# PREVALENCE, AETIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL NEONATAL MENINGITIS AT TIKUR ANBESSA SPECIALIZED HOSPITAL, ADDIS ABABA, ETHIOPIA

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**DECLARATION**

I certify that this Dissertation as my original work and has not been presented for a degree elsewhere.

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**ABSTRACT**

**Background:** Meningitis is inflammation of meninges, which affects all age groups from the newborn to elderly and occurs more commonly during the first month of life. The highest burdens of bacterial meningitis occur in an area of sub- Saharan Africa. Meningitis has been a problem in Ethiopia for the past decade and despite all the management advances the condition has remained constant.

**Objective:** To determine the prevalence, aetiology and antimicrobial susceptibility of bacterial neonatal meningitis at Tikur Anbessa Specialized Hospital.

**Design and Setting:** Descriptive cross sectional study which was conducted within 3 month of period at newborn unit of Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

**Methods:** Study subjects were neonates hospitalized at TASH due to neonatal sepsis with parental/guardian informed consent. Neonates with contraindication to lumbar puncture (LP) and failed LP were excluded. Case definition for sepsis was presence of one or more of the following signs: feeding problem, lethargy, abnormal cardiovascular, respiratory and neurological signs, temperature instability or skin change. Confirmed neonatal bacterial meningitis was defined as isolation of bacterial pathogen from the cerebrospinal fluid (CSF) by culture and/or visualization by gram stain and probable bacterial neonatal meningitis if a neonate had the specified clinical signs of meningitis without a confirmation with culture or gram stain. Neonates were enrolled consecutively until we attained the minimum sample size. Questionnaire was used to collect data on socio demographic characteristics and clinical features of the study subjects. Laboratory result pro-forma was used in collecting CSF analysis. Lumbar puncture was performed on one

hundred and seven neonates with sepsis before they were started on antibiotics or before the change to cephalosporin. Microscopy and cell count, culture and antimicrobial susceptibility tests were performed.

**Results:** We enrolled 115 neonates with suspected meningitis of whom 8 were excluded due to contraindications to LP or failed LP. Male to female ratio was 1.7:1, 71 (66.4%) were admitted into the hospital before or at the age of 7days, and 42 (39.3%) were born with a low birth weight. Median birth weight was 2750gm [Interquantile range (IQR) 2000-3300]; median postnatal age was 3 days (IQR 2-13) and median gestational age 37weeks (IQR 36-38). Feeding intolerance (76.6%), lethargy (49.5%) and abnormal respiratory signs (37.4%) were the most common clinical features observed. White cell count was high in 12(11.2%) of cerebrospinal fluid samples. Bacteria were isolated in Cerebrospinal of six neonates, of which two isolates were *Streptococcus Pneumoniae*, and the other 4 isolates were *Escherichia coli, Pseudomonas Aeruginosa, Klebsella Pneumoniae* and *Acinetobacter*. We diagnosed probable meningitis among 11/107 (10.2%) neonates and bacteriologic confirmed meningitis among 6/107 (5.6%), giving overall prevalence of meningitis of 15.8% [95%CI= 8.8% - 22.7%]. Of the 11 neonates with clinical suspected meningitis 6 (35%) had detectable bacteria in CSF. All isolated bacteria were resistant to ampicillin and gentamycin but were sensitive to ceftriaxone and cefotaxime.

**Conclusion:** Overall prevalence of meningitis among neonates with sepsis hospitalized at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia was 15.8% [95%CI = 8.9% - 22.7%]. Bacteria were detectable in 35.3% of neonates with clinical meningitis, and from 5.6% of all neonates with sepsis. Isolated bacteria were predominantly gram-negative. All bacterial

isolates were resistant to ampicillin and gentamycin, most were sensitive to third generation cephalosporins.

**ABBREVIATIONS**

| | |
|---|---|
| CI | Confidence interval |
| CSF | Cerebral spinal fluid |
| GBS | Group B *streptococcus* |
| gm | Gram |
| HSV | Herpes simplex virus |
| IV | Intravenous |
| L | Liter |
| LBW | Low birth weight |
| LONS | Late onset neonatal sepsis |
| LP | Lumber Puncture |
| LPA | Latex Particle Agglutination |
| MBC | Minimum bactericidal concentration |
| MIC | Minimum inhibitory concentration |
| NICU | Neonatal Intensive care unit |
| PCR | Polymerase chain reaction |
| RBC | Red blood cells |
| TASH | Tikur Anbessa Specialized Hospital |
| WBC | White blood cells |

# 1. INTRODUCTION

## 1.1. Background

Meningitis is inflammation of meninges. It is usually caused by viral, bacterial or fungal pathogens. Bacterial meningitis is potentially a life-threatening infection that is associated with high rates of morbidity and mortality (1,3). The burden of bacterial meningitis in developing countries of 1.1 – 1.9 cases per 1000 live births is very higher when it is compared to 0.2-0.5 cases per 1000 live births in western countries (2).

Meningitis affects all age groups from the newborn to the elderly. From its recognition in 1805 until the early 20$^{th}$ century, bacterial meningitis was invariably fatal. Until recently, up to 50% of patients who survived the acute infection were left with permanent sequelae such as mental retardation and hearing loss (4).

