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BAHIR DAR UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

BACTERIAL ISOLATES, ANTIMICROBIAL SUSCEPTIBILITY PROFILES, AND FACTORS ASSOCIATED WITH MENINGITIS AMONG CHILDREN IN BAHIR DAR, NORTHWEST ETHIOPIA

By: TADESE SISAY (BSc)

A thesis submitted to the Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Bahir Dar University for partial fulfillment of the requirements for the Master of Science degree in Medical Microbiology.

Bahir Dar, Ethiopia

February 2025

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Bacterial Isolates, Antimicrobial Susceptibility Profile, and Factors Associated with Meningitis among Children in Bahir Dar, Northwest Ethiopia

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Declaration

I certify that due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award. The content of the THESIS is the result of work that has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and ethics procedures and guidelines were followed.

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ACRONYMS

BM	Bacterial Meningitis
CABM	Community-Acquired Bacterial Meningitis
CLSI	Clinical Laboratory Standards Institute
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
FHCSH	Felege Hiwot Compressive Specialized Hospital
GBS	Group B S*treptococcus*
Hib	*Haemophilus Influenzae* Type b
HICs	High-Income Countries
HIV	Human Immunodeficiency Virus
LMICs	Low- and Middle-Income Countries
MDR	Multidrug-Resistant
MIC	Minimum Inhibitory Concentration
NBM	Neonatal Bacterial Meningitis
NICU	Neonatal Intensive Care Unit
PBM	Pediatric Bacterial Meningitis
PCR	Polymerase Chain Reaction
SOPs	Standard Operational Definition
TGTH	Tibebe Gion Teaching Hospital
UTI	Urinary Tract Infections
WHO	World Health Organization

ABSTRACT

Background: Meningitis is an inflammation of the meninges. Over two-thirds of all cases of bacterial meningitis occur in children. The case fatality rate of meningitis is as high as 70%, and among survivors, 20% will be left with permanent sequelae. There are limited data on local pathogens and factors contributing to meningitis. The increasing rate of antibacterial resistance is now further complicating this hard to diagnose acute disease and there were knowledge gap. Household overcrowding, absence of immunization, and malnutrition are some risk factors that predispose the individual to meningitis. Few studies on bacterial meningitis at Bahir Dar, and data from pediatric age groups is required to carry out preventive measures.

Objective: To assess bacterial isolates, antimicrobial susceptibility profile, and factors associated with meningitis in children suspected for meningitis in Bahir Dar, Northwest Ethiopia.

Methods: A hospital-based cross-sectional study was conducted at Felege Hiwot Compressive Specialized Hospital (FHCSH) and Tibebe Ghion Specialized Hospital (TGSH) from June 03 to September 05/2024 among 189 children suspected of meningitis. A convenient sampling technique was used to select study participants. Cerebrospinal fluid (CSF) was taken by lumbar puncture and transported to a microbiology laboratory. Blood, chocolate, and MacConkey agars were used for culturing, and the disc diffusion method was used to determine the antimicrobial susceptibility profiles of the bacterial isolates. Data were entered and analyzed using SPSS version 26 software. Fisher's Exact Test was used and P-value <0.05 considered statistically significant.

Results: From 189 CSF samples cultured, bacterial pathogens were isolated from 10 patients with an isolation rate of 5.3% (95% CI=2.6-8.5). The predominant bacterial isolates were S. aureus (30%) and E. coli (30%), followed by K. pneumoniae (20%). Multidrug resistance was observed in 40% of the isolates. A high resistance rate was observed for penicillin; imipenem, gentamicin, and ceftriaxone showed enhanced antibacterial activity. Malnutrition (P=0.005), and tonsillectomy (P=0.003) were associated with childhood bacterial meningitis.

Conclusions: The predominant bacterial isolates were *S. aureus* and *E. coli*, followed by *K. pneumoniae*. The frequency of multiple drug resistance was high. Malnutrition and tonsillectomy were associated with bacterial meningitis.

Keywords: Meningitis, bacteria, antimicrobial susceptibility, children, Ethiopia

1. INTRODUCTION

1.1. Background

Meningitis is an inflammation of the meninges around the brain and spinal cord. Pediatric bacterial meningitis (PBM) is a contagious disease that significantly increases morbidity and mortality from neurological manifestations around the world. Meningitis is divided into two categories: bacterial and aseptic meningitis. A bacterial infection causes acute meningeal inflammation, which is known as bacterial or pyogenic meningitis. Aseptic meningitis is also divided into two categories: nonbacterial meningeal infections (viral or fungal meningitis) and noninfectious meningeal inflammation (Ahmed, 2012, Aksamit Jr and Berkowitz, 2021).

Meningitis affects all age groups, from the newborn to the elderly. Approximately 1 in 6 cases of bacterial meningitis (BM) result in death (WHO, 2023). Children (aged 0-14 years) include infants, neonates, and older children.Children account for more than two-thirds of all instances of bacterial meningitis. Bacterial meningitis has a 70% case fatality rate and leaves one in five survivors permanently disabled, possibly with cognitive impairment, hearing loss, or loss of limb function (Dartey et al., 2020, Amare et al., 2018).

Factors that predispose children to meningitis can be grouped into four categories including age, demographic and socioeconomic factors, exposure to pathogens, and immunosuppression. Patients at the extremes of age, especially pediatric patients (young children) have an increased susceptibility to meningitis. Household overcrowding, HIV infection, absence of immunization, malnutrition, indoor air pollution, and sickle cell disease are some risk factors that predispose the individual to meningitis. The death rate of bacterial meningitis is high, with the number of siblings greater than three (Lundbo and Benfield, 2017, Khan et al., 2021).

The patient's age has a major impact on the etiology of bacterial meningitis. The most frequent etiologic agents in preterm neonates are *Escherichia coli* (*E. coli*) and Group B streptococcus (GBS). The common bacterial isolates in term neonates and infants < three months are GBS, *E. coli, Streptococcus pneumoniae* (*S. pneumoniae*), and *Listeria monocytogenes* (*L. monocytogenes*). The most common causes of bacterial meningitis in children aged ≥ three months to ten years are *S. pneumoniae, Neisseria meningitidis* (*N. meningitidis*), and *Haemophilus influenzae* (*H. influenzae*) (Zainel et al., 2021). GBS is the most frequent cause of bacterial

meningitis in wealthy nations. However, developing countries tend to have a higher prevalence of gram-negative bacteria (Ahmed, 2012, Alamarat and Hasbun, 2020).

Meningitis is more common in the early stages of life. The illness has unique features in newborns. The absence of symptoms allows for confusion between the signs and symptoms of septicemia and other non-infectious conditions such as hypoglycemia, respiratory distress syndrome, and birth asphyxia. The diagnosis of meningitis is challenging in this case. Consequently, a high degree of suspicion is required (Biset et al., 2021).

Antimicrobial resistance (AMR) is one of the biggest problems for global public health. In 2019, bacterial AMR caused 4.95 million fatalities worldwide and was directly responsible for 1.27 million deaths (Ranjbar and Alam, 2023). The antimicrobial drug resistance shown in bacterial meningitis poses a severe threat to the population and creates challenges for healthcare providers. For a very long period, BM was treated empirically with penicillin, cephalosporin, fluoroquinolones, or aminoglycosides. However, these antibiotics are now becoming infective against BM isolates due to drug resistance (Kim, 2015, Ali et al., 2021).

As a medical emergency, bacterial meningitis requires immediate antibiotic therapy. In Ethiopia, there is increased bacterial drug resistance due to the absence of good laboratory facilities to perform culture and antimicrobial susceptibility tests. There is limited data on local pathogens and factors contributing to meningitis. Periodic surveillance of antimicrobial resistance is mandatory to provide effective empirical therapies, to be alert to emerging pathogens, to develop rational prescribing practices, and to make policy decisions (Peng et al., 2021, Runde et al., 2017). Therefore, this study was aimed at determining the bacterial isolates, antimicrobial susceptibility profile, and associated factors among children suspected of meningitis in Bahir Dar, Northwest Ethiopia.

1.2. Statement of the problem

Bacterial meningitis is a serious, often lethal, and disabling infection. This common infection is sometimes misdiagnosed and treated with sepsis to some extent (Yoshizawa et al., 2023). Young newborns and children are especially susceptible to bacterial meningitis because of the immaturity of their immune systems, and a dismal prognosis may result (Ahmed, 2012).

In only 2019 alone, there were more than 236,000 deaths and more than 2.5 million new cases of bacterial meningitis worldwide. Among the leading causes of disability-adjusted life years in children under the age of ten, meningitis came in sixth place. The global estimates of mortality from pneumococcal meningitis and HIV were 45% and 23%, respectively. For meningitis, there was a 20% chance of experiencing sequelae. Compared to Europe, the danger was nearly three times higher in Asia and Africa (Borko et al., 2023, Portnoy et al., 2015).

The meningitis belt, which stretches from west to east Africa in the semiarid sub-Saharan region between 10 and 12 degrees north latitude, is where meningitis is most common in Africa (Mazamay et al., 2021). The term "meningitis belt" indicates a region of sub-Saharan Africa that includes 15 countries with a combined population of over 260 million people. It extends from Ethiopia in the east to the Gambia in the west. It is well known that endemic diseases are highly prevalent in this area and that *N. meningitidis* periodically causes epidemics (Awulachew et al., 2020).

Ethiopia is among the top ten countries in the world with the highest risk of acute bacterial meningitis mortality, accounting for 6–8% of pediatric hospitalizations with case fatality rates ranging from 3.7 to 33.6%. There is still a significant risk to the community's health from the disease, particularly for young people. Approximately 70% of mortality from meningitis/encephalitis occurred in children younger than five years old across 12 nations, including Ethiopia. It needs a comprehensive study to implement targeted intervention programs and perform accurate diagnosis and treatment to reduce rates and ensure healthy and happy children (Awulachew et al., 2020, Borko et al., 2023).

Ethiopia and other resource-constrained nations are susceptible to a variety of factors that increase their risk of developing deadly bacterial meningitis. These comprise: (a) giving antibiotics in primary or private settings without a clear diagnosis, even though many CSF samples do not reveal

the causative agent; (b) receiving late or inadequate CSF culture and Gram-staining results, even though they are essential for an accurate diagnosis and directing treatment. However, because of a lack of well-run laboratories, insufficient immunization distribution and availability, the unavailability of antimicrobial drugs, inadequate surveillance, and antibiotic resistance, bacterial meningitis remains a major issue in Ethiopia (Gudina et al., 2016, Assegu Fenta et al., 2020). There is a need for more research in this area due to the constantly shifting epidemiology, the introduction of novel strains, the possibility of developing better vaccinations, and advancements in laboratory procedures. The age of the patients, their location, the climate, and the season all affect the occurrence of bacterial meningitis. These conditions lead to varied levels of antibiotic resistance and diverse bacterial meningitis organisms (Assegu Fenta et al., 2020, Hibstu et al., 2022).
Although despite bacterial meningitis mostly affects infants and children, there aren't many studies on the condition in Ethiopia. Many these were retrospective studies, which share similar limitations such as recall bias and incomplete data from patient medical records that slowed down the analysis of crucial variables. There was a lack of understanding about the factors associated with an increased occurrence of bacterial meningitis in children. To provide a more thorough understanding of the factors affecting the severity of bacterial meningitis, a study that can correlate the disease's severity and clinical symptoms with test data is necessary (Assegu Fenta et al., 2020, Reta and Zeleke, 2016).

The prevalence of bacterial meningitis in children in Ethiopia is not well-documented, and there is a growing concern about the emergence of multidrug-resistant (MDR) strains of pathogens, complicating treatment protocols and leading to increased morbidity, mortality, and healthcare costs. As data from the pediatric age group required to carry out preventive measures for bacterial meningitis, further research and periodic surveillance of bacterial infections and antibiotic susceptibility profiles from CSF are necessary (Tesera et al., 2020). Therefore, the purpose of this study was to isolate bacteria from cerebrospinal fluid, their antimicrobial susceptibility profile, and associated factors in children suspected of meningitis patients in Bahir Dar, North West Ethiopia.

1.3. Significance of the study

Assessing the prevalence of meningitis and the drug susceptibility profile of isolates is critical to monitoring routine diagnosis and treatment. To explain more, rapid diagnosis, identification, routine monitoring of antibiotic resistance, and ongoing surveillance of meningitis among children are critical steps in mitigating potential epidemic threats.

The result of this study provides real-time epidemiological information on the bacterial profile, distribution, and determinants of meningitis in children in the study area. It provides updated information on the susceptibility profile of the isolates. A comprehensive catalogue of bacterial meningitis disaggregated by age could guide empirical diagnosis and treatment threshold at times of urgency and in peripheral health facilities where there is no microbiology laboratory facility and/or physician who can perform lumbar puncture for CSF. Collectively, this study can be considered an important addition to existing literature.

2. LITERATURE REVIEW

Meningitis develops after the pathogen multiplies in the subarachnoid space and enters the meninges either directly because of sinusitis or mastoiditis or via the hematogenous pathway (bacteremia) (Zainel et al., 2021). The prevalence and antimicrobial susceptibility of bacterial meningitis vary by region, country, pathogen, and age of the patient (Barichello et al., 2023). The majority of the studies on BM were from developed countries, while the burden remains high in developing ones. Most of these investigations are retrospective and focus on etiologic agent and clinical characteristics; only a few, on the other hand, investigate the antimicrobial profile and associated factors (Schiess et al., 2021, WHO, 2023)

2.1. Prevalence of bacterial meningitis in children

Based on a five-year (January 2011 to January 2016) retrospective cross-sectional study at two hospitals in Iran recruiting 89 children, the prevalence of meningitis was 21 (23.6%). The majority of patients (57.1%) with positive cultures were younger than five years old. The most common pathogens were *N. meningitidis* (14.3%), *Haemophilus* species (14.3%), and *S. pneumoniae* (47.6%). Similarly, another multicenter retrospective study was conducted in China. This study included 837 children with ages>3 months. Hence, *S. pneumoniae* (46.9%), GBS (10.0%), and *E.coli* (7.9%) were the major pathogens isolated (Hadi and Bagheri, 2019, Li et al., 2018).

