Addis Ababa University

College of Health Sciences School of Medicine

Department of Microbiology, Immunology, and Parasitology

Comparison of the Efficiency of CSF and Serum PCR in the Diagnosis of Bacterial Meningitis Among Suspected Cases at Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia

By: Lema Ayele (B.Sc, M.Sc candidate)

A Research Thesis Submitted to the Department of Microbiology, Immunology, and Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

February 2025

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences School of Medicine

Department of Medical Microbiology, Immunology, and Parasitology

Comparison of the Efficiency of CSF and Blood PCR in the Diagnosis of Bacterial Meningitis Among Suspected Cases at Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital Medical College, Addis Ababa Ethiopia

Principal investigator: Lema Ayele (BSc, M.Sc. candidate)

Addis Ababa University, Ethiopia. Email: layele2016@gmail.com

Tel: +251-911-35-36-72

Supervisors:

1. Daniel Asrat (MD, MSc, PhD, Professor)
   Consultant Medical Specialist (Medical Microbiologist) Department of Microbiology, Immunology, and Parasitology School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia Tel: +251-911-22-30-19 Email: asratdan@gmail.com, daniel.asrat@aau.edu.et
2. Getachew Tesfaye (PhD)
   Post-doctoral researcher at Communicable and Non-communicable Diseases Research Directorate, Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia, Tel: +251-911347873, Email: Getachew.tesfaye@ahri.gov.et

Co-supervisors:

3. Aminu Seman (M.Sc, Lecturer)
   Department of Microbiology, Immunology, and Parasitology, School of Medicine, College of Health Science, Addis Ababa University, and Addis Ababa, Ethiopia. P.O. Box: 9086 AAU Tel: +251 920 747 176
   E-mail: aminu.seman@aau.edu.et or aminumifta54@gmail.com
4. Dr. Muluwork Tefera (MD) Department of Paediatrics Emergency, College of Health Science Addis Ababa University, Addis Ababa, Ethiopia. Email: muluworktef@yahoo.com

ACKNOWLEDGMENT

Firstly, I would like to express my deep gratitude to Addis Ababa University, College of Health Sciences School of Medicine, Department of Microbiology, Immunology, and Parasitology, for offering such a golden opportunity to conduct this research work. Above all, I would like to express my sincere feelings for my supervisors for supporting me step by step through this research process. Professor Daniel Asrat, and Mr. Aminu Seman deserve special mention for their guidance, both personal and academic, from the beginning to its completion. Their contributions were instrumental in my success. I am deeply indebted to Dr. Getachew Tesfaye. The opportunity he created for myself and my fellow grant recipients, along with the fostering research environment at Armauer Hansen Research Institute, deserves immense appreciation. Dr. Muluwork Tefera deserves special thanks for the supportive environment during data collection at the Pediatrics and Child Health Department of Tikur Anbessa Specialized Hospital. Furthermore, I would also like to thank the staff of the Armauer Hansen Research Institute for the necessary support during my stay. Their commitment and assistance were therefore so important. I would like to extend my great appreciation to all study participants, staff of Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital Medical College, service-dedicated physicians and nurses, and my great friends and families who were very supportive of me, especially during the difficult time of sample collection. Over and above all, I thank the King of the heavens and the earth, my Lord Jesus Christ, Son of the Living God.

TABLE OF CONTENTS **PAGE**

LIST OF TABLES PAGE

LIST OF FIGURES **PAGE**

ABBREVIATIONS/ACRONYMS

AAU	Addis Ababa University
AHRI	Armauer Hansen Research Institute
BM	Bacterial Meningitis
CDC	Centre for Disease Control and Prevention
CLSI	Clinical & Laboratory Standards Institute
CNS	Central Nerve System
CRF	Case Record Form
CRISPER -Cas	Clustered Regularly Interspaced Short Palindromic Repeats
CSF	Cerebrospinal Fluid
Hib	*Haemophilus influenzae* type b
LMIC	Low and Middle-Income Countries
LP	Lumbar Puncture
NAATs	Nucleic Acid Amplification Tests
PCR	Polymerase Chain Reaction
SPSS	Statistical Package for The Social Sciences
STGG	Skim Milk Tryptophan, Glucose, And Glycerol
TASH	Tikur Anbessa Specialized Hospital
Y12HMC	Yekatit 12 Hospital Medical College

ABSTRACT

Background-Early and accurate diagnosis of bacterial meningitis reduces mortality and sequelae. In resource-limited settings like Ethiopia, where laboratory diagnostics are scarce, developing a diagnostic tool based on an easy-to-collect blood sample for the diagnosis of bacterial meningitis is crucial to improving the diagnosis and prompt treatment initiation that has a great impact on patient outcomes.

Objective- To compare the efficiency of cerebrospinal fluid and blood PCR for the diagnosis of bacterial meningitis in patients suspected of meningitis.

Methods- An institutional-based cross-sectional study was conducted at Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital Medical College from August 2023 to February 2024. A total of 202 patients who were suspected of having meningitis were given their consent to collect the demographic, clinical data, CSF, and blood samples. Demographic data and clinical samples (CSF and Blood) were collected by using pre-tested standardized questionnaires and aseptic techniques, respectively. Conventional laboratory analysis and bacterial isolation using aliquots of CSF were performed following the standard microbiology laboratory procedures. The leftover CSF and serum samples were stored at -20°C until transported to the Armauer Hansen Research Inistitute (AHRI) for further molecular tests. Genomic DNA from CSF and serum was extracted by the Bioer NPA-32P automated nucleic acid purification machine. A multiplex PCR technique was used to detect the presence of *Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus agalactiae, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes,* and *Klebsiealla pneumoniae*. The end product of the PCR amplicon was observed under the Vilber machine after staining with ethidium bromide. Data were entered into Epiinfo 7, cleaned, exported to Excel, and loaded into SPSS version 26 software for analysis. Descriptive statistics were presented in tables and bar charts. The agreement between the tests to detect bacterial meningitis was calculated by using an SPSS contingency table.

Result- Out of 202 study participants, 54% (n = 109) were males, and 88.6% (n = 179) of them were from the pediatric population, with an average age of 1.45 ± 0.69 years. Reduced ability to breastfeed was the most common clinical presentation 74.8% (n = 151), followed by high fever 71.3% (n = 144) and a loss of consciousness 66.3% (n = 134). Of 202 CSF, 5.4% (n = 11) and 4.5% (n = 9) of the samples tested were positive for gram stain and culture, respectively.

Overall, bacterial DNA was detected in 26% (n = 54) and 46% (n = 93) CSF and serum samples, respectively. The concordance between CSF and serum PCR was observed in 34 (17%) cases. *E. coli* was the most predominant bacteria detected in both CSF and serum PCR at 55.5% (n = 30) and 61.2% (n = 57), respectively, followed by *S. pneumoniae* detected in both CSF and serum PCR at 15% (n = 8) and 29% (n = 27), respecively.

The overall agreement between CSF and serum PCR is 60.9% with a positive percent agreement, and negative percent agreement between the two tests is 62.9% and 60.1%, respectively.

Conclusion- Bacterial DNA was 20% more frequently detected in serum compared to CSF, and 42% compared to culture in suspected cases of meningitis. Furthermore, these findings are clinically very important to minimise the invasive lumbar puncture for CSF collection. Overall, serum PCR could be considered as a supplementary diagnostic tool, a less invasive diagnostic procedure for the diagnosis of bacterial meningitis. However, further similar studies are important using qPCR and both blood and CSF culture.

Keywords: Bacterial Meningitis, Cerebrospinal fluid, Serum, polymerase chain reaction, Diagnosis, Ethiopia.

CHAPTER 1: INTRODUCTION

1.1. Background

Meningitis is a very serious disease of the central nervous system (CNS) that inflames the defensive membranes of the brain and spinal cord. This inflammation is the result of an infectious agent in the cerebrospinal fluid (CSF) surrounding the brain and spinal cord (Brouwer *et al.*, 2010).

Bacterial meningitis (BM) continues to be a significant infectious disease globally, despite the progress made in treatment and vaccination (Bijlsma *et al.*, 2016). It is a serious and life-threatening condition that requires early diagnosis and treatment (Lien *et al.*, 2019; Ramgopal *et al.*, 2019; Fuentes-Antrás *et al.*, 2019).

Neisseria meningitidis (N. meningitidis), Haemophilus influenzae (H. influenzae Hib*), Streptococcus pneumoniae (S. pneumoniae),* and *Streptococcus agalactiae (S. agalactiae),* are among the four most common bacterial species involved in meningitis (Brouwer *et al.*, 2010; van de Beek *et al.*, 2016). *N. meningitides* and *S. pneumoniae* are the most common organisms causing meningitis in children and adults. *H. influenzae* causes meningitis at all ages, being especially important in children under 5 years of age. *Escherichia coli (E. coli), S. agalactiae,* and *Listeria monocytogene (L. monocytogene*) are the most common bacterial species in neonates (Hamborsky and Kroger, 2015).

The incidence of bacterial meningitis and its mortality rate vary from one area to another, age group, and type of organism (Hamborsky and Kroger, 2015). In areas where human immune deficiency (HIV) is widespread, such as sub-Saharan Africa, the mortality rate could potentially rise to 50% (Wall *et al.*, 2013; Van de Beek *et al.*, 2010). In Ethiopia, bacterial meningitis accounts for 6–8% of hospital admissions and has a fatality rate ranging from 22–28% (Hailu and Muhe, 2001).

The most common clinical symptoms observed in patients with bacterial meningitis include high fever, headache, and loss of consciousness. Two-thirds of adult patients with bacterial meningitis present the triad, although all patients experience at least one of these symptoms (Runde *et al.*, 2017). The classic symptoms may not be observed in neonates and elderly patients (Başpınar *et al.*, 2017).

Diagnosing of bacterial meningitis in low-resource settings like Ethiopia is challenging due to late patient presentation (Desmond *et al.*, 2013), limited diagnostic facilities (Scarborough and Thwaites, 2008), and the high technical skills required to collect CSF samples. Conventional tests such as white blood cell count (WBC), glucose, proteins in CSF, and gram staining are inexpensive and fast but not very specific (Dubos *et al.*, 2006; Neuman *et al.*, 2008). Bacterial cultures require several days for a definitive diagnosis to be made; the need for the best possible environment as well as their sensitivity and specificity are low relative to the molecular method (Obaro, 2019). Generally, studies have shown that the high rates of morbidity and mortality linked to meningitis, particularly in children, are partly caused by delayed detection and diagnosis. Thus, developing a reliable molecular method for the detection of agents of meningitis is clinically relevant. In this regard, molecular methods offer several advantages over culture-based methods, including increased sensitivity, specificity, speed, and efficiency (Albuquerque *et al.*, 2019; Diallo *et al.*, 2021).

On the other hand, while the gold standard for the diagnosis of meningitis is considered to be CSF culture, followed by CSF PCR, bacterial growth, particularly in the patients who have received pre-admission antibiotic treatment, is very low. In addition, to use results from CSF culture for patient management, one has to wait from 24 to 48 hours; when the number of pathogens in the CSF is very low, it may take up to 72 hours. Obtaining a CSF sample, particularly from young infants, is technically very difficult and invasive/painful for the patient. Blood is considered an alternative specimen for the diagnosis of meningitis, despite it being simple to collect. Three decades back a study (Newcombe *et al.*, 1996) showed both the sensitivity and the specificity of detection of meningococcal DNA in serum samples were unaffected compared to patients with confirmed meningococcal diseases determined either by culture or by the presence of gram-negative diplococci in CSF.

Thus, the objective of this study is to compare the efficiency of CSF and serum PCR in detecting bacterial meningitis with the ultimate goal of developing a molecular diagnostic test using an easy to collect blood samples and overcoming the challenges of CSF samples for better patient management.

1.2. Statement of Problem

Bacterial meningitis causes significant mortality and morbidity worldwide; the heaviest burden of disease is experienced in sub-Saharan Africa. According to the global burden of disease study, meningitis caused 318,000 deaths worldwide in 2016 (Naghavi *et al.*, 2018), 48.9 million estimated cases, and 11 million deaths accounted for 20% of all deaths in 2017 (Rudd *et al.*, 2020), and about 236,222 deaths in 2019 (Qu *et al.*, 2023).

In the USA, more than 72,000 people are hospitalized by meningitis every year. Its treatment costs as much as 1.2 billion dollars. Most of the cases were of bacterial infections that accounted for almost 21.8% of total hospitalizations due to meningitis (Holmquist *et al.*, 2006). From 2016 to 2023, the bacteria-related meningitis rate was approximately 1.33 per 100,000 persons, accounting for about 4,100 cases annually. While the annual mortality rate of bacterial meningitis is stable, 500 patients continue to die every year (Wunrow *et al.*, 2023).

Infectious meningitis can result in high mortality and long-term neurological and cognitive impairments (50% of survivors developing neurological complications) (Zainel *et al.*, 2021). Mortality due to bacterial meningitis ranges from 10-20% in high-resource settings to as high as 50% in low-resource settings where these infections are particularly prevalent (Amidu *et al.*, 2019). Because of a lack of infrastructure and appropriate diagnostic facilities, high morbidity and mortality are recorded in these settings (McGloughlin *et al.*, 2018; Kumalo *et al.*, 2016).

Bacterial meningitis is the most severe public health problem for sub-Saharan Africa in every aspect. This is due to poor diagnostic facilities, with proper treatment options not very easily available at the primary hospital level (Scarborough and Thwaites, 2008; Jafri *et al.*, 2013).

Bacterial meningitis is of significant health concern in Ethiopia, as the country stands among those that are most affected by the disease within the African meningitis belt (Memish and Alrajhi, 2002). It is the 9th most common cause of years of life loss and disability-adjusted life years in the country (Bårnes *et al.*, 2018). Despite its high burden, Ethiopia lacks molecular diagnostic tools based on easy-to-collect samples such as blood. The research findings will have a positive impact on enhancing diagnosis and patient outcomes. This project aims to enhance the diagnosis of bacterial meningitis in resource-limited areas by comparing the efficiency of CSF and serum PCR in patients suspected of meningitis.

1.3. Significance of the Study

In Ethiopia, the management of meningitis is primarily based on clinical presentations. However, decisions relying on clinical presentation are often problematic and prone to wrong diagnosis leading to wrong treatment. Hence, we aim to improve patient management by enhancing diagnostics through developing resources and time-saving molecular diagnostic tools that are based on a relatively easy-to-collect blood sample. Thus, this study has significance in that it will involve the development of a rapid PCR diagnostic method based on an easy-to-collect blood or serum sample. This has a very critical advantage both for the patients and healthcare workers, as it avoids the invasive procedure and difficulties of CSF collection. Overall, this study will have the potential to have a significant impact on patient management and relieve patient pain during sample collection.

CHAPTER 2: LITERATURE REVIEW

2.1. Etiology of Meningitis

Gram-negative enteric rods such as *E. coli* and some other organisms such as *L. monocytogenes* and *S. agalactiae* are more common during the neonatal period (Zhai *et al.*, 2022), while S. *pneumoniae, N. meningitides,* and *H. influenzae* are more common in children and young adults (Brueggemann *et al.*, 2021).

2.1.1. *Neisseria meningitidis*

A gram-negative diplococcus is an obligate commensal bacteria residing in the human nasopharynx. The highest incidence of nasopharyngeal carriage of *N. meningitidis* is in adolescents, especially those residing in overcrowded spaces (Harrison *et al.*, 2009). Particularly prone are school-going children and college students, household contacts of meningococcal patients, and also military recruits (Yazdankhah and Caugant, 2004). *N. meningitidis* can exist with or without a polysaccharide capsule. However, nearly all meningococcal meningitis infections are caused by the capsulated form. Based on the polysaccharide characteristics, *N. meningitidis* can be divided into at least 12 different serogroups. Serogroups A, B, C, W, X, and Y are isolated in almost 90% of the infections patients. Serogroup A is frequently isolated from CSF samples of meningitis patients in sub-Saharan Africa, where it causes epidemics (MacNeil *et al.*, 2015).

2.1.2. *Streptococus pneumoniae*

Streptococus pneumoniae is a significant cause of bacterial meningitis. There are more than 90 known serotypes based on the polysaccharide capsule. The most common serotypes causing invasive pneumococcal disease worldwide include 1, 5, 6A, 6B, 14, 19A, 19F, and 23F (Walekhwa *et al.*, 2018). No age group is exempt from pneumococcal meningitis. It usually affects small children under the age of two years. Immunocompromised people are also at a higher risk of acquiring pneumococcal meningitis. *S. pneumoniae* spreads through respiratory droplets, similar to *N. meningitides* and *H. influenzae.*

The high rates of pneumococcal infections may partly be due to the high carriage rates among the general population. Children under 6 years of age have the highest rates of nasopharyngeal carriage (Lan *et al.*, 2024). This high carriage rate among infants and young children explains why pneumococcal disease is the leading cause of vaccine-preventable deaths in this age group (Hussen *et al.*, 2020).

2.1.3. *Haemophilus influenzae* type b (Hib)

The polysaccharide capsule forms the basis for classification into six serotypes-a, b, c, d, e, and f-of *H. influenzae*. Of these, the serotype b, commonly referred to as Hib, is associated with a high incidence of meningitis infections. Indeed, before the introduction of the vaccine, Hib was the leading cause of acute bacterial meningitis in children under the age of 5 years. The introduction of the Hib vaccine has since drastically reduced the incidences of this infection. Vaccination efforts have considerably reduced the diseases, thus showing that the vaccine is effective in maintaining protection for the populations at risk, notably children below five years of age (Gilsdorf, 2021).

2.1.4. *Streptococcus agalactiae*/Group B streptococcus

Streptococcus agalactiae, commonly known as Group B *Streptococcus* (GBS), is a gram-positive bacterium that is the leading cause of bacterial meningitis, particularly in neonates. *S. agalactiae* meningitis is more commonly associated with neonates; it can also occur in adults, particularly in those with chronic medical diseases or co-morbidities, and even in healthy individuals (Al-Bayati *et al.*, 2020; Protonotariou *et al.*, 2015).

2.1.5. *Listeria monocytogenes*

An intracellular facultative anaerobic bacterium that is Gram-positive and can cause meningitis in several people, including the elderly, immunocompromised, and neonates. It is infected in humans by eating contaminated foods, such as prepared foods, deli meats, and soft cheeses (Ranjbar and Halaji, 2018; Jackson *et al.*, 2018). It has 13 distinct serotypes, which differ in flagellar and depend on surface antigens, but almost three serotypes only (1/2a, 1/2b, 4a) cause all human diseases (Choi *et al.*, 2018).

2.1.6. *Esherichia coli*

The most common Gram-negative bacillary organism that causes meningitis, particularly during the neonatal period or in babies under 3 months of age. *E. coli* strains possessing the K1 capsular polysaccharide are predominant (approximately 80%) among isolates from neonatal *E. coli* meningitis, and most of these K1 isolates are associated with a limited number of O serotypes (Ku *et al.*, 2017). The infection is a serious condition, especially in newborns and premature babies (Kim, 2016).

2.1.7. *Klebsiella pneumoniae*

Klebsiella pneumoniae strains causing meningitis contain distinct resistance genes, capsular serotypes, virulence genes, and related clones, which might eventually lead to plasmid-mediated resistance and virulence transmission, which can have a negative influence on patient prognosis (Ku *et al.*, 2017). Individuals with meningitis caused by hypervirulent carbapenem-resistant *K. pneumoniae* (Hv-CRKP) had higher mortality than the other patients. The high percentage and fatality of *K. pneumoniae* caused meningitis particularly by Hv-CRKP strains, should be of significant concern (Huang *et al.*, 2022).

2.1.8. Other bacterial etiologies

Other uncommon causes of bacterial meningitis include *Staphylococcus aureus, Pseudomonas aeruginosa,* and some *Enterococci*. They are usually associated with health care associated infections and may be acquired after trauma or some surgical interventions (Tunkel *et al.*, 2004).

2.2. Epidemiology

Bacterial meningitis has been a serious health concern since several years ago. *N. meningitidis, H. influenzae* type b, and *S. pneumoniae* were the most common causes of bacterial meningitis between 1950 and 1970; they accounted for 70% of cases of meningitis in the United States. In 1977, Centre for Disease Control and Prevention (CDC) national surveillance showed 3 cases per 100,000, with the highest case rate among children under one year old (Brouwer *et al.*, 2010; Calabrese, 1977).

Since the introduction of vaccination programs for both *N. meningitidis* and *H. influenzae* type b, incidence rates have drastically declined. From 2.00 cases per 100,000 in 1998, the number of cases of bacterial meningitis declined to 1.38 cases per 100,000 in 2007. During this time, the median age of patients affected increased from 30.3 to 41.9 years (Cohn *et al.*, 2010). According to the report from 2017, the number of cases of meningococcal disease had decreased, indicating the efficacy of the immunization campaign (Thigpen *et al.*, 2011; Runde *et al.*, 2017).

In developed countries, the overall incidence of BM has stabilized. For example, in England, the mean annual incidence from 2012 to 2019 remained at 1.49 cases per 100,000, with variations in causative pathogens by age group. *S. agalactiae* meningitis was predominant among the infants (Doran *et al.*, 2016), though that of pneumococcal meningitis increased among the older adults (Koelman *et al.*, 2021).

Bacterial meningitis remains a big health problem, especially in the so-called "meningitis belt" of Africa that stretches from Senegal to Ethiopia. This region suffered from high incidences of the disease caused by various bacterial pathogens for several decades. It is estimated that from 1928 to 2018, there were approximately 2.6 million bacterial meningitis cases and more than 151,000 deaths attributable to Africa. The three major causative agents for meningitis in Africa include *N. meningitidis, S. pneumoniae*, and *H. influenzae* type b. Until 2010, sero-group A of Nm accounted for an estimated 80–85% of meningococcal diseases occurring in the meningitis belt. Since its introduction in 2010, the MenAfriVac vaccine has been introduced, and there has been a marked decline in NmA, while other serogroups such as NmW and NmX have emerged to be a great threat to public health. Despite the vaccination, the disease burden is high; it mainly affects children under five years of age (Mazamay *et al.*, 2021).