Meningitis occurs more commonly during first month of life (25). The disease has special characteristics during neonatal period. Signs and symptoms are non specific and indistinguishable from those of septicemia and other non-infective causes such as respiratory distress syndrome, birth asphyxia, and hypoglycemia, among others. This situation makes the diagnosis of meningitis difficult. Therefore, a high index of suspicion is necessary.

In developed countries, group B streptococci are found to be the most common aetiology of bacterial meningitis. Therefore, identifying and treating maternal genitourinary infection is being used as a prevention strategy. In the developing countries, gram-negative bacilli are more common than *Group B streptococcus*. The mortality varies based on the treatment, with survival rates being 17% to 29% and complication rates being 15%

to 68%. Despite the preventive measures and the availability of medicines, the incidence of newborn bacterial meningitis for the last 30yrs has remained constant (2, 5).

## 1.2. Statement of the Problem

Bacterial meningitis is a serious often disabling and fatal infection, which causes 170,000 deaths worldwide each year. It is a common infection often unrecognized and partially treated with sepsis. Due to immaturity of their immune systems, young infants are particularly vulnerable to bacterial meningitis and poor outcomes may occur.

Despite the development of, effective vaccines, useful tools for rapid identification of pathogens and potent antimicrobial drugs, neonatal meningitis continues to contribute substantially to neurological disability (7).

Africa experiences a disproportionally large burden of meningitis due to its young population. Bacterial meningitis in Africa is associated with high case fatality and frequent neuropsychological sequelae. Neonatal meningitis remains a serious problem with the high mortality of 60%. (8)

Though bacterial meningitis mostly occurs in neonates, only two studies on bacterial neonatal meningitis have been done in Ethiopia. One of the studies was done in 1998 and while the other was done in 2011.Both of these studies were retrospective studies with similar limitations, namely missing of some data in patients' medical records, as a result of which some of relevant variables in those studies were not studied (3,4).

Therefore, the purpose of this study is to determine the prevalence, aetiologic agents and antimicrobial susceptibility of neonatal bacterial meningitis.

## 2. LITERATURE REVIEW

### 2.1. Prevalence of Bacterial Neonatal Meningitis

Because of testing limitations, the worldwide incidence of neonatal meningitis is difficult to determine with accuracy. However, a study of neonatal infections in Asia (based on data collected from China, Hong Kong, India, Iran, Kuwait, and Thailand) reported estimated incidences of neonatal meningitis that ranged from 0.48 per 1000 live births in Hong Kong to 2.4 per 1000 live births in Kuwait  Another study that looked at neonatal infections in Africa and South Asia reported figures ranging from 0.8 to 6.1 per 1000 live births. These numbers are believed to be underestimates of the true incidence of neonatal meningitis in underdeveloped countries, given the lack of access to health care facilities in these areas. (7, 22)

### 2.2. Aetiology of Bacterial Neonatal Meningitis

Both the probable organisms and their likely mode of acquisition vary with the age at presentation with meningitis. Presentation in the first week of life (early onset infection) and particularly in the first two days of life reflects vertical transmission, while late onset infection suggests nosocomial or community acquired infection. The corresponding organisms are different; early onset meningitis is more likely to be caused by *group B streptococcus (GBS)*, *Escherichia coli*, and *Listeria monocytogenes*, while other Gram-negative organisms as well as *staphylococcal* species may cause late onset meningitis. Unfortunately, most case series on neonatal meningitis do not distinguish cases according to their age at onset (14).

The microorganisms causing neonatal meningitis not only vary between different countries, but also show temporal changes within the same country. In developed countries, infection with gram-negative bacilli accounts for 30-40% of meningitis cases, with *Escherichia coli* constituting the most common organism isolated (50%) of all gram-negative isolates, followed by *Klebsella Pneumoniae* (15). In developing countries, Gram-negative enteric organisms appear to account for the majority of early onset, and *Streptococcus pneumoniae* for late onset meningitis and *GBS* is less prominent compared to developed countries (14).

### 2.3. Antibiotic Susceptibility Pattern of Causative Agents

Appropriate antibiotic therapy is a critical aspect of management. The initial choice of antibiotics is empirical, based on age at onset, likely pathogens, and antibiotic susceptibility patterns, with a focus on *GBS*, *Escherichia coli*, other gram-negative organisms, and *Listeria monocytogenes*. Antibiotics are subsequently modified according to culture and antibiotic susceptibility result. Delay in CSF sterilization is a particular feature of gram-negative meningitis and may in part account for its higher mortality compared with the mortality from *GBS* infection. Sterilization of CSF is influenced by the dose *Group A streptococcus* of antibiotic that can be administered safely, the penetration of antibiotics into the CSF, and the minimum bactericidal concentration of the causative organisms (16)

*Group B streptococcus* is uniformly susceptible to penicillin, ampicillin and cephalosporin. It is usually resistant to aminoglycosides. It seems prudent to use the narrower spectrum agent, penicillin, in order to minimize any potential impact on antibiotic resistance among

other pathogens. Because *GBS* has a minimum bactericidal concentration tenfold higher than, and the inoculums in the CSF of neonates with meningitis is generally much higher than that in older infants and children with meningitis, it is recommended that large doses of antibiotics are administered. For ampicillin, the recommended dose is up to 300mg/kg/daily divided 8 hourly in infants<7 days of age or 4-6 hourly in infants >7 days of age. Penicillin or ampicillin is initially combined with gentamycin 4mg/kg/dose daily in 32-35 weeks' gestation babies or 5 mg/kg/dose daily in >35 weeks' gestation babies. The recommended doses of cefotaxime are 50mg/kg/dose 12 hours in babies <7days of age, 8 hourly in 7-21days old babies and 6-8 hourly in >21days old babies (14).