A descriptive study conducted in Nepal on 252 suspected cases of meningitis. Showed that gram staining and culture techniques were able to identify 7.2% of cases of bacterial meningitis. The majority of cases (72.2%) were caused by gram-negative organisms (Shrestha et al., 2015). Another retrospective descriptive study was conducted in Indonesia at Sanglah Hospital Pediatric Ward in March 2019. Gram-positive bacteria made up 24 (46.1%) and gram-negative bacteria made up 28 (53.8%) of the 52 samples that were collected. The study did not explain the number of negative cultures in children with bacterial meningitis. So a prospective study is needed to clearly describe the characteristics of bacterial culture in bacterial meningitis children (Kristianti et al., 2020).

From 2010 to 2016, routine surveillance was conducted on children who were admitted to the Sylvanus Olympio University Hospital Center (CHU SO) in Lomé, along with a suspected case of

bacterial meningitis. According to this study, the majority of infections (67.3%) were caused by *S. pneumoniae*, followed by *H. influenzae* (23.5%) and *N. meningitidis* (9.2%; 9/98) (Tsolenyanu et al., 2019).

A retrospective study was conducted on 542 children at the National Hospital Abuja (NHA). Based on this study, 14 (50%) of the culture-positive samples had turbid appearances, although 5.2% of the samples were positive for culture. Of the culture-positives, 12 (43%) were obtained by direct smear and culture, and 16 (57%) by culture alone. The four most often isolated pathogens were *Staphylococcus aureus (S. aureus)* (32.2%), *E.coli* (14.3%), *Klebsiella pneumoniae* (*K. pneumoniae*) (21.5%), and *S. pneumoniae* (17.6%) (Iregbu and Abdullahi, 2015).

In Ethiopia, a cross-sectional study of 8,274 suspected acute bacterial meningitis was reported from three sentinel surveillance hospitals from 2002–2015. The study shows that 8.8 % of samples tested positive for bacteria that cause meningitis. More than 90% of cases were due to vaccine-preventable bacteria, with *S. pneumonia* accounting for 46.9% of cases, *H. influenzae* accounting for 31.7% of cases, and *N. meningitides* accounting for 21.4% of cases (Asefa et al., 2020).

A hospital-based cross-sectional study design was also conducted in Dilla, Ethiopia. Of the CSF samples collected, 203 (70.7%) had a clear appearance, 11 (3.8%) had xanthochromic characteristics, and 73 (25.4%) had purulent characteristics with apparent turbidity. The overall prevalence of bacterial meningitis was 13.2%. From this, 7.3% were detected by gram stains. The most frequent isolates from culture-positive cases were *N. miningitidis* (16%), *E.coli* (16%), *S. aureus* (18.4%), and *S. pneumoniae* (34.2%) (Awulachew et al., 2020).

A retrospective study carried out in Gondar demonstrates that 1.73% of samples tested positive for bacteria that cause meningitis (Biset et al., 2021). Another retrospective study from Gondar in the post-pneumococcal vaccine era shows 1.6% of community-acquired bacterial meningitis (CABM) was identified. *S. pneumoniae* was the most frequent pathogen identified (70%) in children. The most common age group were infants 2–12 months old (40%) (Amare et al., 2018).

2.2. Antimicrobial susceptibility of bacteria causing meningitis in children

A retrospective descriptive study was carried out in March 2019 at the Pediatric Ward of Sanglah Hospital in Indonesia. The study found that the antibiotics vancomycin, linezolid, and tipecyclin

had high sensitivity against gram-positive bacteria, while the antibiotics carbapenem, aminoglycoside, and the fourth generation of cephalosporine still had high sensitivity against gram-negative bacteria. The majority of the bacteria (78.6%) were gram-positive, and about 26.9% of the bacteria were multidrug-resistant organisms (MDRO). Ceftriaxone, when used as an empirical therapy, had low sensitivity (30%) to gram-positive bacteria and moderate sensitivity to gram-negative bacteria (53.57%) (Kristianti et al., 2020).

A five-year retrospective cross-sectional study conducted in Iran showed that vancomycin and chloramphenicol were shown to be the most efficient antibiotics against *S. pneumoniae* isolates based on the results of antibacterial susceptibility tests. Additionally, ceftriaxone (100%) was the most effective in vitro medication for *N. meningitides* and *H. influenzae* isolates (Hadi and Bagheri, 2019).

A three-year retrospective research on 542 children ages 0 to 15 was conducted at the National Hospital Abuja (NHA), in Nigeria. Both *S. aureus* and *S. pneumoniae* tested were 100% susceptible to cefuroxime and amoxicillin-clavulanic acid; ceftriaxone also showed the same level of sensitivity in *S. pneumoniae*. 83% of *K. pneumoniae* was sensitive to ceftriaxone, but 100% sensitive to imipenem. 100% of the identified *E. Coli* strains were responsive to imipenem, but only 75% of them were sensitive to ceftriaxone, amoxicillin-clavulanic acid, and amikacin (Iregbu and Abdullahi, 2015).

A retrospective study conducted in Gondar collected data from the bacteriological results of the cerebrospinal fluid of suspected meningitis neonates from 2013 to 2019. Resistance rates among the antimicrobials were cephalosporin (90%), cotrimoxazole (88.9%), penicillin (77.3%), and aminoglycoside (54.54%). A hospital-based cross-sectional study was also conducted in Dilla, Ethiopia, by collecting CSF samples from 287 children. Multidrug resistance was present in approximately 38.5% of *S. pneumoniae*, 33.3% of *N. meningitidis*, 50% of *H. influenzae*, 57.1% of *S. aureus*, and 40% of *E. coli* (Awulachew et al., 2020) (Biset et al., 2021).

2.3. Factors associated with bacterial meningitis (BM)

A cross-sectional study was carried out in the Gaza Strip at three hospitals. Based on this study, malnutrition (low hemoglobin levels) and household overcrowding were statistically related (P-value <0.05) to meningitis (Jarousha and Afifi, 2014). An institution-based, cross-sectional study was conducted among newborns with meningitis from May 1, 2021, to October 30, 2021.

According to this study, there was a strong correlation between infant meningitis and prolonged labor and prolonged rupture of membranes. 88.3% of newborns had less than 24 hours of labor, while 11.7% had more than 24 hours of labor. On the other hand, 9.4% of newborn's mothers had a positive urinary tract infection, and the remaining 90.6% had a negative urinary tract infection (Wondimu et al., 2023).

A retrospective study from four teaching hospitals in different locations in Ethiopia was conducted in a hospital setting with the participation of 425 patients. The age and sex of the patient were investigated as possible sociodemographic risk factors for bacterial meningitis, according to the study. Clinical variables including nutritional status, duration of illness prior to admission, and vaccination status correlate with positive culture results. The age and favorable cultural traits of the individuals were significantly correlated (Gudina et al., 2016).

A hospital-based retrospective cross-sectional study was conducted in a referral hospital, and 83 (45.4%) of the children had their vaccinations. Of the children with meningitis cases, 124 (79%) showed no evidence of a dietary issue, and 72 (36.7%) of them used corticosteroids prior to starting antibiotics (Abiy et al., 2020).

At Gondar University Hospital, a retrospective analysis of 4994 admissions over the two years was carried out. During this time, the proportion of dwellings in urban regions was substantially larger than in rural areas (53% vs. 47%). Meningitis may have been predisposed by upper respiratory tract infection (25%), prior hospitalization (10%), vaccination (10%), and moderate to severe malnutrition (25%) (Amare et al., 2018).

A prospective cross-sectional study was conducted at Debre Markos Referral Hospital with 152 study participants between March 1, 2021, and May 30, 2021. This study's findings showed that there was no significant correlation between any sociodemographic factor and the existence of BM. There was a significant correlation between the absence of immunization and tonsillectomy with BM. At Felege Hiwot Comprehensive Specialized Hospital, a retrospective cross-sectional study was also carried out between January and May 2016. The study evaluated the risk factors and clinical outcomes of pediatric meningitis using samples from 179 pediatric patients. Of the moms with children, 26 (45.6%) had jobs, and 65.6% had no formal education. 49.2% of the patients, or 88, had all of the vaccinations. None of them had a history of receiving anti-meningitis medication. Two (1.1%) of them had a history of urinary tract infections (UTI) before admission, 17 (9.5%) were malnourished, and ten (5.6%) were on immunosuppressive agents (Hibstu et al.,

2022, Tewabe et al., 2018). The following table (table 1) summarizes some important literature with their findings.

Table 1: Summary of Literature Review on Bacterial Meningitis in Children

No	Author. Year	Study design	Country	Sample size	Finding
1	(Hadi and Bagheri, 2019)	Retrospective cross-sectional	Iran	89	23.6% were culture-positive for BM. Vancomycin and chloramphenicol were efficient against *S. pneumoniae*. ceftriaxone (100%) effective for *N. meningitides* and *H. influenza*
2	Li *et al*., 2018	Multicenter retrospective Study	China	837	305 confirmed cases (36.4%) of meningitis. *S. pneumoniae* (46.9%), GBS (10.0%), and *E. coli* (7.9%) were the major pathogens isolated.
3	Jarousha, 2014	Cross-sectional study	Palestine	1853	Relationship (P-value <0.05) between malnutrition and household overcrowding with meningitis
4	(Iregbu and Abdullahi, 2015).	Retrospective cross sectional study	Nigeria	542	5.2% of the samples were positive for culture. *S. aureus and S. pneumoniae* tested were 100% susceptible to cefuroxime amoxicillin-clavulanic and ceftriaxone. 83% of *K. pneumoniae* was sensitive to ceftriaxone.
5	Amare *et al*., 2018	Hospital-based retrospective cross-sectional	Ethiopia	4994	1.6% CABM. Meningitis is predisposed by upper respiratory tract infection (25%), malnutrition (25%), vaccination (10%), and prior hospitalization (10%).
6	Wondimu et al., 2023	Cross-sectional study	Ethiopia	185	88.3% of newborns <24 hours of labor, while 11.7% >24 hours of labor. 9.4% of newborns' mothers had positive urinary tract infections.

2.4. Conceptual framework

To show the link between associated factors and Childhood BM, a conceptual framework is developed based on the findings of the literature review (Figure 1). The framework broadly explains the associated factors of Childhood BM and the link among them by classifying them in to three group's namely socio-demographic factors, health related factors, and maternal factor.

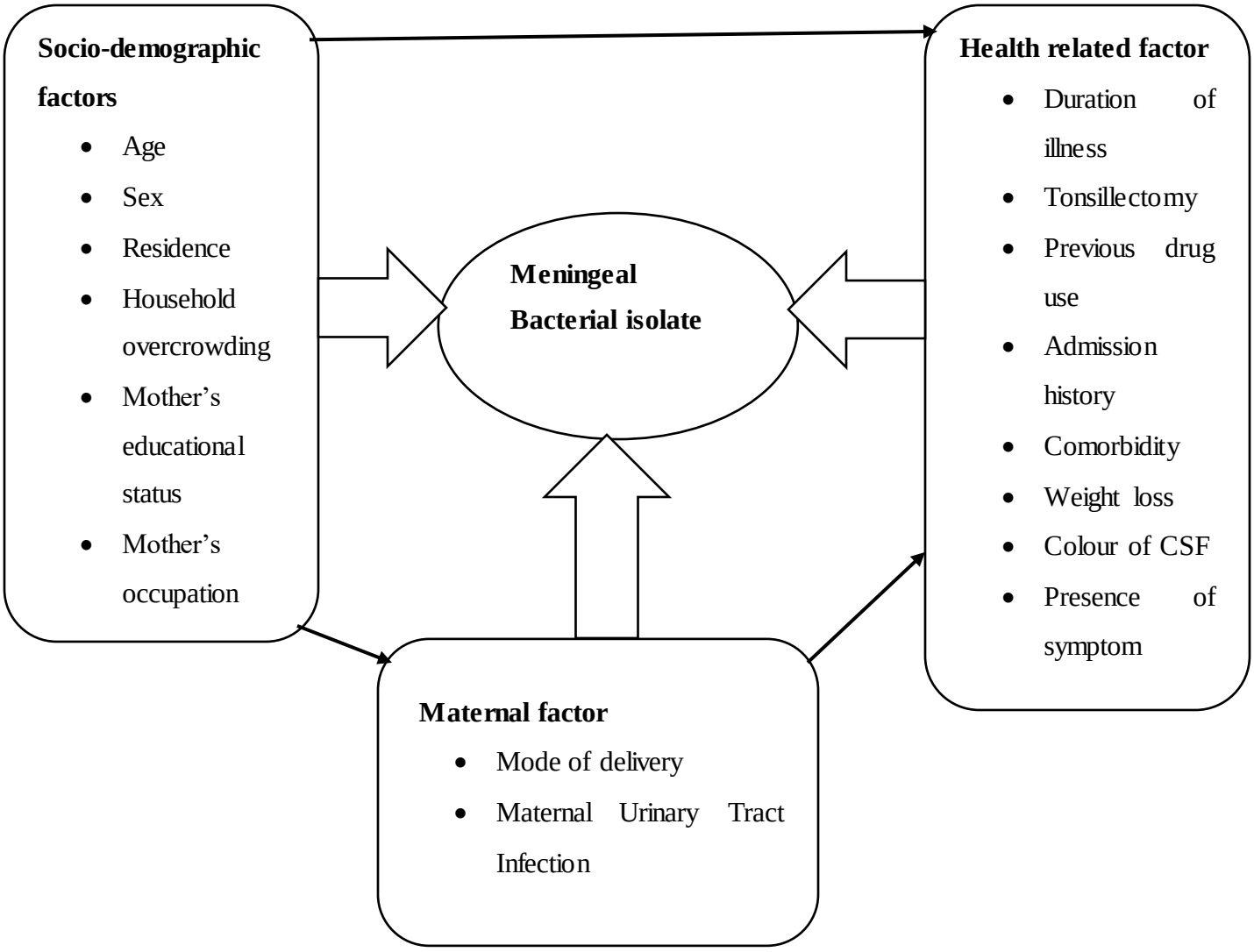


Figure 1: Conceptual framework for bacterial isolates, antimicrobial susceptibility profile, and associated factors among children suspected for meningitis.