Meningitis remains endemic in Ethiopia, as in many parts of the meningitis belt, though a significant health issue, particularly during the dry season from December to June. *N. meningitidis* is the primary causative agent alongside *S. pneumonae* and *H. influenzae* type b (Mihret *et al.*, 2019; Mazamay *et al.*, 2021). New serogroups, such as *N. meningitidis* serogroup W and X, added to the geographical variation in disease incidence, require constant adaptation of monitoring and vaccination activities (WHO, 2022).

2.3. Clinical Presentation

The clinical presentation of a patient with meningitis constitutes a classical triad found in 41% of cases: fever, nuchal rigidity, and altered mental status. Moreover, additional symptoms like being sensitive to light, confusion, rash, joint pain, and cold hands and feet will be experienced in patients with bacterial meningitis. Presentation may also differ with bacterial meningitis based on age factor. Infants usually present with poor feeding, irritability, fever, stiff neck, bulging fontanelle, high-pitched crying, and abnormal reflexes, while in older adults, symptoms can be atypical; lethargy is often presenting without prominent meningeal signs (Bijlsma *et al.*, 2016).

2.4. Complications

Bacterial meningits can cause complications such as hearing defects, speech abnormalities, intellectual impairment, learning difficulties, and seizures (Koomen *et al.*, 2003). Complications are more common in small children, thus causing serious neurological defects, which often tend to be long-standing. These complications are local neurological deficits, paralysis of the limbs, developmental disabilities, seizures, cerebral abscesses, and hydrocephalus. Most of these complications in children usually resolve within 2-3 years, but 10% of the children may develop complications that persist throughout their lives (Bodilsen *et al.*, 2016).

2.5. Laboratory Diagnosis

2.5.1. Conventional Methods

Bacteria meningitis is best diagnosed by combining clinical assessment with laboratory evidence of the causative organism. The presence of bacteria in the CSF forms the basis of the diagnosis. The CSF characteristics highly suggestive of BM include an elevated CSF cell count (>500/µL), predominantly neutrophils, increased protein levels in the CSF (>1 g/l), increased levels of CSF lactate (31.53 mg/dL), and a lowered CSF/blood ratio of glucose (<0.4). Gram staining and culture are conventional approaches for identifying bacteria in CSF samples. However, the yield may be decreased if there is previous antibiotic treatment. The Gram stain and colony appearance can give hints to the specific organism (Berwal *et al.*, 2017) (Table 1).

Table 1: Classification of bacteria causing acute meningitis based on Gram-reaction and colony appearance (Murray *et al.*, 2011).

A. Gram-Positive Bacteria		
Bacterium	**Gram-reaction**	**Colony appearance**
S. aureus	Gram-positive cocci (clusters)	Yellow and round, opaque colonies may exhibit hemolysis on blood agar
S. pneumoniae	Gram-positive diplococci (lanceolate, short chains)	Little, clear, dome-shaped colonies with a "fried egg" look
S. agalactiae	Gram-positive cocci (pairs or chains)	Small, grayish-white colonies exhibits beta-hemolysis on blood agar
L. monocytogenes	Gram-positive bacilli (small rods)	Tiny, translucent, grayish-white colonies; shows beta-hemolysis on blood agar
B. Gram-Negative Bacteria		
N. meningitidis	Gram-negative diplococci ("coffee beans")	Small, greyish-white colonies with a wet, slimy appearance
H. influenzae	Small Gram-negative pleomorphic rods	Small, transparent, grayish-white colonies; requires special growth factors (X and V factors).

E. coli	Gram-negative rods (single or paired)	Large, lactose-fermenting colonies that are pink or red on MacConkey agar.
K. pneumoniae	Gram-negative bacilli (singles or pairs)	Large, mucoid colonies with a distinct "jelly" appearance due to capsule formation.

2.5.2. Molecular Methods

Nucleic acid amplification tests (NAATs) such as multiplex PCR testing can provide rapid and accurate diagnoses for bacterial meningitis (De Zoysa *et al.*, 2012; Bhagchandani *et al.*, 2016). Depending on the method used, results can be available in as little as 15 minutes and no longer than 3 hours for a single sample (Başpınar *et al.*, 2017; Albuquerque *et al.*, 2019). The time to results is greatly increased by shipping if the PCR test is not conducted on-site. Although the sensitivity and specificity of commercial NAATs are frequently greater than 90% for each bacterial pathogen, test performance differs depending on the pathogen and assay (Bhagchandani *et al.*, 2016; Tunkel *et al.*, 2004; Başpınar *et al.*, 2017; Albuquerque *et al.*, 2019). For example, the BioFire Film Array meningitis/encephalitis panel (FilmArray ME) is a PCR panel that rapidly (1 hour) detects 14 pathogens, including six bacteria, seven viruses, and one fungus, directly from CSF (Leber *et al.*, 2016; Wootton *et al.*, 2016). However, it is limited to pathogens designed for, infrastructure requirements, easy contamination of samples, the need for additional laboratory personnel training, and the lack of an isolate on which traditional antimicrobial susceptibility testing can be performed (Leber *et al.*, 2016).

Recently, advancement of new molecular techniques such as clustered regularly interspaced short palindromic repeats (CRISPER –Cas) (Batista and Pacheco, 2018; Barrangou and Horvath, 2017), Next-generation sequencing (NGS) (Zhang *et al.*, 2019; Guo *et al.*, 2019b) and Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) were the promising molecular techniques for detection of nucleic acid from CSF (Davis, 2018; Croxatto *et al.*, 2012).

Multiplex-PCR is a very useful tool for early diagnosis in bacterial meningitis. It not only provides the identification of infection but also important epidemiological data useful for vaccination programs to prevent transmission of diseases (Bårnes *et al.*, 2018; Albuquerque *et al.*, 2019).

Table 2: Summary of PCR Study for Diagnosing Bacterial Meningitis in Clinical Samples

Study Area	Years	Specimen	Approach	Remark	Reference
India	2022	CSF Samples (n=113)	Culture, multiplex PCR	Multiplex PCR detected 15% positive samples; culture detected only 10%.	(Sharma *et al.*, 2022)
United Kingdom	1996	Blood Samples (n=80)	PCR for meningococcal meningitis	PCR showed 100% sensitivity and specificity for diagnosed cases. PCR on blood is practical for patients where lumbar puncture is contraindicated.	(Newcombe *et al.*, 1996)
India	2022	CSF Samples (n=178)	Real-time PCR and culture	Real-time PCR exhibited 100% sensitivity and 81.3% specificity for compared to culture.	(Raza *et al.*, 2022a)
Egypt	2022	CSF Samples (n=48)	Multiplex PCR	94% sensitivity and 100% specificity for diagnosing bacterial meningitis.	(Ahmed *et al.*, 2022)
Ethiopia	2018	CSF Samples (n=218)	PCR and culture	21 (10%) of CSF samples were PCR-positive; only 1 positive for culture.	(Bårnes *et al.*, 2018)
Ethiopia	2019	CSF and Serum Samples (n=23 pairs)	PCR technique	22 (96%) CSF samples had detectable NmDNA; 6 (26%) serum samples were positive for NmDNA.	(Mihret *et al.*, 2019)
Ethiopia	2020	CSF Samples (n=72)	PCR technique and culture	*S. agalactiae* was detected in 64% of CSF samples by PCR with culture zero detection.	(Geteneh *et al.*, 2020)

2.6. Gaps in Literature

Laboratory-based surveillance of meningitis by the application of advanced molecular tools is essential for the proper diagnosis of bacterial meningitis and identifying various causative agents. Even if there were studies conducted previously on meningitis, most of the studies employed classical microbiological methods, where their pathogen detection and/or recovery is very low compared to advanced molecular methods. Most of the studies have focused on meningococcal meningitis, and very few studies have focused on other types of bacterial meningitis. Therefore, studies targeting the majority of meningitis agents are essential in settings like Ethiopia, where bacterial meningitis with high morbidity and mortality rates is common. While meningitis is diagnosed by PCR, the recommended sample to utilize is CSF, and there was no prior study that relied on serum samples to do PCR for the diagnosis of bacterial meningitis.

2.7. Objectives of the study

2.7.1. General Objective

- To compare the efficiency of CSF and blood PCR in the diagnosis of bacterial meningitis among suspected cases in Addis Ababa, Ethiopia.

2.7.2. Specific Objectives

- To assess the efficiency of CSF PCR in the diagnosing bacterial meningitis
- To assess the efficiency of blood PCR in the diagnosis of bacterial meningitis

CHAPTER 3: METHODOLOGY

3.1. Study Area

The study was conducted at Tikur Anbessa specialized hospital (TASH) and Yekatit 12 Hospital Medical College (Y12HMC).

Tikur Anbessa specialized hospital, which was established in 1972, is considered Ethiopia's largest specialized hospital. It has around 800 beds and serves a large patient population of 370,000 to 400,000 per year. TASH, as a linked teaching hospital of the School of Medicine under Addis Abeba University's College of Health Sciences, provides training in a variety of medical fields. Furthermore, it is noted for providing highly specialized clinical services that are uncommon in the country, such as cancer therapy and heart surgery. TASH has a sophisticated diagnostic laboratory with sections for immunohematology, clinical chemistry, quality regulation, molecular biology, and microbiology.

Yekatit 12 Hospital Medical College, under the supervision of the Addis Ababa City Administration Health Bureau, offers routine healthcare services to the city's population, is a referral institution for other regions' patients, and acts as a catchment referral for nearby health centers. Y12HMC was established in 1923 E.C.Currently, it has a capacity of 350 beds. This capacity allows the hospital to serve a large number of patients, providing a range of medical services, including outpatient and inpatient care, emergency treatments, and specialized services. In addition to this, they offer specific care in Anesthesia and Critical Care Services, internal medicine, cardiology, gastroenterology, neurology, dermatology, endocrinology, pediatrics and child health, psychiatry, orthopedics, and HIV/AIDS and TB treatment. Y12HMC has a diagnostic laboratory equipped to perform a diverse array of tests, including hematology, biochemistry, microbiology, immunology, and pathology. The selection of these study sites was mainly due to the accessibility of microbiological laboratory facilities and pediatric services.

3.2. Study Design and Period

An institutional-based cross-sectional study was conducted from August 2023 to February 2024

3.3. Study Population

All patients who were clinically suspected of meningitis in TASH and Y12HMC during the study period.

3.4. Sample Size and Sampling Technique

3.4.1. Sample Size Calculation

The sample size was calculated based on a single-population proportion formula using a prevalence of 10% from a previous study conducted at Jimma University Specialized Hospital, Southwest Ethiopia, in 2018 (Bårnes *et al.*, 2018).

By using the formula $n = \frac{Z^2\, p(1-p)}{d^2}$ P=0.10; from a previous study; Z = 1.96

(i.e., for a 95% C.I.) And d=0.04

This gives 217. The sample size was corrected $N = \frac{1}{1-\text{non responce reate}} \text{x } n$

With a 10% non-response rate, a total of 241 patients were planned.

A proportional allocation of samples was made by considering prior patient flow in each study site in the past three months. In the prior three months, about 60 suspected meningitis patients have visited Y12HMC, and about 40 patients have visited TASH. By looking at this number, the total samples were allocated to each site accordingly: 145 patients to Y12HMC and 96 patients to TASH. A total of 57 suspected patients of meningitis from TASH and 145 suspected patients were interviewed from Y12 HMC. Thirty-nine patients who did not provide both samples and had insufficient sample volume were excluded at TASH (Figure 1).

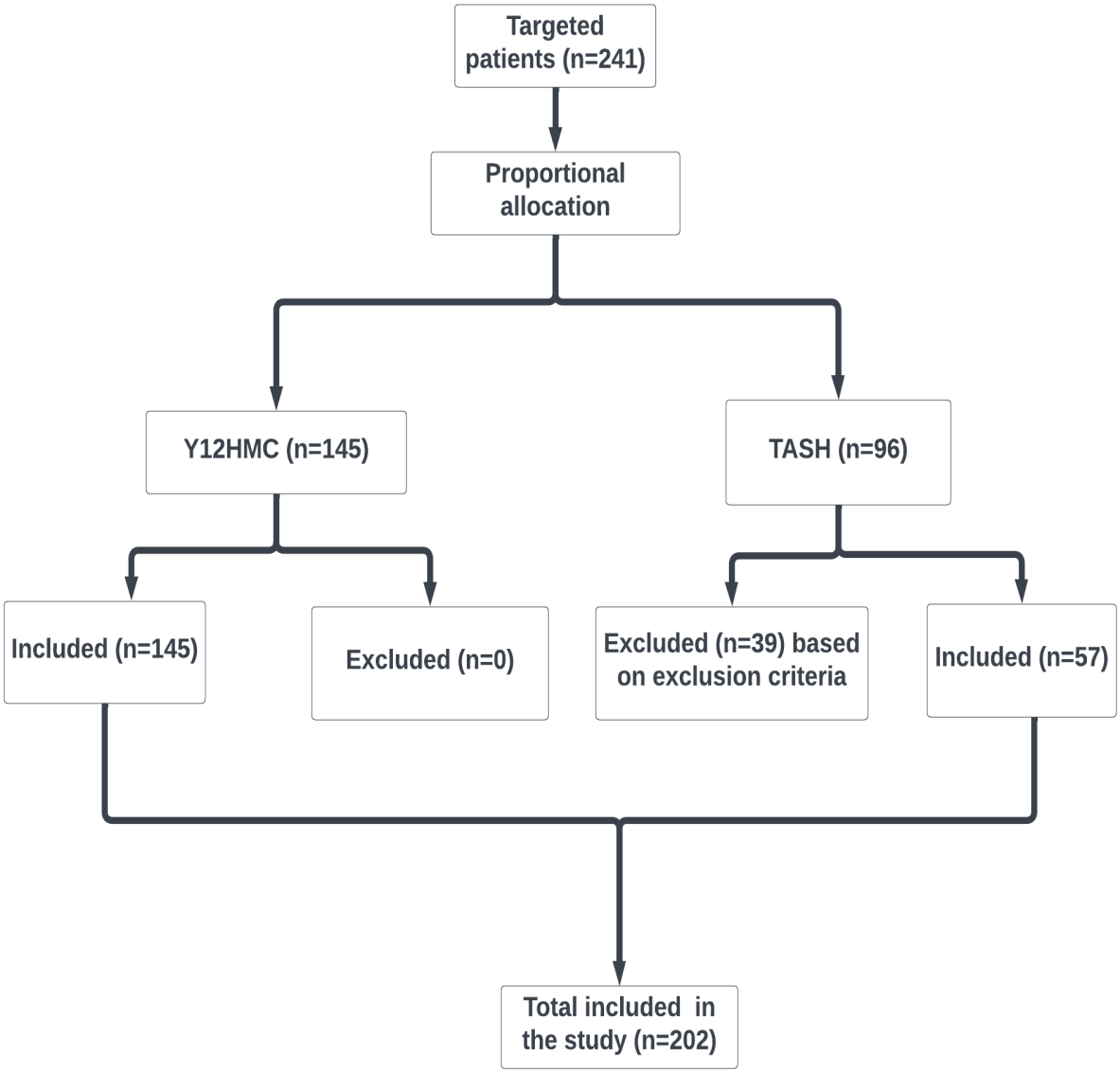

Figure 1: A chart of patient recruitment, inclusion and exclusion, and institutional allocation.

3.4.2. Sampling Technique

A convenient non-probability sampling technique was applied.

3.5. Eligibility

3.5.1. Inclusion Criteria

- All patients suspected of meningitis and willing to take part in the study were included.

3.5.2. Exclusion Criteria

- Patients who were not eligible for lumber puncture (contraindication)
- Patients who were so severely ill that taking blood samples was impractical
- Inappropriate sample volume
- Neonates (< 30 days)

3.6. Study Variables

3.6.1. Dependent Variables

- CSF PCR and serum PCR result
- Types of organisms detected

3.6.2. Independent Variables

- Socio-Demographic Characteristics
- Clinical Presentations

3.7. Data Collection Techniques and Tools

3.7.1. Demographic and Clinical Data Collection

A questionnaire addressing clinical investigation details defining bacterial meningitis was developed before the recruitment of the study participants. First, the questionnaire was developed in English, then translated into the local language, and returned to English (Annex III). Personal information about the patient, including clinical data at admission, was documented. The questionnaire is filled out by the attending physician or a supporting healthcare professional using data from the patient's clinical investigation. Patients who provided informed consent or assent (parent/guardian consent) were assigned a unique research identifier by the order in which they were enrolled in the study. Furthermore, one microbiologist was recruited from each hospital's microbiology laboratory. The completeness and consistency of the data entered into the computer software were checked daily.

3.7.2. CSF Sample Collection

The procedure was explained to the patient by the attending physician/nurses, including the benefits and risks of the procedure. An antiseptic (tincture iodine) was applied to the puncture site. The appropriate site L3-L4 was identified by the attending physician, and a spine needle was inserted into the subarachnoid space. One up to three millilitre of CSF was aspirated into a sterile plane tube (Tube 1 for chemistry and immunology, Tube 2 for Microbiology, and Tube 3 for cell count and differential) by the attending physician. The CSF tube was labeled with patient information, collection time, and tube number. Collected samples were transported to the microbiology laboratory of TASH and Y12HMC at ambient temperature within ten minutes, and laboratory analysis was started within 30 minutes. A minimum of 1 ml of CSF samples collected into tube 2 were transferred by sterile pasture pipet to a cryotube in the safety cabinet, coded, documented in the logbook, and stored at -20 °C for PCR before conventional laboratory analysis started. The samples for PCR were transported to AHRI in a cold box and, upon arrival, were stored at -80 °C until thawed for PCR analysis (Annex IV).

3.7.3. Blood Sample Collection

The procedure was explained, and consent was taken from the patients. The appropriate vein was selected, and the region was cleaned with an alcohol swab and allowed to air-dry. A minimum of 1 mL of blood samples were collected in serum separator tubes by nurses as soon as CSF was collected. Collected blood samples were labeled with patient information and transported to the microbiology laboratory immediately with CSF samples. The blood samples were placed at room temperature to allow them to clot. The clotted samples were centrifuged at 6000 rpm for 10 minutes. The separated serum was transferred by sterile pasture pipet to a cryotube in the safety cabinet and stored at -20 °C for PCR analysis. The serum for PCR was coded, documented on the logbook, and stored at -20 °C at the hospital microbiology laboratory. The serum for PCR was transported to AHRI in a cold box and stored at -80 °C until thawed for PCR analysis (Annex IV).

3.7.4. Conventional Laboratory Analyses of CSF

A CSF specimen was transported to the microbiology laboratory within 10 minutes. The samples were processed by hospital microbiologists as per the standard procedure at TASH and Y12HMC for Gram staining and bacterial culture. All specimens were centrifuged at 3000 RPM for 15 minutes. The supernatant was discarded, and the sediment was re-suspended by pipetting. After performing gram staining, a drop of suspension was inoculated into blood agar, chocolate agar, and MacConkey agar plates. MacConkey agar plates were incubated at 37^0C overnight, while blood agar plates and chocolate agar plates were incubated overnight at 37^0C in a 5% CO2 atmosphere within a candle jar. Positive culture results before 72 hours were further confirmed by biochemical tests and gram staining, but culture-negative results were reported after three days. Gram-positive cocci were identified by biochemical tests, catalase and coagulase positivity, optochin disk sensitivity, bile solubility test, and bacitracin sensitivity tests. Gram-negative bacteria were identified based on phenotypic characteristics and a series of biochemical tests such as carbohydrate utilization, indole production, citrate utilization, H2S production, triple sugar iron utilization, and motility testing. The isolates from both hospitals were transported in skim milk tryptophan, glucose, and glycerol (STGG) median to AHRI, for confirmation and stored at -80°C for further study (Annex V).

3.7.5. Molecular Methods

3.7.5.1. Protocol optimization

The optimization of the multiplex PCR procedure was performed with ATCC strains of the target bacteria, namely *N. meningitidis, S. pneumoniae, H. influenzae, S. agalactiae, E. coli, S. aureus, L. monocytogenes,* and *K. pneumoniae.* Nucleic acid was extracted by a Bioer NPA-32P automated nucleic acid purification machine, and the extract was checked for purity by Nano drop spectrophotometry. Pathogens were assigned into two groups: Group 1: *N. meningitidis, S. pneumoniae, H. influenzae, S. agalactiae*, Group 2: *E. coli, S. aureus, L. monocytogenes,* and *K. pneumoniae* based on amplicon size, and two multiplex PCR master mixes, each containing specific primers (Table 3) of the pathogens were prepared. The PCR-conditions, including annealing temperature, were optimized to obtain the maximum sensitivity and specificity by using gradient PCR. The amplified products were visualized on agarose gels stained with ethidium bromide, confirming the successful amplification of target genes. The protocol for multiplex PCR optimized because all the positive controls amplified successfully and the four bands were visualized in a single reaction for both groups of bacteria, and there were no bands for the negative controls. These optimized protocols were then used in the subsequent analysis of actual clinical samples (Annex V).

3.7.5.2. Multiplex PCR Assays Using the Actual Clinical Samples

3.7.5.3. Samples and Reagent Preparation

CSF and serum samples stored at -80 ºC were thawed and centrifuged briefly in a microcentrifuge to allow homogenous mixing. The ATCC (American Type Culture Collection) control strains of all targeted bacterial species stored at AHRI were thawed along with the samples. A 96-well plate pre-loaded with magnetic beads and extraction reagent was equilibrated to room temperature and centrifuged briefly to ensure proper mixing (Annex V).

3.7.5.4. Nucleic Acid Extraction and Nano Drop Measurement

A volume of 300 µL of the CSF and serum samples was transferred to the pre-loaded 96-well plate. As directed by the manufacturer, the nucleic acid was extracted using the Bioer NPA-32P automated nucleic acid purification machine (HANGZHOU BIOER TECHNOLOGY CO., LTD. CHINA). The positive control for all targeted bacteria was extracted from the corresponding ATCC strains (*N. meningitidis* ATCC 13090, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 10211, *S. agalactiae* ATCC 12386, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 19115, and *K. pneumoniae* ATCC 13883) using the same procedure applied to the samples. Nuclease-free water was extracted as a negative control along with the samples. The purity and concentration of the extracted nucleic acid was measured using 1 µL of the eluate by Nano drop spectrophotometric at an absorbance of 260/230 and 260/280. The extract was kept at -20 °C until use (Annex V).