*Listeria monocytogenes* is not susceptible to cephalosporin. Ampicillin is the mainstay of therapy, and the combination of ampicillin and gentamicin is synergistic in vitro and provides more rapid bacterial clearance in animal models of infection. Thus, this combination is favored for initial therapy, with cessation of the aminoglycoside when the CSF has been sterilized and the patient has improved clinically (14).

Gram- negative enteric bacteria include *E.coli, klebsella, enterobacter, citrobacter, salmonella, proteus, pseudomonas, and serratia*. The infection caused by these organisms have for several decades been treated with the combination of ampicillin and aminoglycoside. However, these gram-negative organisms are frequently resistant to ampicillin; CSF aminoglycoside concentration is often minimally above their MICs and CSF culture remains positive longer than with *GBS* meningitis. This necessitates other therapeutic strategies such as intrathecal and intraventricular administration of antibiotics such as gentamicin especially in certain infants with obstructive ventriculitis complicating gram-negative meningitis that may require administration of intraventricular

aminoglycoside to assist in sterilization of the CSF, though this therapy is not recommended routinely. The introduction of cefotaxime and ceftazidime has provided an attractive option for therapy of gram-negative meningitis. This is based on the lower MBCs of gram-negative bacteria to cefotaxime compared to penicillin and aminoglycosides; high CSF concentration can also be safely achieved with cefatoxime. (14).

*Streptococcus pneumonia* is empirically treated with a combination of penicillin or ampicillin and cefotaxime although penicillin resistance does occur and may be increasing in frequency. Once *Streptococcus pneumoniae* is identified and susceptibility-testing results are available, therapy may be completed with the appropriate agent. *Streptococcus pneumoniae* infection is usually treated with a 2-week course of IV antibiotics, namely penicillin G for penicillin sensitive bacteria, ceftriaxone or cefotaxime for Penicillin-intermediate bacteria and ceftriaxone or cefotaxime plus vancomycin for penicillin-resistant bacteria (14).

## 2.4. Risk Factors of Bacterial Neonatal Meningitis

Neonates are at a greater risk of sepsis and meningitis than other age groups because of deficiencies in humeral and cellular immunity and phagocytic function, lower integrity of barriers, and immature defense mechanism. Infants born before 32 weeks of gestational age receive much less of the maternal immunoglobulin than the full-term infants. The defense against encapsulated bacteria is compromised in neonates because they have an immature and inefficient alternative complement pathway (7).

The development of sepsis and meningitis in the neonate depends on several risk factors in both the infant and the mother, as well as on the virulence of the pathogen. Prematurity, prolonged rupture of membranes, low birth weight, perinatal and intrauterine infections and maternal urinary tract infections are strongly associated with neonatal meningitis. The mode of infection of the neonate may be either hematogenous or directly through aspiration or inhalation of the pathogen. An early onset of neonatal bacterial meningitis (within the first week of life) indicates vertical transmission, whereas later onset is mainly caused by nosocomial infection (17).

## 2.5. Diagnosis of Neonatal Meningitis

### *2.5.1. Clinical Diagnosis of Neonatal Meningitis*

Symptoms seen with the neonatal meningitis are often unspecific that point to several conditions including sepsis that makes the diagnosis of meningitis difficult. Therefore, a high index of suspicion is needed. Neonatal meningitis can present with one or more signs and symptoms of sepsis, these include temperature instability, lethargy/irritability, feeding intolerance, abnormal cardiovascular and/or respiratory signs, Central nervous system abnormality signs/symptoms like seizure, bulging anterior fontanel, neck stiffness, abnormal posture and impaired neonatal reflexes (24).

To make a definitive diagnosis of meningitis CSF analysis is mandatory, but probable neonatal meningitis can be made if a neonate present with one or more of these signs and symptoms. These include convulsion, impaired neonatal reflexes, bulging anterior

fontanel and neck retraction, with no culture isolation or microscopic visualization of bacteria in the CSF (28).

Delayed diagnosis of neonatal meningitis is a potentially critical pitfall. Failure to perform a lumbar puncture and detect infection in a neonate with mild fever and minimal, nonspecific clinical findings is problematic. All neonates in whom meningitis might be the cause of symptoms should undergo CSF examination. Delay in treatment because of equivocal laboratory screening tests or because the findings altered by prior partial treatment may cause significant harm (7).

### *2.5.2. Microbiologic Assay of Cerebrospinal Fluid*

Suspected bacterial infection is often, but not uniformly, confirmed by positive results from cultures of cerebrospinal fluid (CSF) or blood. CSF cultures should be obtained in all symptomatic infants; despite the close relationship between bacterial sepsis and meningitis, it has been estimated that 15-30% of infants with CSF-proven meningitis will have negative blood cultures (18).

### *2.5.3. Immunologic Assay of Cerebrospinal Fluid*

Polymerase chain reaction (PCR) assay is a powerful diagnostic tool with excellent sensitivity and specificity. It permits identification of *GBS* antigen in urine or CSF, and it is the standard for identification of *herpes simplex virus (HSV)* and *enterovirus* in CSF. In neonates, PCR has a sensitivity of 71-100% for *HSV* and a specificity of 98-99% (19). For GBS antigen, PCR has sensitivity of 99.6% and 100% specificity and has a sensitivity of 85% and a specificity of 100% for *enterovirus* (29). Rapid screening is available with

latex particle agglutination (LPA) testing of urine, which can be performed for *GBS, Escherichia coli*, and *Streptococcus pneumonia* (7).