3. OBJECTIVES

3.1. General objective

- To assess bacterial isolates, antimicrobial susceptibility profile, and factors associated with meningitis in children suspected for meningitis in Bahir Dar, Northwest Ethiopia.

3.2. Specific objectives

- To determine bacterial isolate among children with presumptive meningitis in Bahir Dar, Northwest Ethiopia.
- To determine the antimicrobial susceptibility profile of the isolates.
- To identify factors associated with bacterial meningitis.

4. METHODS AND MATERIALS

4.1. Study design, area and period

A hospital-based cross-sectional study was conducted from June 03 to September 05/2024 at Felege Hiwot Comprehensive Specialized Hospital (FHCSH) and Tibebe Ghion Specialized Hospital (TGSH), which are found in Bahir Dar, Northwest Ethiopia. Bahir Dar is the capital city of Amhara Regional State, which is located 565 km away from Addis Ababa, the capital city of Ethiopia. Based on the 2023 Census conducted by the Central Statistical Agency of Ethiopia (CSA), Bahir Dar has about 474,743 populations. The city administration comprises nine rural and nine urban kebele. Within the city, there are two specialized hospitals (FHCSH and TGTH), one public primary hospital (Addisalem Hospital), and ten public health centers (CSA, 2023).

The FHCSH was established in 1952 and provides services in outpatient, inpatient, and operation theaters for people in Bahir Dar City and the surrounding zones and regions. FHCSH is currently providing comprehensive healthcare services. It serves a population of more than 7 million in the region and neighboring regions (CSA, 2023). Currently, clinical and non-clinical departments and service units to provide diagnostic, curative, and rehabilitation services at outpatient and inpatient units have been established in the hospital. It also provides disease prevention and health promotion services. The hospital has pediatric wards with a total of 375 beds 561 nurses and 70 laboratory staff. At this ward, there are 62 nurses, 14 general practitioners, three pediatricians, and other supportive staff currently staffing the department. There were two and above childhood meningitis cases reported daily. Meningeal diagnoses like gram staining and other related tests are performed in these hospital laboratories.

The TGSH is another hospital located in Bahir Dar. TGSH was established in 2019. TGSH is a tertiary-level hospital that serves over 7 million populations. At present, the hospital has 12 wards and 503 beds. Additionally, it houses around 1,000 healthcare professionals. There were three and above childhood meningitis cases reported daily. Meningeal diagnoses like culture, gram staining, and other related tests are performed in these hospital laboratories.

4.2. Population

4.2.1. Source population

Children who were suspected to have bacterial meningitis in FHCSH and TGSH

4.2.2. Study population

Children who were suspected to have bacterial meningitis and whose parents/guardians willing to give CSF samples for their diagnostic purpose during the study period

4.3. Inclusion and exclusion criteria

4.3.1. Inclusion criteria

The patients fulfilling the following criteria were included in the study:

- All infants and children 1 day to 14 years with suspected of meningitis ((WHO Meningitis case definition): an illness admitted to a hospital with sudden-onset fever (>38.5°C rectal or 38°C axillary) and one of the following signs: neck stiffness, altered consciousness, or other meningeal sign.

4.3.2. Exclusion criteria

Patients exhibiting the following characteristics were not included in the study:

- Children with suspected meningitis who were not able to undergo lumbar puncture due to contraindications, such as significant respiratory distress and widespread skin lesions on the puncture site.
- Children whose lumbar punctures were unsuccessful.

4.4. Variables

4.4.1. Dependent variable

- Bacterial isolate
- AST profile of the isolates

4.4.2. Independent variables

- Age
- Sex
- Residence
- Mother's/guardians educational status
- Mother's/guardians occupation
- Duration of illness

- Tonsillectomy
- Previous drug use
- Admission history
- Comorbidity
- Mode of delivery
- Presence of symptom
- Weight loss
- Maternal UTI

4.5. Operational definition

- ✓ Children: Based on the treatment protocol, older infants and children were characterized in this study as 2 months to 14 years of age, and young infants as <2 months of age. Consequently, the current study's definition of children included newborns and children from 1 day to 14 years of age (Amare et al., 2018).
- ✓ Suspected meningitis: An illness with sudden onset of fever (> 38.5 °C rectal or > 38.0°C axillary) and one or more of the following: neck stiffness, altered consciousness, another meningeal sign, or petechial rash. In patients less than one (1) year old, suspect meningitis when fever is accompanied by bulging fontanelle.
- ✓ Underweight: Individuals whose body mass index (BMI) is below 18.5, indicating that their body weight is considered too low for their height, which may pose health risks.

4.6. Sample size determination

Since the study is based on a single population proportion, the sample was calculated as follows; $n = (Z\alpha/2)^2 P(1 - P)/ d^2$ where; n is the sample size to be determined, Za/2 is 1.96, p is the prevalence (14.3 %) from children at Debre Markos Hospital (Abiy et al., 2020) and d (5%) is margin of allowable error. Using the above formula and considering a 95% confidence interval, the final sample size (n) was 189. The sample were allocated randomly from the two hospitals by assume that equal patients flow.

4.7. Sampling technique and procedure

A convenience sampling technique was used. All children who fulfilled the inclusion criteria during the study period were enrolled in the study consecutively until the desired sample size was attained.

4.8. Data collection

4.8.1. Socio-demographic data

A structured questionnaire was used to collect sociodemographic and clinical data from the patient and caregivers.

4.8.2. Laboratory data

4.8.2.1. Specimen collection and transportation

The CSF specimen was taken by an experienced physician using a lumbar puncture as part of the diagnosis of meningitis. After collection, the volume and gross appearance of CSF, i.e., clear, bloody/traumatic, cloudy, and xanthochromic, were recorded. The CSF samples were immediately transported to the Medical Microbiology Laboratory of the Department of Medical Laboratory Science, College of Medicine and Health Sciences, Bahir Dar University, within two hours of collection, using trans-isolate transport for further analysis. The inoculation, incubation, identification (biochemical and gram stain techniques), antimicrobial susceptibility testing (AST), and AST interpretation were done using standard operating procedure (SOP) and the 2023 CLSI guideline (CLSI, 2023).

4.8.2.2. Isolation and identification of bacterial meningitis

The collected CSF specimens were inoculated on three solid media including blood agar, chocolate agar, and MacConkey agar. The blood and chocolate agar plates were incubated at 35-37°C in a candle jar with a capnophilic (5-10% CO_2) environment. The MacConkey agar was incubated aerobically for the isolation of gram-negative aerobic bacteria and examined for the presence of any bacterial growth after 24 hours. Those plates showing no growth were incubated for another 24 hours.

The plates were examined macroscopically for morphological and presumptive identification. The growths were identified by colonial morphology such as hemolytic reactions (on a blood agar plate) pigment production or color changes surrounding carbohydrate fermenting colonies (on a MacConkey agar plate). Other identification methods were colony characteristics (white, golden yellow, pink, and grey in color or mucoid in appearance) and gram stain under microscopic examination. Conventional biochemical tests (such as catalase, coagulase, oxidase, hydrogen sulfide test, urease, indole, citrate, methyl red test, sugar utilization tests, susceptibility to optochin disc and Satellitism test for *H.influenzae* isolation) were used to identify bacteria. Collectively,

isolation and identification were done according to standard microbiological methods (Murray et al., 2020) and charts (Figure 2).

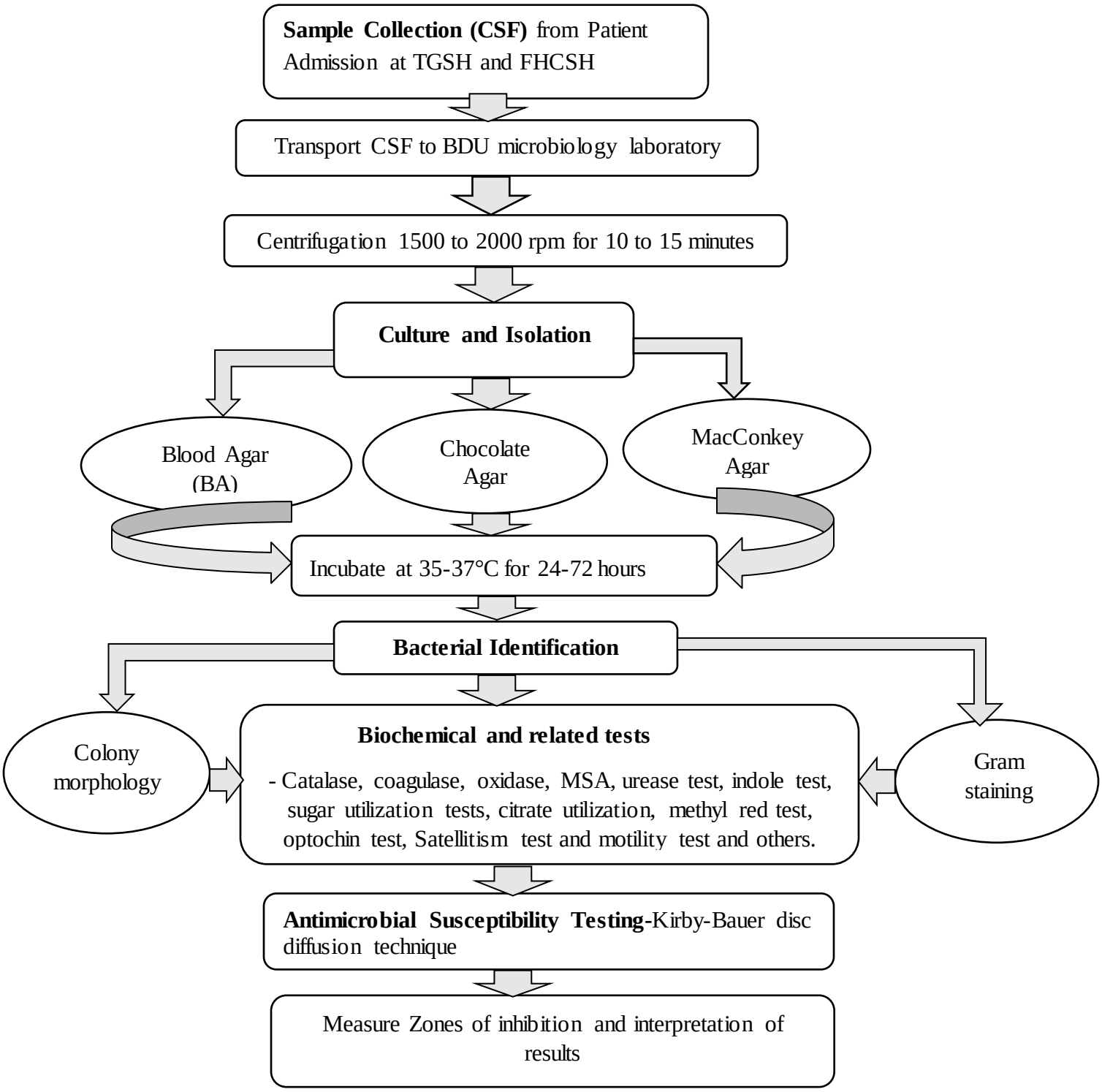


Figure 2: Flow chart for CSF processing, identification of bacteria, and AST test

4.8.2.3. Antimicrobial susceptibility testing

The antimicrobial susceptibility profile of the isolates was determined by using the Kirby-Bauer disc diffusion technique as per the standards of the CLSI guideline on Muller-Hinton Agar (MHA) (Oxoid, Ltd., England) for isolates. Mueller-Hinton agar supplemented with 5% sheep blood was used for fastidious bacteria such as *S. pneumoniae*. From a pure fresh culture, 3-5 colonies were taken and transferred into a tube containing 5 ml of sterile nutrient broth (Oxoid) and mixed gently. Then the suspension was adjusted to McFarland standard equivalent to 0.5 ($1x10^8$ cfu/mL) (CLSI, 2023).

Using a sterile cotton swab, the bacteria were seeded evenly over the entire surface of Mueller Hinton agar (Oxoid). Similarly, fastidious bacteria like *S. pneumoniae* were seeded on Mueller-Hinton agar supplemented with 5% sheep blood agar by rotating at 60°C at least three times (Murray et al., 2020) within less than 15 minutes of inoculum preparation for AST.

The plates were left at room temperature to dry for 3 to 5 minutes. Then, a set of antibiotic discs with the recommended concentrations was placed on the surface of inoculated MHA. While ampicillin (10µg), chloramphenicol (30µg), imipenem (10µg), erythromycin (15µg) penicillin (10µg) (Oxoid Ltd, Basingstoke, Hampshire, UK) was used to assess the antimicrobial susceptibility profiles (ASP) of gram-positive bacteria; gentamicin (10µg), trimethoprim-sulphametoxazole (25µg), chloramphenicol (30µg), erythromycin (15µg), ampicillin (10µg), and ceftriaxone (30µg) discs were used for gram-negative isolates. These discs are selected based on the local prescription pattern for meningitis and the recommendations of the CLSI (CLSI, 2023). Antimicrobial disks were placed on inoculated MHA using sterile forceps at 15 mm distance from each other. The plates were incubated (plates placed side up or inverted) at 37°C for 18–24 hours, and the zone of inhibition around the disk was measured to the nearest millimeter using a graduated caliper in millimeters. Finally, the isolates were classified as sensitive, intermediate, and resistant to the tested drugs (CLSI, 2023).