3.7.5.5. Master Mix Preparation: Platinum Taq Master Mix for Multiplex PCR

For the multiplex PCR for the detection of the pathogens, the pathogens were assigned into two groups, 1 and 2, based on the primer's properties, mainly based on their amplicon size.

Group 1: In this group, the primers for the four pathogens included, (*N.meningitidis, S. pneumoniae, H. influenzae,* and *S. agalactiae*), to be detected simultaneously in one reaction by optimizing the reaction condition for multiplex PCR assay. The multiplex PCR master mix reaction was prepared in a volume of 20 µL using 2.5 µL of 10x PCR buffer, 1.2 µL $MgCl_2$, 1 µL dNTPs, 1 µL each primer (1 µL forward and 1 µL reverse) of the above four organisms, 7.1 µL nuclease-free water and 0.2 µL platinum Taq polymerase (Invitrogen Product USA) (Annex V).

Group 2: Primers assigned for *E. coli, S. aureus, L. monocytogenes,* and *K. pneumoniae*. Similarly, the multiplex PCR master mix reaction was prepared in a final volume of 20 µL, constituting 2.5 PCR buffer, 1.2 µL Mgcl2, 1 µL dNTP, 1µL each primer (1µL forward and 1µL reverse) of the above four organisms, 7.1 µL of nuclease-free water, 0.2 µL Platinum Taq polymerase (Invitrogen Product USA) (Annex V).

3.7.5.6. Preparation of Template DNA and Assembly of a PCR Reaction

Nucleic acid extracts from CSF and serum samples, previously stored at -20°C, were thawed, vortexed, and centrifuged before adding to the prepared master mix.

Group 1: 5 µL of nucleic acid extract from CSF and serum samples were used as template and added to the master mix containing the primer of *N. meningitidis, S. pneumoniae, H. influenzae,* and *S. agalactiae* and make a final volume of 25 µL. 5 µL ATCC control extracts of these four bacteria were combined in one Eppendorf tube and used as a positive control for the group 1 reaction.

Group 2: Again 5 µL of Nucleic acid extracts from CSF and serum samples were used as template and added to the master mix containing the primer of *E. coli, S. aureus, L. monocytogenes,* and *K. pneumoniae* and make a final volume of 25 µL. 5 µL ATCC control strain extracts of these four bacteria were combined in a single Eppendorf tube and used as positive controls for group 2 reactions.

Both the master mixes (Groups 1 and 2) were subjected to thorough vortexing to ensure homogeneous mixing, and brief centrifugation was performed to ensure the collection of the liquid at the bottom. Aliquots of 5 µL of mixed ATCC strain extracts were added to the master mix, making the final reaction volume of the multiplex PCR 25 µL. The mixtures were vortexed, centrifuged briefly, and run along with the samples. To monitor the potential cross-contamination of the PCR, a separate reaction tube containing all components of the master mix but with nuclease-free water instead of template DNA was included (Annex V).

3.7.5.7. Multiplex PCR Assay

Finally, the reaction mixtures in both groups 1 and 2 was amplified in a thermocycler (T100 LASEC; BIO-RAD) for 35 cycles using the following program: initial denaturation at 94°C for 3 min, final denaturation at 94°C for 10 s, annealing step at 60°C, for 30 s, extension step at 72°C for 30 sec, final extension at 72°C for 5 min, and infinite hold at 12°C. The lid temperature of the thermal cycler was 105 °C, and the program ran for around 1 hour and 20 minutes. Nuclease-free water from extraction and Master Mix was used as negative controls. The amplified product was placed at 2–8 °C until gel electrophoresis was performed (Annex V).

3.7.5.8. Gel Electrophoresis and Visualization

A 2.5% agarose gel was prepared and stained with 2 µL of ethidium bromide. 5 µL of loading dye were added to the PCR product to achieve a final volume of 30 µL. The products were thoroughly mixed with the loading dye, and then 10 µL of the PCR product containing the loading dye were loaded into the wells of the gel. A 100-bp DNA ladder was also loaded into a separate well for size comparison. Electrophoresis was run on the gel at 400 amps and 120 volts for 45 minutes. After electrophoresis, the gel was visualized under VILBER machine to see the separated DNA bands according to amplicon sizes for the targeted bacteria (Table 3) (Annex V).

Table 3: Primers specific the target organisms used in this study

Organism	Target gene	Oligo name	Nucleotide Sequence (5' to 3')	Amplicon size (bp)	References
N. meningitidis	*Ctr A*	*CtrA*-F	GCTGCGGTAGGTGGTTCAA	111	(De Almeida *et al.*, 2019)
		CtrA-R	TTGTCGCGGATTTGCAACTA		
S. pneumoniae	*Lyt A*	*LytA*-F	ACGCAATCTAGCAGATGAAGCA	75	(Ouattara *et al.*, 2020; Tavares *et al.*, 2019)
		LytA-R	TCGTGCGTTTTAATTCCAGCT		
H. influenzae	*Hpd*	*Hpd*-F	GGTTAAATATGCCGATGGTGTTG	151	(Ouattara *et al.*, 2020)
		Hpd-R	TGCATCTTTACGCACGGTGTA		
E. coli	*rRNA*	*Eco*-F*rRNA*	GGGAGTAAAGTTAATACCTTTGC	204	(Bingen *et al.*, 1996)
		Eco-R*rRNA*	CTCAAGCTTGCCAGTATCAG		
L. monocytogenes	*Hly*	*Lm*-F*hly*	CAT GGCACCACCAGCATCT	64	(Le Monnier *et al.*, 2011; De Almeida *et al.*, 2019)
		Lm-R*hly*	ATC CGCGTGTTTCTTTTCGA		
S. aureus	*Fem A*	*Sau*-F*femA*	TGCTGGTGGTACATCAAA	97	(Meng *et al.*, 2020)
		Sau-R*femA*	ACGGTCAATGCCATGATTTAA		
S. agalactiae	*Cfb*	*Sag*-F*cfb*	ATGATGTATCTATCTGGAACTCTAGTG	260	(Guo *et al.*, 2019a)
		Sag-R*cfb*	CGCAATGAAGTCTTTAATTTTTC		
K. pneumoniae	*rcsA*	*Kpn*-F*rcsA*	GGATATCTGACCAGTCGG	176	(Ku *et al.*, 2017)
		Kpn-R*rcsA*	GGGTTTTGCGTAATGATCTG		

Key: bp represents the base pair.

3.8. Data quality

A pre-test was performed to assess the quality of the questionnaires before the actual data collection on 5% of the study population, and amendments were made accordingly. Data collectors received training on filling out questionnaires, sample protocols, sample handling, and transportation. Clear, detailed, and standard sample collection protocols were developed. All data and samples collected from study participants were labeled with a unique identifier. The samples were transported to the microbiology laboratory of TASH and Y12HMC as soon as possible after collection. The proper labeling of the samples by unique identifier was checked at the microbiology laboratory. Samples were processed by a laboratory microbiologist and stored under appropriate conditions according to hospital guidelines. Quality controls of culture media and biochemical test reagents were performed. The standard protocols were followed for the inoculation and incubation of cultures. The isolates were stored in skim milk, tryptone, glucose, and glycerin (STGG) media (Annex V) at -20°C for short-term storage at hospitals, transported in a cold box, and stored at -80°C for confirmation and further study at AHRI. The samples for the PCR were kept at -20 °C at hospitals until transported to the AHRI molecular laboratory. On arrival at AHRI, the samples were stored at -80°C until used for PCR analysis. An appropriate extraction kit was used, and quality controls for the extraction kit were made. Extraction controls (ATCC and nuclease-free water) were used to monitor extraction efficiency. Standardized PCR protocols and a master mix were used. Positive (verifies that the PCR reagents, including primers and enzymes, are functioning correctly) and negative (for checking contamination in PCR setup) controls for each PCR run were implemented. To avoid contamination, the biosafety cabinet was cleaned with 70% alcohol and 10% bleach both before and after each master mix preparation.

Each laboratory procedure (DNA extraction, master mix preparation, template addition, and sample storage) was carried out in a separate room to avoid any cross-contamination. Validate software for data entry and analysis was used. A quality control measure for data analysis software was implemented. The result was interpreted according to established criteria. The results were reported clearly and concisely.

3.9. Data analysis

The collected data was reviewed, coded, and entered into Epi Info version 7. Following data entry, the data was cleaned to remove mistakes such as duplicates and missing values. After cleaning, the data was exported to Microsoft Excel for a preliminary review before being loaded into SPSS version 26.0 for analysis. Frequencies and percentages were calculated to summarize the data. Some additional analyses were performed, such as cross-tabulations, to explore the relationships between variables. The results of the analysis were presented by using tables and bar charts. SPSS contingency tables were used to show the agreement between two variables.

3.10. Ethical Considerations

The ethical approval for this study was obtained from the Department Research Ethics Review Committee (DRERC/006/2023), DMIP, Addis Ababa, and AHRI/ALERT ethics review committe (PO-55-22). In addition to these, it got ethical clearance from the institutional review board of Y12HM College no. 322/23 and TASH. The information sheet was prepared in different translations. Informed consent was obtained from parents/guardians and assent was obtained from children participated in the study (Annex II). The participants were told the purpose of the study, the right to refuse or participate in the study, and the anonymity and confidentiality of the information collected. Study participants gained detailed information concerning the purposes of the study, and they were asked about the study to check whether they understood it correctly or not, and their concerns were cleared, if any. Any relevant finding during the course of the study was communicated with the attending physician for better management. The finding of the study will also be presented at national and international conferences and published in peer-reviewed journals.

3.11. Project management

Data cleaning and cross-checking were done, and the questionnaire was revisited to gather any missing information. Throughout the study period, all laboratory and clinical data were documented on the proper documents and archived on a CD, memory flash, or email attachment.

3.12. Operational Definitions

Meningitis: is an inflammation of the protective membranes covering the brain and spinal cord.

Probable meningitis: is defined as having clinical findings suggestive of BM, such as the sudden onset of fever, stiff neck, or consciousness changes, besides microscopic and chemical analysis of cerebrospinal fluid. Culture-negative results but with the typical finding in cerebrospinal fluid: leukocytosis (>100 cells/mm3), predominant cell neutrophils (10-100 cells/mm3) and either elevated protein (> 100 mg/dL) or decreased glucose (< 40 mg/dL) Confirmed meningitis: Laboratory-confirmed by culture or identifying by Gram stain or antigen detection methods of a bacterial pathogen in the CSF or from the blood in clinical suspected with bacterial meningitis (Okike *et al.*, 2018).

CHAPTER 4: RESULTS

4.1. Socio-Demographic Characteristics of the Study Participants

A total of 202 patients suspected of meningitis were included in the study; 54% (n = 109) were males. Of the study participants, 134 (66.3%) were between the age group of 1–12 months, 45 (22.3%) from 1–17 years, and 23 (11.4%) were adults (≥18 years). Sixty eight percent (n = 137) of study participants received antibiotics before admission, and about 29.2% (n = 59) were vaccinated (Table 4).

Table 4: Sex and Age, Antibiotic Use, and Vaccination History of Study Participants at TASH and Y12HMC from August 2023 to February 2024.

Sex		Frequency	Percent (%)
Male		109	54
Female		93	46
Total		**202**	**100**
Age			
Infant and Children			
1-12 months		134	66.3
1-17 Years		45	22.3
Adults			
≥18 Years		23	11.4
Total		**202**	**100**
Antibiotic Use Before sample collection	Yes	137	68
	No	65	32
	Total	**202**	**100.0**
Vaccination	Yes	59	29.2
	No	143	70.8
	Total	**202**	**100.0**

4.2. Clinical Findings of the Study Participants

The most common clinical presentation shown by study participants were reduced ability to breastfeed 74.8% (n = 151), followed by high fever 71.3% (n = 144) and loss of consciousness 66.3% (n = 134) (Table 5).

Table 5: Clinical Presentation of Study Participants at TASH and Y12HMC from August 2023 to February 2024.

Clinical Presentation		Frequency	Percent (%)
Reduced Breast Feeding (Poor Feeding)	Yes	151	74.8
	No	51	25.2
	Total	**202**	**100.0**
High Fever ($\geq$ 38.5^{o}C)	Yes	144	71.3
	No	58	28.7
	Total	**202**	**100.0**
Loss of Consciousness	Yes	134	66.3
	No	68	33.7
	Total	**202**	**100.0**
Neck Stiffness	Yes	58	28.7
	No	144	71.3
	Total	**202**	**100.0**
Seizures	Yes	60	29.7
	No	142	70.3
	Total	**202**	**100.0**
Bulging Fontanelle of Infant	Yes	23	11.4
	No	179	88.6
	Total	**202**	**100.0**
Petechiae (Skin Rash) (>5mm)	Yes	5	2.5
	No	197	97.5
	Total	**202**	**100.0**

4.3. Laboratory findings of CSF Gram-Reaction, and Culture

Of 202 CSF samples Gram staining showed the presence of bacteria in 11 (5.4%) samples. Of the total of 202 samples cultured, only 9 (4.5%) were positive. *N. meningitidis, S. pneumoniae* (2 cases), *E. coli* (2 cases), *S. aureus, Acinetobacter* species (2 cases), and *Entrobacter* species were culture-isolated organisms (Table 6).

Table 6: CSF Findings of Gram Staining and Culture of Study Participants at TASH and Y12HMC from August 2023 to February 2024.

Conventional Laboratory Analysis	Appearance	Frequency	Percent (%)
Gram Stain	Gram-Positive Cocci	2	1.0
	Gram-Positive Diplococci	2	1.0
	Gram-Negative Bacilli	6	2.9
	Gram-Negative Diplococci	1	0.5
	Negative	191	94.6
	Total	**202**	**100.0**
Culture Result	Positive	9	4.5
	Negative	193	95.5
	Total	**202**	**100.0**

4.4. Findings of the Multiplex PCR assays

4.4.1. CSF PCR Results

Two hundred and two CSF samples were analyzed using conventional multiplex PCR. DNA from 54 (26%) of the samples was successfully amplified. Of the total 54 CSF samples that tested positive for PCR, 37 samples (69%) were from infants aged 1 to 12 months, 10 samples (19%) were from pediatric patients aged 1 to 17 years, and 7 samples (13.0%) were from adults aged ≥ 18 years (Table 7).

Table 7: CSF-PCR Result Obtained from Study Participants at TASH and Y12HMC from August 2023 to February 2024.

Test Results	CSF (n = 202)	Percent (%)
Positive	54	26
Negative	148	74
Total	**202**	**100**

4.4.2. Frequency of Bacterial Pathogens Detected in CSF-PCR

E. coli was the most common pathogen detected accounting for 30 (55.5%) of PCR-positive, followed by *S. pneumoniae* detected in 8 (15%) of PCR-positive samples. Of the 30 *E. coli* positive samples, 21 were from infants aged 1 to 12 months, 6 were from pediatric patients aged 1 to 17 years, and 3 were from adults ≥18 years old. Of the 8 *S. pneumoniae* positive samples, 6 were from infants aged 1 to 12 months, and 2 were from adults ≥ 18 years old. Multiplex PCR identified double infections in 1 CSF samples (*E. coli* with *N. meningitidis*) (Figure 2).

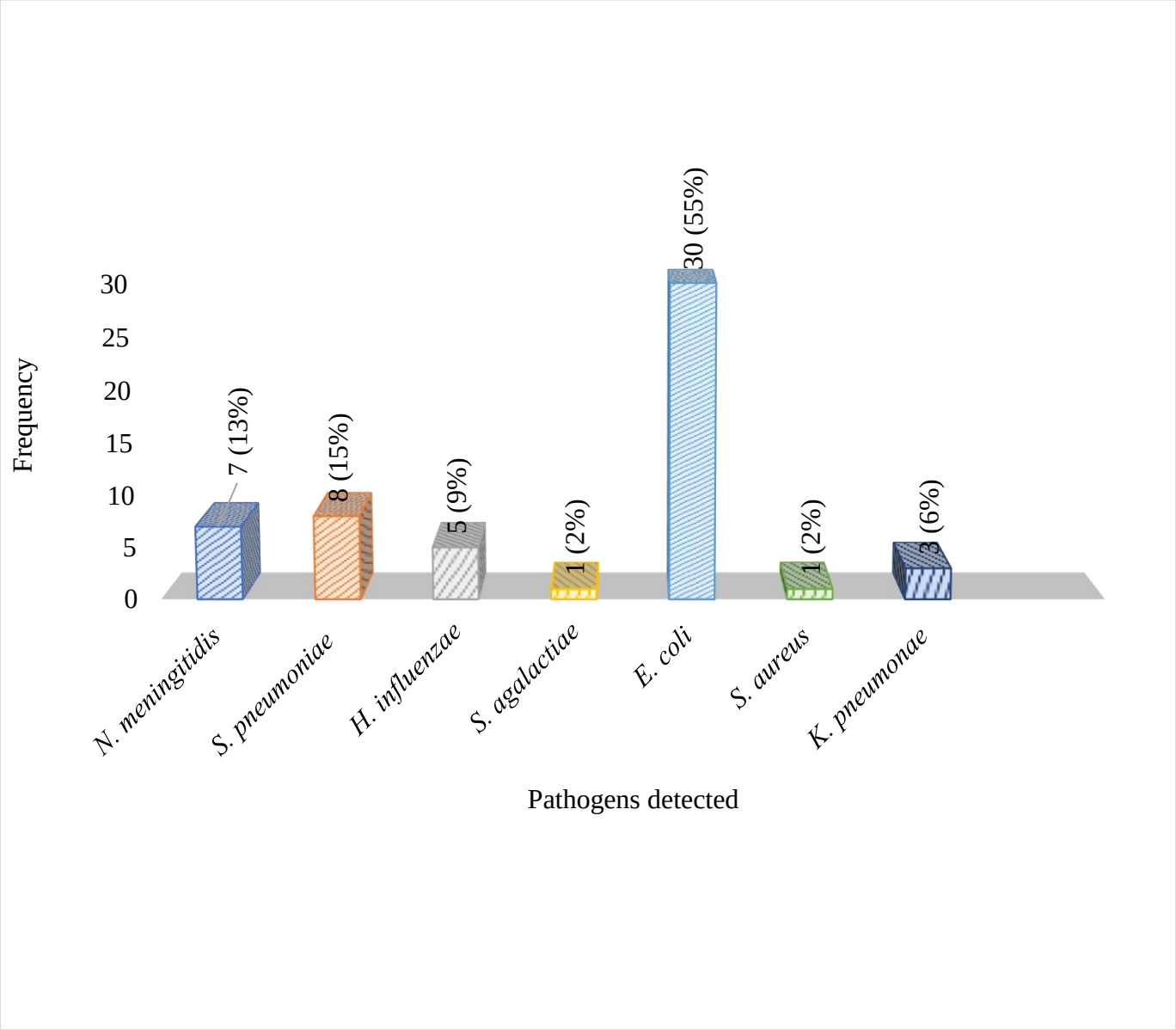

Figure 2: The Frequency of Bacterial Pathogen Detected in CSF-PCR.

4.4.3. Serum PCR Results

Two hundred and two serum samples were analyzed using conventional multiplex PCR, and DNA from 93 (46%) of the samples was successfully amplified. Of these, 64 samples (69%) were from infants aged 1 to 12 months, 23 samples (25%) were from pediatric patients aged 1 to 17 years, and 6 samples (6%) were from adults aged $\geq$ 18 years (Table 8).

Table 8: Serum PCR Results of Study Participants from TASH and Y12HMC from August 2023 to February 2024.

Test Result	Serum (n = 202)	Percent (%)
Positive	93	46
Negative	109	54
Total	**202**	**100**

4.4.4. Frequency of Bacterial Pathogens Detected in Serum-PCR

Among the serum PCR-positive samples, *E. coli* was identified in 57 samples, representing 61.2% of PCR-positive. *S. pneumoniae* was identified in 27 samples, which accounted for 29% of the total positive samples by PCR. *E. coli* was most prevalent in infants (39/57), followed by children (14/57) and adults (4/57). *S. pneumoniae* was also primarily detected in infants (18/27) followed by children (7/27). Multiplex PCR revealed double-infections in 10 serum samples, including *E. coli* with *S. pneumoniae* (3 samples), *K. pneumoniae, H. influenzae, N. meningitidis*, and *S. aureus*, and *S. pneumoniae* with *S. aureus* (2 samples) and *K. pneumoniae* (Figure 3).