### *2.5.4. Biochemical Analysis of Cerebrospinal Fluid*

Cerebrospinal fluid glucose is normally approximately two-thirds of the fasting plasma glucose. A glucose level below 40mg/dL is significant and occurs in bacterial and fungal meningitis and in malignancy. Total protein levels in CSF are normally very low, and albumin makes up approximately two-thirds of the total. High levels (above 0.2-0.4gm/L) are seen in many conditions including bacterial and fungal meningitis, subarachnoid hemorrhage and traumatic tap (24).

The classic finding of decreased CSF glucose, elevated CSF protein, and pleocytosis is seen in gram-negative meningitis and in late gram-positive meningitis; these findings are also suggestive of viral meningitis, especially HSV. Only if all 3 parameters are normal does the lumbar puncture provide evidence against infection; no single CSF parameter exists that can reliably exclude the presence of meningitis in a neonate (20).

### *2.5.5. Microscopic Analysis of Cerebrospinal Fluid*

The number of white blood cells (WBCs) found in the CSF in healthy neonates varies according to gestational age. Many authors use a cut off value of 20-30cells/μL. Bacterial meningitis commonly causes CSF pleocytosis greater than 100cells/μL, predominantly polymorph nuclear leukocytes (PMNs). In neonates with viral meningitis, the picture may be similar but with a less marked pleocytosis. HSV meningitis may be particularly associated with a large number of red blood cells (RBCs) in the CSF (7).

### *2.6. Review of Published Research on Neonatal Meningitis*

#### *2.6.1. African Studies on Neonatal Meningitis*

In Nigeria, a three-year prospective study on clinical spectrum and characteristics of neonatal meningitis in a tertiary hospital was carried out. That study showed a high incidence of 1.9 per 1000 live births, and that infection was significantly more frequent among low-birth weight babies than among term babies. Non-specific signs and symptoms were common, and temperature instability was a constant finding. Specific neurological manifestations noted differed from those of other reports in the literature and contributed significantly to outcome. The most common aetiological gram- positive pathogen isolated was *Staphylococcus aureus* while the most common gram-negative organisms were *Klebsiella* species. *Group B streptococci* were not isolated. The mortality rate was 33 per cent and was higher for females. There was no significant difference in outcome between babies born in the hospital and referred infants, nor between early onset and late onset disease. Gentamicin and ceftazidime were the most appropriate antibiotics (21).

There was one retrospective study conducted in Addis Ababa University Teaching Hospital, Ethiopia. In a community-based retrospective study of neonatal meningitis, 55 cases were identified over a period of 10 years. The prevalence of meningitis for preterm and term newborns were 3.66 and 0.97 per 1000 live birth respectively ($p<0.01$) that means that the preterm birth was significantly associated with occurrence of neonatal meningitis. The overall prevalence was 1.37 per 1000 live births. 22(40%) babies with meningitis died, more preterm than term babies (13/22 Vs9/33; $p<0.05$). Known maternal risk factors for neonatal meningitis were observed in 15 (27%) babies. The risk factors

were more common in preterm than in term newborns (10/22 Vs 5/33; $p<0.05$). The common causative organisms were *Klebsiella pneumonia, Escherichia coli* and *enterobacter* species. These organisms together accounted for 67% of all CSF isolates. These organism were evenly distributed among early and late-onset meningitis, and among term and preterm newborns.7 of 33(21%) of the surviving newborns developed neurological complications (3)

A descriptive cross sectional study was carried out between August 8 and December 1 1999 at the newborn unit of Kenyatta national hospital, Nairobi, Kenya. The prevalence of meningitis amongst cases of suspected sepsis was 17.9%. The male: female ratio was 1.5:1 mean birth weight 2116.7 grams with a range of 1682.2-2551.2. The mean gestational age was 35.7 weeks (32.6-38.8) and the mean postnatal age was 4.1 days (2.7-5.4). Among the patients with meningitis, none of the CSF parameters were significantly different from those among patients without meningitis. Feeding difficulties or refusal to feed and lethargy were the most common clinical features, present in 73.3% and 60% of patients with meningitis respectively. Neonates with meningitis had a higher mean CSF protein value 2.67 g/L as compared to 1.97 g/L in neonates without meningitis,($p=0.367$) and a significantly higher mean CSF white cell count 21 cells/mL as compared to 7 cells/mL in neonates without meningitis ($p=0.001$). The most common aetiological agents were *Escherichia coli (46.7%). Group B. streptococci (26.7%) and Klebsiella pneumonia (13.3%).* Most blood and CSF isolates were resistant to ampicillin and gentamicin but showed good in-vitro sensitivities to amikacin, cefuroxime and the third generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime). Blood cultures were positive in only 53.3% of neonates with meningitis (2).