4.9. Quality control

A site assessment and 10% pre-test were conducted at Debre Tabor Hospital prior to the actual data collection, which gave diagnostic accuracy and identified gaps. Data collection was done after the data collectors (microbiologists and pediatricians) received on-site training. The data were checked for completeness and representativeness prior to entry. SOPs for specimen collection were applied. The specimens (CSF) were collected and handled properly. CSF was collected in a sterile

tube by attending physicians. Each specimen was clearly labelled. As much as possible, these specimens were received in the laboratory and processed soon after collection.

The samples (CSF) were spread on a solid culture medium, which was first checked for sterility and the performance of the culture medium. The sterility of culture media was checked by incubating 3–5% of the batch at 35–37°C overnight and observing for bacterial and fungal growth. The performance of culture media was evaluated by using standard quality control strains, such as *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, obtained from APHI and FHCSH. The data entry was done by two individuals and checked for agreement. Cross-checking of the data with the results paper was conducted.

4.10. Data processing and analysis

Data were double-entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 26 software. Descriptive statistics such as frequency and crosstabs were used to summarize the data. Normality and multi-collinearity test ensured the validity and reliability of our statistical analyses. Logistic regression models were applied to determine the association between predictors and outcome variables with a 95% confidence interval. A p values ≤ 0.05 were considered statistically significant.

4.11. Ethical considerations

The study protocol was approved by the Institutional Review Board (IRB) of the College of Medicine and Health Sciences (CMHS), Bahir Dar University (BDU), with protocol number 1039/2024. Then, a support letter was obtained from the department of Medical Laboratory Science (DMLS), CMHS-BDU. Further support letters and permission were secured from the Amhara Public Health Institute (APHI) and the administrators of the study hospitals. Consent was obtained from the parent or guardian, and assent was obtained from the child whenever possible. The purpose and importance of the study, as well as the rights of participants' parents or guardians, were clearly explained. The participants were coded and identified for anonymity. The data were put in a secure place, and confidentiality was maintained. Critical results important for the management of the patients were given to the treating physician.

5. RESULTS

5.1 Socio-demographic and clinical characteristics of the participants

Of the 189 children enrolled, 89 (47.1%) were male, resulting in an overall male-to-female ratio of 1:1.12. Of these enrolled, 103 (54.5%) were rural residents. The mean age of the study participants was 2.66 years. Sixty-five (34.4%) children's age was found between 28 days and 12 months. As shown in Table 2, most of the mothers were unable to read and write, 69 (36.5%). Ninety-nine (52.4%) of the mothers were farmers.

Thirty-eight (20.1%) of children are underweight. Ten (5.3%) of mothers have delivered by Caesarian section. One hundred forty-three (75.7%) of the children were fully vaccinated for their age as per WHO guidelines; conversely, 24.3% were not fully vaccinated, and 78 (41.3%) of the study participants had comorbidities like pneumonia and otitis media. The characteristics of 14 (7.4%) CSF samples collected were purulent with visible turbidity, while 136 (72%) CSF samples were clear in appearance (Table 2).

Table 2: Socio-demographic and clinical characteristics of study participants at FHCSH and TGSH, Bahir Dar, north-west Ethiopia, 2024

Variables	Characteristics	Frequency (%)	Culture positive
Sex	Male	89 (47.1)	5
	Female	100 (52.9)	5
Age	≤28 day	53 (28.0)	2
	29 day-≤12 month	65 (34.4)	4
	1-5 years	34 (18)	2
	6-10 years	23 (12.2)	1
	11-14 years	13 (6.9)	1
Residence	Urban	86 (45.5)	4
	Rural	103 (54.5)	6
Mother's educational status	Cannot read and write	69 (36.5)	6
	Primary	60 (31.7)	1
	Secondary	35 (18.5)	2
	Diploma and above	25 (13.2)	1
Mother occupation	Farmer	99 (52.4)	6

	House wife	49 (25.9)	1
	Merchant	32 (16.9)	2
	Others	9 (4.8)	1
Number of house partitions	≤four	92 (48.7)	6
	>four	97 (51.3)	4
No of people sharing the same room with the child	≤2	73 (38.6)	2
	3-4	102 (54)	4
	>4	14 (7.4)	4
Mode of delivery	Spontaneous vaginal	104 (55)	3
	Assisted vaginal	75 (39.7)	5
	Caesarian section	10 (5.3)	2
Duration of suspected meningeal illness	≤ a week	94 (49.7)	3
	> a week	95 (50.3)	7
Tonsillectomy	Yes	49 (25.9)	7
	No	140 (74.1)	3
Admission history	Yes	80 (42.3)	6
	No	109 (57.7)	4
Previous drug use	Yes	34 (18)	1
	No	155 (82)	9
Comorbidity	Yes	78 (41.3)	5
	No	111 (58.7)	5
Underweight	Yes	38 (20.1)	6
	No	151 (79.9)	4
Maternal UTIs/STIs during pregnancy	Yes	34 (18)	1
	No	155 (82)	9
Characteristics of CSF	Clear	136 (72)	3
	Yellow	27 (14.3)	2
	Bloody	12 (6.3)	1
	Turbid	14 (7.4)	4

Clinical data

Clinical variables were collected, and the findings are summarized below (Figure 3). Briefly, loss of appetite (63%), and headache (60.8%) were the most common clinical complaints. On the other hand, rash was the least recognized sign among meningitis presumptive children (Figure 3).

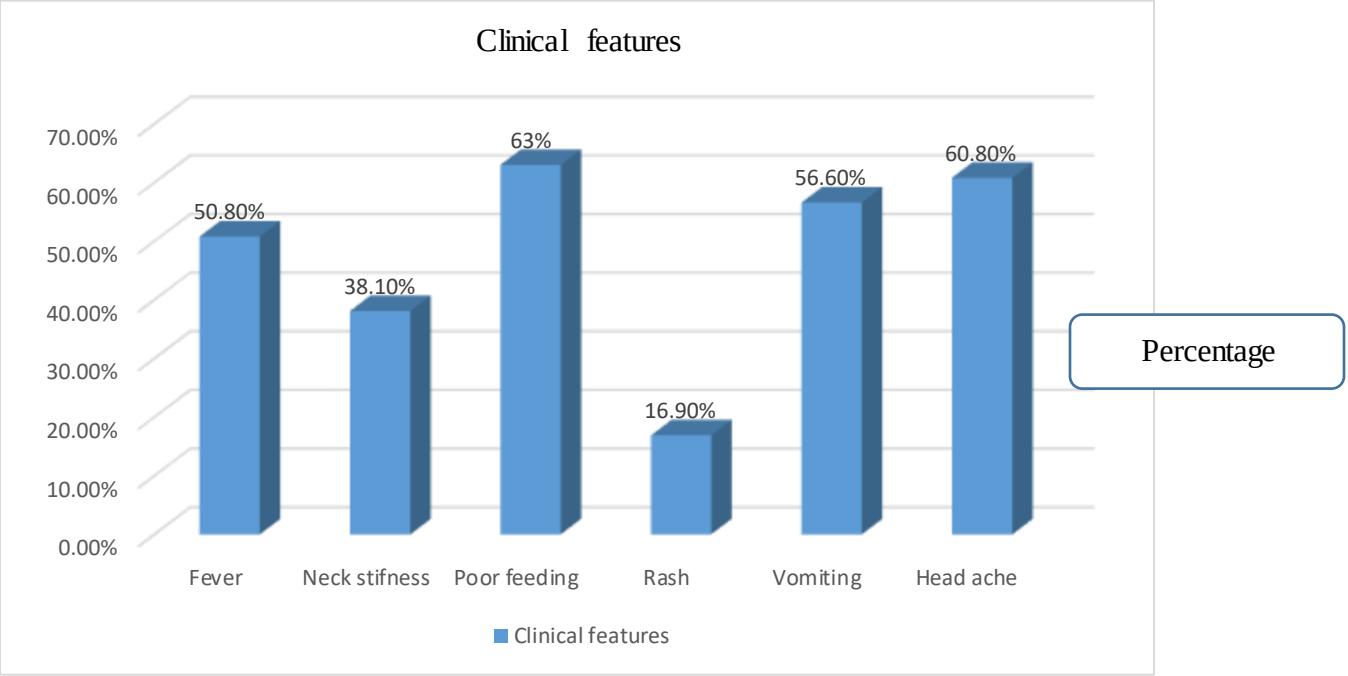


Figure 3: Clinical sign and symptom of study participants at FHCSH and TGTH, Bahir Dar, north west Ethiopia, 2024

5.2 Prevalence of bacterial meningitis

Among the total 189 CSF samples processed, bacterial meningitis was identified using bacteriological culture in 10 (5.3%) (95% CI=2.6-8.5) children. From these positive samples, five different bacteria were identified. The prevalence of BM was 5.8% (6/103) in rural residents and 5.6% (5/89) in males. Four (6.1%) cases of BM were observed in the age group of 28 days–12 months. Our study found that 2.9% of the isolates were from patients who had a history of previous drug use. Six (7.5%) of the isolates were from patients who had an admission history, as shown in the above Table 2.

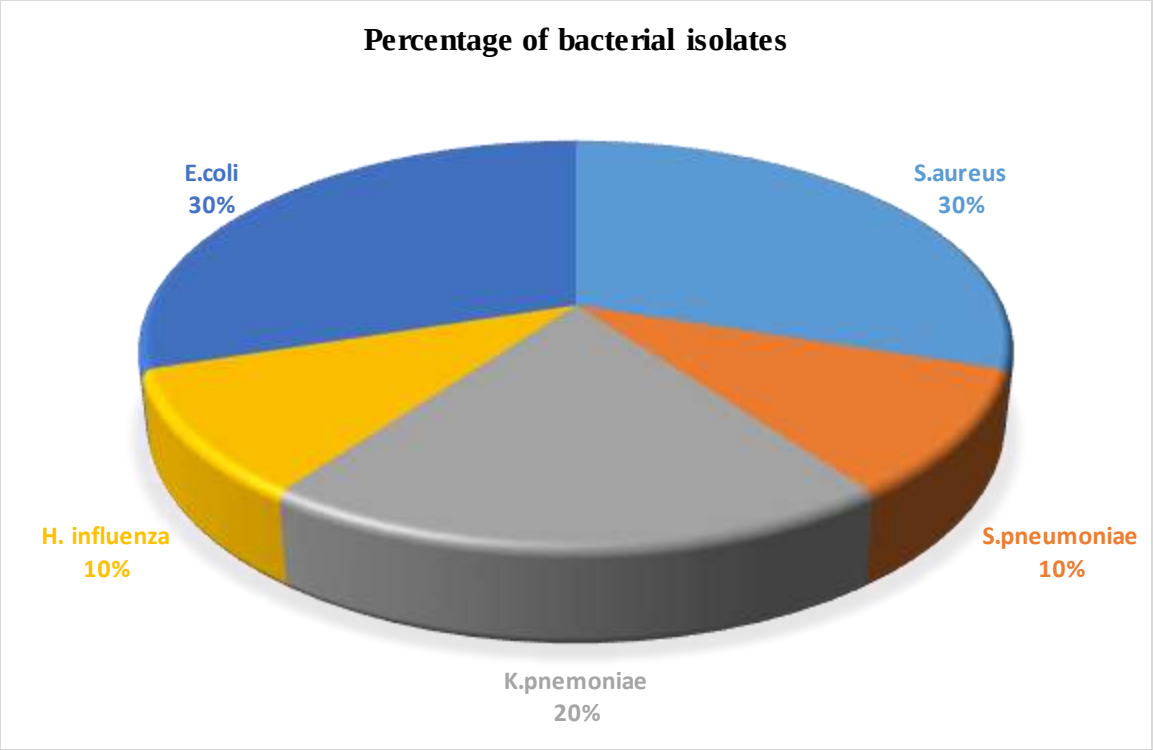
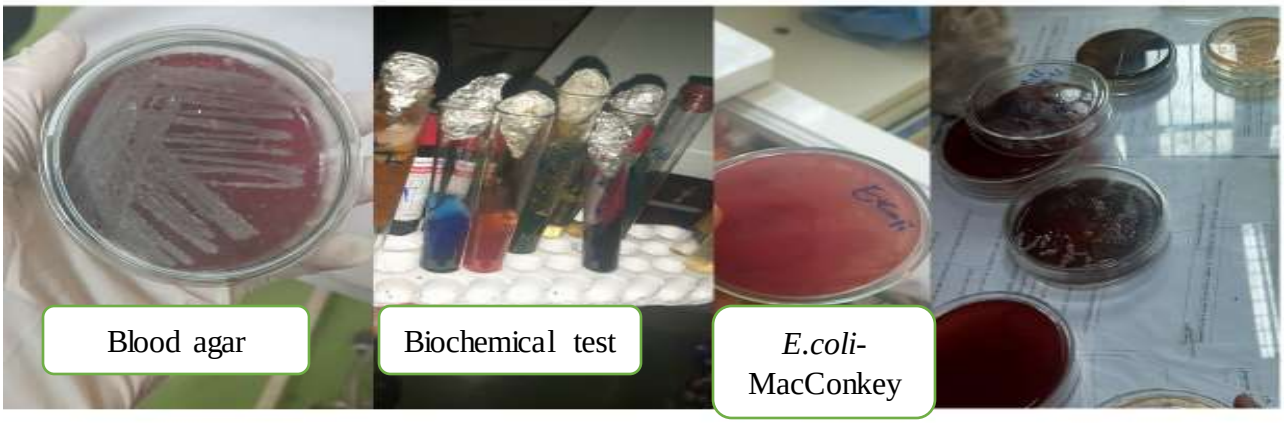


Figure 4: Types and frequency of bacterial isolates from CSF cultures of children suspected of having meningitis at TGSH and FHSCH, Bahir Dar, north-west Ethiopia, 2024.

Among the 10 culture-positive cases, six (60%) were gram-negative and four (40%) were gram-positive. *S. aureus* 3 (30%) and *E. coli* 3 (30%) were the predominant isolates, followed by *K. pneumoniae* 2 (20%) (Figure 4). No multiple isolates were identified from a single specimen or patient.