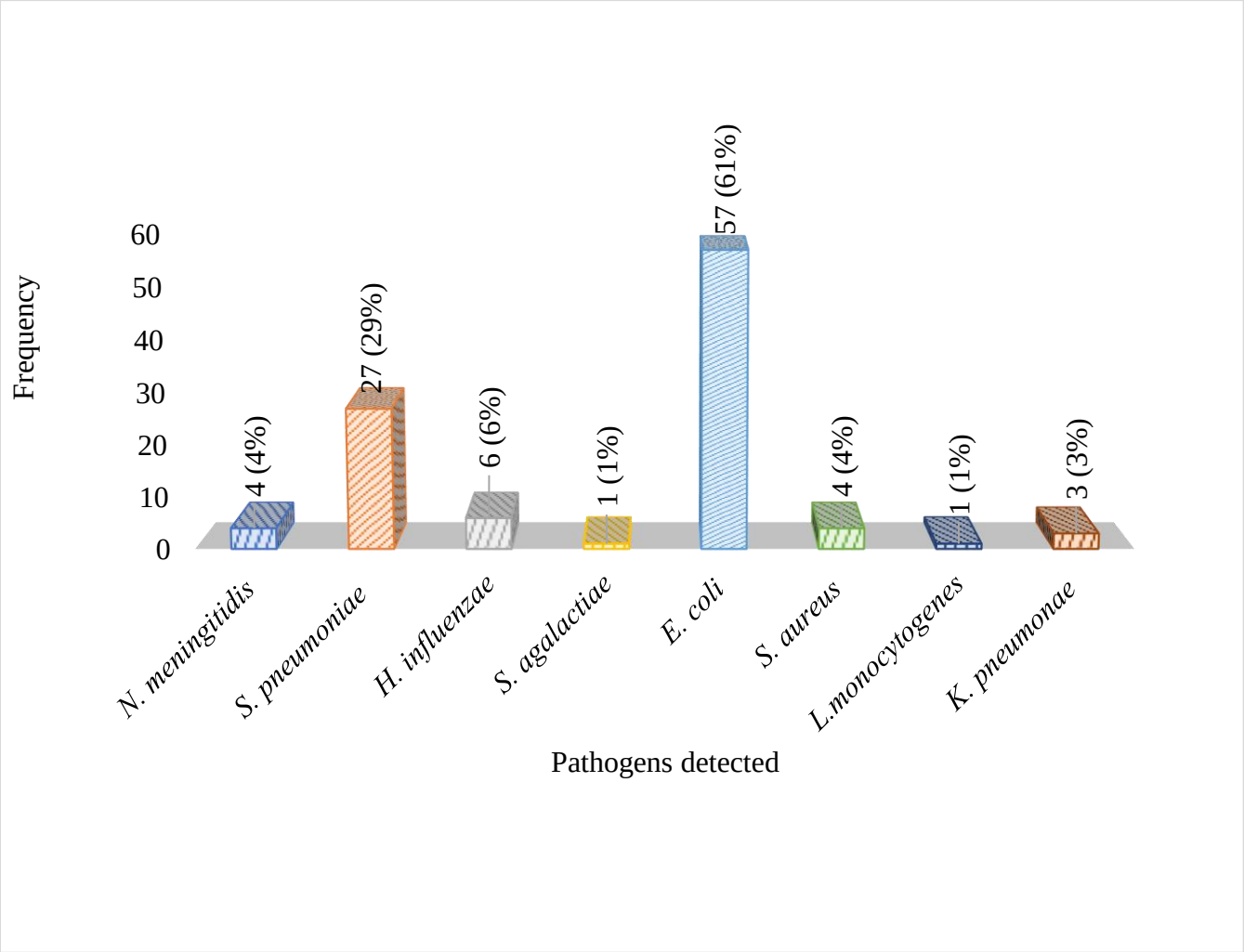

Figure 3: Frequency of Bacterial Pathogens Detected in Serum Samples

4.4.5. Comparison of CSF and Serum PCR Results

Two hundred and two CSF and serum samples were analyzed. From the total of 202, 93 (46%) serum and 54 (26%) CSF samples were tested positive for bacterial DNA. Serum-based PCR is 20% more efficient than CSF-based PCR (Table 9).

Table 9: Comparison of CSF and Serum PCR Findings Obtained from Study Participants at TASH and Y12HMC from August 2023 to February 2024.

Test Result	CSF PCR (n = 202)	Percent (%)	Serum PCR (n=202)	Percent (%)
Positive	54	26	93	46
Negative	148	74	109	54
Total	**202**	**100**	**202**	**100**

4.4.6. Comparison of Pathogens Detected in CSF and Serum PCR

Thirty E. *coli* was detected in CSF samples, compared to 57 detected in serum samples. Notably, concordance of 17 *E. coli* was found in both serum and CSF samples. Eight cases of *S. pneumoniae* were detected in CSF samples, compared to a total of 27 cases detected in serum. Concordance of 5 *S. pneumoniae* was found in both serum and CSF samples (Table 10).

Table 10: Comparison of Pathogens Detected in CSF and Serum PCR Tests at TASH and Y12HMC from August 2023 to February 2024.

		Serum -PCR		
	CSF-PCR	Negative	Positive	Total
N. meningitidis	Negative	194	1	195
	Positive	4	3	7
	Total	198	4	202
S. pneumoniae	Negative	172	22	194
	Positive	3	5	8
	Total	175	27	202
H. influenzae	Negative	192	5	197
	Positive	4	1	5
	Total	196	6	202
S. agalactiae	Negative	200	1	201
	Positive	1	0	1
	Total	201	1	202
E.coli	Negative	132	40	172
	Positive	13	17	30
	Total	145	57	202
S. aureus	Negative	197	4	201
	Positive	1	0	1
	Total	198	4	202
L. monocytogen	Negative	201	1	202
	Total	201	1	202
K. pneumoniae	Negative	196	3	199
	Positive	3	0	3
	Total	199	3	202

4.4.7. Agreement Between CSF and Serum PCR Tests

The overall percentage of agreement/concordance of the PCR tests between serum and CSF is about 60.9%, or 100%x (34 + 89)/202. The negative percent agreement was 100%x89/148 = 60.14%, and the positive percent agreement is about 62.96%, 100%x(34/54) (Table 11).

Table 11: Statistical Calculation of Concordance, Positive and Negative Percent Agreement Between CSF and Serum PCR Tests of Study Participants at TASH and Y12HMC from August 2023 to February 2024.

Results	Serum Positive	Serum Negative	Total
CSF Positive	34	20	**54**
CSF Negative	59	89	**148**
Total	**93**	**109**	**202**

CHAPTER 5: DISCUSSION

In the present study, two hundred and two patients suspected to have bacterial meningitis were included, with notable male predominance (54%). This agrees with the earlier studies that showed males to be more prone to bacterial meningitis than females, as identified in various settings (Tigabu *et al.*, 2021; Niemelä *et al.*, 2023).

This study showed that 88.6% of the participants were pediatric patients aged between 1 month and 17 years, with 66.3% of the total number of patients being below the age of 1 year. This agrees with the global epidemiological data of the disease, where there is a predominance of infections in the younger age groups. A study from Ethiopia has also shown similar trends in the distribution of age among affected persons, emphasizing the need for targeted preventive measures in younger infants (Gudina *et al.*, 2018; Kokeb, 2023). In this study, the distribution across age groups reflects the dynamic change in bacterial meningitis epidemiology, where pediatric cases remain prevalent due to immature immune system (Intra *et al.*, 2024).

The major clinical presentations in this study are decreased ability to breastfeed 74.8%, high fever 71.3%, and loss of consciousness 66.3%. These symptoms are consistent with those reported in other studies, where fever and loss of consciousness were common indicators of bacterial meningitis (Feldman, 1976; Tunkel *et al.*, 2004). However, only 28.7% of the cases were reported to have neck stiffness, which is lower than expected, considering that this is one of the classical signs and symptoms for diagnosing meningitis. Among the suspected meningitis cases in Ethiopia, a clinical presentation analysis study stated that fever, neck stiffness, and irritability were rated at 83.8%, 76.02%, and 62.4%, respectively (Bekele *et al.*, 2021). This finding underlines the variability in clinical presentation and suggests a wider range of clinical presentations should be taken into consideration when diagnosing bacterial meningitis. Seizure 29.7%, bulging fontanelle 11.4%, and petechial 2.2% were observed in patients suspected of meningitis in lower frequency.

A retrospective analysis at Gondar University Hospital from 2012 to 2021 found that 62.7% of children presented with seizures, and 48.3% exhibited a bulging fontanelle (Kokeb, 2023). The presence of variation in symptoms implicates the importance of considering diverse presentations for the diagnosis and treatment of bacterial meningitis (Kumar *et al.*, 2015).

In this study, only 5.5% showed positivity for Gram stain. This is relatively low sensitivity and may indicate that Gram stain does not effectively capture all the cases of infection. From a total of 202 meningitis-suspected patients, CSF samples were cultured, and the bacteria were identified in only 9 samples, which is a 4.5% recovery yield. This is comparable to the previous studies conducted in Gondar, where an isolation rate was (5.2%) (Tegene *et al.*, 2015), Tikur Anbessa (4.4%) (Reta and Zeleke, 2016), Hawassa Ethiopia (6.4%) (Daka *et al.*, 2011), and (6.9%) (Assegu Fenta *et al.*, 2020). However, this finding was higher than the studies reported from another study in Gondar (1.28%) (Tigabu *et al.*, 2021).

On the other hand, this finding is lower than studies done in Tikur Anbessa (10.8%) (Mihret *et al.*, 2016), Dabra Markos university (11.2%) (Hibstu *et al.*, 2022), Dilla University Referral Hospital (13.2%) (Awulachew *et al.*, 2020), Uganda (44.12%) (Sonavane *et al.*, 2008), and India (62.5%) (Modi *et al.*, 2012), Kenya (11.20%) (Gituro *et al.*, 2017), Netherlands (13%) (van Veen *et al.*, 2016), and Qatar (53.60%) (Khan *et al.*, 2017).

The discrepancies in culture results may be attributed to various factors not explored in this study, such as viral, fungal, and other non-culturable microbial causes of meningitis. Other potential reasons for the differing results include unnecessary lumbar punctures, the non-availability of specialized media for specific pathogen isolation in laboratory settings, bacterial endemicity, the characteristics of the study population, study design, sampling techniques, and the initiation of antibiotic treatment prior to lumbar puncture. The study indicated that approximately 68% of patients received antibiotics before sample collection, which may account for the notably low rate of culture isolation.

In this study, a higher number of bacterial DNA was detected in CSF by PCR. Using CSF PCR assay we tested the common bacterial causative agents of meningitis specifically, *N. meningitidis, S. pneumoniae, H. influenza* type b*, S. agalatiae, E. coli, L. monocytogence, S. aureus,* and *K. pneumoniae*. Overall, the CSF PCR yield was 26% of the 202 tested samples. In agreement with the present study, other studies in Turkey detected (10% vs. 59.6%) (Ceyhan *et al.*, 2008), Brazil (3.8% vs. 29.2%) (Ferreira *et al.*, 2018), Iran (27.7% vs. 43.8%), (Bidgani *et al.*, 2016), China (0% vs. 50.7%) (Dmitriev *et al.*, 2004), Jimma, Ethiopia (2% vs. 33%) (Bårnes *et al.*, 2018), and Addis Ababa, Ethiopia (0 vs. 64%) (Geteneh *et al.*, 2020). This implies that PCR is the most sensitive method compared to culture. In this study, the PCR method successfully detected all culture-positive bacteria for which it was designed. PCR may identify bacteria even when they are present after antibiotic treatment, which significantly enhances its diagnostic capabilities.

In the present study, the most common etiological agent identified in CSF was *E. coli* (55.5%) of positive samples. The predominance of *E. coli* was similar to what was reported in Nigeria and Kenya, where both studies reported *E. coli* being the most common bacteria isolated (Airede *et al.*, 2008; Laving *et al.*, 2003). However, in Taiwan (Lin *et al.*, 2015) and Niger (Odedina and Emumwen, 2008), *E. coli* was the second most common bacterial isolate; it has also been infrequently reported in Bangladesh (Sultana *et al.*, 2008), Nigeria (Nwadioha *et al.*, 2013), and Ghana (Owusu *et al.*, 2012).

The identification of a higher predominance of *E. coli* in the current study may also be related to the pediatric age group (66.3% being a pediatric population below the age of 1 year), in which *E. coli* is the predominant pathogen. Furthermore, there are no licensed vaccines against *E. coli*, despite these pathogens being the major causes of bacterial meningitis in infants. Thus, the predominance of *E. coli* in this study is consistent with emerging trends in bacterial meningitis, particularly among infants, where it has been increasingly recognized as a significant pathogen.

The second-highest bacteria identified by PCR analysis from CSF was *S. pneumoniae* (15%). *S. pneumonia* is one of the most common bacteria that cause meningitis in Africa, as shown in studies in Ghana (Owusu *et al.*, 2012) and Ethiopia (Mihret *et al.*, 2019; Gudina *et al.*, 2018; Tegene *et al.*, 2015; Amare *et al.*, 2018). The possible reason for *S. pneumoniae* prevalence in the study population could be a high carriage rate among infants and young children.

The emergence of multiple serotypes of *S. pneumoniae* that the existing vaccines do not cover, or low vaccine coverage (Tamir *et al.*, 2024) might lead to an increase in the prevalence of this bacteria.

The detection of *N. meningitides* (12.9%) and *H. influenzae* (9.3%) in this study aligns with findings from other regions of Ethiopia (Mihret *et al.*, 2019; Gudina *et al.*, 2018; Amare *et al.*, 2018), emphasizing their role as primary pathogens in bacterial meningitis. *N. meningitidis* and *H. influenzae* were among the most frequently detected organisms using multiplex PCR techniques (Bårnes *et al.*, 2018; Raza *et al.*, 2022b). Among the study participants, only 29.2% received vaccination, indicating significantly low coverage compared to the established vaccination guidelines. The persistence of *N. meningitidis* as a detection emphasizes the need for ongoing surveillance and vaccination efforts, particularly since outbreaks can occur rapidly in densely populated areas. While the continued detection of *H. influenzae* suggests that vaccination coverage (Zemariam *et al.*, 2024) may not be universal or effective enough to eliminate this pathogen.

The detection rate of *S. agalactiae* (1.8%) in this study is notably low compared to findings from other studies conducted in Ethiopia (64% of 72 study participants) (Geteneh *et al.*, 2020). Previous studies have included neonates in their research populations, as *S. agalactiae* is primarily recognized for causing neonatal meningitis and other serious infections in this vulnerable group.

The detection of this bacterium in such studies shows the critical importance of implementing effective screening and prophylaxis strategies during pregnancy. *S. aureus* (1.8%) and *K. pneumoniae* (5.5%) add complexity to the bacterial profile observed in the study. *S. aureus* and *K. pneumoniae* may indicate secondary infections or complications following other primary infections, highlighting the necessity for comprehensive clinical assessments and diagnosis in suspected meningitis patients.

There is limited data on the use of serum PCR for diagnosing bacterial meningitis, as most molecular tests focus on CSF samples. A study from three decades ago indicated that the sensitivity and specificity of detecting meningococcal DNA in serum were comparable to those in confirmed meningococcal cases identified through culture or CSF analysis. Both the sensitivity and the specificity of the test were 100% for patients with confirmed cases of meningococcal disease when the blood buffy coat and serum were used (Newcombe *et al.*, 1996).

To the authors' knowledge, this is the first study that used and compared serum PCR with CSF PCR for diagnosing the eight common bacteria causing meningitis. The findings from this study revealed that 46% of serum samples tested positive for bacterial DNA, while only 26% of CSF samples. This suggests that serum PCR is 20% more efficient compared to CSF PCR for diagnosing bacterial meningitis. Contrastingly, another study reported a much higher detection rate of 91.9% for meningococcal DNA in CSF samples, compared to 45.9% in serum samples (Bronska *et al.*, 2006).

The current study also demonstrates a higher detection rate of meningococcal DNA in CSF samples (3.5%) compared to serum samples (1.9%) for individual bacteria. However, the overall positivity rate for all bacteria was lower in CSF samples. This discrepancy suggest the complexity of diagnosing bacterial meningitis, where CSF is considered the best specimen for pathogen identification due to its direct association with central nervous system infections. The microorganisms have variable potential to cross the BBB, which may form the basis of differences (van Sorge and Doran, 2012).

Serum samples from patients suspected of bacterial meningitis revealed the presence of several bacteria. The most frequently detected bacteria were *E. coli* (61.2%), followed by *S. pneumoniae* (29.0%), again highlighting the significance of *E. coli* as a leading cause of bacterial meningitis. Other bacteria identified include *N. meningitidis* (4.3%), *H. influenzae* type b (6.5%), *S. agalactiae* (1.1%), *L. monocytogenes* (1.1%), *S. aureus* (4.3%), and *K. pneumoniae* (3.2%).

A significant difference was observed when comparing bacteria detected in serum and CSF samples; many bacteria, including *E. coli* and *S. pneumoniae*, were detected in serum samples but not in CSF samples. This difference emphasizes the potential value of serum samples for the diagnosis of bacterial meningitis. The difference highlights the limitations of relying solely on CSF analysis for diagnosing bacterial meningitis. For instance, while 27 cases of *S. pneumoniae* were identified in serum samples, only 8 were detected in CSF samples. This discrepancy suggests that a significant number of pneumococcal meningitis cases may be missed if CSF analysis is the primary diagnostic method. Similarly, although 57 cases of *E. coli* were detected in serum samples, only 30 were identified in CSF samples, indicating a potential underestimation of *E. coli* as a pathogen of meningitis using CSF as primary sample.

The differences observed in the detection of bacterial pathogens between serum and CSF PCR may stem from several factors. First, the timing of CSF sampling can affect bacterial detection; if collected too early, the bacterial load may be low, resulting in false negatives (Brink *et al.*, 2015). Second, the blood-brain barrier (BBB) can hinder bacteria from entering the CSF, with some species more capable of crossing it than others (van Sorge and Doran, 2012). An association between high-level bacteremia and development of meningitis has been suggested for *E. coli*, *S. pneumoniae*, GBS and Hib from experimental models of hematogeneous meningitis (La Scolea Jr and Dryja, 1984; Petersdorf *et al.*, 1962; Kim, 2016; Bell *et al.*, 1985). Lastly, technical limitations in CSF sampling and laboratory methods (Mlinarić *et al.*, 2018). These findings highlight the importance of a comprehensive diagnostic approach that includes both serum and CSF analyses for accurate identification and treatment of bacterial meningitis.

The study found a 60.9% overall agreement between CSF and serum PCR results for diagnosing bacterial meningitis, with a negative percent agreement (NPA) of 60.14% and a positive percent agreement (PPA) of 62.96%. The agreement between two samples was not compared with another study, as there is no study comparing the agreement of both samples else were. The PPA suggests a likelihood of confirming or ruling out the disease when both tests are positive, while the NPA indicates excluding bacterial meningitis when both tests yield negative results. This highlights the importance of using paired samples to avoid missing cases in the diagnosis of bacterial meningitis.

Bacterial DNA was 20% more frequently detected in serum compared to CSF, and 42% compared to culture in suspected cases of meningitis. Furthermore, these findings are clinically important to minimise the invasive lumbar puncture for CSF collection. Overall, serum PCR could be considered as a supplementary diagnostic tool, a less invasive diagnostic procedure for the diagnosis of bacterial meningitis. However, further similar studies are important using qPCR and both blood and CSF culture.

5.1. Limitations and Strength

5.1.1. Strength

This is the first study from Ethiopia comparing the efficiency of CSF and serum PCR in diagnosing bacterial meningitis. The rate of detection in serum PCR was higher when compared to CSF PCR; therefore, its source of data as serum may be used as the best specimen for diagnosis of bacterial meningitis. This study has highlighted the importance of serum PCR for diagnosis in cases of bacterial meningitis, even when CSF samples turn out to be negative. The use of multiple targets of bacteria in the multiplex PCR-based study raises the possibility of identification of several pathogens at one time. Briefly, these findings support the inclusion of serum PCR in routine diagnostic practices for bacterial meningitis, which might improve diagnosis and treatment decisions.

5.1.2. Limitations

Due to the low sensitivity of culture, the diagnostic accuracy of CSF and serum PCR cannot be calculated. Culture is considered the gold standard, but low sensitivity may affect the calculation of the diagnostic accuracy of CSF and serum PCR.

5.2. Conclusion

Bacterial DNA was more frequently detected in serum-PCR compared to CSF-PCR and culture. It can be concluded that the development of rapid PCR diagnostic tools that utilize serum samples will improve the diagnosis of bacterial meningitis, patient management, and minimize patient discomfort during sample collection.

5.3. Recommendation

Based on the findings of the present study, the following recommendations are made:-

- Future studies should be conducted in order to address the reasons for observed differences in the detection rates of bacterial agents in serum-PCR and CSF-PCR.
- Establish or improve the existing diagnostic facilities for serum PCR that help in managing patients suspected of meningitis.
- Based on the present data, we recommend for the clinicians to consider serum PCR as the supplementary diagnostic tool for the diagnosis of bacterial meningitis.
- Multiplex PCR, which detects the most common bacterial pathogens simultaneously, is recommended as it allows for rapid availability of results and facilitates early and appropriate treatment.

REFERENCES

Ahmed, M. A., Askar, G. A., Farghaly, H. S., Ahmed, A. O., Kamal, D. T., Ahmed, S. S. & Mohamad, I. L. J. A. N. T. 2022. Accuracy Of Cerebrospinal Fluid C-Reactive Protein And Multiplex Polymerase Chain Reaction And Serum Procalcitonin In Diagnosis Of Bacterial And Viral Meningitis In Children. *Acta Neuropathol,* 31(2)**,** 61-71.

Airede, K. I., Adeyemi, O. & Ibrahim, T. 2008. Neonatal Bacterial Meningitis And Dexamethasone Adjunctive Usage In Nigeria. *Niger J Clin Pract,* 11**,** 235-45.

Al-Bayati, A., Douedi, S., Alsaoudi, G., Mosseri, M., Albustani, S., Upadhyaya, V., Gornish, N. & Elsawaf, M. J. I. 2020. Meningitis From Invasive Streptococcus Agalactiae In A Healthy Young Adult. *Idcases,* 21.

Albuquerque, R. C., Moreno, A. C. R., Dos Santos, S. R., Ragazzi, S. L. B. & Martinez, M. B. 2019. Multiplex-Pcr For Diagnosis Of Bacterial Meningitis. *Braz J Microbiol,* 50**,** 435-443.

Amare, A. T., Kebede, Z. T. & Welch, H. D. 2018. Epidemiology Of Bacterial Meningitis In Children Admitted To Gondar University Hospital In The Post Pneumococcal Vaccine Era. *Pan Afr Med J,* 31**,** 193.

Amidu, N., Antuamwine, B. B., Addai-Mensah, O., Abdul-Karim, A., Stebleson, A., Abubakari, B. B., Abenyeri, J., Opoku, A. S., Nkukah, J. E. & Najibullah, A. S. 2019. Diagnosis Of Bacterial Meningitis In Ghana: Polymerase Chain Reaction Versus Latex Agglutination Methods. *Plos One,* 14**,** E0210812.

Assegu Fenta, D., Lemma, K., Tadele, H., Tadesse, B. T. & Derese, B. 2020. Antimicrobial Sensitivity Profile And Bacterial Isolates Among Suspected Pyogenic Meningitis Patients Attending At Hawassa University Hospital: Cross-Sectional Study. *Bmc Microbiol,* 20**,** 125.

Awulachew, E., Diriba, K. & Awoke, N. 2020. Bacterial Isolates From Csf Samples And Their Antimicrobial Resistance Patterns Among Children Under Five Suspected To Have Meningitis In Dilla University Referral Hospital. *Infect Drug Resist,* 13**,** 4193-4202.