Retrospective analysis of 390 cerebrospinal fluid specimens submitted for culture and antibiotic susceptibility patterns to the bacteriology laboratory of Gondar University Teaching Hospital in Ethiopia was conducted between September 2002 and August 2003. Bacterial pathogens were isolated from 22 patients. The isolation rate was 5.6%. The most commonly isolated bacteria were *Neisseria meningitidis* 10(45.5%) and *Streptococcus pneumonia* 7(31.8%). Among gram-positive organisms, *Streptococcus pneumoniae* showed a high level of resistance to chloramphenicol 4(57%), tetracycline 3 (43%), co-trimoxazole 3(43%), ampicillin 3(43%), and gentamicin 1(14%). Among gram-negative bacteria, *Neisseria meningitidis* was found to be resistant to co-trimoxazole 5(50%), chloramphenicol 3(30%), gentamicin 3(30%) and ampicillin 2(20%). A single isolate of Proteus species was found to be resistant to co-trimoxazole and tetracycline. *Escherichia coli* was found to be resistant to all antibiotics except gentamicin and ciprofloxacin. Multiple drug resistance was observed in more than 50% of the isolates (*streptococcus pneumonia*, *Neisseria meningitidis* and *Escherichia coli*). No organism was found to be resistant to ciprofloxacin (1).

A ten years (2001-2010) review was carried out in 2011 at Tikur Anbessa Specialized Hospital in Addis Ababa, Ethiopia. Of 2510 culture specimens, 1321(52.63 %%) were from blood and 1189(47.37%) were from CSF. The study reported a bacteria isolation in 414(16.49) of the total 2510 suspected meningitis cases; 358(27.10%) were isolated from blood, while 56(4.71%) were isolated from CSF. The numbers of bacterial meningitis cases in each year from 2001 to 2010 were 41, 18, 16, 50, 54, 46, 44, 45, 40 and 56, respectively and the positive isolation rates in the same years were 13.6%, 14.6%, 17.0%, 25.1%, 20.8%, 26.1%, 15.5%, 15.5%, 12.8% and 12.6% respectively. The highest isolation rates were observed from the year 2004 to 2006. From the 414 cases of neonatal bacterial meningitis,

the isolated pathogens were *Coagulase-negative-staphylococcus* 148(35.7%), *Staphylococcus aureus* 65(15.7%), *Klebsiella pneumoniae* 50(12.8%), *Acinetobacter* 45(10.8%) and *Escherchia coli* 28(6.76%). *Coagulase negative staphylococcus* was the most predominant pathogen, it accounting for 148(35.75%) of all cases. *Staphylococcus aureus* and *klebsiella pneumoniae* accounted for 65(15.7%), 50(12.1%) respectively. More than 50% of the pathogens were isolated from preterm and low birth weight neonates (4).

### *2.6.2. Neonatal Meningitis in the Rest of the World*

During the period January 1980 to December 1990 (11 years), a retrospective study of patients with bacterial meningitis who were admitted into Bangkok Children's Hospital was carried out. There were 618 patients with 77 cases (12.5%) occurring below the age of one month (neonatal meningitis). *Pseudomonas aeruginosa* was the most common pathogenic organism (16.9%) in neonatal meningitis, other causative agents in this age group being *Klebsiella pneumoniae* (13.0%), *group B streptococcus* (11.7%), *Escherichia coli* and *Enterobacter s*pecies (10.4% each) (11).

A 3-year retrospective study on neonatal meningitis in the neonatal intensive care unit was conducted from 1988-1990 at the Mount Hope Women's Hospital, Trinidad, West Indies. Neonates were included in the study if organisms were cultured in their cerebrospinal fluid (CSF) and /or if there was a pleocytosis (>/=100/mm3) in their CSF. There were 49 neonates with meningitis out of a total of 17,048 live born infants during the 3-year period. The overall incidence of neonatal meningitis was 2.87/1000 live births. There were 34 male (63%) with mean birth weight of 2389g. The risks included preterm delivery (50%), and prolonged rupture of amniotic membranes (37%). Associated maternal conditions

included hypertension and ante-partum hemorrhage (9%). In contrast to other reported studies, there was early onset of the condition (mean age at presentation was 4 days) and the commonest organism found was *Group B streptococcus* while the least common organisms were Gram- negative bacteria (9).

A two and a half year prospective study of neonatal meningitis in the two main referral Hospitals in Northern Jordan was carried out during the period between January 1992 and July 1994 to determine the clinical and particular characteristics of meningitis in the newborn. During the two and half year study period, there were 47,669 live births in the catchment areas of the two Hospitals. There were 53 infants with neonatal meningitis, giving an incidence of 1.1 per 1000 live births. 42 patients had microorganisms cultured in their CSF, whilst the remaining 11 had positive blood cultures and significant pleocytosis despite their CSF cultures being sterile. Twenty-nine were boys and 24 were girls with a male to female ratio of 1.2:1. There were 24 preterm and /or LBW infants, whilst the rest were term infants. The mean age at presentation was 7 days (range 1-28).15 neonates were seen within 48 hours of birth (early-onset) while the remaining 38 patients presented more than 48hours after birth(late-onset). Gram-negative organisms were isolated most frequently (87%) with a predominance of *Klebsiella pneumonaie* (40%). 17 of the neonates died and 22 survived without any residual disability. Rates of mortality and neurological sequelae were higher among the preterm/LBW patients when compared with the rates among full term/normal birth weight group (38% v 28%) and (53% v 29%) respectively (12).