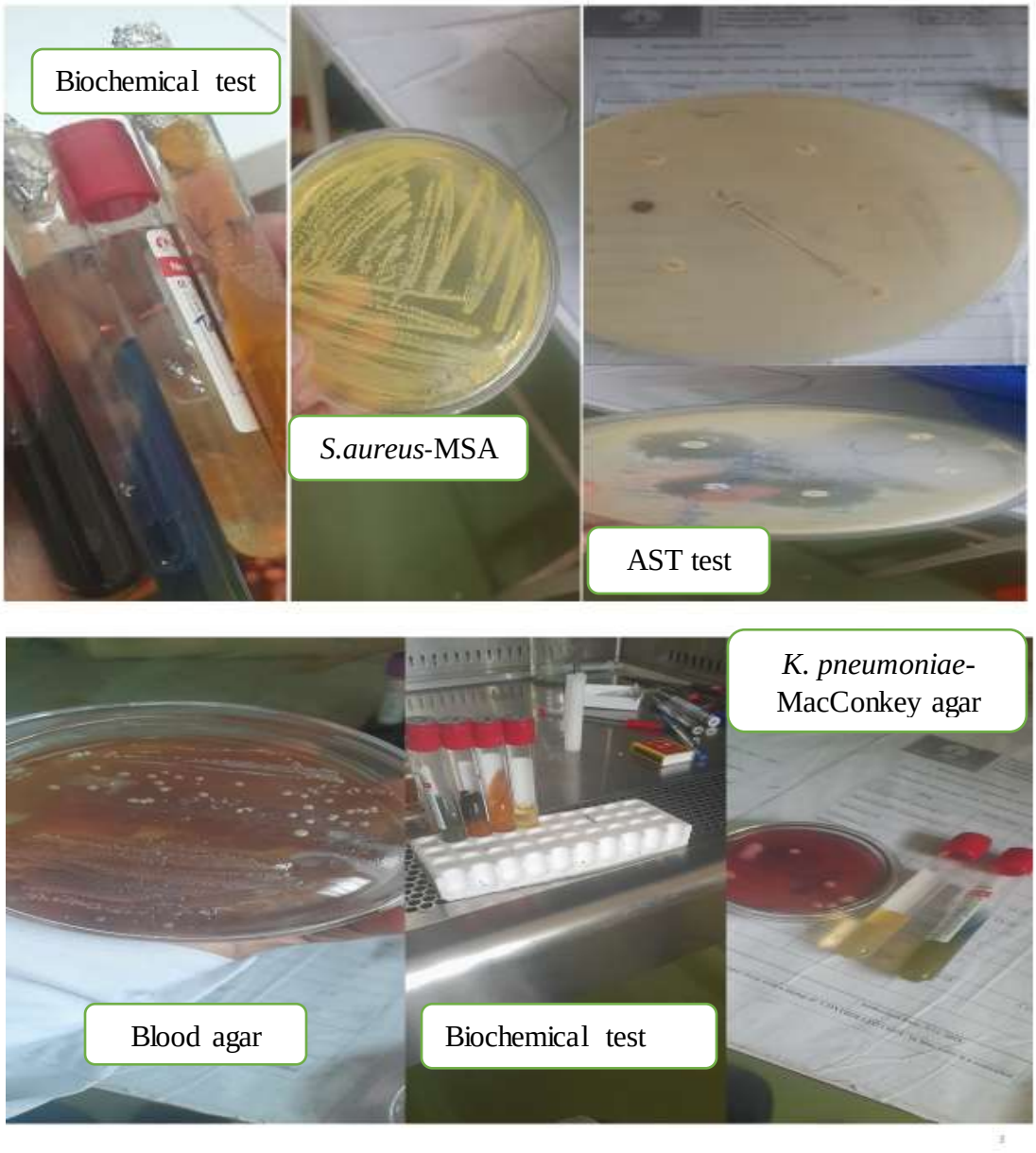


Figure 5: Some photos taken in our laboratory during data collection from CSF cultures of children suspected of having meningitis at TGSH and FHSCH, Bahir Dar, north-west Ethiopia, 2024.

5.3 Distribution of bacterial isolates

Among the 10 bacterial isolates, four (40%) of the isolates were found in children with the age group between 28 days and 12 months. From this age group, two isolates of *S. aureus*, one isolate of *E. coli*, and one isolate of *K. pneumoniae* were found. In the age group below 28 days, there were only two (20%) bacterial isolates found. *H. influenzae*, one isolate *of E. coli*, one isolate *of*

S. aureus, and *S. pneumoniae* isolates were found in the age group above one year. Two (20%) *E. coli* isolates were found below the 12-month age (Figure 6).

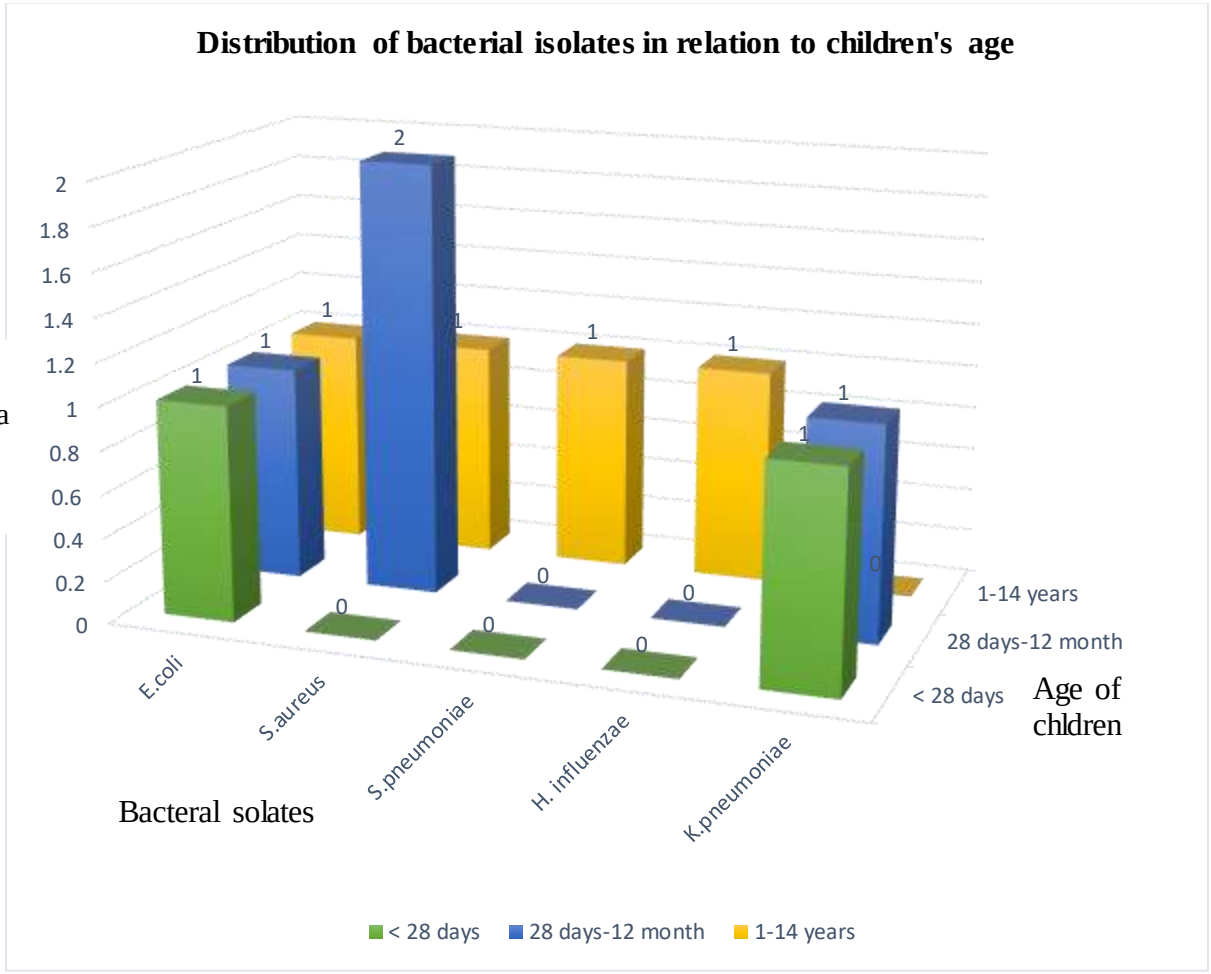


Figure 6: Distribution of bacterial isolates in relation to children's age group suspected of having meningitis at TGSH and FHSCH, Bahir Dar, north-west Ethiopia, 2024

Regarding the residence, four (40%) children at urban have bacterial meningitis, and the remaining six (60%) isolates were found among rural residents. All *E. coli* isolated in this study were found in rural residents. However, of three *S. aureus*, two of them were found among urban residents. From the total ten isolates, six (60%) of the child's meningitis cases were found in children who have illiterate mothers. Five (50%) of female children had positive bacterial meningitis. Likewise, six (60%) children whose mothers were farmers have confirmed bacterial meningitis (Table 3).

Table 3: Distribution of bacterial isolates suspected of having meningitis at TGSH and FHSCH, Bahir Dar, north-west Ethiopia, 2024

Variables	Characteristics	Distribution of bacterial isolates					
		E.coli	*S.aureus*	*K.pneumoniae*	*S.pneumoniae*	*H.influenzae*	*Total*
Sex	Male	1	2	1	1	0	5
	Female	2	1	1	0	1	5
Residence	Urban	0	2	1	1	0	4
	Rural	3	1	1	0	1	6
Mother's educational status	Cannot read write	2	2	2	0	0	6
	Primary	1	0	0	0	0	1
	Secondary	0	0	0	1	1	2
	Diploma and above	0	1	0	0	0	1
Mother occupation	Farmer	2	3	1	0	0	6
	House wife	1	0	0	0	0	1
	Merchant	0	0		1	1	2
	Other	0	0	1	0	0	1

5.4 Antibiotic susceptibility profile of bacterial isolates

Table 4 shows the resistance rates of bacterial isolates causing bacterial meningitis. *Klebsiella pneumoniae* showed a high rate of resistance at 2(100%) to ampicillin, 1(50%) to chloramphenicol, and 2(100%) to amoxicillin clavulanate. In contrast, *K. pneumoniae* was 2(100%) sensitive to imipenem and gentamycin. Similarly, *S. aureus* was found to be resistant to 3(100%) ampicillin. One isolate of *S. aureus* also show resistance to trimethoprim sulfamethoxazole, tetracycline, erythromycin, and clindamycin.

Moreover, one isolate of *E. coli* were found to be resistant to ciprofloxacin and ceftriaxone. While 3(100%) of these isolates were found susceptible to gentamicin and imipenem. *S. pneumoniae* showed high resistance 1(100%) to chloramphenicol, ampicillin, amoxicillin clavulanate, and penicillin. While *S. pneumoniae* showed 1(100%) sensitivity to clindamycin and ciprofloxacin from tested antibiotics. *H. influenzae* showed a high rate of resistance at 1(100%) to ampicillin, penicillin G, and tetracycline (Table 4).

Table 4: Antibiotic Susceptibility Profile of Bacterial Isolates from Meningeal Infections at FHCSH and TGSH, North West, Ethiopia, 2024

Drugs		**Bacterial isolates**				
		S. aureus (3), n (%)	***H. influenzae (1)*** n (%)	***E. coli*** (3) n (%)	***K. pneumoniae (2)*** n (%)	***S. pneumoniae*** (1) n (%)
Tetracycline	S	2(66.6%)	0	1(33.3%)	1(50%)	1(100%)
	R	1(33.3%)	1(100%)	2(66.6%)	1(50%)	0
Erythromycin	S	2(66.6%)	1(100%)	3(100%)	2(100%)	1(100%)
	R	1(33.3%)	0	0	0	0
Ampicillin	S	0	0	1(33.3%)	0	0
	R	3(100%)	1(100%)	2(66.6%)	2(100%)	1(100%)
Chloramphenicol	S	3(100%)	1(100%)	2(66.6%)	1(50%)	0
	R	0	0	1(33.3%)	1(50%)	1(100%)
Ciprofloxacin	S	2(66.6%)	1(100%)	2(66.6%)	1(50%)	1(100%)
	R	1(33.3%)	0	1(33.3%)	1(50%)	0
Clindamycin	S	2(66.6%)	N/A	N/A	N/A	1(100%)
	R	1(33.3%)	N/A	N/A	N/A	0
Ceftriaxone	S	3(100%)	1(100%)	2(66.6%)	2(100%)	1(100%)
	R	0	0	1(33.3%)	0	0
Trimethoprim-Sulphamethoxazole	S	2(66.6%)	1(100%)	2(66.6%)	1(50%)	1(100%)
	R	1(33.3%)	0	1(33.3%)	1(50%)	0
Amoxicillin-clavulanate;	S	2(66.6%)	N/A	1(33.3%)	0	0
	R	1(33.3%)	N/A	2(66.6%)	2(100%)	1(100%)
Penicillin G	S	1(33.3%)	0	N/A	N/A	0
	R	2(66.6%)	1(100%)	N/A	N/A	1(100%)
Imipenem	S	3(100%)	N/A	3(100%)	2(100%)	1(100%)
	R	0	N/A	0	0	0
Gentamycin	S	N/A	1(100%)	3(100%)	2(100%)	N/A
	R	N/A	0	0	0	N/A

S=Sensitive, R=Resistant, n=frequency, N/A=Not applied

5.5 Multidrug resistance patterns of bacterial isolates

The overall prevalence of multidrug resistance (bacteria resistant to three or more classes of antibiotics tested) was 40%. However, one isolate (10%) of the bacteria was sensitive to all antibiotics tested. Among gram-negative bacteria, 3/6 (50%) and among gram-positive bacteria,

1/4 (25%) were MDR. Among three *S. aureus*, one isolate had multidrug resistance. One *K. pneumoniae* (50%), and two isolate *E. coli* (66.6%) were MDR. A high level of bacterial resistance to different antibiotics, particularly ampicillin, was observed in almost all gram-negative bacterial isolates (Table 5). However, because there was only a single isolate of *H. influenzae* and *S. pneumoniae*, it was not possible to characterize the species in depth, and no significant inferences about its antibiotic sensitivity could be made.