Bårnes, G. K., Gudina, E. K., Berhane, M., Abdissa, A., Tesfaw, G., Abebe, G., Feruglio, S. L., Caugant, D. A. & Jørgensen, H. J. J. B. I. D. 2018. New Molecular Tools For Meningitis

Diagnostics In Ethiopia–A Necessary Step Towards Improving Antimicrobial Prescription. *Bmc Infectious Diseases,* 18(1):684**,** 1-14.

Barrangou, R. & Horvath, P. 2017. A Decade Of Discovery: Crispr Functions And Applications. *Nat Microbiol,* 2**,** 17092.

Başpınar, E. Ö., Dayan, S., Bekçibaşı, M., Tekin, R., Ayaz, C., Deveci, Ö. & Hoşoğlu, S. J. B. J. O. M. 2017. Comparison Of Culture And Pcr Methods In The Diagnosis Of Bacterial Meningitis. *Pan Afr Med J,* 48**,** 232-236.

Batista, A. C. & Pacheco, L. G. C. 2018. Detecting Pathogens With Zinc-Finger, Tale And Crispr-Based Programmable Nucleic Acid Binding Proteins. *J Microbiol Methods,* 152**,** 98-104.

Bekele, F., Ahmed, A., Kedir, A. & Sheleme, T. 2021. Treatment Outcome And Associated Factors Of Bacterial Meningitis At Pediatric Wards Of Southwestern Ethiopian Hospital: A Prospective Observational Study. *J Pharm Health Care Sci,* 7**,** 41.

Bell, L. M., Alpert, G., Campos, J. M. & Plotkin, S. A. 1985. Routine Quantitative Blood Cultures In Children With Haemophilus Influenzae Or Streptococcus Pneumoniae Bacteremia. *Pediatrics,* 76**,** 901-4.

Berwal, A., Chawla, K., Vishwanath, S. & Shenoy, V. P. J. J. O. L. P. 2017. Role Of Multiplex Polymerase Chain Reaction In Diagnosing Tubercular Meningitis. *J Lab Physicians,* 9**,** 145-147.

Bhagchandani, S. P., Kubade, S., Nikhare, P. P., Manke, S., Chandak, N. H., Kabra, D., Baheti, N. N., Agrawal, V. S., Sarda, P., Mahajan, P., Ganjre, A., Purohit, H. J., Singh, L., Taori, G. M., Daginawala, H. F. & Kashyap, R. S. 2016. Nested Pcr Assay For Eight Pathogens: A Rapid Tool For Diagnosis Of Bacterial Meningitis. *Mol Diagn Ther,* 20**,** 45-54.

Bidgani, S., Navidifar, T., Najafian, M. & Amin, M. 2016. Comparison Of Group B Streptococci Colonization In Vaginal And Rectal Specimens By Culture Method And Polymerase Chain Reaction Technique. *J Chin Med Assoc,* 79**,** 141-145.

Bijlsma, M. W., Brouwer, M. C., Kasanmoentalib, E. S., Kloek, A. T., Lucas, M. J., Tanck, M. W., Van Der Ende, A. & Van De Beek, D. 2016. Community-Acquired Bacterial Meningitis In Adults In The Netherlands, 2006-14: A Prospective Cohort Study. *Lancet Infect Dis,* 16**,** 339-47.

Bingen, E., Denamur, E., Brahimi, N. & Elion, J. J. C. I. D. 1996. Genotyping May Provide Rapid Identification Of Escherichia Coli K1 Organisms That Cause Neonatal Meningitis. *Clin Infect Dis,* 22**,** 152-156.

Bodilsen, J., Dalager-Pedersen, M., Schønheyder, H. C. & Nielsen, H. J. B. I. D. 2016. Time To Antibiotic Therapy And Outcome In Bacterial Meningitis: A Danish Population-Based Cohort Study. *Bmc Infect Dis,* 16**,** 1-7.

Brink, M., Welinder-Olsson, C. & Hagberg, L. 2015. Time Window For Positive Cerebrospinal Fluid Broad-Range Bacterial Pcr And Streptococcus Pneumoniae Immunochromatographic Test In Acute Bacterial Meningitis. *Infect Dis (Lond),* 47**,** 869-77.

Bronska, E., Kalmusova, J., Dzupova, O., Maresova, V., Kriz, P. & Benes, J. 2006. Dynamics Of Pcr-Based Diagnosis In Patients With Invasive Meningococcal Disease. *Clin Microbiol Infect,* 12**,** 137-41.

Brouwer, M. C., Tunkel, A. R. & Van De Beek, D. 2010. Epidemiology, Diagnosis, And Antimicrobial Treatment Of Acute Bacterial Meningitis. *Clin Microbiol Rev,* 23**,** 467-92.

Brueggemann, A. B., Jansen Van Rensburg, M. J., Shaw, D., Mccarthy, N. D., Jolley, K. A., Maiden, M. C. J., Van Der Linden, M. P. G., Amin-Chowdhury, Z., Bennett, D. E., Borrow, R., Brandileone, M. C., Broughton, K., Campbell, R., Cao, B., Casanova, C., Choi, E. H., Chu, Y. W., Clark, S. A., Claus, H., Coelho, J., Corcoran, M., Cottrell, S., Cunney, R. J., Dalby, T., Davies, H., De Gouveia, L., Deghmane, A. E., Demczuk, W., Desmet, S., Drew, R. J., Du Plessis, M., Erlendsdottir, H., Fry, N. K., Fuursted, K., Gray, S. J., Henriques-Normark, B., Hale, T., Hilty, M., Hoffmann, S., Humphreys, H., Ip, M., Jacobsson, S., Johnston, J., Kozakova, J., Kristinsson, K. G., Krizova, P., Kuch, A., Ladhani, S. N., Lâm, T. T., Lebedova, V., Lindholm, L., Litt, D. J., Martin, I., Martiny, D., Mattheus, W., Mcelligott, M., Meehan, M., Meiring, S., Mölling, P., Morfeldt, E., Morgan, J., Mulhall, R. M., Muñoz-Almagro, C., Murdoch, D. R., Murphy, J., Musilek, M., Mzabi, A., Perez-Argüello, A., Perrin, M., Perry, M., Redin, A., Roberts, R., Roberts, M., Rokney, A., Ron, M., Scott, K. J., Sheppard, C. L., Siira, L., Skoczyńska, A., Sloan, M., Slotved, H. C., Smith, A. J., Song, J. Y., Taha, M. K., Toropainen, M., Tsang, D., Vainio, A., Van Sorge, N. M., Varon, E., Vlach, J., Vogel, U., Vohrnova, S., Von Gottberg, A., Zanella, R. C. & Zhou,

F. 2021. Changes In The Incidence Of Invasive Disease Due To Streptococcus Pneumoniae, Haemophilus Influenzae, And Neisseria Meningitidis During The Covid-19 Pandemic In 26 Countries And Territories In The Invasive Respiratory Infection Surveillance Initiative: A Prospective Analysis Of Surveillance Data. *Lancet Digit Health,* 3**,** E360-E370.

Calabrese, V. P. J. M. Q., Medical College Of Virginia Quarterly 1977. Bacterial Meningitis: Some Epidemiologic And Clinical Factors In Diagnosis. *Mcv/Q, Medical College Of Virginia Quarterly,* 13**,** 123-130.

Ceyhan, M., Yildirim, I., Balmer, P., Borrow, R., Dikici, B., Turgut, M., Kurt, N., Aydogan, A., Ecevit, C., Anlar, Y., Gulumser, O., Tanir, G., Salman, N., Gurler, N., Hatipoglu, N., Hacimustafaoglu, M., Celebi, S., Coskun, Y., Alhan, E., Celik, U., Camcioglu, Y., Secmeer, G., Gur, D. & Gray, S. 2008. A Prospective Study Of Etiology Of Childhood Acute Bacterial Meningitis, Turkey. *Emerg Infect Dis,* 14**,** 1089-96.

Choi, M. H., Park, Y. J., Kim, M., Seo, Y. H., Kim, Y. A., Choi, J. Y., Yong, D., Jeong, S. H. & Lee, K. J. A. O. L. M. 2018. Increasing Incidence Of Listeriosis And Infection-Associated Clinical Outcomes. *Ann Lab Med,* 38**,** 102-109.

Cohn, A. C., Macneil, J. R., Harrison, L. H., Hatcher, C., Theodore, J., Schmidt, M., Pondo, T., Arnold, K. E., Baumbach, J. & Bennett, N. J. C. I. D. 2010. Changes In Neisseria Meningitidis Disease Epidemiology In The United States, 1998–2007: Implications For Prevention Of Meningococcal Disease. *Clin Infect Dis,* 50**,** 184-191.

Croxatto, A., Prod'hom, G. & Greub, G. 2012. Applications Of Maldi-Tof Mass Spectrometry In Clinical Diagnostic Microbiology. *Fems Microbiol Rev,* 36**,** 380-407.

Daka, D., Loha, E. & Giday, A. 2011. Streptococcus Pneumonia And Antimicrobial Resistance, Hawassa Referral Hospital, South Ethiopia. *J Med Lab Diagn,* 2**,** 27-30.

Davis, L. E. 2018. Acute Bacterial Meningitis. *Continuum (Minneap Minn),* 24**,** 1264-1283.

De Almeida, S. M., Dalla Costa, L. M., Siebra, C., Arend, L. N. V. S. & Nogueira, K. D. S. J. A. D. N.-P. 2019. Validation Of Multiplex Pcr For The Diagnosis Of Acute Bacterial Meningitis In Culture Negative Cerebrospinal Fluid. *Arquivos De Neuro-Psiquiatria,* 77**,** 224-231.

De Zoysa, A., Edwards, K., Gharbia, S., Underwood, A., Charlett, A. & Efstratiou, A. J. J. O. M. M. 2012. Non-Culture Detection Of Streptococcus Agalactiae (Lancefield Group B Streptococcus) In Clinical Samples By Real-Time Pcr. *J Med Microbiol,* 61**,** 1086-1090.

Desmond, N. A., Nyirenda, D., Dube, Q., Mallewa, M., Molyneux, E., Lalloo, D. G. & Heyderman, R. S. J. P. O. 2013. Recognising And Treatment Seeking For Acute Bacterial Meningitis In Adults And Children In Resource-Poor Settings: A Qualitative Study. *Plos One,* 8**,** E68163.

Diallo, K., Feteh, V. F., Ibe, L., Antonio, M., Caugant, D. A., Du Plessis, M., Deghmane, A. E., Feavers, I. M., Fernandez, K., Fox, L. M., Rodrigues, C. M. C., Ronveaux, O., Taha, M. K., Wang, X., Brueggemann, A. B., Maiden, M. C. J. & Harrison, O. B. 2021. Molecular Diagnostic Assays For The Detection Of Common Bacterial Meningitis Pathogens: A Narrative Review. *Ebiomedicine,* 65**,** 103274.

Dmitriev, A., Suvorov, A., Shen, A. D. & Yang, Y. H. 2004. Clinical Diagnosis Of Group B Streptococci By Scpb Gene Based Pcr. *Indian J Med Res,* 119 Suppl**,** 233-6.

Doran, K. S., Fulde, M., Gratz, N., Kim, B. J., Nau, R., Prasadarao, N., Schubert-Unkmeir, A., Tuomanen, E. I. & Valentin-Weigand, P. 2016. Host-Pathogen Interactions In Bacterial Meningitis. *Acta Neuropathol,* 131**,** 185-209.

Dubos, F., Lamotte, B., Bibi-Triki, F., Moulin, F., Raymond, J., Gendrel, D., Bréart, G. & Chalumeau, M. J. A. O. D. I. C. 2006. Clinical Decision Rules To Distinguish Between Bacterial And Aseptic Meningitis. *Arch Dis Child,* 91**,** 647-650.

Feldman, W. E. J. T. J. O. P. 1976. Concentrations Of Bacteria In Cerebrospinal Fluid Of Patients With Bacterial Meningitis. *J Pediatr,* 88**,** 549-552.

Ferreira, M. B., De-Paris, F., Paiva, R. M. & Nunes, L. S. 2018. Assessment Of Conventional Pcr And Real-Time Pcr Compared To The Gold Standard Method For Screening Streptococcus Agalactiae In Pregnant Women. *Braz J Infect Dis,* 22**,** 449-454.

Fuentes-Antrás, J., Ramírez-Torres, M., Osorio-Martínez, E., Lorente, M., Lorenzo-Almorós, A., Lorenzo, O. & Górgolas, M. 2019. Acute Community-Acquired Bacterial Meningitis: Update On Clinical Presentation And Prognostic Factors. *New Microbiol,* 41**,** 81-87.

Geteneh, A., Kassa, T., Alemu, Y., Alemu, D., Kiros, M., Andualem, H., Abebe, W., Alemayehu, T., Alemayehu, D. H. & Mihret, A. J. P. O. 2020. Enhanced Identification Of Group B Streptococcus In Infants With Suspected Meningitis In Ethiopia. *Plos One,* 15**,** E0242628.

Gilsdorf, J. R. 2021. Hib Vaccines: Their Impact On Haemophilus Influenzae Type B Disease. *J Infect Dis,* 224**,** S321-S330.

Gituro, C. N., Nyerere, A., Ngayo, M. O., Maina, E., Githuku, J. & Boru, W. 2017. Etiology Of Bacterial Meningitis: A Cross-Sectional Study Among Patients Admitted In A Semi-Urban Hospital In Nairobi, Kenya. *Pan Afr Med J,* 28.

Gudina, E. K., Tesfaye, M., Wieser, A., Pfister, H. W. & Klein, M. 2018. Outcome Of Patients With Acute Bacterial Meningitis In A Teaching Hospital In Ethiopia: A Prospective Study. *Plos One,* 13**,** E0200067.

Guo, D., Xi, Y., Wang, S. & Wang, Z. J. B. I. D. 2019a. Is A Positive Christie-Atkinson-Munch-Peterson (Camp) Test Sensitive Enough For The Identification Of Streptococcus Agalactiae? *Bmc Infect Dis,* 19**,** 1-5.

Guo, L. Y., Li, Y. J., Liu, L. L., Wu, H. L., Zhou, J. L., Zhang, Y., Feng, W. Y., Zhu, L., Hu, B., Hu, H. L., Chen, T. M., Guo, X., Chen, H. Y., Yang, Y. H. & Liu, G. 2019b. Detection Of Pediatric Bacterial Meningitis Pathogens From Cerebrospinal Fluid By Next-Generation Sequencing Technology. *J Infect,* 78**,** 323-337.

Hailu, M. & Muhe, L. J. E. M. J. 2001. Childhood Meningitis In A Tertiary Hospital In Addis Ababa: Clinical And Epidemiological Features. *Ethiop Med J,* 39**,** 29-38.

Hamborsky, J. & Kroger, A. 2015. *Epidemiology And Prevention Of Vaccine-Preventable Diseases, E-Book: The Pink Book*, Public Health Foundation.

Harrison, L. H., Trotter, C. L. & Ramsay, M. E. J. V. 2009. Global Epidemiology Of Meningococcal Disease. *Vaccine,* 27**,** B51-B63.

Hibstu, Z., Mullu, A., Mihret, A. & Mengist, H. M. 2022. Prevalence, Antibiogram, And Associated Factors Of Bacteria Isolated From Presumptive Meningitis Patients At Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia. *Cureus,* 14**,** E28500.

Holmquist, L., Russo, C. A. & Elixhauser, A. 2006. Meningitis-Related Hospitalizations In The United States, 2006. *Healthcare Cost And Utilization Project (Hcup) Statistical Briefs.* Rockville (Md): Agency For Healthcare Research And Quality (Us).

Huang, N., Jia, H., Zhou, B., Zhou, C., Cao, J., Liao, W., Liu, S., Wang, L., Chen, L. & Chen, L. J. F. I. P. H. 2022. Hypervirulent Carbapenem-Resistant Klebsiella Pneumoniae Causing Highly Fatal Meningitis In Southeastern China. *Frontiers In Public Health,* 10**,** 991306.

Hussen, S., Asnake, S., Wachamo, D. & Tadesse, B. T. J. F. 2020. Pneumococcal Nasopharyngeal Carriage And Antimicrobial Susceptibility Profile In Children Under Five In Southern Ethiopia. *F1000res,* 9.

Intra, J., Carcione, D., Sala, R. M., Siracusa, C., Brambilla, P. & Leoni, V. 2024. Surveillance Of Bacterial Meningitis In The Italian Hospital Of Desio: A Twenty-Year Retrospective Study. *Applied Microbiology,* 4**,** 481-485.

Jackson, K. A., Gould, L. H., Hunter, J. C., Kucerova, Z. & Jackson, B. J. E. I. D. 2018. Listeriosis Outbreaks Associated With Soft Cheeses, United States, 1998–2014. *Emerg Infect Dis,* 24**,** 1116.

Jafri, R. Z., Ali, A., Messonnier, N. E., Tevi-Benissan, C., Durrheim, D., Eskola, J., Fermon, F., Klugman, K. P., Ramsay, M. & Sow, S. J. P. H. M. 2013. Global Epidemiology Of Invasive Meningococcal Disease. *Popul Health Metr,* 11**,** 1-9.

Khan, F. Y., Abu-Khattab, M., Almaslamani, E. A., Hassan, A. A., Mohamed, S. F., Elbuzdi, A. A., Elmaki, N. Y., Anand, D. & Sanjay, D. 2017. Acute Bacterial Meningitis In Qatar: A Hospital-Based Study From 2009 To 2013. *Biomed Res Int***,** 2975610.

Kim, K. S. J. E. P. 2016. Human Meningitis-Associated Escherichia Coli. *Ecosal Plus,* 7**,** 10.1128/Ecosalplus. Esp-0015-2015.

Koelman, D. L. H., Van Kassel, M. N., Bijlsma, M. W., Brouwer, M. C., Van De Beek, D. & Van Der Ende, A. 2021. Changing Epidemiology Of Bacterial Meningitis Since Introduction Of Conjugate Vaccines: 3 Decades Of National Meningitis Surveillance In The Netherlands. *Clin Infect Dis,* 73**,** E1099-E1107.

Kokeb, M. 2023. Surveillance On Pediatric Bacterial Meningitis In Gondar University Hospital, Ethiopia From 2012 To 2021: Retrospective Analysis. *Ethiopian Medical Journal,* 61.

Koomen, I., Grobbee, D., Jennekens-Schinkel, A., Roord, J. & Van Furth, A. M. J. A. P. 2003. Parental Perception Of Educational, Behavioural And General Health Problems In School-Age Survivors Of Bacterial Meningitis. *Acta Paediatrica,* 92**,** 177-185.

Ku, Y.-H., Chuang, Y.-C., Chen, C.-C., Lee, M.-F., Yang, Y.-C., Tang, H.-J. & Yu, W.-L. J. S. R. 2017. Klebsiella Pneumoniae Isolates From Meningitis: Epidemiology, Virulence And Antibiotic Resistance. *Sci Rep,* 7**,** 6634.

Kumalo, A., Kassa, T., Mariam, Z. S., Daka, D. & Tadesse, A. H. J. H. S. J. 2016. Bacterial Profile Of Adult Sepsis And Their Antimicrobial Susceptibility Pattern At Jimma University Specialized Hospital, South West Ethiopia. *Health Science Journal,* 10**,** 1.

Kumar, A., Debata, P. K., Ranjan, A. & Gaind, R. 2015. The Role And Reliability Of Rapid Bedside Diagnostic Test In Early Diagnosis And Treatment Of Bacterial Meningitis. *Indian J Pediatr,* 82**,** 311-4.

La Scolea Jr, L. J. & Dryja, D. J. J. O. C. M. 1984. Quantitation Of Bacteria In Cerebrospinal Fluid And Blood Of Children With Meningitis And Its Diagnostic Significance. *J Clin Microbiol,* 19**,** 187-190.

Lan, Y., Liu, L., Hu, D., Ge, L., Xiang, X., Peng, M., Fu, Y., Wang, Y., Li, S., Chen, Y., Jiang, Y., Tu, Y., Vidal, J. E., Yu, Y., Chen, Z. & Wu, X. 2024. Limited Protection Of Pneumococcal Vaccines Against Emergent Streptococcus Pneumoniae Serotype 14/St876 Strains. *Infection,* 52**,** 801-811.

Laving, A. M., Musoke, R. N., Wasunna, A. O. & Revathi, G. 2003. Neonatal Bacterial Meningitis At The Newborn Unit Of Kenyatta National Hospital. *East Afr Med J,* 80**,** 456-62.

Le Monnier, A., Abachin, E., Beretti, J.-L., Berche, P. & Kayal, S. J. J. O. C. M. 2011. Diagnosis Of Listeria Monocytogenes Meningoencephalitis By Real-Time Pcr For The Hly Gene. *J Clin Microbiol,* 49**,** 3917-3923.

Leber, A. L., Everhart, K., Balada-Llasat, J. M., Cullison, J., Daly, J., Holt, S., Lephart, P., Salimnia, H., Schreckenberger, P. C., Desjarlais, S., Reed, S. L., Chapin, K. C., Leblanc, L., Johnson, J. K., Soliven, N. L., Carroll, K. C., Miller, J. A., Dien Bard, J., Mestas, J., Bankowski, M., Enomoto, T., Hemmert, A. C. & Bourzac, K. M. 2016. Multicenter Evaluation Of Biofire Filmarray Meningitis/Encephalitis Panel For Detection Of Bacteria, Viruses, And Yeast In Cerebrospinal Fluid Specimens. *J Clin Microbiol,* 54**,** 2251-61.

Lien, C. Y., J., L. J., Tsai, W. C., Chen, S. Y., Huang, C. R., Chien, C. C., Lu, C. H. & Chang, W. N. 2019. The Clinical Characteristics Of Spontaneous Gram-Negative Bacterial Meningitis In Adults: A Hospital-Based Study. *J Clin Neurosci,* 64**,** 101-105.

Lin, M.-C., Chiu, N.-C., Chi, H., Ho, C.-S. & Huang, F.-Y. 2015. Evolving Trends Of Neonatal And Childhood Bacterial Meningitis In Northern Taiwan. *J Microbiol Immunol Infect,* 48**,** 296-301.

Macneil, J. R., Bennett, N., Farley, M. M., Harrison, L. H., Lynfield, R., Nichols, M., Petit, S., Reingold, A., Schaffner, W. & Thomas, A. J. P. 2015. Epidemiology Of Infant Meningococcal Disease In The United States, 2006-2012. *Pediatrics,* 135**,** E305-E311.