Prospective surveillance study was conducted from 1992–2002, in 20 neonatal units in Australia and New Zealand. Early onset neonatal bacterial meningitis was defined as

meningitis occurring within 48 hours of delivery. There were 852 babies with early onset sepsis, of whom 78 (9.2%) had early onset neonatal bacterial meningitis. The incidence of early onset group B streptococcal meningitis fell significantly from a peak of 0.24/1000 live births in 1993 to 0.03/1000 in 2002 (p =0.002). There was no significant change over time in the incidence of *Escherichia coli* meningitis. The rate of early onset neonatal bacterial meningitis among very low birth weight babies was 1.09/1000 live births compared with the rate of 0.11/1000 live births in all infants. Case-fatality rates for early onset neonatal bacterial meningitis did not change significantly with time. Birth weight less than 1500 g and Gram-negative bacillary meningitis were significant risk factors for mortality. Sixty two percent of the 129 babies who died from early onset sepsis or suspected sepsis did not have a lumbar puncture performed (10).

**Table 1: Summary of Literature Review on Neonatal Meningitis in the Rest of the World**

| **No** | **Author, Year** | **Study Design** | **Country** | **Sample Size** | **Findings** |
|---|---|---|---|---|---|
| 1 | May M, 2005 (10) | Prospective Surveillance | Australia | 852 | Prevalence of neonatal meningitis among sepsis - 9.2/1000 live births compared to 0.1 for other neonates |
| 2 | Chotpitayasunondh,1994 (11) | Retrospective Surveillance | Bangkok | 618 | Prevalence of neonatal meningitis among admitted meningitis -12.5%, *Pseudomonas aeruginosa* common bacteria isolated |
| 3 | Daoud A.S,1996 (12) | Retrospective Surveillance | Jordan | 47,669 | Incidence – 1.1/1000 live births. *Klebsiella Pneumoniae* was common bacteria isolated |

**Table 2: Summary of Literature review on Neonatal Meningitis in Africa**

| No | Author, Year | Study Design | Country | Sample Size | Findings |
|---|---|---|---|---|---|
| 1 | Airede,1993 (21) | Prospective Descriptive cross sectional | Nigeria | 36 | Incidence 1.9/1000 live births. Meningitis was seen more among LBW. *Staphylococcus aureus* & *Klebsiella pneumoniae* were common pathogen isolated. Gentamycin and ceftazidime were appropriate antibiotics. |
| 2 | Laving, 2003 (2) | Descriptive Cross Sectional | Kenya | 84 | Prevalence 17.9% among suspected neonatal sepsis. *Escherichia coli*, *GBS* & *klebsiella pneumoniae* isolated. Most isolates were resistant to ampicillin & gentamycin |
| 3 | Andargachew,2005 (1) | Retrospective Descriptive Cross sectional | Ethiopia | 390 | Prevalence of neonatal meningitis 5.6%. *Neisseria meningitides & streptococcus pneumoniae* were common pathogens isolated & were resistant to ampicillin, gentamycin & cotrimoxazole. |

## 2.7. Study Justification

It is known that microorganisms causing neonatal bacterial meningitis with their antimicrobial susceptibility vary from place to place as many studies have shown. So, this study will provide certain information on the prevalence, aetiologic agents and antimicrobial susceptibility of neonatal bacterial meningitis in this specified hospital.

Thus, the information on the sensitivity of organisms to antibiotics used empirically to treat neonatal bacterial meningitis will be an important input for developing effective treatment protocols with the aim of decreasing mortality and morbidity.

## 3. STUDY OBJECTIVES

### 3.1 Overall Objective

To determine the prevalence, aetiology and antimicrobial susceptibility of bacterial neonatal meningitis at Tikur Anbessa Specialized Hospital

### 3.2 Specific Objectives

i. To determine prevalence of neonatal bacterial meningitis among neonates admitted with clinically diagnosed sepsis at Tikur Anbessa specialized Hospital.
ii. To establish the aetiology of neonatal bacterial meningitis among neonates admitted with meningitis at Tikur Anbessa Specialized Hospital
iii. To determine the antimicrobial susceptibility of bacteria isolated among neonates with meningitis at Tikur Anbessa Specialized Hospital

# 4. MATERIALS AND METHODS

## 4.1. Study Site

This study was conducted at Tikur Anbessa Specialized Hospital (TASH), located in Addis Ababa, Ethiopia. Founded in 1964 Ethiopian calendar, TASH is a university teaching centre and a referral institution. It provides health services to more than 500,000 people from Addis Ababa and other parts of Ethiopia.

The Department of Pediatrics has 100-pediatric beds and admits approximately 2500 inpatients per year. Ambulatory services handle approximately 110,000 visits annually. Currently, there are21 permanent teaching staff and two part-time staff responsible for overseeing undergraduate and postgraduate medical education in the field of paediatrics. Among these university lecturers, only three are neonatologist.

The neonatal ward can accommodate as many as 60 patients. On average, it serves 20-40 patients daily and an additional 3-4 infants receiving Kangaroo Mother Care, with an annual average of 5000-6000newborn admissions. Nurse/patient ratio generally averages 1:4-5, with approximately 3-7 nurses on duty at any given time. Fifty percent of admissions are from outlying birth centres. Many referrals are premature and low birth weight infants. There is a facility for rooming in for mothers and a 5-bed Kangaroo mother care unit which serves as a teaching centre for Kangaroo mother care for preterm babies. The maternity ward is located close to the neonatal ward and delivers 4000-5000 babies annually.