Table 5: Multi-drug resistance (MDR) profile of bacterial isolates from meningeal infections at FHCSH & TGSH, North West, Ethiopia, 2024.

Multi-drug resistance pattern	*S. aureus* N (%)	*E.coli* N (%)	*K. pneumoniae* N (%)	*H. influenzae* N (%)	*S. pneumoniae N (%)*
PEN, CIP, TET	0	1 (33.3)	0	0	0
PEN, CIP, TET, TR	0	0	1 (50)	0	0
PEN, TET, TR, CEF, CHL	0	1 (33.3)	0	0	0
PEN, CLN, TET, TR, ERY, CIP	1 (33.3)	0	0	0	0

PEN=Penicillin, CLN=Clindamycin, TET=Tetracycline, TR=Trimethoprim, CEF=Ceftriaxone, ERY=Erythromycin, CHL=Chloramphenicol, CIP=Ciprofloxacin, CXT=Cefoxitin, AZT=Azithromycin, GEN=Gentamycin.

5.6 Factors associated with bacterial meningitis

In this study, Fisher's Exact Test in SPSS was used to explore the association between bacterial meningitis and associated factors. The Fisher's Exact Test indicates that there were associations between bacterial meningitis and underweight (malnutrition) (P = 0.005), and tonsillectomy (P = 0.003).

Table 6: Factors associated with the presence of bacterial meningeal infections at FHCSH and TGSH, Bahir Dar, north-west Ethiopia, 2024.

Variables	**Characteristics**	**Meningitis Negative (%)**	**Meningitis Positive (%)**	**Fisher's Exact Test P−value**

Sex	Male	84	5	0.551
	Female	95	5	
Residence	Urban	82	4	0.758
	Rural	97	6	
Number of house partition	≤four	86	6	0.529
	>four	93	4	
Duration of suspected meningeal illness	≤ a week	91	3	0.330
	> a week	88	7	
Tonsillectomy	Yes	42	7	**0.003**
	No	137	3	
Admission history	Yes	74	6	0.328
	No	105	4	
Previous drug use	Yes	33	1	0.693
	No	146	9	
Comorbidity	Yes	73	5	0.743
	No	106	5	
Weight loss	Yes	32	6	**0.005**
	No	147	4	
Maternal UTIs/STIs	Yes	33	1	0.693
	No	146	9	

6. DISCUSSION

In countries with limited resources like Ethiopia, bacterial meningitis is a significant cause of both mortality and morbidity, particularly in newborns (Biset et al., 2021). Worldwide, acute bacterial meningitis is a serious health issue for children and newborns, calling for an enhanced vaccination program, prompt diagnosis, and intensive treatment (Jiang et al., 2017). This study was aimed at determining bacterial isolates, an antimicrobial susceptibility profile, and associated factors among children suspected of having meningitis in Bahir Dar, Northwest Ethiopia.

The isolation rate of bacteria found in this study was found to be 5.3% (95% CI = 2.6-8.5). This is comparable to previous studies conducted in Ethiopia (4.7%) (Reta and Zeleke, 2016), in Addis Ababa (5%) (Yeshidinber, 2020), and 5.2% in Nigeria (Iregbu and Abdullahi, 2015). On the other hand, our finding is lower than a study in India (28%) (Gahlot and Kasana, 2021), 13.2% in Dilla (Awulachew et al., 2020), and 8.5% in Hawasa (Ali, 2024). The fastidious nature of the organisms or prior exposure to antibiotics, over diagnosis of meningitis in all feverish neonates, infants, and children has resulted in unnecessary lumbar punctures that may lead to a low isolation rate of bacteria for our study.

On the other hand, the rate of isolation in our study is higher than studies conducted in Gondar (1.7%) and Ghana (0.8%) (Biset et al., 2021, Renner et al., 2019). It is important to keep an eye on the trends of an isolate.

This study shows the prevalence of bacterial meningitis in the rural regions was higher than that in the urban regions, similar to a study conducted in Vietnam (Nguyen-Huu et al., 2022). The majority of meningitis cases were found in children younger than one year which was parallel finding with a study conducted in North India (Raza et al., 2019, Bari et al., 2016). The higher rate of meningitis in young children may be because of their underdeveloped immune systems (Awulachew et al., 2020). The majority of etiological agents for meningitis in our study were gram-negative organisms (60%). This is comparable to studies in Indonesia (53.8%) and Nepal (72.2%) (Kristianti et al., 2020, Shrestha et al., 2015). Contrary to our study, gram-positive bacteria were the predominant isolate in the CSF culture of the study in Gondar (Dagnew et al., 2013). This may be due to a slight change in the shift of pathogens from gram-positive to gram-negative.

In this study, the major bacteria responsible for child bacterial meningitis were *S. aureus* (3) (30%), *E. coli* (3) (30%), and *Klebsiella pneumoniae* (2) (20%). Parallel to this, several studies suggested

that *S. aureus*, *S. pneumoniae*, *E. coli*, and *K. pneumoniae* were commonly isolated pathogens from the CSF of children (Ali et al., 2021, Iregbu and Abdullahi, 2015). Similar results reported from Nigeria, Addis Ababa and Bahir Dar show that *S. aureus* and *E. coli* were the predominant isolates (Reta and Zeleke, 2016, Hadi and Bagheri, 2019, Tesera et al., 2020). Despite variations in the frequency of each pathogen, these agents remain the prime cause of BM in our population. Numerous factors, including immunization status, age, patient geography, differences in socioeconomic status, and environmental factors, affect the unique etiology of the disease. For instance, low socioeconomic status, skin colonization, crowded living conditions, or close contact with infected individuals can facilitate transmission of *E. coli* and *S. aureus*.

A low number of *Haemophilus influenzae* (10%), and *S. pneumoniae* (10%) were found in our study. However, widespread WHO research carried out in Ethiopia and other meningitis belt nations has revealed similar trends, with *S. pneumoniae*, *N. meningitides*, and *H. influenzae* being the most prevalent pathogens (Reta and Zeleke, 2016, Hadi and Bagheri, 2019, Dagnew et al., 2013, Awulachew et al., 2020). The introduction of conjugate vaccinations may be the cause of this notable shift in the epidemiology of acute bacterial meningitis.

The medications with the highest efficacy against *S. aureus* in our investigation were chloramphenicol and ceftriaxone (100% sensitivity) with ciprofloxacin (66.6% sensitivity). Nonetheless, two of the three *S. aureus* isolates demonstrated the greatest degree of antibiotic resistance to penicillin; and 100% resistance to ampicillin. A study carried out in Nepal and Bahir Dar reveals nearly identical findings in this respect (Tesera et al., 2020, Shrestha et al., 2015). Due to self-medication, exposure to a wide range of medicines, and insufficient dosages of antibiotics, *S. aureus* develops novel cellular mechanisms to withstand the effects of medications, which contributes to the high resistance.

Of the gram-negative isolates, *E. coli* showed 100% sensitivity to erythromycin, rifampicin, imipenem, and gentamycin. Comparable findings have also been reported in China and Ethiopia (Tesera et al., 2020, Jiang et al., 2017). In this study, 66.6% of *E. coli* was resistant to ceftriaxone, amoxicillin clavulanate and tetracycline. But a study from Iran shows *E. coli* was 100% resistant to ceftriaxone (Hadi and Bagheri, 2019). Geographical and policy variations regarding the use of antibiotics may be the cause of the variation. Furthermore, this investigation demonstrates that 66.6% of the *E. coli* isolates were ampicillin-resistant. This is consistent with research from Dilla,

Ethiopia, and China, which found that over 50% of *E. coli* isolates had ampicillin resistance (Awulachew et al., 2020, Li et al., 2018). Using antibiotics prior to hospital admission is a prevalent practice in many developing countries, which may have contributed to the rising occurrence of antibiotic-resistant meningitis.

H. influenzae showed 100% sensitivity to chloramphenicol, ceftriaxone, and trimethoprim sulphiomoxazole. A comparable study from Zimbabwe showed that *H. influenzae* was sensitive to ceftriaxone, ampicillin, chloramphenicol, and ciprofloxacin (Gudza-Mugabe et al., 2017). The *S. pneumoniae* isolates utilized in our investigation were resistant to ampicillin, chloramphenicol and penicillin (100%), similar to those from Pakistan (Bhatti et al., 2024), Cameroon (Tiabou et al., 2019), Beijing (95.7%) (Peng et al., 2021) and Hawassa (100%) (Assegu Fenta et al., 2020). In contrast, a study from Debre Markos Comprehensive Specialized Hospital shows that *S. pneumoniae* was 100% susceptible to penicillin (Hibstu et al., 2022). However, because there was only a single isolate of *H. influenzae and S. pneumoniae*, it was not possible to characterize the species in-depth and no significant inferences about its antibiotic sensitivity could be made.

The most effective antibiotics against both Gram-positive and Gram-negative isolates in this investigation were tetracycline, erythromycin clindamycin, and chloramphenicol, while ceftriaxone proved to be the most effective against Gram-negative isolates. Comparable research was reported from Ethiopia, Nepal and Iran (Hadi and Bagheri, 2019, Awulachew et al., 2020, Shrestha et al., 2015). All gram-negative isolates were also sensitive to gentamicin and imipenem, which were comparable with reports from Iran and India (Khan et al., 2014, Motamedifar et al., 2015).

According to the results of this study, penicillin and ampicillin had the highest rates of antibiotic resistance. Over 90% of the bacteria that were identified in this study were ampicillin-resistant. Similar investigations were conducted in Ethiopia, Pakistan and Iran (Tesera et al., 2020, Ali et al., 2021, Assegu Fenta et al., 2020, Jiang et al., 2017). This could be because there are no guidelines guiding drug selection, and it is usual for prescribed pharmaceuticals to be misused in society. Because ampicillin is used often, there is a significant rate of ampicillin resistance in many published researches, which highlights the necessity for ongoing surveillance for ampicillin resistance in bacterial isolates.

In our study, 40% of the isolates had multiple antibiotic resistance, which shows this is a serious issue currently. MDR accounted for 26.9% in Indonesia (Kristianti et al., 2020), 41.2% in Debre Markos (Hibstu et al., 2022), and 42.1% in Dilla Ethiopia (Awulachew et al., 2020) were in line with our study. In contrast to this, MDR accounted (58%) in Gondar (Biset et al., 2021), Bahir Dar (75%) (Tesera et al., 2020), and Gondar 61.9% (Dagnew et al., 2013). Because of this high level of resistance, therapy becomes more difficult and frequently requires the use of more expensive or difficult-to-access alternatives, which may not always be successful. Exposure to a wide variety of medicines, indiscriminate and irrational use of antimicrobials, self-medication, and insufficient dosages of antibiotics may cause the high resistance.

Based on our study, among gram-negative bacteria, 3/6 (30%) and among gram-positive bacteria, 1/4 (25%) were MDR. This shows gram-negative bacteria were more resistant to antibiotics due to several structural and biochemical features. In a similar manner, previous studies from Debre Markos show that 85.7% of gram-negative bacteria were MDRO (Hibstu et al., 2022). In contrast to our result, a study from Indonesia showed that 78.6% of gram-positive bacteria were MDRO (Kristianti et al., 2020). Factors such as antibiotic use, infection control practices, geographical variations, and transmission dynamics may contribute to these differeences.

Multidrug resistance was present in approximately 57.1% of *S. aureus*, and 40% of *E. coli* (Awulachew et al., 2020). In the same way as the above study, our study showed that 33.3% of *S. aureus* and 66.6% of *E. coli* had multidrug resistance. In this study, among gram-negative bacteria, 50% of *K. pneumoniae* isolates were MDR, but *K. pneumoniae* also showed that it was 100% sensitive to imipenem and gentamycin. In contrast study from Iraq showed that there was 100% multidrug resistance in *K. pneumoniae* (Saadi et al., 2017). Generally, this study justify, the dynamics of bacterial meningitis in the context of MDR highlight the complexity of managing infections in the face of resistance, especially in regions with limited healthcare resources like Ethiopia. Addressing both the incidence of bacterial meningitis and the emergence of antimicrobial resistance is crucial for improving public health outcomes in affected populations.

Based on this study, malnutrition (underweight) was statistically related (P-value <0.05) to meningitis, calculated by Fisher's Exact Test. Similar results were reported from research conducted in the African meningitis belt and in the Gaza Strip (Jarousha and Afifi, 2014, Pinilla-Monsalve et al., 2023, Ali et al., 2021). Poverty, malnourishment, overcrowding, and insufficient

health care seem to be closely linked to meningitis. Low-income patients had a higher propensity to carry and disseminate the bacterium that causes meningitis (Khan et al., 2014).

The other key identified factors associated with culture positivity were tonsillectomy. This result is in line with research in Debre Markos that showed a strong correlation between tonsillectomy with BM, as well as a study carried out in Ethiopia and Africa (Hibstu et al., 2022, Consortium et al., 2015). The occurrence of a wider CI may be due to the small sample size and low number of culture-positive bacterial meningitis cases in our study.

7. LIMITATIONS OF THE STUDY

Due to resource limitations, this study could not be performed on genotypic drug resistance patterns and was unable to be performed vancomycin drug susceptibility test.

Due small positive sample, we cannot perform logistic regression and may affects associated factor analysis.