Mazamay, S., Guégan, J.-F., Diallo, N., Bompangue, D., Bokabo, E., Muyembe, J.-J., Taty, N., Vita, T. P. & Broutin, H. 2021. An Overview Of Bacterial Meningitis Epidemics In Africa From 1928 To 2018 With A Focus On Epidemics "Outside-The-Belt". *Bmc Infect Dis,* 21**,** 1027.

Mcgloughlin, S., Richards, G. A., Nor, M. B. M., Prayag, S., Baker, T. & Amin, P. J. J. O. C. C. 2018. Sepsis In Tropical Regions: Report From The Task Force On Tropical Diseases By The World Federation Of Societies Of Intensive And Critical Care Medicine. *J Crit Care,* 46**,** 115-118.

Memish, Z. A. & Alrajhi, A. A. J. N. J. 2002. Meningococcal Disease. *Pediatr Rev,* 7**,** 77-82.

Meng, X., Zhang, G., Sun, B., Liu, S., Wang, Y., Gao, M., Fan, Y., Zhang, G., Shi, G. & Kang, X. J. F. I. M. 2020. Rapid Detection Of Meca And Fema Genes By Loop-Mediated Isothermal Amplification In A Microfluidic System For Discrimination Of Different Staphylococcal Species And Prediction Of Methicillin Resistance. *Front Microbiol,* 11**,** 1487.

Mihret, W., Brusletto, B., Øvstebø, R., Troseid, A., Norheim, G., Yared Merid, Y. M., Afework Kassu, A. K., Workeabeba Abebe, W. A., Samuel Ayele, S. A. & Mezgebu Silamsaw Asres, M. S. A. 2019. Molecular Studies Of Meningococcal And Pneumococcal Meningitis Patients In Ethiopia. *Innate Immun,* 23(3)**,** 158-167.

Mihret, W., Lema, T., Merid, Y., Kassu, A., Abebe, W., Moges, B., Tenna, A., Woldegebriel, F., Yidnekachew, M., Mekonnen, W., Ahmed, A., Yamuah, L., Silamsaw, M., Petros, B., Oksnes, J., Rosenqvist, E., Ayele, S., Aseffa, A., Caugant, D. A. & Norheim, G. 2016. Surveillance Of Bacterial Meningitis, Ethiopia, 2012-2013. *Emerg Infect Dis,* 22**,** 75-8.

Mlinarić, A., Vogrinc, Ž. & Drenšek, Z. 2018. Effect Of Sample Processing And Time Delay On Cell Count And Chemistry Tests In Cerebrospinal Fluid Collected From Drainage Systems. *Biochem Med (Zagreb),* 28**,** 030705.

Modi, G. B., Patel, K. D., Soni, S., Patel, K. J., Mangukiya, J. D. & Jain, P. S. 2012. Bacteriological Profile Of Pyogenic Meningitis In Tertiary Care Hospital, Ahmedabad. *National Journal Of Medical Research,* 2**,** 313-317.

Murray, P. R., Rosenthal, K. S., Pfaller, M. A. & Kłuciński, P. 2011. *Mikrobiologia*, Elsevier Urban & Partner.

Naghavi, M., Marczak, L. B., Kutz, M., Shackelford, K. A., Arora, M., Miller-Petrie, M., Aichour, M. T. E., Akseer, N., Al-Raddadi, R. M. & Alam, K. J. J. 2018. Global Mortality From Firearms, 1990-2016. *Jama,* 320**,** 792-814.

Neuman, M. I., Tolford, S. & Harper, M. B. J. T. P. I. D. J. 2008. Test Characteristics And Interpretation Of Cerebrospinal Fluid Gram Stain In Children. *Pediatr Infect Dis J,* 27**,** 309-313.

Newcombe, J., Cartwright, K., Palmer, W. H. & Mcfadden, J. J. J. O. C. M. 1996. Pcr Of Peripheral Blood For Diagnosis Of Meningococcal Disease. *J Clin Microbiol,* 34**,** 1637-1640.

Niemelä, S., Lempinen, L., Löyttyniemi, E., Oksi, J. & Jero, J. 2023. Bacterial Meningitis In Adults: A Retrospective Study Among 148 Patients In An 8-Year Period In A University Hospital, Finland. *Bmc Infect Dis,* 23**,** 45.

Nwadioha, S. I., Nwokedi, E. O., Onwuezube, I., Egesie, J. O. & Kashibu, E. 2013. Bacterial Isolates From Cerebrospinal Fluid Of Children With Suspected Acute Meningitis In A Nigerian Tertiary Hospital. *Niger Postgrad Med J,* 20**,** 9-13.

Obaro, S. J. T. L. I. D. 2019. Updating The Diagnosis Of Bacterial Meningitis. *Lancet Infect Dis,* 19**,** 1160-1161.

Odedina, E. & Emumwen, E. 2008. Bacterial Meningitis Among Children In Federal Medical Centre. *African Journal Of Clinical And Experimental Microbiology,* 9**,** 152-156.

Okike, I. O., Ladhani, S. N., Johnson, A. P., Henderson, K. L., Blackburn, R. M., Muller-Pebody, B., Cafferkey, M., Anthony, M., Ninis, N. & Heath, P. T. 2018. Clinical Characteristics And Risk Factors For Poor Outcome In Infants Less Than 90 Days Of Age With Bacterial Meningitis In The United Kingdom And Ireland. *Pediatr Infect Dis J,* 37**,** 837-843.

Ouattara, M., Tamboura, M., Kambire, D., Sanou, M., Ouattara, K., Congo, M., Kaboré, A., Sanou, S., Kabré, E. & Sharpley, S. J. E. I. D. 2020. Identification Of Streptococcus Suis Meningitis By Direct Triplex Real-Time Pcr, Burkina Faso. *Emerg Infect Dis,* 26**,** 2223.

Owusu, M., Nguah, S. B., Boaitey, Y. A., Badu-Boateng, E., Abubakr, A.-R., Lartey, R. A. & Adu-Sarkodie, Y. 2012. Aetiological Agents Of Cerebrospinal Meningitis: A Retrospective Study From A Teaching Hospital In Ghana. *Ann Clin Microbiol Antimicrob,* 11**,** 1-8.

Petersdorf, R. G., Swarner, D. R. & Garcia, M. 1962. Studies On The Pathogenesis Of Meningitis. Ii. Development Of Meningitis During Pneumococcal Bacteremia. *J Clin Invest,* 41**,** 320-7.

Protonotariou, E., Arampatzi, A., Ourailoglou, V., Diza, E. & Skoura, L. J. H. 2015. An Unusual Case Of Streptococcus Agalactiae Meningitis In A Patient With Sys-Temic Lupus Erythematosus. *Hippokratia,* 19**,** 372.

Qu, C., Wang, Y., Wang, X., He, R., Cao, H., Liu, B., Zhang, H., Zhang, N., Lai, Z., Dai, Z. & Cheng, Q. 2023. Global Burden And Its Association With Socioeconomic Development Status Of Meningitis Caused By Specific Pathogens Over The Past 30 Years: A Population-Based Study. *Neuroepidemiology,* 57**,** 316-335.

Ramgopal, S., Walker, L. W., Vitale, M. A. & Nowalk, A. J. 2019. Factors Associated With Serious Bacterial Infections In Infants ≤60 Days With Hypothermia In The Emergency Department. *Am J Emerg Med,* 37**,** 1139-1143.

Ranjbar, R. & Halaji, M. J. B. P. H. 2018. Epidemiology Of Listeria Monocytogenes Prevalence In Foods, Animals And Human Origin From Iran: A Systematic Review And Meta-Analysis. *Bmc Public Health,* 18**,** 1-12.

Raza, S., Das, B., Chaudhry, R., Goyal, V., Lodha, R., Sood, S., Gautam, H., Kapil, A. J. J. O. M. & Diseases, I. 2022a. Efficiency Of Real-Time Pcr In The Diagnosis Of Community-Acquired Bacterial Meningitis In Children. *Journal Of Microbiology And Infectious Diseases,* 12**,** 47-53.

Raza, S., Das, B., Chaudhry, R., Goyal, V., Lodha, R., Sood, S., Gautam, H., Kapil, A. J. J. O. M. & Diseases, I. 2022b. Efficiency Of Real-Time Pcr In The Diagnosis Of Community-Acquired Bacterial Meningitis In Children. *Journal Of Microbiology And Infectious Diseases,* 12**,** 47-53.

Reta, M. A. & Zeleke, T. A. 2016. Neonatal Bacterial Meningitis In Tikur Anbessa Specialized Hospital, Ethiopia: A 10-Year Retrospective Review. *Springerplus,* 5**,** 1-6.

Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., Colombara, D. V., Ikuta, K. S., Kissoon, N. & Finfer, S. J. T. L. 2020. Global, Regional, And National Sepsis Incidence And Mortality, 1990–2017: Analysis For The Global Burden Of Disease Study. *The Lancet* 395**,** 200-211.

Runde, T. J., Anjum, F. & Hafner, J. W. 2017. Bacterial Meningitis. *Lancet Infect Dis*.

Scarborough, M. & Thwaites, G. E. J. T. L. N. 2008. The Diagnosis And Management Of Acute Bacterial Meningitis In Resource-Poor Settings. *Lancet Neurol,* 7**,** 637-648.

Sharma, N., Gautam, H., Tyagi, S., Raza, S., Mohapatra, S., Sood, S., Dhawan, B., Kapil, A., Das, B. K. J. J. O. F. M. & Care, P. 2022. Clinical Use Of Multiplex-Pcr For The Diagnosis Of Acute Bacterial Meningitis. *J Family Med Prim Care,* 11**,** 593-598.

Sonavane, A. E., Baradkar, V. & Mathur, M. 2008. Pattern And Antibiotic Susceptibility Of Bacteria Isolated In Clinically Suspected Cases Of Meningitis In Children. *Journal Of Pediatric Neurosciences,* 3**,** 131-133.

Sultana, N., Husain, M. A., Mazed, M. A., Akter, N., Sultan, M. T. & Hoque, M. E. 2008. Antimicrobial Sensitivity Pattern Of Bacterial Isolates Causing Meningitis. *Journal Of Chittagong Medical College Teachers' Association,* 19**,** 28-32.

Tamir, T. T., Terefe, B., Wassie, M., Workneh, B. S. & Zegeye, A. F. 2024. Prevalence, Spatial Distribution And Determinants Of Complete Childhood Pneumococcal Conjugate Vaccination In Ethiopia: Spatial And Multilevel Analyses. *Bmc Public Health,* 24**,** 2433.

Tavares, D. A., Handem, S., Carvalho, R. J., Paulo, A. C., De Lencastre, H., Hinds, J. & Sá-Leão, R. J. S. R. 2019. Identification Of Streptococcus Pneumoniae By A Real-Time Pcr Assay Targeting Sp2020. *Sci Rep,* 9**,** 3285.

Tegene, B., Gebreselassie, S., Fikrie, N. J. B. R. & Therapy 2015. Bacterial Meningitis: A Five-Year Retrospective Study Among Patients Who Had Attended At University Of Gondar Teaching Hospital, Northwest Ethiopia. *Biomedical Research And Therapy,* 2**,** 1-9.

Thigpen, M. C., Whitney, C. G., Messonnier, N. E., Zell, E. R., Lynfield, R., Hadler, J. L., Harrison, L. H., Farley, M. M., Reingold, A. & Bennett, N. M. J. N. E. J. O. M. 2011. Bacterial Meningitis In The United States, 1998–2007. *N Engl J Med,* 364**,** 2016-2025.

Tigabu, A., Jember, A., Nega, T., Wubishet, G., Misganaw, H., Goshu, T. & Negash, M. 2021. Bacterial Meningitis Among Adult Patients At University Of Gondar Comprehensive Specialized Referral Hospital. *Infect Drug Resist,* 14**,** 565-574.

Tunkel, A. R., Hartman, B. J., Kaplan, S. L., Kaufman, B. A., Roos, K. L., Scheld, W. M. & Whitley, R. J. J. C. I. D. 2004. Practice Guidelines For The Management Of Bacterial Meningitis. *Clin Infect Dis,* 39**,** 1267-1284.

Van De Beek, D., Brouwer, M., Hasbun, R., Koedel, U., Whitney, C. G. & Wijdicks, E. 2016. Community-Acquired Bacterial Meningitis. *Nat Rev Dis Primers,* 2**,** 16074.

Van De Beek, D., Farrar, J. J., De Gans, J., Mai, N. T. H., Molyneux, E. M., Peltola, H., Peto, T. E., Roine, I., Scarborough, M. & Schultsz, C. J. T. L. N. 2010. Adjunctive Dexamethasone In Bacterial Meningitis: A Meta-Analysis Of Individual Patient Data. *Lancet Neurol,* 9**,** 254-263.

Van Sorge, N. M. & Doran, K. S. 2012. Defense At The Border: The Blood-Brain Barrier Versus Bacterial Foreigners. *Future Microbiol,* 7**,** 383-94.

Van Veen, K. E., Brouwer, M. C., Van Der Ende, A. & Van De Beek, D. 2016. Bacterial Meningitis In Diabetes Patients: A Population-Based Prospective Study. *Sci Rep,* 6**,** 36996.

Walekhwa, M., Muturi, M., Gunturu, R., Kenya, E. & Kabera, B. 2018. Streptococcus Pneumoniae Serotype Epidemiology Among Pcv-10 Vaccinated And Unvaccinated Children At Gertrude's Children's Hospital, Nairobi County: A Cross-Sectional Study. *F1000res,* 7**,** 879.

Wall, E. C., Cartwright, K., Scarborough, M., Ajdukiewicz, K. M., Goodson, P., Mwambene, J., Zijlstra, E. E., Gordon, S. B., French, N. & Faragher, B. J. P. O. 2013. High Mortality Amongst Adolescents And Adults With Bacterial Meningitis In Sub-Saharan Africa: An Analysis Of 715 Cases From Malawi. *Plos One,* 8**,** E69783.

Who 2022. Thirteen Die As Eleven Regions In Ethiopia See Meningitis Outbreak: . *Who Report*.

Wootton, S. H., Aguilera, E., Salazar, L., Hemmert, A. C. & Hasbun, R. 2016. Enhancing Pathogen Identification In Patients With Meningitis And A Negative Gram Stain Using The Biofire Filmarray(®) Meningitis/Encephalitis Panel. *Ann Clin Microbiol Antimicrob,* 15**,** 26.

Wunrow, H. Y., Bender, R. G., Vongpradith, A., Sirota, S. B., Swetschinski, L. R., Novotney, A., Gray, A. P., Ikuta, K. S., Sharara, F., Wool, E. E., Aali, A., Abd-Elsalam, S., Abdollahi, A., Abdul Aziz, J. M., Abidi, H., Aboagye, R. G., Abolhassani, H., Abu-Gharbieh, E., Adamu, L. H., Adane, T. D., Addo, I. Y., Adegboye, O. A., Adekiya, T. A., Adnan, M., Adnani, Q. E. S., Afzal, S., Aghamiri, S., Aghdam, Z. B., Agodi, A., Ahinkorah, B. O., Ahmad, A., Ahmad, S., Ahmadzade, M., Ahmed, A., Ahmed, A., Ahmed, J. Q., Ahmed, M. S., Akinosoglou, K., Aklilu, A., Akonde, M., Alahdab, F., Al-Ahdal, T. M. A., Alanezi, F. M., Albelbeisi, A. H., Alemayehu, T. B. B., Alene, K. A., Al-Eyadhy, A., Al-Gheethi, A. A. S.,

Ali, A., Ali, B. A., Ali, L., Ali, S. S., Alimohamadi, Y., Alipour, V., Aljunid, S. M., Almustanyir, S., Al-Raddadi, R. M., Alvis-Guzman, N., Al-Worafi, Y. M., Aly, H., Ameyaw, E. K., Ancuceanu, R., Ansar, A., Ansari, G., Anyasodor, A. E., Arabloo, J., Aravkin, A. Y., Areda, D., Artamonov, A. A., Arulappan, J., Aruleba, R. T., Asaduzzaman, M., Atalell, K. A., Athari, S. S., Atlaw, D., Atout, M. M. D. W., Attia, S., Awoke, T., Ayalew, M. K., Ayana, T. M., Ayele, A. D., Azadnajafabad, S., Azizian, K., Badar, M., Badiye, A. D., Baghcheghi, N., Bagheri, M., Bagherieh, S., Bahadory, S., Baig, A. A., Barac, A., Barati, S., Bardhan, M., Basharat, Z., Bashiri, A., Basnyat, B., Bassat, Q., Basu, S., Bayileyegn, N. S., Bedi, N., Et Al. 2023. Global, Regional, And National Burden Of Meningitis And Its Aetiologies, 1990–2019: A Systematic Analysis For The Global Burden Of Disease Study 2019. *The Lancet Neurology,* 22**,** 685-711.

Yazdankhah, S. P. & Caugant, D. A. J. J. O. M. M. 2004. Neisseria Meningitidis: An Overview Of The Carriage State. *J Med Microbiol,* 53**,** 821-832.

Zainel, A., Mitchell, H. & Sadarangani, M. 2021. Bacterial Meningitis In Children: Neurological Complications, Associated Risk Factors, And Prevention. *Microorganisms,* 9**,** 535.

Zemariam, A. B., Abebe, G. K., Kassa, M. A., Alamaw, A. W., Molla, R. W., Abate, B. B., Tilahun, B. D., Wondie, W. T., Shimelash, R. A. & Fentanew, M. 2024. Immunization Coverage And Its Associated Factors Among Children Aged 12-23 Months In Ethiopia: An Umbrella Review Of Systematic Review And Meta-Analysis Studies. *Plos One,* 19**,** E0299384.

Zhai, Q., Li, S., Zhang, L., Yang, Y., Jiang, S. & Cao, Y. 2022. Changes In Pathogens Of Neonatal Bacterial Meningitis Over The Past 12 Years: A Single-Center Retrospective Study. *Transl Pediatr,* 11**,** 1595.

Zhang, J. Z., Zheng, P., Sun, H. M., Dong, J. J., Li, S. L., Fan, S. Y., Ren, H. T., Wu, H. L., Guan, H. Z. & Chen, Q. 2019. Next-Generation Sequencing Combined With Routine Methods To Detect The Pathogens Of Encephalitis/Meningitis From A Chinese Tertiary Pediatric Neurology Center. *J Infect,* 78**,** 409-421.

ANNEXES

Annex-I: Information Sheet

English Version

Name of the Organization: Addis Ababa University, college of health sciences, school of medicine, department of microbiology, immunology, and parasitology**.**

Title of the Research: 'Comparison of the efficiency of CSF and blood PCR in diagnosis of bacterial meningitis from patients suspected of meningitis attending TASH, and Y12HMC.

Name of researcher: Lema Ayele (MSc candidate) Tel: +251-911-35-36-72 Email:layele2016@gmail.com

Introduction

You are invited to participate in this research study conducted by an M.Sc. student at Addis Ababa University College of Health Sciences, School of Medicine, Department of Microbiology, Immunology, and Parasitology. This study will be carried out for comparing CSF and blood PCR samples for diagnosis of bacterial meningitis. The proposed study will be conducted at TASH and Y12HMC, Addis Ababa, Ethiopia, on patients suspected of meningitis, from August 2023 to February 2024.

Purpose of the study

The aim of this study is to compare the efficiency of CSF and serum PCR samples for diagnosis of bacterial meningitis among patients who are suspected to have it. Participation in this study is completely voluntary. If you do not wish to participate or later change your mind at any stage after initially agreeing to participate, there will be no consequence for your responsibilities. If you are willing to participate, you will then need to sign a consent form, and you will be given a copy of this information sheet for your own records.

What will be expected from you as a participant in the study?

In this study, you will be asked to provide 1 mL to 3 mL of CSF and 10 mL of blood samples as a participant. Also, you will be required to answer questions regarding your health and socio-demographic information. Please note that the results may be discussed with qualified persons who can give you proper consultation if the findings are important. However, your name, address, and telephone number will remain confidential and will not be used for such discussions, and an identification code will be used instead. If you decide to participate in this study, you will get your identification code.

How much time will I spend participating in this study?

This will only take a few minutes, and all you will be required to do is sign the consent form, fill out the questionnaire, and provide the necessary specimens.

What will be the risks of participating in this study?

There is no risk associated with the specimen collection and these specimens would follow the routine procedures for the laboratory investigation.

How our information will be kept confidential?

All the information you provide and the findings alone will be used in this research. Such information will only be accessed by a few authorized professionals. Besides, all information will be encoded in the computer and password-protected to ensure confidentiality.

What will be the benefits of participation?

Since this is an MSc student study, there is no payment for participants. Also, you will not be requested to pay for the laboratory examination. The result will be given to you; in case it will be clinically significant, it will help you with further diagnosis and treatment.

What will be your rights as a participant in this study?

You may withdraw from the study at any time, and there will be no consequence for that action. If you have any questions now or later on about the research, you are encouraged to ask.

What can I do if I have a problem or question?

In case of any questions or problems while performing the study, refer directly to the investigator and advisors.

