International Medical and applied* . 2014; 3(9):768.
32. Oliviero R, Stefania A. Ascitic fluid analysis for diagnosis and monitoring of spontaneous bacterial peritonitis. *J.World.Gastroenterol*. 2009; 15(31): 3845–3850.
33. Saleem F A, Shaikh SA, Khan SR, Khan F, Faruque VA, Muhammad Khan MA. Empyema thoracis in children, clinical presentation, management, and complications**.** *J. of the college of physicians, and surgeons Pakistan*. 2014; 24:30-35.
34. Kerr D N S , Pearson D T. , Read A E. Infection of ascitic fluid in patients with hepatic cirrhosis.*Medical journal* .2012;5(8):45.
35. Rezaeizadeh G, Pourabar B, Mohamed H A. Antimicrobial susceptibility of bacteria isolated from cerebrospinal fluids in an Iranian referral pediatric center. *Medical. J. Clin Medicin.* 2013:79(20): 2012- 131.
36. Kurien T, Mukkai K, Balaji V, Sudha J, John J, Mitra S, etal. Invasive pneumococcal disease associated with high case fatality in India. *J.Clini. Epedemology* .2013; 66: 36-43.
37. Varghese T. Routine analysis of cirrhotic ascites for evidence of infection not worth the effort?. *J. Internation. Gastroenrto*. 2011) 30(5):201.

38. Yagupsky P, Press J. Using of the isolator 1.5 Microbial tubes for culture of synovial sluid from patients with septic arthritis. *J.Clinical. Medicin*.1997; 35(9): 2410–2412.
39. Ryan MJ, Kavanagh R, Wall PG, Hazleman BL. Bacterial joint infection in England and wales analysis of bacterial isolates over four year period. *J.of Rheumatology.*1997;36: 370-375.
40. Syed VA, Ansari JA, Karki P, Regmi M, Khanal B. Spontaneous bacterial peritonitis (SBP) in cirrhotic ascites a prospective study in a tertiary care hospital Nepal. *J.Kathmandu University Medical.* 2007; 5 (17): 48-59.
41. Shrestha G R,Tandukar S, Ansari S, Subedi A, Shrestha A, Adhikari N P, Basnyat R S and Jeevan Sherchand B. Bacterial meningitis in children under 15 years of age in Nepal. *BMC Pediatrics*.2015;15(94): 2 – 7.
42. Yoon SH, Choi NW**.** Detecting bacterial growth in continuous ambulatory peritoneal dialysis using two culture methods. *J.of internal medicine*. 2010; 25(1):65.
43. Sujatha R, Nidhi P, Arunagiri D, Narendran D. Bacteriological profile and antibiotic sensitivity pattern from various body fluids of patients attending Rama medical college hospital, kanpura. *J.International of Advances in Case Reports*. 2015; 2(3):119-124.
44. Daur A. V, Klimak J.F, CogoL L, Botão D. G, Monteiro B. L , Costa D .M. Enrichment methodology to increase the positivity of cultures from body fluids. *J.Brazilian of infectious diseases*. 2006; 10(6):372-373.
45. Thiago J B R , Andrade J M O, César L M, Antonieta L, Milena A M, Pagliarelli M, Antonangelo L. Characteristics of ascitic fluid from patients with suspected spontaneous bacterial peritonitis in emergency units at a tertiary hospital. *J.Sao Paulo Med*. 2011; 129 (5): 1516.
46. Langra FM, Palac M, Paiva F M, Veki MV, Martins M C, Sato N D. Bacterial agents isolated from cerebrospinal fluids fluids of patients with acquired immunodeficiency syndrome and neurological complication. *J.Med . trop. S. Poul*. 1994;36(6):491-496.
47. Heikki P, Tuula P , Luis B, Lurdes M , Silvestre DC, Anjos E, *et al.* Emerging infectious diseases. *J.Medic.Health.Scince*. 2014; 20(11):76.