8. CONCLUSIONS

The prevalence of culture based bacterial meningitis among children hospitalized with clinically diagnosed meningitis in FHCSH and TGTH, Ethiopia, was 5.3%. Relatively higher numbers of bacteria were isolated from children under the age of one year. Isolated bacteria were predominantly gram-negative. *S. aureus*, *E. coli*, and *K. pneumoniae* were the predominant bacterial isolates. The *S. pneumoniae* and *H. influenza* have been isolated in small numbers. Both gram-positive and gram-negative isolates were resistant to the penicillins tested. Imipenem, Gentamycin, and Ceftriaxone had the most effective antibacterial activity across bacterial isolates tested in the study. The frequency of multiple drug resistance was high among some bacterial isolates. Malnutrition and tonsillectomy were associated with childhood bacterial meningitis.

9. RECOMMENDATIONS

- Strengthen Surveillance and Diagnostic Capacity
- Treatment of suspected bacterial infection should be guided by culture and susceptibility whenever possible.
- Strengthen Infection Control and Prevention
- Strict guidelines and antibiotic stewardship programs should be in place for the prevention and control of antimicrobial resistance.
- It would be valuable to conduct more in-depth similar studies with a large number of individual participants, more study sites and molecular characterization of the bacterial meningitis isolates.
- Improve Public Awareness and Education

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11. ANNEXES

Annex I: Information Sheet (for mothers or guardians, English version)

Name of the Organization: Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Laboratory Sciences.

Title of the Research Project: 'Bacterial Isolates, Antimicrobial Susceptibility Profile, and Factors Associated with meningitis among Children in Bahir Dar, Northwest Ethiopia.'

Name of Investigator: Tadese Sisay (BSc)

Introduction: You are invited to participate in a study to be conducted by an MSc student at Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Laboratory Science. It is aimed at determining the spectrum of bacterial isolates, their drug susceptibility patterns, and associated factors for children suspected of meningitis at Felege Hiwot and Tibebe Gion Hospitals. It is important to know the distribution of the bacteria to control the disease. Knowing risk factors will be important for designing preventive strategies and useful for drug choice. Please read the following statements and ask any unclear questions before you agree to participate.

Participation in this study is exclusively voluntary. If you are not interested in participating with your child, or if you once decide to participate and draw your children at any time, there will be no consequences, and your child will get all the services provided in the hospital with no problems. If you decide to enroll your child, you have to sign the consent form, and you may obtain a copy of this information sheet.

What is expected from you and your child as a participant of the study?

As a participant in this study, your children are expected to agree that after a CSF specimen is collected for diagnostic purposes by a responsible physician using a lumbar puncture, it will be collected from your child. In addition, you are expected to give answers to some questions about your and your child's health and sociodemographic conditions. You need to know that the results might be discussed with appropriate individuals outside of this hospital. However, the name of you or your child, address, and phone number will not be disclosed, and rather than identification, a code will be used in such conditions.

How much time will my child and I spent to participate in this study?

You will spend 20-25 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

What are the risks of participating in this study?

There are no anticipated risks to your participation. Because I will took CSF specimen drawn for your children diagnostic test purpose. Therefore, there is no risk.

How our information is to be kept in secret?

All information that you give and the results from your child specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

What are the benefits from participation?

Since this study is MSc student research, there will be no payment for participants. However, your child participation is important for studying the prevalence bacteria's isolated from CSF specimen and their drug susceptibility to know the distribution of this bacteria and associated risk factors.

What are your rights as a participant of this study?

You have the right to withdraw your child from the study at any time and all the services provided in the hospital will not be discontinued. You have also welcomed if you have any question for further explanations about the study. You can get the results of the analysis.

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

- ✓ Please direct any questions/problems you may encounter during this study to Tadese Sisay
- ✓ Department of medical laboratory science, College of medicine and health sciences, Bahir Dar University, Bahir Dar, Ethiopia.
- ✓ Mobile: +251943399977; Email: tadesis2015@gmail.com
- ✓ For additional information, please contact Bahir Dar University, Medical faculty institutional review board (IRB) office.

Agree to participate? □ Yes □ No

Annex II- Subject information sheet (for mothers /guardians, Amharic version)

ባህር ዳር ዩኒቨርሲቲ,የጤና ሣይንስ ኮሌጅ, የሕክምና ላቦራቶሪ ሣይንስ ት/ት ክፍል

እድሜያቸው አስራ አራት አመት እና በታች በሆኑ ልጆቸ ላይ የህብለ ሰረሰር ፈሳሽ ተወስዶ ለሚሰሩ ባክቴርያወች ምን ያህል የተሰራጩ እንደሆነ ለማወቅ ጥናት ለሚሳተፉ ልጆች እናቶች/አሳዳጊዎች የተዘጋጀ መረጃ

በባህር ዳር ዩኒቨርሲቲ ህክምና እና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል የማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነዉን/ትን ማንኛዉም ሃሳብ ይጠይቁ፡

መግቢያ፡ የጥናቱ ርዕስ ‹‹ እድሜያቸው ≤14 አመት እና በታች በሆኑ ልጆች ላይ የህብለ ሰረሰር ፈሳሽ ተወስዶ ባክቴሪያ ምን ያህል የተስፋፉ እንደሆነ ለማወቅና ለህክምና ስኬታማነት የሚያሳድረው ተፅእኖ/ችግር/ ለማጥናት ነው፡፡ አላማዉም ይህ ባክቴሪያ ምን ያህል የተስፋፉ እንደሆነና ለህክምና ስኬታማነት የሚያሳድረው ተፅእኖ/ችግር/ ለማግኘት አማራጭ መንገድ ይሆናል፡፡ እርስዎ እና ልጅዎ በዚህ ጥናት ላይ የሚኖራችሁ ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡ከፈለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ፡፡

ልጄ የጥናቱ ተሳታፊ በመሆኑ የሚጠበቅበት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ለልጅዎ ለራሱ ምርምራ የህብለ ሰረሰር ፈሳሽ ናሙና ከልጅዎ ላይ ከተወሰደ የታዘዘለትን የላቦራቶሪ ምርመራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና እንደሚወሰድና ለጥናቱ እንዲዉል መስማማት ይጠበቅብወታል፡፡ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን እንዲሁም የልጅዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል ልጅዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎና ስለልጅዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

የተዘጋጀዉን መጠይቅ ለመሙላት፤የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ከ20-25 ደቂቃ ያስፈልጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡ናሙና የሚሰበሰበዉ ከልጅዉ ከተቀዳዉ የህብለ

ሰረሰር ናሙና ሃኪሙ ያዘዘለት ቤተ-ሙከራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና ነዉ።

የልጄ የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለ ራስዎ እና ስለ ልጅዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው፡፡ከዚያም በላይ ስለ እርስዎና ስለ ልጅዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም፡፡ ነገር ግን የእርስዎ ተሳትፎ ህጻናትን ለመርዳትና ባክቴሪያ ምን ያህል የተስፋፋ እንደሆነና ለህክምና ስኬታማነት ይረዳል፡፡

የልጄ በዚህ ጥናት ተሳታፊ መሆኑ መብቱ ምንድን ናቸው ?

በጥናቱ ውስጥ ያላችሁን ተሳትፎ በማንኛውም ጊዜ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ ልጅን ከጥናቱ በማግለል ምክንያት የሚቀርበት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ፡፡ከዚህም በተጨማሪ ጥናቱ በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለበት፡፡የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡ ታደሰ ሲሳይ

የህክምና ላባራቶሪ ሳይንስ ት/ክፍል: ባህር ዳር ዩኒቨርሲቲ

ሞባይል: Mobile: +251943399977

ኢሜይል: Email: tadesis2023@gmail.com

ለመሳተፍ ይስማማሉ? □ እስማማለሁ □ አልስማማም

ለተጨማሪ መረጃዎች የባህር ዳር ዩኒቨርሲቲ ህክምና ፋኩልቲ ኢንስቲትዩሽናል ሪቪው ቦርድ ይጠይቁ፡፡

Annex-III- Consent Form (for mothers/guardians, English version)

I have been informed about the study, which is aimed at determining the prevalence of bacterial isolates from CSF of suspected meningitis patients, and their drug susceptibility pattern and associated risk factors among children. For this study CSF sample is required from my children. The aims of the study were explained to me.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to keep hold of information, decline to cooperate and make my child withdraw from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use the CSF taken from my child for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my child sample analyzed free.

Participant code: _____ Signature: _____

Date: _____

For those who can't read the information

Advisor nurse name _____ Signature: _____

Date _____

Annex-IV - Consent form (for mothers/guardians, Amharic Version)

የተሳታፊወች ስምምነት ማረጋገጫ ቅጽ

እኔ ከላይ የተጠቀሰኩት የተሳታፊዉ እናት/ተንከባካቢ ‹‹**እድሜያቸው አስራ አራት አመት እና በታች በሆኑ ልጆች ላይ የህብለሰረሰር ፈሳሽ ተወስዶ ለሚሰራው ባክቴሪያ ምን ያህል የተሰራጩ እንደሆነ እና እነዚህ ታህዋስን/ባክቴሪያ/ ለምን ያህል ለተለያዩ መድሃኒቶች የተለማመዱ/የተቋቋሙ መሆናቸዉን ለማሳየት የሚጠና**›› ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ለጥናቱም ከልጆች የተወሰደ የህብለሰረሰር ፈሳሽ ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎችም ተረድቻለሁ።

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡ ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡፡ ከሰረሰርጌ አንጎል ላይ የሚወሰደው ለምርምር እንደሚውልም ተረድቻለው፡፡በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

የሚስጥር ቁጥር ---------- የተሳታፊዉ ወላጅ ወይም አሳዳጊ ፊርማ /የጣት አሻራ ---------- ቀን----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

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

Annex-V **Questionnaire (English version)**

Questionnaire for the study on 'Bacterial Isolates, Antimicrobial Susceptibility Profile, and Factors Associated with meningitis among Children in Bahir Dar, Northwest Ethiopia.'

Instruction: fill the information, either thick in the appropriate boxes by using "right" or a word or phrases where required. Code number ____

I. Socio-demographic factors and Patient Identification

S. no	Characteristics	Answer	Code
101	Age (in years)	----------	
102	Sex	Male	
		Female	
103	Residence	Urban	
		Rural	
104	Mother's educational level	Unable to write and read	
		Grade 1-8th	
		9-12th	
		Diploma	
		First degree and above	
105	Mother's occupation	Student	
		Employed	
		Jobless	
		Farmer	
		Daily laborer	
		Merchant	
		Others	
106	No of house partition	≥4	
		<4	
107	No of people sharing the same room with the child	<2	
		2-3	
		>4	
108	Does the child attend daycare or school?	Yes	

		No	

II. Health related information

S. No	Characteristics	Answer	Code
109	Mode of delivery	Spontaneous vaginal	
		Assisted vaginal	
		Caesarian section	
110	Duration of illness	≤a week	
		>a week	
111	Tonsillectomy	Yes	
		No	
112	Vaccination	Yes	
		No	
113	Previous drug use	Yes	
		No	
114	Admission history	Yes	
		No	
115	Comorbidity	Yes	
		No	
116	Malnutrition	Yes	
		No	
117	Maternal UTI	Yes	
		NO	
118	Duration of labor	<24hours	
		> = 24 hour	
119	Symptoms		

Annex-VI **Questionnaire (Amharic version)**

ባህር ዳር ዩኒቨርሲቲ: የጤና ሳይንስ ኮሌጅ: የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

እድሜያቸው ከ14 አመት እና በታች በሆኑ ልጆቸ ላይ የህብለ ሰረሰር ፈሳሽ ተወስዶ ለሚሰራው ባክቴሪያ ምን ያህል የተሰራጩ እንደሆነ እና እነዚህ ታህዋስን/ባክቴሪያ/ ለምን ያህል ለተለያዩ መድሃኒቶች የተለማመዱ/የተቋቋሙ መሆናቸዉን ለማሳየት የሚጠና ጥናት ለሚሳተፉ ልጆች እናቶች የተዘጋጁ መጠይቆች

የሚስጥር ቁጥር --------

ክፍል ፩፡

ተ.ቁ	አካባቢያዊና ማህበረሰባዊ ሁኔታዎች	መልስ	Code
101	እድሜ		
102	ጾታ	ወንድ	
		ሴት	
103	አሁን የሚኖሩበት አካባቢ?	ከተማ	
		ገጠር	
104	የእናት ወይም የአሳዳጊ የትምህርት ደረጃ	ማንበብ መጻፍ የማትችል	
		ከ1-8ኛ	
		ከ9-12ኛ	
		ዲፕሎማ	
		የመጀመሪያ ዲግሪ እና ከዛ በላይ	
105	የእናት የስራ ሁኔታ	ተማሪ	
		ተቀጣሪ	
		ነጋዴ	
		የቀን ሰራተኛ	
		ስራ የሌላት	
		ግብርና	
		ሌላ	
106	የቤታችሁ ክፍል ብዛት ስንት ነዉ?	≥4	
		<4	
107	ከልጅዎ ጋር አንድ ክፍል የሚጋሩ ሰዎች ቁጥር ስንት ነዉ?	<2	
		2-3	
		>4	
108	ልጁ በመዋዕለ ሕፃናት ወይም በትምህርት ቤት ይማራል?	ይማራል	
		አይማርም	

ክፍል ፪-የሰነ-ጤና መረጃ

ተ. ቁ	የሰነ-ጤና መረጃ	መልስ	Code
109	**ልጁ እንዴት ተወለደ?**	**ያለምንም እርዳታ**	
		እርዳታ ተደርጎለታል	
		በቀዶ ህክምና	
110	ልጅዎ ለምን ያህል ግዝ ታሞ/ታማ ነበር?	≤ አንድ ሳምንት	

		>አንድ ሳምንት	
111	ልጅዎ እንጥል ተቆርጦ/ተቧጦ ያውቃል	አዎ	
		የለም	
112	ልጅዎ ክትባት ወስዶ ነበር?	አወ	
		አይደለም	
113	ልጅዎ መድሃኒት በመዉሰድ ላይ ነዉ/ነች?	አዎ	
		አይወስድም	
114	ልጅዎ ከዚህ በፊት በጤና ተቋም መጥቶ ያውቃል	መጥቶ ያውቃል	
		መጥቶ አያውቅም	
115	ልጅዎ ተጨማሪ ህመም አለበት ወይ	አዎ	
		የለበትም	
116	ልጅዎ የምግብ እጥረት አለበት ወይ	አዎ	
		የለም	
117	እናት የሽንት ቧንቧ ህመም ነበረባት ወይ	አዎ	
		የለም	
118	በወሊድ ጊዜ የምጥ እርዝማኔ	<24 ሰአት	
		>24 ሰአት	
119	ምልክቶች		

መጠይቁን ጨርሰዋል::አመሰግናለሁ::

Annex VII– Laboratory data collection format

1. Patient ID. ----- 2. Appearance of specimen----------

3. Gram stain result----------4. Culture growth: Yes----------No----------

5. Name of bacteria, if isolated----------6. Biochemical identification test results-------

7. Antimicrobial susceptibility testing

Antibiotics	S (mm)	I (mm)	R (mm)
Amoxicilin-clavulanic acid			
Imipenem			
Ampicillin			
Ciprofloxacin			

Chloramphenicol			
Trimethoprim sulphonamde			
Penicillin			
Gentamycin			
Erythromycin			
Tetracycline			
Clindamycin			

Annex VIII-SOP for preparation of culture media, collection, and processing of specimens

1. CSF samples should be collected in a sterile tube by a responsible physician using a lumbar puncture and should be labeled properly.
2. Record the volume and gross appearance of the CSF, i.e., clear, bloody, cloudy, or xanthochromic.
3. Centrifuge the CSF specimen for Gram stain; centrifuge for 10 to 15 min at 1500–2000 rpm.
4. Using a sterile pipette, inoculate the media and streak for isolated colonies. In addition, add a single drop of fluid to the 4th quadrant of the plate and allow it to air dry.
5. Interpretation of Cultures: Examine the MacConkey, blood, and chocolate agar plates. Daily for 72 hours (Murray et al., 2020).