PI: Lema Ayele (M.Sc candidate at Addis Ababa University in the field of Medical Microbiology)

Email:layele2016@gmail.com Phone number +251911353672

Supervisors:

1. Daniel Asrat (MD, Ph.D., Professor) Consultant Medical Specialist (Medical Microbiologist) Department of Microbiology, Immunology, and Parasitology School of Medicine, College of Health Sciences, Addis Ababa University Addis Ababa, Ethiopia Tel: +251-911-22-30-19
   Email: asratdan@gmail.com, daniel.asrat@aau.edu.et

Co-supervisors

2. Aminu Seman (M.Sc., Ph.D. Fellow) Department of Microbiology, Immunology, and Parasitology, School of Medicine, College of Health Science, Addis Ababa University, Addis Ababa, Ethiopia. P.O Box: 9086 AAU, Tel: +251 920 747 176
   E-mail: aminu.seman@aau.edu.et or aminumifta54@gmail.com

የአማርኛ ቅጂ (Amharic version)

የድርጅቱ ስም፡- አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ፣ የሕክምና ትምህርት ቤት፣ የማይክሮ ባዮሎጂ፣ የበሽታ መከላከያ እና ፓራሲቶሎጂ ክፍል።

የጥናቱ ርዕስ፡ 'የ CSF እና የደም PCR ቅልጥፍና ማነጻጸር የባክቴሪያ ገትር ገትር በሽታ በቲኤሽ ከሚካፈሉ ታማሚዎች በተጠረጠሩ ታማሚዎች እና Y12HMC።

የተመራማሪው ስም፡ አቶ ለማ አየለ (ኤምኤስሲ እጩ) በስልክ፡ +251-911-35-36-72 ኢሜል፡ layele2016@gmail.com

መግቢያ

በኤም.ኤስ.ሲ በተካሄደው በዚህ የምርምር ጥናት ላይ እንድትሳተፉ ተጋብዘዋል። በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ፣ የሕክምና ትምህርት ቤት፣ የማይክሮ ባዮሎጂ፣ የበሽታ መከላከያ እና ፓራሲቶሎጂ ትምህርት ክፍል ተማሪ። ይህ ጥናት የ CSF እና የደም PCR ናሙናዎችን የባክቴሪያ ማጅራት ገትር በሽታን በመመርመር ያለውን ውጤታማነት ለማነጻጸር ያለመ ይሆናል። ጥናቱ የማጅራት ገትር በሽታ አለባቸው ተብለው የተጠረጠሩ ታማሚዎችን ከነሐሴ 2023 እስከ የካቲት 2024 በአዲስ አበባ፣ ኢትዮጵያ ውስጥ TASH እና Y12HMC የሚማሩትን ያካትታል።

የጥናቱ ዓላማ

የዚህ ጥናት ዓላማ የ CSF እና የደም PCR ናሙናዎች የባክቴሪያ ገትር ገትር በሽታ እንዳለባቸው ከተጠረጠሩ ታካሚዎች መካከል ያለውን ውጤታማነት ማወዳደር ነው። በዚህ ጥናት ውስጥ መሳተፍ ሙሉ በሙሉ በፈቃደኝነት ነው. በመጀመሪያ ለመሳተፍ ከተስማሙ በኋላ በማንኛውም ደረጃ ላይ ለመሳተፍ ካልፈለጉ ወይም በኋላ ላይ ሀሳብዎን ከቀየሩ, ለኃላፊነትዎ ምንም ውጤት አይኖርም. ለመሳተፍ ፍቃደኛ ከሆናችሁ፣የፈቃድ ፎርም መፈረም አለባችሁ፣እና ለራሳችሁ መዝገቦች የዚህን የመረጃ ወረቀት ቅጂ ይሰጥዎታል።

በጥናቱ ውስጥ እንደ ተሳታፊ ከእርስዎ ምን ይጠበቃል?

የዚህ ጥናት ተሳታፊ እንደመሆንዎ መጠን ከ 1 ሚሊ እስከ 3 ሚሊ ሊትር CSF እና 10 ml የደም ናሙናዎችን እንዲያቀርቡ ይጠየቃሉ. እንዲሁም የእርስዎን የጤና እና የማህበራዊ-ስነ-ሕዝብ መረጃን በተመለከተ ጥያቄዎችን መመለስ ይጠበቅብዎታል። እባክዎን ውጤቶቹ አስፈላጊ ከሆኑ ተገቢውን ምክር ሊሰጡዎት ከሚችሉ ብቃት ካላቸው ሰዎች ጋር ሊወያይ እንደሚችል ልብ ይበሉ። ሆኖም ስምህ፣ አድራሻህ እና የስልክ ቁጥርህ ሚስጥራዊ ሆነው ይቆያሉ እና ለእንደዚህ አይነት ውይይቶች ጥቅም ላይ አይውሉም እና በምትኩ የመታወቂያ ኮድ ስራ ላይ ይውላል። በዚህ ጥናት ለመሳተፍ ከወሰኑ የመታወቂያ ኮድዎን ያገኛሉ።

በዚህ ጥናት ውስጥ ለመሳተፍ ምን ያህል ጊዜ አሳልፋለሁ?

ይህ ጥቂት ደቂቃዎችን ብቻ ነው የሚወስደው፣ እና እርስዎ የሚፈለጉት የፈቃድ ፎርሙን መፈረም፣ መጠይቁን መሙላት እና አስፈላጊዎቹን ናሙናዎች ማቅረብ ነው።

በዚህ ጥናት ውስጥ የመሳተፍ አደጋዎች ምን ምን ይሆናሉ?

ከናሙና ስብስብ ጋር የተያያዘ ምንም አይነት ስጋት የለም እና እነዚህ ናሙናዎች ለላቦራቶሪ ምርመራው መደበኛውን ሂደት ይከተላሉ.

የእኛ መረጃ እንዴት በሚስጥር ይጠበቃል?

ያቀረቡት መረጃ እና ግኝቶቹ ብቻ በዚህ ጥናት ውስጥ ጥቅም ላይ ይውላሉ። እንዲህ ዓይነቱ መረጃ በጥቂት የተፈቀደላቸው ባለሙያዎች ብቻ ይደርሳል. በተጨማሪም፣ ሁሉም መረጃዎች በኮምፒዩተር ውስጥ ይቀመጣሉ እና ሚስጥራዊነትን ለማረጋገጥ በይለፍ ቃል ይጠበቃል።

የመሳተፍ ጥቅሙ ምን ይሆን?

ይህ MSc የተማሪ ጥናት ስለሆነ ለተሳታፊዎች ምንም ክፍያ የለም። እንዲሁም ለላቦራቶሪ ምርመራ ክፍያ እንዲከፍሉ አይጠየቁም. ውጤቱ ይሰጥዎታል; ክሊኒካዊ ጠቀሜታ ያለው ከሆነ, ለተጨማሪ ምርመራ እና ህክምና ይረዳዎታል.

በዚህ ጥናት ውስጥ እንደ ተሳታፊ የእርስዎ መብቶች ምን ይሆናሉ?

በማንኛውም ጊዜ ከጥናቱ መውጣት ይችላሉ፣ እና ለድርጊቱ ምንም ውጤት አይኖርም። ስለ ጥናቱ አሁን ወይም በኋላ ማንኛቸውም ጥያቄዎች ካሉዎት እንዲጠይቁ ይበረታታሉ።

ችግር ወይም ጥያቄ ካጋጠመኝ ምን ማድረግ እችላለሁ?

ጥናቱን በምታደርጉበት ጊዜ ማንኛቸውም ጥያቄዎች ወይም ችግሮች ካሉ በቀጥታ ወደ መርማሪው እና አማካሪዎች ያመልክቱ።

PI፡ አቶ ለማ አየለ (በአዲስ አበባ ዩኒቨርሲቲ በህክምና ማይክሮባዮሎጂ ዘርፍ ኤም.ኤስ.ሲ እጩ)

ኢሜል፡layele2016@gmail.com ስልክ ቁጥር +251911353672

ተቆጣጣሪዎች፡-

1. ዳንኤል አስራት (ኤም.ዲ.፣ ፒኤችዲ፣ ፕሮፌሰር) አማካሪ ሜዲካል ስፔሻሊስት (ሜዲካል ማይክሮባዮሎጂስት) የማይክሮ ባዮሎጂ፣ ኢሚውኖሎጂ እና ፓራሲቶሎጂ ሕክምና ትምህርት ቤት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ አዲስ አበባ፣ ኢትዮጵያ ስልክ፡ +251- 911-22-30-19 እ.ኤ.አ ኢሜል፡ asratdan@gmail.com, daniel.asrat@aau.edu.et

ተባባሪ ተቆጣጣሪዎች

2. አሚኑ ሰማን (ኤም.ኤስ.ሲ.፣ ፒኤችዲ ፌሎው) የማይክሮ ባዮሎጂ፣ የበሽታ መከላከያ እና ፓራሲቶሎጂ ክፍል፣ የሕክምና ትምህርት ቤት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ፣ አዲስ አበባ፣ ኢትዮጵያ። P.O Box: 9086 AAU, Tel: +251 920 747 176 ኢሜል፡ aminu.seman@aau.edu.et ወይም aminumifta54@gmail.com

Annex-II: Consent Form

A. Adult Participant Informed Consent Form (≥18 years of Age)

English Version

Participant Code Number: _____

I have been fully informed, in a language I understand, about the purpose of the research study titled Comparison of the Efficiency of CSF and serum PCR in Diagnosing Bacterial Meningitis in Patients Suspected of Meningitis. I understand that this research will involve taking CSF and blood. I have been reassured that there is no injury with the collection of these specimens. I am also aware that any data collected in the course of this research will be treated as confidential. I understand that my current or future responsibilities will not be affected whether I refuse to participate in this study or withdraw myself at any time. Having been fully informed about this study, I hereby give consent to participate in this study and confirm by signing below.

Participant Name: _____

Signature: _____

Date: _____

Investigator Name: _____

Signature: _____

Date: _____

<u>የአማርኛ ቅጂ (Amharic Version)</u>

የአዋቂዎች ተሳታፊ በመረጃ የተደገፈ የስምምነት ቅጽ (≥የ 18 ዓመት ዕድሜ)

የተሳታፊ ኮድ ቁጥር፡- _____

የማጅራት ገትር በሽታ በተጠረጠሩ ታማሚዎች ላይ የሲኤስኤፍ እና የደም PCR ውጤታማነት ንጽጽር በሚል ርዕስ ስለተደረገው የምርምር ጥናት ዓላማ በምረዳው ቋንቋ ሙሉ መረጃ ተሰጥቶኛል። ይህ ጥናት CSF እና ደም መውሰድን እንደሚጨምር ተረድቻለሁ። በእነዚህ ናሙናዎች ስብስብ ላይ ምንም አይነት ጉዳት እንደሌለ አረጋግጦልኛል. በዚህ ጥናት ሂደት ውስጥ የሚሰበሰብ ማንኛውም መረጃ ሚስጥራዊ ሆኖ እንደሚቆጠርም አውቃለሁ። በዚህ ጥናት ለመሳተፍ ፈቃደኛ ባልሆን ወይም በማንኛውም ጊዜ ራሴን ለማንሳት የአሁን ወይም የወደፊት ኃላፊነቶቼ ላይ ተጽእኖ እንደማይኖራቸው ተረድቻለሁ። ስለዚህ ጥናት ሙሉ በሙሉ ከተረዳሁ በኋላ፣ በዚህ ጥናት ለመሳተፍ ፍቃድ ሰጥቻለሁ እና ከዚህ በታች በመፈረም አረጋግጣለሁ።

የተሳታፊ ስም፡ _____

ፊርማ፡ _____

ቀን፡- _____

የመርማሪ ስም፡ _____

ፊርማ፡ _____

ቀን፡- _____

B. Consent Form for Parents/Guardians (For children ≤ 17 years)

Participant Code Number: _____

I have been fully informed of the research study described in the research protocol titled "Comparison of the Efficiency of CSF and serum PCR in Diagnosing Bacterial Meningitis in Patients Suspected of Meningitis." I hereby affirm that this study would require the collection of CSF and blood from my child. The purposes of this research have been explained to me, and it has also been explained to me that everything in this questionnaire is strictly confidential. I am also aware that I do have the right to withhold information, refuse to cooperate, or withdraw the child from this study at any point. I hereby give my informed consent to the researcher to use the CSF and blood samples drawn from my child in this study, and of the facts I understand. Opportunity has been provided to me to ask questions, and I have received satisfactory clarification on the study. I have also been informed that one of the benefits of participating is that the results of the analysis of samples of my child will be provided free.

Research participant code: _____

Signature: _____

Date: _____

For those who cannot read the information:

Advisor Nurse's name: _____

Signature: _____

Date: _____

<u>የአማርኛ ቅጂ (Amharic Version)</u>

ለወላጆች/አሳዳጊዎች የስምምነት ቅጽ (ለህፃናት ≤17 አመት)

የተሳታፊ ኮድ ቁጥር፡- _____

"በማጅራት ገትር በሽታ በተጠረጠሩ ታማሚዎች ላይ የባክቴሪያ ገትር ገትር በሽታን ለመመርመር የ CSF እና Blood PCR ውጤታማነት ንፅፅር" በሚል ርዕስ ስለተደረገው የምርምር ጥናት ሙሉ በሙሉ መረጃ ደርሶኛል። ይህ ጥናት CSF እና ደም ከልጄ መሰብሰብ እንደሚፈልግ በዚህ እውቅና እሰጣለሁ። የዚህ ጥናት ዓላማዎች ተብራርተውልኛል, እና በዚህ መጠይቅ ውስጥ ያለው ሁሉም ነገር በጥብቅ ሚስጥራዊ እንደሆነም ተብራርቷል. በተጨማሪም በማንኛውም ጊዜ መረጃን የመከልከል፣ ለመተባበር ፈቃደኛ አለመሆን ወይም ልጁን ከዚህ ጥናት የማስወጣት መብት እንዳለኝ አውቃለሁ። በዚህ ጥናት ውስጥ ከልጄ የተወሰዱትን CSF እና የደም ናሙናዎችን እና የተረዳሁትን እውነታዎች ለተመራማሪው በመረጃ የተደገፈ ፈቃዴን እሰጣለሁ። ጥያቄዎችን እንድጠይቅ እድል ተሰጥቶኛል፣ እናም በጥናቱ ላይ አጥጋቢ ማብራሪያ አግኝቻለሁ። በተጨማሪም በመሳተፍ ውስጥ ካሉት ጥቅሞች አንዱ የልጄ ናሙናዎች ትንተና ውጤቶች በነጻ እንደሚሰጡ ተነግሮኛል.

የጥናት ተሳታፊ ኮድ፡ _____

ፊርማ፡ _____

ቀን፡- _____

መረጃውን ማንበብ ለማይችሉ፡-

የአማካሪ ነርስ ስም፡ _____

ፊርማ፡ _____

ቀን፡- _____

C. Assent Form for Child Participant (For children ≥ 12 years)

Participant Code Number: _____

I have been informed in a language that I understand the purpose of participating in the research study titled "Comparison of the Efficiency of CSF and serum PCR in Diagnosing Bacterial Meningitis in Patients Suspected of Meningitis." I understand that this study involves the collection of CSF and blood from me. I have been advised that specimen collection is not associated with any harm. I also understand that all information obtained during the course of the research will remain confidential. I understand that I am free to decide not to take part or to withdraw from the study at any time without penalty. I have been fully informed about the nature of this study, and to the best of my ability, hereby give my assent to participate by signing below.

Participant Name: _____

Signature: _____

Date: _____

Investigator Name: _____

Signature: _____

Date: _____

<u>የአማርኛ ቅጂ (Amharic Version)</u>

የድጋፍ ቅጽ ለህፃናት ተሳታፊ (ለህፃናት ≥ 12 ዓመት)

የተሳታፊ ኮድ ቁጥር፡- _____

"በማጅራት ገትር በሽታ በተጠረጠሩ ታማሚዎች ላይ የባክቴሪያ ገትር ገትር በሽታን ለመመርመር የ CSF እና Blood PCR ውጤታማነትን ማነፃፀር" በሚል ርዕስ በተደረገው የምርምር ጥናት ውስጥ የመሳተፍ አላማ እንደገባኝ በአንድ ቋንቋ ተነግሮኛል። ይህ ጥናት CSF እና ደም ከእኔ መሰብሰብን እንደሚያካትት ተረድቻለሁ። የናሙና ስብስብ ከምንም ጉዳት ጋር እንደማይገናኝ ተረድቻለሁ። በጥናቱ ወቅት የተገኘው መረጃ ሁሉ ሚስጥራዊ እንደሚሆንም ተረድቻለሁ። በማንኛውም ጊዜ ያለ ምንም ቅጣት በጥናቱ ላለመሳተፍ ወይም ላለመሳተፍ የመወሰን ነፃነት እንዳለኝ ተረድቻለሁ። ስለዚህ ጥናት ምንነት ሙሉ በሙሉ ተረድቻለሁ፣ እና በተቻለኝ መጠን፣ ከዚህ በታች በመፈረም ለመሳተፍ ፍቃድ ሰጥቻለሁ።

የተሳታፊ ስም፡ _____

ፊርማ፡ _____

ቀን፡- _____

የመርማሪ ስም፡ _____

ፊርማ፡ _____

ቀን፡- _____

Annex-III: Questionnaire

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY

English version

Questionnaire format for patients suspected of bacterial meningitis attending Tikur Anbessa specialized hospital and Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia.

1. Medical record number: _____
2. sample ID: _____
3. Phone number _____
4. Site data collection: _____
5. Date of Interview: ____/____/_____

Part I. Addressees

6. Region_____
7. Zone_____
8. Woreda_____
9. City_____
10. Kebele_____
11. Gote (Sub-kebele) _____
12. House No_____
13. Phone No_____

Part 2: Socio-demographic characteristics					
No	Question	Response			
14	Age	_____year In months (if <12 months) _____			
15	Sex	1. ☐ Male		2. ☐ Female	
16	Residence	☐ Residence: _____			
17	Family size				
18	Educational status	No formal education	Grade 1-8	9-12	College or University
	Part 3: Patient medical history				
19	Fever ≥38.5^{O}C	Yes ☐		No ☐	
20	Headache	Yes ☐		No ☐	
21	Nacke stiffness	Yes ☐		No ☐	
22	Lowered consciousness	Yes ☐		No ☐	
23	Seizures	Yes ☐		No ☐	
24	Vomiting or poor breastfeeding	Yes ☐		No ☐	
25	Bulging fontanel	Yes ☐		No ☐	
26	Antibiotics before admission	Yes☐		No ☐	
27	If Q24 "Yes" what type of antibiotics He/she took?				
28	Meningitis vaccination status	☐ vaccinated		☐ Not vaccinated	
	Part 4: Laboratory test				
29	Type of sample	☐CSF ☐Blood ☐Both			
30	CSF appearance	☐Clear ☐Hematic(blood stained)		☐Turbid ☐Xanthochromic (yellowish)	

31	Gram stain	☐Gram negative ☐Gram positive ☐Negative
32	Cell in CSF (≥5cell/mm3)	☐Yes ☐No
33	CSF Culture result	☐Positive ☐Negative
34	If Q33 "Positive" Type of organism	*N. meningitides*☐, *S. pneumoniae*☐, *H. influenza*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogence*☐, *K. pneumoniae*☐, others ☐
35	Blood culture result	☐Positive ☐Negative
36	If Q35 is "Positive" type of organism	*N. meningitides*☐, *S. pneumoniae*☐, *H. influenza*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogence*☐, *K. pneumoniae*☐,others ☐
37	CSF PCR result	☐Positive ☐Negative
38	If Q37 is a "positive" type of organism detected	*N. meningitidis*☐, *S. pneumoniae*☐, *H. influenzae*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogenes*☐, *K. pneumoniae*☐
39	Blood PCR results	☐Positive ☐Negative
40	If Q39 is a "positive" type of organism detected	*N. meningitidis*☐, *S. pneumoniae*☐, *H. influenzae*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogenes*☐, *K. pneumoniae*☐

<u>የአማርኛ ቅጂ (Amharic version)</u>

ይህ መጠይቁ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል ለሚማሩ በማህበረሰብ አቀፍ የባክቴሪያ ገትር ገትር በሽታ ለተጠረጠሩ ታማሚዎች እና የየካቲት 12 ሆስፒታል ሜዲካል ኮሌጅ፣ አዲስ አበባ፣ ኢትዮጵያ 2023 ነው።

1. የሕክምና መዝገብ ቁጥር፡- _____
2. የናሙና መታወቂያ፡_____
3. ስልክ ቁጥር _____
4. የጣቢያ መረጃ መሰብሰብ፡ _____
5. የቃለ መጠይቁ ቀን፡_____

ክፍል 1 አዲራሳ

6. ክልል_____
7. ዞን_____
8. ወረዳ_____
9. ከተማ_____
10. ቀበሌ_____
11. ጎጤ (ንዑስ ቀበሌ) _____
12. የቤት ቁጥር_____
13. ስልክ ቁጥር_____

ክፍል 2፡ ማህበራዊ ሁኔታ					
ተ.ቁ	ጥያቄ	መልስ			
14.	እድሜ	_____በአመት በወራት(ከ12 ወራት በታች ሲሆኑ) _____			
15.	ፆታ	☐ ወንድ		☐ ሴት	
16.	መኖርያ	ክልል/የመኖሪያ፡ _____			
17.	የበተሰብ ኩጥር				
18.	የትምህርት ደረጃ	ምንም መደበኛ ትምህርት የለም	1-8	9-12	ኮሌጅ ወይም ዩኒቨርሲቲ

	ኪፍል 3: የታካሚ የሕክምና ታሪክ		
19.	ሙቀት _____ ^{O}C		
20.	ራስ ምታት	☐ አዎ	አይ ☐
21.	የአንገት ህመም	☐ አዎ	አይ ☐
22.	ራስን መቆጣጠር አለመቻል	☐ አዎ	አይ ☐
23.	የሚጥል	☐ አዎ	አይ ☐
24.	የቆዳ መላላጥ	☐ አዎ	አይ ☐
25.	ማስታወክ ለህጣናት	☐ አዎ	አይ ☐
26.	ጡት መጥባት አለመቻል	☐ አዎ	አይ ☐
27.	ባለፈው ሁለት ሳምንት ውስጥ አንቲባዮቲኮችን ትጠቀማለህ?	☐ አዎ ከሆነ አንቲባዮቲክ ዓይነት	አይ ☐
28.	የማጅራት ገትር ክትባት	☐ ተከትቢያለሁ	☐ አልተከተብሁም
	ክፍል 4: የላብራቶሪ ምርመራ		
29.	የናሙና ዓይነት	CSF ☐,ደም ☐,ሁለቱም ☐	
30.	የሲኤስኤፍ ገጽታ	ግልጽ☐, ተርቢድ☐, ግልጽ☐, ሄማቲክ(በደም የተበከለ)☐	
31.	Gram stain	☐Gram negative ☐Gram positive ☐Negative	
32.	ሕዋስ በCSF (>=5cell/mm3)	☐አዎ	☐አይ
33.	የሲኤስኤፍ ባህል ውጤት	☐አዎንታዊ	☐አሉታዊ
34.	If Q33 "Positive" Type of organism	*N. meningitidis*☐, *S. pneumoniae*☐, *H. influenza*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogence*☐, *K. pneumoniae*☐,others ☐	
35.	Blood culture result	☐Positive	☐Negative