48. Ahmed S L, Omaima T M, Waleed I O. Bacteriological, and serological study on synovial fluid in septic arthritis patient. *J.Egypt. Acad*. 2010; 2(2):27-35.
49. Omer I, Hummeida, M.A, Mus H A. Improved conventional method for the laboratory diagnosis of peritonitis from peritoneal dialysate in Sudan. *J.Peritoneal dialysis internati* . 2011 ; 31 (4):495-498.
50. Balan K, Sujitha K, Vijayalakshmi TS. Antibiotic susceptibility pattern of gram-negative clinical isolates in a teaching tertiary care hospital. *Sch. J. App. Med. Sci*. 2013; 1(2):76-79.
51. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests; Twenty-Second Informational Supplement. *CLIS 2012; 32(3): M100-S22.*

AnnexI:Laboratory procedures for biochemical reactions and drug susceptibility testing (CLIS guidelines).

I. Specimen Collection, Transport and Handling

A Specimen collection:

All body fluid were collected by physicians using aseptic procedure, and send in to labratorys for analysis of protein, glucose, WBC and culture.

B. Specimen transport and handling

All body fluids collected 3-5 ml , and centrifuging at 2,500 rpm for 10 minutes to concentrate any organisms of present. If less < 1 ml of body fluids were received directly, samples were inoculated in to the media. CSF stored at 37 C^{O}-degree incubator to maintain body temperature or keep at room temperature. CSF never be refrigerating because fastidious organisms may not survive at lowered temperatures.

a. Cloudy specimens were not centrifuged.

b. The sediment is plated to media, and did for gram stain, and AFB.

II. Direct Examination

- Appearance of fluids,WBC,Gram stain , and AFB

White Cell Count (WBC)

Body fluid were counted for leukocytes and differential by manual method and authomated sysmex (kx-5014) machine.

Procedure of Manual method

- Two type of body fluids clear(no need dilution) and cloud / tramatic(diluited by 2% acetic acid)
- Dilute the cloudy body fluids 1:10
- Mix and Place the diluited fluids in to the cover glass and the counting chamber
- Allow the mixture to flow under the cover glass until the chamber is completely charged and allow the cells to settle
- Count the white cells in the four corner area
- WBC / mm3= No. of cells X dilution factor/volume
- Differentials by wright stains report for poymorphic feature and monomorphic feature

Procedure of gram Stain

Organisms are classified according to their Gram stain reaction - Gram positive and Gram negative. Gram-positive bacteria have thicker and denser peptidoglycan layers in their cell walls. Iodine penetrates the cell wall in these bacteria and alters the blue dye to inhibit its diffusion through the cell wall during decolourisation. Gram-negative cells, which do not retain the methyl/crystal violet, are stained by a counterstain of safranine.

- Prepare a smear and heat gently to fix.
- Flood the slide with 0.5% crystal violet and leave for 30sec.
- Tilt the slide, and rinse slide gently with water.
- Flood on sufficient (1%) Lugol's Iodine.
- Tilt the slide and wash off the iodine with water.
- Decolourise with 95 - 100% ethanol or acetone.
- Rinse with water.
- Flood the slide with 0.1% counterstain safranin.
- Rinse with water and dry obserb using 100x(immersion oil)

Interpretation

Gram- positive organisms stain deep blue/purple.

Gram- negative organisms stain pink/red.

Ziehl-Neelsen stain (for acid fast bacilli)

This staining technique is used to demonstrate the presence of acid and alcohol fast bacilli (AAFB) which have waxy envelopes that make them difficult to stain and decolourise. In this method heat is used to help drive the primary stain into the waxy cell walls of these difficult to stain cells.

- Flood the slide with strong carbol fuchsin.
- Heat the underside of the slide gently until steam rises but not boiling.
- Leave for 3-5min keeping the slide moist with stain.
- Rinse the slide well in a gentle and indirect stream of deionised water until no colour appears in the water.
- Decolourise for 10-20sec with a (3% v/v) acid-alcohol solution and then rinse well with water. Repeat the decolourising and the washing until the stained smear appears faintly. pink and the water washing off the slide runs clear.