## 4.2. Study Design and Period

This was a descriptive cross sectional study, which was conducted over a 3 month period between December 2013 and February 2014.

## 4.3. Source Population

Study subjects were drawn from neonates receiving care at TASH, and have included neonates born at the hospital, and sick neonates referred from other health facilities in Ethiopia. In general, neonates came from families of low and middle socio-economic status who are living in or close to Addis Ababa.

## 4.4. Study Population

### i. Inclusion Criteria

- Age: - from birth to 28days of life
- Hospitalized at Tikur Anbessa Specialized Hospital – Newborn ward
- Parental/guardian informed consent
- Disease condition – clinically diagnosed neonatal sepsis

***Case definition of neonatal sepsis*** – case was defined as neonatal sepsis if a neonate present with one or more of the following signs (24).

- Feeding problem – poor feeding or refusal to feed
- Vomiting, diarrhea, abdominal distension (any of these)
- Lethargy
- Cardiovascular signs- tachycardia, hypotension, bradycardia

- Respiratory signs - tachypnea, apnea, cyanosis, grunting
- Temperature instability (hyper/hypothermia)
- Skin change - pallor, petechiae, purpura
- CNS signs - Seizure, impaired neonatal reflexes, irritability, bulging fontanel, hypotonia, neck retraction (any of these).

**ii. Exclusion Criteria**

-All neonates with suspected neonatal sepsis without lumber puncture done because of contraindications to LP (severe cardio respiratory distress, extensive skin lesion on the LP site.)

-Parents/guardian refusal

-Neonates with failed LP.

**iii. Outcomes of Interest**

- Neonatal Bacterial Meningitis

✓ **Confirmed Meningitis**

Was defined as neonates who presented with one or more clinical signs and symptoms of sepsis with detectable bacteria from the CSF by culture and/or visualization by Gram stain

✓ **Probable Meningitis**

Was defined as neonates who presented with one or more clinical signs and symptoms of meningitis (convulsions, impaired neonatal reflexes, bulging anterior fontanel, neck retraction,) with no culture isolation or microscopic visualization of bacteria in the CSF

## 4.5 Sample Size

We used Fisher's formula for prevalence studies to estimate the required sample size

$$N=\frac{(Z_{1-\alpha/2})^2 \times P(1-P)}{D^2}$$

N = minimum sample size

P= estimated prevalence of neonatal bacterial meningitis

For this study P estimated at 7.5% by taking an average of local prevalence 9% and 5.6% done by Gebremariam and Andargachew respectively, in a similar population (4,1).

$Z_{(1-\alpha/2)}$ = 1.96 for 95% confidence interval

D=5% margin of precision error.

$$N = \frac{(Z_{1-\alpha/2})^2 \times P(1-P)}{D^2}$$

$$= \frac{(1.96)^2 \times 0.075(0.925)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.0693}{0.0025}$$

$$N = 107$$

We required a minimum of 107 neonates with suspected sepsis.

## 4.6. Sampling Technique

All neonates who fulfilled the inclusion criteria during the study period were enrolled into the study consecutively until the desired sample size was attained

## 4.7. Study Tools

Data collection questionnaire and Laboratory result pro-forma were the study tools. The questionnaire was used to collect important information on demographic characteristics and clinical features of the study subjects. Laboratory result pro-forma was used in collecting CSF analysis result microscopy and cell count, culture and antimicrobial susceptibility tests (Appendix I).

## 4.8. Study Procedures

This study was conducted at neonatal ward of TASH. The research assistants (Two medical interns who were doing their clinical rotation in the newborn unit during data collection), were oriented by the principal investigator on taking detailed history and performing a proper physical examination to attain the information required and trained on how to fill the questionnaire. The orientation and training took half a day.

The principal investigator selected neonates who satisfied the inclusion criteria, fully explained the study protocol to the parents/guardians of the neonates and got an informed written consent (Appendix II). Then the principal investigator with two medical interns who were doing their rotation in this ward during the study period performed a physical examination of eligible neonates and took a detailed history using the data collection questionnaire prepared for this study. The principal investigator reviewed, identified eligible neonates, interviewed the parents and examined the eligible neonates in the NBU daily accompanying the admitting intern.

### 4.8.1 Clinical Procedures

***Lumbar Puncture and Cerebrospinal Fluid Specimen Collection***

The principal investigator performed a lumbar puncture on eligible neonates as follows:

- A lumbar puncture tray was availed and had two specimen bottles (a plain sterile and a fluoride bottle), #22 or #23 gauge lumbar puncture spinal needles.
- The infant was placed in a lateral position with spine flexed by one of medical intern or nurse.
- The principal investigator scrubbed.
- The principal investigator wore a gown and sterile gloves
- The skin was disinfected along a line drawn between the crests of the two iliac crests with 70% alcohol and povidone-iodine to clean the surface and remove debris and oils, and was then allowed to dry.
- The infant was draped with sterile towels.
- At the level of the iliac crest, the intervertebral space was palpated between L4-L5
- Spinal needle gauge 22 or 23 was inserted slowly with stylet in place into the intervertebral space, toward the umbilicus. Two fingers were used to guide the needle and thumbs to slowly advance. One millimeter at a time and stylet was withdrawn frequently to check for CSF flow.
- One ml of CSF fluid was collected in each of the two sterile bottles, the stylet was reinserted then the needle was removed. One bottle for cell count and gram stain while the other one was for culture and sensitivity.
- Pressure was applied at the puncture site, antiseptics were cleaned from the skin and band-aid was placed over site.