36.	If Q35 is "Positive" type of organism	*N. meningitidis*☐, *S. pneumoniae*☐, *H. influenza*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogence*☐, *K. pneumoniae*☐,others ☐
37.	CSF PCR result	☐Positive ☐Negative
38.	If Q37 is a "Positive" type of organism detected	*N. meningitidis*☐, *S. pneumoniae*☐, *H. influenza*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogence*☐, *K. pneumoniae*☐
39.	Blood PCR results	☐Positive ☐Negative
40.	If Q39 is a "Positive" type of organism detected	*N. meningitidis*☐, *S. pneumoniae*☐, *H. influenza*☐, *S. agalactiae*☐, *E. coli*☐, *S. aureus*☐, *L. monocytogence*☐, *K. pneumoniae*☐

Annex-IV: Sample Collection Procedures

I. CSF sample collection (Lumber puncture)

A. Material required

- CSF collection kit
- Spinal needles (22-25 gauge)
- Sterile plane tubes for CSF collection
- Disinfectant (70% alcohol and povidone-iodine)
- Sterile gauze pads
- Adhesive bandages

B. Procedure

1. Collect all necessary materials from the CSF collection kit and a puncture-resistant auto-cleavable container
2. Wear a surgical mask and sterile latex or nitrile gloves that are impermeable to liquids.
3. Label the collection tubes with the patient's name, date and time of specimen collection, and Unique Identification Number. Be sure this number matches the number on both the request and report forms
4. Ensure that the patient is kept motionless during the lumbar puncture procedure, either sitting up or lying on the side, with his or her back arched forward so that the head almost touches the knees to separate the lumbar vertebrae during the procedure
5. Disinfect the skin along a line drawn between the crests of the two ilia with 70% alcohol and povidone-iodine to clean the surface and remove debris and oils. Allow drying completely
6. Position the spinal needle between the 2 vertebral spines at the L3-L4 level and introduce it into the skin with the bevel of the needle facing up
7. Remove CSF (1 ml minimum, 3-4 ml if possible) and collect into sterile screw-cap tubes. If 3-4 ml CSF is available, use 3 separate tubes and place approximately 1ml into each tube.
8. Withdraw the needle and cover the insertion site with an adhesive bandage. Discard the needle in a puncture-resistant, autoclavable discard container
9. Remove the mask and gloves and discard them in an autoclavable container
10. Wash hands with antibacterial soap and water immediately after removing gloves
11. Transport the CSF to a microbiology laboratory within 10 minute for culture and analysis

II. Blood Sample Collection

A. Materials and reagents

- Tourniquets
- Alcohol swabs
- Gauze pads
- Blood collection tubes (serum separator tubes)
- Needles (20-22 gauge for adults; 23 gauge for pediatrics)
- Clean gloves
- Band-Aids
- Biohazard sharps container
- Patient identification labels

B. Procedure

1. Prepare the materials that are needed: tourniquet, alcohol swabs, gauze, blood collection tubes, and needles.
2. Identify the client to verify his identity.
3. Explain the procedure to the patients and obtain consent.
4. Choose the best vein to be used for the venipuncture.
5. Wrap a tourniquet around, proximal to the site of the venipuncture, constricting the flow of blood to make the vein more prominent.
6. Clean the site of venipuncture using the alcohol swab and allow it to air-dry.
7. Grasp the patient's arm and secure the vein with traction on the skin below the site of venipuncture.
8. The needle is inserted into the vein at an angle of 15-30 degrees, bevel up, and gently pull back on the plunger to aspirate blood into the tube.
9. Obtain at least 1 mL of blood in a serum separator tube
10. Remove the tourniquet and ask the patient to gently press on the venipuncture site with gauze.
11. Discard used needles and other contaminated items into appropriate sharps containers.
12. Label the blood collection tube with identification, date, and time of the blood collection.
13. Take the blood sample to the laboratory for processing.

Annex-V: Laboratory Procedures

I. Gram staining technique

1. Label the slides clearly with the date and the patient's name and number.
2. Making smears by spreading evenly and covering an area on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or alcohols (methanol).
5. Cover the fixed smear with crystal violet stain for 1 minute.
6. Rapidly wash off the stain with clean water.
7. Cover the smear with lugol's iodine for 1 minute.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (5 seconds) with 3% acetone alcohol. Wash immediately with clean water.
10. Cover the smear with safranin for 1 minute.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials, and then with the oil immersion objective to look for bacteria and cells.

II. Culture and identification

❖ 0.5% STGG (Skim milk-Tryptone-Glucose-Glycerine) Media preparation

STGG media is commonly used for the transport and storage of bacteria.

❖ Materials Required

a. Ingredients

- 3.0 g Tryptone Soya Broth (Oxoid CM 0129)
- 0.5 g Glucose (Sigma)
- 2.0 g Skim Milk Powder (Oxoid LP0031)
- 10.0 mL Glycerol
- Distilled Water (up to 100 mL)

b. Equipment

- 500 mL Duran bottle or similar container
- Magnetic stirrer
- Autoclave
- Sterile cryotubes (1.8 mL or similar)
- Vortex mixer

c. Procedure

1. Dissolve Ingredients
2. In a clean 500 mL Duran bottle, add approximately 90 mL of distilled water.
3. Using a magnetic stirrer, gradually add:

- 3 g of Tryptone Soya Broth
- 0.5 g of Glucose
- 2.0 g of Skim Milk Powder
- 10.0 mL of Glycerol

4. Stir the mixture until all powders are completely dissolved and the solution is clear.
5. Adjust Volume: After the powders have dissolved, add distilled water to bring the total volume up to 100 mL.
6. Sterilization: Autoclave the mixture at 121°C for 15 minutes at a pressure of 15 psi.

7. Cooling: After autoclaving, carefully remove the bottle and allow it to cool in a water bath or at room temperature.
8. Aliquoting: Once cooled, dispense the STGG medium into sterile cryotubes in 1 mL aliquots.
9. Storage: Store the prepared STGG media at −20°C or lower for long-term storage, or at 4°C for short-term use (up to six months).
10. Quality Control: Perform sterility testing by plating a small volume from each batch onto an appropriate agar medium and incubating it to check for contamination before use

A. Media preparation

- Weighing and dissolving of culture media
- Sterilization
- In the case of blood agar, add heat-sensitive chemicals or materials, such as blood and some antibiotic additives.
- PH testing of culture media
- Dispensing of culture media
- Quality control test of culture media
- Labeling and Storage of culture media

B. Inoculation

The most common culture media used for the inoculation of CSF samples includes; MacConkey, chocolate, and Blood agar. After inoculation of the sample in the appropriate media

1. Before beginning the workday and after it is over, clean the workbench.
2. When handling dangerous diseases, use a safety cabinet and put on gloves, a face mask, and protective clothes.
3. Before and after usage, flame sterilizes metal forceps, wire loops, and straight wires.

4. After removing and reinstalling caps or plugs, flame the necks of tubes, culture bottles, and specimen bottles.
5. Avoid letting the caps or tops of bottles and tubes come into contact with the non-sterile surface while inoculating. Holding the top cap between your fingers will help you avoid this.

C. Incubation

Incubation will be carried out after inoculation because we need to establish an environment that is conducive to the growth of pathogenic organisms after the sample has been placed on the proper media. Since human-like environments are the most common and suitable for pathogenic bacteria, we must incubate the bacteria overnight at 37 oC in an aerobic or microaerophilic environment or in an atmosphere with 5% CO2 enriched (Fastidiouse growth) based on the expected bacterial species.

D. Biochemical testing

I. Gram-positive bacteria

Catalase test: The purpose of this test is to distinguish between bacteria that generate the catalase enzyme, like staphylococci (+ve), and those that do not, like streptococci (-ve). The enzyme catalase facilitates the conversion of hydrogen peroxide into oxygen and water.

A. Procedure

- Fill a slide with two to three milliliters of hydrogen peroxide solution. Colonies of the test organism are selected using a sterile wooden stick. Add hydrogen peroxide solution and stir until bubbling occurs.
- Interpretation: Active bubbling--positive test, No release of bubbles-negative test

B. **Coagulase test:** This test is used to differentiate S. aureus which produces the enzyme coagulase from other Staphylococcus spps. Coagulase causes the plasma to clot by converting fibrinogen to fibrin.

Procedure

1. Place a drop of physiological saline on two separate slides.
2. Emulsify the test organism in each drop to make a thick suspension.
3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds.
4. Interpretation: Clumping within 10 seconds ----------S.aureus

No clumping within 10 seconds ----------other staphylococcusspecies

C. Mannitol salt agar test

Principle

Inoculate isolated colonies onto media for differentiation, or inoculate specimens on medium for primary isolation. Incubate for 24 hours at 37ºC. Examine the morphology of the colony.

Positive outcome: yellow colony, possibly surrounded by a yellow halo.

Negative: neither the growth of colorless or pink colonies (CONs) nor the growth of bacteria.

II. Gram-negative bacteria

A. **Indole test:** To detect the ability of an organism to degrade the amino acid tryptophan and produce indole. It is important in the identification of Enterobacteriaceae.

Procedure

The bacteria to be examined are cultured at 37 ^{o}C for the entire night after being infected with peptone water, which includes the amino acid tryptophan. Kovac's reagent is added in little drops after incubation. Isoamyl alcohol, concentrated HCl, and para-dimethyl aminobenzaldehyde make up Kovac's reagent. The synthesis of indole in anaerobes and non-fermenters can be detected more sensitively using Ehrlich's reagent. The development of a pink or red ring at the top is interpreted as a sign of positive.

B. Triple sugar iron agar

Procedure

- Stabbing the butt and dragging the stick across the slope's surface allowed for the inoculation of the triple sugar iron agar slant, which was then
- incubated for 18 to 24 hours at 35 to 37 oC.
- Checked for the formation of black precipitate.

C. **Urease test**

Methods

- Obtain two urease broths from the refrigerator.
- Inoculate one broth using an aseptic technique. Leave the other broth uninoculated (this will be used as a control).
- Incubate at an appropriate temperature for 24 to 48 hours (do not exceed 48 hours for this test). Observe the color to indicate the result.

D. Oxidase test

The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme.

Procedure

- Dip a piece of filter paper into the mixture of the reagents.
- Utilizing a disposable loop or stick, scrape some new growth from the culture plate and rub it onto the filter paper. Alternatively, use the paper's edge to touch a colony.
- Check for blue within ten seconds.

E. Citrate utilization test

Procedure: Bacterial colonies are picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37°C. If the organism can utilize citrate, the medium changes its color from green to blue.

F. Motility testing

To check for motility, choose a well-isolated colony and stab the motility medium within 1 cm of the tube's bottom with a sterile straight wire. When removing the straight wire from the medium, make sure it stays in the same line as it entered. For 24 hours or until growth is noticeable, incubate at 35°C. A murky area that extends away from the line of inoculation indicates a positive motility test. Growth at the injection line but not beyond indicates a negative test.

Methods

- Use motility agar tube.
- An inoculating pick should be used. Make the choice as straight as you can.
- Using your unidentified stock culture, do a stab inoculation about two thirds of the way into the agar. Make the stab as straight as you can, both in and out. Since you will be assessing the quantity of development away from the stab, straightness is crucial. An untidy stab will be hard to assess.
- For 24 to 48 hours (up to 72 hours), incubate at the proper temperature.
- old your culture up to a light source to observe it.

III. Molecular Method

A. Samples and reagent preparation

a. Material and reagents

- CSF and Blood samples
- 96-well plate containing magnetic beads and extraction reagent
- 96-well plates
- Pipettes and tips
- Personal protective equipment (PPE)

b. Procedure

1. Thaw CSF and blood samples at room temperature.
2. Shortly Centrifuge at 6000 rev/min for a few seconds
3. Bring all centrifuged samples to room temperature.
4. Equilibrate a 96-well plate containing magnetic beads and extraction reagent to room temperature.
5. Centrifuge the equilibrated plate according to the manufacturer's instructions.

B. DNA extraction

a. Automation purification

1. Reagent Preparation

Add 500µL Lysis Buffer to columns 1 and 7 of the 2.2 mL. 96-deep-well plate, 500µL Wash Buffer I to the columns 2 and 8, 500µL Wash Buffer II to the columns 3 and 9, 70µL Elution Buffer to the columns 5 and 11, 175µL pure water, and 25µL MagaBio Reagent to the columns 6 and 12 (the magnetic beads should be mixed thoroughly before use). Put the 96 well-prepacked reagents at room temperature. Shake the 96-well plate upside down three times, and tear off the plastic bag. Centrifuge the pre-packed reagent for a few seconds (or swing by hand a few times) to avoid reagent adhering to the wall of the tubes. Tear off the aluminum foil film of the 96-well plate and identify the direction of the plate (magnetic beads in columns #6 and #12).Add 300µL sample to the 96 well plate columns #1 and #7.

2. Place a 96-deep well plate on the instrument, and install the 8-strip tips on the instrument.

3. Run the program according to the following procedures:

Step	Well	Name	Waiting time (min:ss)	Mixing time (min:ss)	Magnetic time (min:ss)	Adsorption	Speed	Volume (microliter)
1	6	Beads	0:0	0:0	0:15		M	200
2	1	Binding	0:0	3:00	0:35	√	F	700
3	2	Wash 1	0:0	0:30	0:20	√	F	500
4	3	Wash 2	0:0	0:30	0:20	√	F	500
5	5	Elution	1:0	2:00	0:25	√	F	70
6	6	Discard	0:0	0:0	0:0		M	200

Temperature settings: elution temperature: 80 °C. Heating during elution begins at Step 5.

4. After the automatic purification is over, elution buffer in columns 5 and 11 is transferred to a clean nuclease-free 0.5 mL centrifuge tube; store at -20° if not using.

C. Nano drop procedures

a. Materials and reagents

- Nanodrop spectrophotometer
- Nuclease-free water
- Extracted nucleic acid sample

b. Procedure

1. First, make sure the volume of the nucleic acid extracted sample falls within the range recommended by the Nano Drop device.
2. Blank the Nano Drop device using nuclease-free water following the manufacturer's directions.
3. Add the 1 µL of the extracted sample to the Nano Drop measuring surface.
4. Run the device and obtain the measure at both 260/230 and 260/280 nm
5. Calculate the ratio:

- A ~1.8 ratio indicates a relatively pure DNA sample.
- A ~2.0 ratio indicates that it is pure RNA.

D. Master Mix preparation

a. Material and reagents

- Materials Required
- PCR Buffer (10x): 2.5 µL
- $MgCl_2$: 1.2 µL
- dNTPs: 1.0 µL
- Primers: 1.0 µL of each primer (2 primers total = 2.0 µL)
- Molecular Grade Water: 13.1 µL
- Platinum Taq Polymerase: 0.2 µL
- Template DNA: 5.0 µL (added later)
- PCR Tubes
- Pipettes and Tips

b. Procedure

1. Calculate the total volume for the master mix (without template DNA) is 20 µL.
2. Combine all reagents in single PCR tube according volume requered (2.5 µL of 10x PCR Buffer, 1.2 µL of $MgCl_2$, 1.0 µL of dNTPs, 2.0 µL of primers (1.0 µL of each primer), 13.1 µL of molecular grade water, 0.2 µL of Platinum Taq Polymerase) for one PCR reactions.
3. Gently pipette vortex to mix the components without introducing bubbles.
4. Briefly centrifuge the tube to ensure all components are at the bottom.
5. To PCR tube containing the master mix, add 5.0 µL of the DNA template.
6. Mix gently by vortex to ensure the template is well incorporated into the master mix.
7. Ensure the PCR tube is properly sealed to prevent evaporation during the PCR process.

E. PCR Procedure

a. Protocol

1. Set the Program of thermal cycler (T100 LASEC BIO-RAD) with the following cycling conditions

- Initial denaturation: 94°C for 3 minutes
- Cycling (35 cycles):
- Denaturation: 94°C for 10 seconds
- Annealing: 60°C for 30 seconds
- Extension: 72°C for 5 minutes
- Final extension: 72°C for 10 seconds
- Lid temperature: 105°C

2. Place the PCR tubes in the thermal cycler and start the program.
3. Run the PCR program (approximately 1 hour and 20 minutes).
4. Store the amplified products at 2-8°C until gel electrophoresis is performed.

F. Reagent preparation procedures

I. 50x TAE Buffer Preparation

a. Materials and reagents

- Tris Base: 484 g
- Glacial Acetic Acid: 114.2 mL
- EDTA (0.5 M): 200 mL
- Deionized Water: Approximately 2L
- pH Meter or pH Indicator Strips: For pH adjustment
- Beaker or Mixing Container
- Magnetic Stirrer: For mixing
- Volumetric Flask or Graduated Cylinder

b. Procedure

1. Weigh 484 g. Tris base and dissolve it in distilled water.
2. Add 114.2 ml of glacial acetic acid (100% stock) and 200 ml of 0.5 M EDTA (pH 8).
3. Adjust the final volume to 2 L with distilled water.
4. Stir the solution overnight for complete dissolution.
5. Measure the pH on the second day using a universal indicator.
6. Label the container and store the 50x TAE buffer.

II. 1x TAE buffer from 50x stock

1. Dilute 20 ml of 50x TAE stock solution with 980 ml of distilled water.
2. Mix the solution thoroughly.
3. Label the container and use the 1x TAE buffer for the experiment.

III. 100-bp DNA Ladder Preparation

a. Materials and reagents

- Thermo Scientific GeneRuler 100 bp DNA Ladder
- Nuclease-free water
- 6X DNA loading buffer (containing blue tracking dye)
- Microcentrifuge tubes
- Pipettes and pipette tips
- Vortex mixer
- 20°C freezer for storage

b. Procedure

1. ALIQUOT 1µL of the Thermo Scientific GeneRuler 100 bp DNA Ladder.
2. 4µL of nuclease-free water is added to the 1 µL DNA ladder. The ease of adding nuclease-free water makes the DNA ladder reduce to a working concentration best suited for the gel electrophoresis process.
3. Add 1µL of 6X DNA loading buffer with blue tracking dye. This loading buffer is used for viewing the DNA during electrophoresis and for proper loading into the gel wells.
4. Vigorously mix the solution using a vortex for a short period of time. The solution is supposed to be uniform now; there should not be any extra bubbles present in the solution.
5. The DNA ladder tube is marked with an identifying label. The date of preparation as well as the contents are indicated.
6. The mixture of the DNA ladder solution was stored at -20 °C. The tube should be tightly sealed to protect against contamination and degradation.
7. When ready to use, thaw the DNA ladder solution on ice. Mix gently before loading onto the agarose gel for electrophoresis.

IV. 2.5% Agarose Gel Preparation

a. Materials and reagents

- Agarose powder
- Analytical balance
- TAE (Tris-Acetate-EDTA) buffer
- Heating device (hot plate)
- Flask
- Graduated cylinder
- Ethidium bromide solution
- Casting tray with well-forming comb
- Nitrile Gloves

b. Procedure

1. Weigh 2.5 grams of agarose powder with an analytical balance.
2. Add measured agarose powder to 100 milliliters of TAE buffer in a flask.
3. Gently mix the mixture to make sure the agarose powder is uniformly distributed in the buffer.
4. Use a hot plate to gently heat the agarose-buffer mixture until the agarose powder is fully dissolved.
5. Monitor closely the content of the solution in order to prevent overboiling.
6. Keep heating until the solution gets clear and all the agarose particles have dissolved.
7. Let the hot agarose solution cool down.
8. While the agarose solution is still hot, add about 2 microliters of ethidium bromide solution to it. Ethidium bromide is a fluorescent dye that intercalates with DNA, thus permitting DNA bands to be visualized under UV light. Besides, gloves must be worn and working must be done in a hood when handling ethidium bromide due to the mutagenic properties of it.
9. Carefully pour the warm agarose solution into a casting tray.
10. Place the comb in the tray to form wells for loading samples.
11. Leave the gel to solidify for 20 min at RT.
12. It will solidify and should be firm but opaque when chilled correctly.
13. Gently remove the comb from the solidified gel, taking care not to tear the wells.

Safety Note: Ethidium bromide is a mutagen, so wear gloves and handle it with care. Dispose of waste containing ethidium bromide according to your institution's guidelines. Wear gloves and safety goggles throughout the procedure to protect yourself from potential hazards. The concentration of agarose used may vary depending on the size of DNA fragments to be separated. Always use nuclease-free materials and work in a clean environment to prevent contamination.

G. Gel Electrophoresis and Visualization

a. Materials and reagent

- PCR product
- Loading dye
- 100-bp DNA ladder
- Agarose gel
- UV machine (Vilber)
- Gel electrophoresis equipment (gel tray, comb, power supply)
- Pipettes and tips
- Micro centrifuge tube

b. Procedure

1. To mix 25 µL of the PCR product in a micro centrifuge tube and then add 5 µL of loading dye. Pipette it gently up and down to mix evenly
2. Centrifuge briefly for a few seconds to collect this mixture at the bottom
3. Load 10 µl of your PCR product carefully into the wells of submerged agarose gel prepared for each well, taking care not to introduce air bubbles
4. In another well load 5 µL of 100 bp DNA ladder to compare the size
5. Connect the power supply after closing the gel electrophoresis chamber
6. Set the voltage at 120 V, 400 amp, and run it for an appropriate time of around 45 minutes
7. For observing loading dye migration, monitor the progress of electrophoresis.
8. Remove the gel from the chamber when the electrophoresis is complete.
9. Observe DNA bands under a Vilber machine.
10. Compare the size of the PCR product with those of the DNA ladder
11. Photograph the gel, recording the outcome.

Annex-VI: Declaration Sheet

I, the undersigned, a candidate for an MSc in Medical Microbiology, hereby declare that this research work is my original work in partial fulfillment of the requirements for the degree of Master Science in Medical Microbiology. Where the work of others has been used, it has been fully acknowledged and referenced according to requirements.

Name of the principal investigator

1. Lema Ayele Signature _____ Date_____

Approved by advisors;

1. Daniel Asrat (MD, M. Sc, Ph.D., Professor) Signature _____ Date_____
2. Aminu Seman (M.Sc., Ph.D. Fellow) Signature _____ Date_____