- Counter stain with (1% w/v) methylene blue or malachite green for 20-30sec.
- Rinse with water and allow to dry.
- 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

III. Culture Setup

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

IV. Culture Interpretation

All body fluid are sterile so any organisms that grow, regardless of the quantification, are going to be process for identification and sensitivity.

C. Culture and identification

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

Positive/present □ Negative/absent □

b) Identification steps for suspected colonies

Gram stains/AFB stains/Oxidase test positive □ /negative □/Catalase test positive □ /negative □/Coagulase test positive □ /negative □/Lactose

c) fermentation from MacConkey agar

□ Lactose Fermenter/□ Late Lactose fermenter/□ Non lactose fermente

d) Biochemical reactions

Identification of bacterial isolates involves the use of biochemical screening Medias. Indole, Urease, Mannitol, Triple sugar iron (TSI), Citrate, Motility, Lysine Decarboxylase, Mannolet , and Oxidase tests.

Biochemical reactis	Indo	Urea	Man	TSI	Cit	MA	X
Result positive/negative							
Gram negative rods							

Key: LDC = Lysine decarboxylase, Man = Mannitol (mannite), Triple sugar iron (TSI), Ox = Oxidase test, Cit = Citrate test, Mot = Motility, Ind = Indole test, Urea = Urease, H2S = Hydrogen sulphide (blackening), R = Red-pink (alkaline reaction), Y = Yellow (acid reaction), d = different strains give different results.

A. **Indole test**: Few colonies of the culture will be inoculated into peptone water and incubated at 37oC for 24 hours. Few drops of indicator (Kovac's reagent) will be added and gently shake to mix well. Colour change will be then observed. If the layer of indicator reagent turns to red within 1 minute, it is Indole positive (positive result). If the layer of indicator reagent remains yellow within 1 minute, it is indole negative (negative result).

B. **Urease test (Christensen's (modified) urea broth)**: Urea agars will be inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap will be loosened and then incubated at 37oC for 3-12 hours. A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish red color of the Medium. Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.

C. Triple Sugar Iron (TSI) Agar Slant: Using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism.

Incubate at 37◦C for 18 to 24 h. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only. If alkaline slant–alkaline butt (red–red): sucrose and glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

D. **Citrate utilization test using Simmon's citrate agar**: Simmon's citrate slopes will be prepared in bijou bottles as recommended by the manufacturer (stored at 2-8oC). And slopes will be then stabbed and incubated at 37oC aerobically for 48 hours. Blue colour indicates a positive reaction and if Simmon's citrate agar slopes remained as green in colour indicate negative reaction.

E. **Motility Test (using motility agars)**: Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35-37oC for 24 hour. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

F. Lysine decarboxylase: Decarboxylation of lysine can be detected by culturing bacteria in a medium containing the desired amino acid, glucose, and a pH indicator bromcresol purple. The acids produced by the bacteria from the fermentation of glucose will initially lower the pH of the medium and cause the pH indicator to change from purple to yellow. The acid pH activates the enzyme that causes decarboxylation of lysine to amines and the subsequent neutralization of the medium. This results in another color change from yellow back to purple. Bacteria that decarboxylate lysine turn the medium purple. Inaddition bacteria that produce H2S appear as black colonies.

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

3. Antibiotics susceptibility result for Bacteria isolates

Isolated bacterial	antibiotics		AML	AMC	C	GM	CTX	NOR	TE	CRO	SXT	FOX	OX	IMI	MEM
	Susceptibility Patern	S													
		I													
		R													

NOTE: Amoxacillin(AML), Chloramphericol(C), Ceftriaxone(CRO), Cefotaxime(CTX), Tetracycline(TE), Gentamicine(GM), Amoxicilin-Clavulanic acid(AmC), Cefoxitine(FOX) , Norfloxacin(NOR), Oxacillin(OX), Imipenem(IMI) and Merepenem(MEM)

Inoculation of Test Plates

- Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level.
- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.
- The lid may be left ajar 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 battery of antimicrobial discs is 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

After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers which is held on the back of the inverted plate.