Laboratory procedures

Gram Stain for the CSF specimen

Purpose: Used to classify bacteria as gram positive and gram negative based on their gram reaction.

Principle: Gram's stain is that cells are first fixed to slide by heat or alcohol and stained with a basic dye (e.g. crystal violate), which is taken up in similar amounts by all bacteria. The slides are then treated with a Gram's iodine (iodine KI mixture) to fix (mordant) the crystal violet stain on

Gram positive bacteria, decolorized with acetone or alcohol, and finally counter stained with Safranin.

Procedure

1. Place the fixed slide on the staining rack.

2. Cover the heat-fixed smear with crystal violet; leave for one minute. Wash off with water and drain well.

3. Cover the smear with Gram's iodine; leave for one minute. Wash off promptly with water and drain.

4. Decolorizing agent (acetone/alcohol) over the slide for 30 seconds.

5. Cover the smear with basic fuchsin or safranin (red counterstain); leave for one minute. Wash with water and drain; air-dry in an upright position.

6. Leave until dry and look for a microscope (100 xs)

7. Report as gram positive (blue coccii or rods) and gram negative (red rods or coccii) and others accordingly (Murray et al., 2020).

Biochemical testing procedures

Identification of gram-positive bacteria

Catalase test: This test is used to differentiate those bacteria that produce the enzyme catalase, such as *staphylococci*, from non-catalase-producing bacteria, such as streptococci.

Principle: Catalase acts as a catalyst in the breakdown of hydrogen peroxide into oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old. **Method:**

1. Pour 2-3 ml of the H2O2 solution into a test tube.
2. Using sterile wooden sticks (swabs) remove several colonies of the test organism and immerse them in the hydrogen peroxide solution.

3. Look for immediate bubbling.

Bile solubility test: This helps to differentiate *S. pneumoniae,* which is soluble in bile and bile salts, from other alpha hemolytic *streptococci* (*viridian streptococci,* which are insoluble).

Principle: Heavy inoculums of a test organism are emulsified in saline, and bile salt sodium deoxycholate is added. This dissolves *S. pneumoniae* by clearing the turbidity within 10–15 minutes. Viridians and other *streptococci* are not dissolved, and therefore there is no clearing of turbidity. **Method:**

1. Emulsify several colonies on the test organism in a tube containing 2 ml of saline to give a turbid suspension.
2. Divide the bacterial suspension between two tubes.
3. In one tube, add two drops of the sodium deoxycholate reagent and mix.
4. To the other tube (the negative control), add two drops of sterile distilled water and mix.
5. Leave both tubes for 10–15 minutes at 35–37 °C.
6. Look for clearing of turbidity in the tube containing the sodium deoxycholate.

Coagulase test: The test is used to differentiate staphylococcus aureus (+ve) from other *staphylococcus* spp. (-ve).

Principle: In the presence of the enzyme coagulase, the addition of commercial rabbit plasma produces a clumping reaction. **Procedure**

1. Place a drop of physiological saline on two separate slides.
2. Emulsify the test organism in each of the drops 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.

Mannitol fermentation test

Mannitol salt agar is a differential and selective medium. When mannitol is fermented, acid products are produced, and the pH drops. Phenol red is yellow in color below pH 6.8. Thus,

mannitol fermenters such as *Staphylococcus aureus* will have a yellow halo around them. Mannitol non-fermenters such as *Staphylococcus epidermidis* will leave the MSA media unaltered (pink).

Identification of gram-negative bacteria:

Identification of gram-negative bacteria will be based on their test results through a series of biochemical tests. **Procedure**

1. Prepare a suspension of the test organism in nutrient broth by adding 3–4 colonies of the test organism to 5 ml of nutrient broth.
2. A loop full of the bacterial suspension is inoculated into indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, oxidase, urea agar, and motility medium. See them in detail below, one by one.
3. Incubate at 35–37 °C for 18–24 hours.
4. Look for color change (turbidity for motility) in the medium.
5. Identify the test organism by considering the results of biochemical tests.

Indole test

Principle: Some bacteria can produce indole from the amino acid tryptophan using the enzyme typtophanase. Production of indole is detected using Ehrlich's reagent or Kovac's reagent. Indole reacts with the aldehyde in the reagent to give it a red color. An alcoholic layer concentrates the red color as a ring at the top.

Procedure: The bacteria to be tested is inoculated in peptone water, which contains amino acid tryptophan, and incubated overnight at 37 oC. Following incubation, a few drops of Kovac's reagent are added. Kovac's reagent consists of para-dimethyl aminobenzaldehyde, isoamyl alcohol, and concentrated HCl. Ehrlich's reagent is more sensitive for detecting indole production in anerobes and non-fermenters. The formation of a red or pink-colored ring at the top is taken as positive.

Citrate utilization test:

Principle: This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator, bromthymol blue. The medium also contains inorganic ammonium salts, which are utilized as the sole source of nitrogen. Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down into pyruvate and CO2. Production of Na2CO3 as well as NH3 from the utilization of sodium citrate and ammonium salt, respectively, results in alkaline PH. This results in a change in the medium's color from green to blue.

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

Triple sugar iron agar (TSI) is used to determine if bacteria can ferment glucose and/or lactose and if they can produce hydrogen sulfide or other gases. In addition, TSI detects the ability to ferment sucrose. These characteristics help distinguish various *Enterobacteriaceae*, including *Salmonella* and *Shigella*, which are intestinal pathogens.

Procedure: Bacterial colonies are picked up from a straight wire, and the tube is inoculated by stabbing into the agar butt (bottom of the tube) with an inoculating wire and then streaking the slant in a wavy pattern. Results are read after 18 to 24 hours of incubation. TSI contains three sugars: glucose, lactose, and sucrose. Lactose and sucrose occur at 10 times the concentration of glucose (1.0% versus 0.1%). Ferrous sulfate, phenol red (a pH indicator that is yellow below pH 6.8 and red above it), and nutrient agar are present. A yellow slant on TSI indicates the organism ferments sucrose and/or lactose. A yellow butt shows that the organism fermented glucose. The black precipitate in the butt indicates hydrogen sulfide production. Production of gases other than hydrogen sulfide is indicated by either cracks or bubbles in the media or the media being pushed away from the bottom of the tube (Murray et al., 2020).

Test for Oxidase Using Filter Paper

A cytochrome oxidase test can be used to determine whether an organism possesses the enzyme.

1. Submerge a piece of filter paper in the reagent solution.

2. Scrape some new growth from the culture plate and place it on the filter paper using a disposable loop or stick. The edge of the paper can also be used to touch a colony.

3. In 10 seconds, check for the color blue.

Check for urease by testing: The enzyme Urease, which is produced by certain bacteria, catalyzes the hydrolysis of urea to create carbon dioxide and ammonia. Urea cannot be metabolized by organisms lacking this enzyme. Together with urea, a small amount of yeast extract is also present in urea broth. For organisms unable to metabolize urea, the nutrients required for growth will be inadequate. The hydrolysis of urea will occur.Because ammonia is created during urea hydrolysis, the pH will rise. The pH indicator phenol red turns pink as a result of this pH rise. However, because of the medium's strong buffering, a considerable quantity of ammonia production is required to cause a color shift. Consequently, the broth will only turn pink when using strong urea hydrolyzers, indicating a successful reaction. This should happen within the next day or two.

Techniques

1. Remove the two urease broths that were refrigerated.

2. Inoculate one broth using the aseptic technique. Leave the other broth uninoculated as it will serve as a control.

3. Decide on an appropriate incubation temperature (the one that your organism responds to the best). Incubate for 24 to 48 hours (but don't let it go past 48 hours) for this test.

4. Take out the broths from the incubator and examine their color.

Motility test: To test for motility, select a well-isolated colony and stab the motility medium with a sterile straight wire until it is within 1 cm of the tube's bottom. Make sure the straight wire stays in the same line as it entered the medium when you remove it. Incubate at 35°C for 24 hours or until growth is evident.A red, muddy area that spreads outward from the injection site signifies a

successful motility test. A negative test is shown by red development up to the injection line but not past it. Check out the in-depth methods listed below (Murray et al., 2020).

Methods

1. Obtain a motility agar tube from the back shelf.

2. Use an inoculating pick. Straighten the pick as much as possible.

3. Make a stab inoculation (about 2/3 of the way into the agar) from your unknown stock culture. Try to make the stab (in and out) as straight as possible. Straightness is important because you will be evaluating the amount of growth away from the stab. A messy stab will be difficult to evaluate.

4. Incubate at an appropriate temperature for 24 to 48 hours (up to 72 hours).

5. Observe your culture by holding it up to a light source.

H2S production: - Principle: Hydrogen sulfide (H2S) is produced by bacterial anaerobic degradation of the two sulfurcontaining amino acids, cysteine and methionine. Hydrogen sulfide is released as a byproduct when carbon and nitrogen atoms in the amino acids are consumed as nutrients by the cells. Under anaerobic conditions the sulfhydryl (-SH) group on cysteine is reduced by cysteine desulfurase. The agar contains high levels of peptones (sources of cysteine and methionine) and ferrous sulfate as an indicator. When H2S is produced, the ferrous ion reacts with it to give ferrous sulfide, an insoluble black precipitate.

Required: TSI Agar Slant, Inoculating loop and Incubator.

Procedure: 1. the triple sugar iron agar slant was inoculated by stabbing the butt and drawing the stick over the surface of the slope.

2. Incubated at 35-37oC for 18 to 24 hours. 3. Looked for black precipitate formed.

Result

- Acid deep (yellow)/alkaline slant (red):- glucose fermented, lactose and/or sucrose not fermented.
- Acid deep (yellow)/acid slant (yellow):- lactose and/or sucrose fermented.
- Alkaline deep and slant (all red):- glucose, sucrose, and lactose not fermented.
- Deep split or displaced: - gas production.

- Deep blackened: - H2S production.

VI. Antimicrobial susceptibility testing: - Procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth.

2. Much the turbidity of suspension with turbidity standard.

3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).

4. Spread the inoculums evenly over the Muller-Hinton agar plate with the swab

5. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate.

6. Incubate the plate aerobically at 35-37oC for 18-24 hours.

7. Read the test after checking that the bacterial growth is neither heavy nor light.

Measure the radius of the inhibition zone

8. Interpret the sensitivity reaction of the test organism to each antibiotics (CLSI, 2023).

Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm

Antibiotics	**Interpretation**	**Clinical Laboratory Standards Institute Minimum Inhibitory Concentration breakpoint/millimeter**			
		S. pneumonia	*N. meningitides*	*S. aureus*	*H. influenza*
Erythromycin (15ug)	Sensetive (S)	≥ 21		≥ 23	
	Intermediate (I)	16–20		14-22	
	Resistance (R)	≤ 15		≤ 13	
Tetracycline (30ug)	Sensetive	≥ 28		≥ 19	
	Intermediate	25–27		15-18	
	Resistance	≤ 24		≤ 14	
Ciprofloxacin(5 ug)	Sensetive	-	≥ 35	≥ 21	≥ 21
	Intermediate	-	33–34	16-20	-

	Resistance	-	≤ 32	≤ 15	-
Clindamycin (2ug)	Sensetive	≥ 19		≥ 21	≥ 29
	Intermediate	16–18		15-20	26-28
	Resistance	≤ 15		≤ 14	≤ 25
Chloramphenicol(30ug)	Sensetive	≥ 21	≥ 26	≥ 18	
	Intermediate	-	20–25	13-17	
	Resistance	≤20	≤ 19	≤ 12	
Ampicillin/Penicillin (10ug)	S				≥ 22
	I				19-21
	R				≤ 18
Ceftriaxone(30ug)	S		≥ 34		≥ 26
	I		-		-
	R		-		-

12. DECLARATION

I, Tadese Sisay, declare that this thesis, titled "Bacterial Isolates, Antimicrobial Susceptibility Profile, and Factors Associated with meningitis among Children in Bahir Dar, Northwest Ethiopia, "is my own original work, that it has not been presented for a degree at any other university, and that all sources of materials used for the thesis have been duly acknowledged.

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Date of Submission: ---------/----------/----------

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Chairperson:

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