Annex II: English version of participant information sheet, consent form and questioner

Participant information sheet

Department of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title

Bacterial isolate and there susceptibility pattern from body fluid at Tikur Anbessa specialazed Hospital, Addis Ababa, Ethiopia.

First, we would like to thank you 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. If you have any question regarding the study, please ask free

Background information

Body fluids serve as a medium for carrying nutrients and waste products from the cells as well as among cells. Different types of body fluids are included in our body like Urine, Amniotic fluid, seminal, saliva, and sweet, Pleural, Peritoneal, Cerebrospinal, Synovial, and Pericardial etc. The most common types of samples that are routinely encountered in the clinical laboratory such as; Pleural, Peritoneal, Cerebrospinal, Synovial, and Pericardial etc. All these body fluids are usually sterile microorganisms like bacteria, fungi, virus and parasites may invade and infect the body fluids and results in severe disease like; meningitis, spontaneous bacterial peritonitis (SBP), pleural effusion (PE) , Pericardial effusions, and Septic arthritis. To be able to diagnose and manage properly, those patients who had body fluids cased by bacteria's, it is necessary to treat patient using antibiotics to reduce the morbidity and mortality.

Aim of the study

The purpose of the study is to determine the profiel of bacterial isolate and their susceptibility pattern from body fluid at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, results have bee given to their physician for treatment or to get counseling. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policy makers.

Risks and complication

There is no considerable risk to the participants during their participating in the study other than the painful at the site of puncture during collection of body fluid ,especially collection of CSF it is boring and painful such like CSF sample must be handle with carefully and samples not be discarded until all investigation completed ,if there is any dalliance the sample shoud be stored at room temperatures, and it doesn't need refrigerator.

Confidentiality

In order to maintain the confidentiality of participants' information, the name were not be given and the samples were be coded. Participants were not be prohibited to stop or withdraw at any time from the study. No personal information were be disclosed to third party or was not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Frehiwot T/haymanot (PI): Signature: _____ Date: _____

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

PI Address: Frehiwot T/haymanot : Department of Medical Laboratory Sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: frehiwo2005@gmail.com; T\el.: +251911864285

Informed consent

I have been informed about the study, which plans to determine the prevalence and antimicrobial resistance of bacterial isolates from body fluid at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. The objective and the application of the study was briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give for the mentioned study. I agreed that the specimen were be tested for any bacteria. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the

analysis of body fluid isolates of any organism will be given to the health facility and that I may ask the information if I want.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participantcode:_____Signature:_____Date:_____

Socio demographic factors and patient identification

A. Patient Identification

1. Patient name----------

2. Hospital registration no----------

3. Age----------

4. Sex ----------

Annex III: Amharic Version of the participant information sheet, Consent form and questioner (የተሳታፊዎች መረጃ ቅጽ፤ የፈቃደኝነት ማረጋጫ ና መጠይቅ)

3.1 የተሳታፊወች መረጃ ቅጽ

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮላጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንትአርስት፣ በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ ና ለሽንት መሽንያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድሐኒት ያለውን የግትርነት ደረጃ፤ እና መንስኤዎቹን ማሳየት፡፡

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ::

መግቢያ

ሰፕቲሴሚያ ና የሽንት መሽኒያ በሽታ በተለያዩ ተህዋስያን የሚመጡ የጤና ችግር ሲሆኑ በዓለም ላይ የሚያደርሰት የሞት እና የኢኮኖሚ ቀውስ ከፍተኛውን ደረጃ ይይዛል፡፡ በተለይም በታዳጊ ሀግሮች ውስጥ ለሚገኙ ህዝቦች የሞት እና ጉዳት ዋነኛ ምክንያት ነው፡፡ ለዚህም ዋነኛ መንስኤው ኢንተሮባክተሪሴ የተባለት ተህዋስያን የሚያመጡት በሽታ ምክንያት የሚሞቱት ሰዎች ከፍተኛውን ቁጥር ይይዛሉ፤ በተጨማሪም እነዚህ ባክቴሪያዎች ለመድሐኒቶች በከፍተኛ ደረጃ የግትርነት ባህሪ እያሳየ መሆኑ ችግሩን ውስብስብ አድረጎታል፡፡ ሥለሆነም ይህን በሽታ ከፍተኛ ጉዳት ከማስከተሉ በፊት በመከላከል የሕብረተሰቡን ሞት ለመቀነስ ያለውን ስርጭት እንዲሁም ለመድሃኒቶችም የለዉን የግትርነት ማዎቅ በጣም አስፈላጊ ነው፡፡

የጥናቱ አላማ

በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ ና ለሽንት መሽንያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድሐኒት ያለውን የግትርነት ደረጃ፤ እና መንስኤዎቹን ማሳየት፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም:: ነገር ግን በጥናቱ መሰረት ተህዋስያኑ ለተገኘባቸው ተሳታፊዎች ከሚመለከታቸው የሆስፒታሉ ባለሙያዎች ጋር በመነጋገር አስፈሊጊ የሆነ ህክምና እንዲደረግላቸው ይደረጋል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ ለዚህ ጥናት የምንጠቀምበት የደም ናሙና

ከክንድዎ የሚዎሰድ ሲሆን መጠኑም ከ 5 ሚሊ ሊትር (ከ 1 -2 የሻይ ማንኪያ) ነው፡፡ ይህም ከመጠነኛ ስሜት በስተቀር

በጤናዎ ላይ ምንም አይነት ጉዳት አያደርስም፡፡ ለሽንት ምርመራ የሚሆን ሽንት በሚያመጡበት ስአትም ቢሆን ምንም የተለየ ስሜት የለዉም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ ሊያገኙ የሚችሉ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማ ብቻ የሚገለፅ ይሆናል፡፡

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የዋና ተመራማሪው አድራሻ፤

መለሰ ኃይሉ ለገሰ፤ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስ አበባ፣ ኢትዮጵያ

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3.2 የፈቃደኝነት ማረጋገጫ ቅፅ

በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ ና ለሽንት መሽንያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድሐኒት ያለውን የግትርነት ደረጃ፤ እና መንስኤዎቹን ማሳየት በሚል ርዕስ ለይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ ሙሉ መረጃም ሆነ የደም ናሙና ውጤቴ በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠዉ የደም ናሙና እና የሽንት ናሙና ለኢንተሮባክቴርሴባክቴሪያዎች ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች ለተቋሞች እንደሚሰጥና ዉጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡ ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ_____

መጠይቅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ዲፓርትመንት

ይሕ የመጠየቅ ቅጽ ስለተሳታፊዎች አጠቃላይ መረጃ ና ለበሽታው (ለኢንትሮባክተርሴዎች) መንስኤ ሊሆኑ የሚችሉትን ምክንያቶች ለማወቅ የሚደረግ መጠይቅ ነው፡፡ መጠየቁን የሚያስሞላው በጥናቱ ጊዜ

የተመረጠው(ችው) የነርስ ባለሙያ ሲሆን መጠየቁ የሚሞላው ጥናቱ በሚከሄድበት ቦታ ነው፡፡ ለጥናቱ መሳካት ና ትክክለኛነት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ አንብበዉ በታማኝነት ያለምንም ፍረቻ እንዲሞሉልን በትህትና እንጠይቃለን፡፡

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

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

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

ሀ. ስለበሽተኛዉ አጠቃላይ መረጃ

1. የበሽተኛዉ ስም----------

2. እድሜ----------

3. ፆታ----------

4. የሕክምና መለያ ቁጥር----------