The CSF pressure was not measured because of unavailability of manometer in the facility. The infant's cardiac and respiratory status was monitored throughout the procedure, as airway obstruction could have occurred due to positioning for the procedure.

### 4.8.2 Laboratory Procedures

#### i. *Cerebrospinal Fluid Specimen Handling*

CSF containing bottles were transported to a microbiology laboratory as soon as possible and not later than 30 minutes after the lumbar puncture. The appearance of the CSF was recorded even before taking it to the laboratory. CSF was termed as turbid if one could not read well a letter through the CSF bottle.

#### *ii. Cerebrospinal Fluid Cell Count and Microscopy*

CSF cell count examination was done following the standard procedure and using the counting chamber of an improved Neubauer chamber. Gram stain was also prepared following the standard procedure.( Appendix III ).

Gram-positive organisms appeared dark violet or purple. Gram-negative organisms appeared red or pink (from the counter stain) and were reported accordingly.

#### *iii. Cerebrospinal Fluid Bacterial Culture*

CSF culture was done according to the following procedure:

- The fresh CSF was centrifuged for 10 minutes at 3000 revolutions per minute to get the sediment of centrifuged CSF.
- At least 20-50μL of the sediment was inoculated with a sterile pipette on to chocolate, blood agar.

- The solid culture media was incubated for at least 72hrs at 35-37$^0$C in candle extinction jars to provide 5-8% carbon dioxide.
- Growth was checked every 24 hours for 3days.

### *iv. Antimicrobial Susceptibility Testing*

Antimicrobial susceptibility testing was performed using the modified disc diffusion method (modified Kirby-Bauer technique). This method used Müeller-Hinton agar. (Appendix IV)

Antibiotics tested in this study included, ampicillin, Gentamycin, chloramphenicol, ceftazidime, ceftriaxone and cefotaxime. Results were interpreted based on criteria of NCCLS (National committee on clinical laboratory standards) (23).

## 4.9. Data Management and Analysis

The data collected using the data collection questionnaire (Appendix I), was coded and entered into computer using Statistical package for Social Science (SPSS) windows version 20. The data was checked for completeness and analysis was done using the same statistical software program. Maternal and infant characteristics were converted to Categorical format. Post- natal age was categorized into age group ≤ 7 days and 8-28 days. Birth weight was categorized into weight group <2500gm and ≥2500gm. Gestational age was categorized into <37weeks and ≥37weeks and place of birth was categorized into home/on the way and health facility. Maternal literacy was categorized into literate and illiterate, rupture of membrane duration was categorized as into <24 hours and≥24 hours.

Maternal fever was categorized into a group with fever during pregnancy/intra-partum and a group without fever.

The overall prevalence of neonatal bacterial meningitis was computed by adding the prevalence of confirmed neonatal meningitis and probable neonatal meningitis. The prevalence of confirmed neonatal meningitis was computed by taking the number of neonates with CSF culture proven meningitis as a numerator and all neonates with sepsis enrolled in this study as denominator. Prevalence of probable neonatal meningitis was computed by using number of neonates with probable meningitis as numerator and all neonates with sepsis as a denominator. 95% CI for each prevalence was computed using confidence interval calculator for proportion.

Aetiology data was analysed by stating the frequency of all isolates and the isolates were categorized into gram positive and gram negative when reporting. Antimicrobial susceptibility test was performed for each isolate and was reported accordingly. A 2x2 chi- square statistical analysis was used to compare selected variables (Preterm, LBW, LONS, Sex, maternal PROM, maternal fever) that were thought to be associated with developing neonatal meningitis and odds ratio was determined.

Then results were presented in descriptive form using frequency tables and figures from which conclusion and recommendation were made. Results were compared with the findings in other studies and discussed.

### 4.10. Ethical Considerations

This research project work was approved by ethical and review committee of University of Nairobi and Departments of Pediatrics and Child Health of both University of Nairobi

and Addis Ababa University. Permission was obtained from Tikur Anbessa Specialized Hospital Administrator to conduct the study. All essential ethical considerations to ensure the confidentiality of the identity of the patients were taken. A letter informing the medical director of TASH about the objective of the study was written from the Department of Pediatrics and Child Health of University of Nairobi prior to data collection.

***Informed consent***

The parents/guardians of the patients had the details of the study fully explained to them before recruitment followed by consent through signing of the written informed consent form (Appendix II)

***Autonomy***

The study was carried out only after informed consent was obtained. Participants were free to withdraw from the study at any stage without penalty. There were no additional costs for participation in this study.

***Risks***

The study had no major risks except some discomfort during positioning for lumbar puncture, during this procedure we monitored cardio respiratory condition of the neonate.

***Safety***

This study didn't interfere with or delay management of a severely ill neonate.

***Benefit***

This study helped the doctors specially the research assistants (two medical interns) to know and explore more about neonatal meningitis. Principal investigator in addition to the primary care provider followed CSF laboratory results of eligible neonates.

***Confidentiality***

Eligible neonates were assigned a study number at the beginning of the study, which was used as identification of the patient throughout the study rather than the patients name or inpatient number. Written data was stored in a cupboard, which has a key accessible to only research assistants and the principal investigator. Data entry was done daily using SPSS Windows version 20 into a computer which was password protected to restrict access.

***Data Sharing Plan***

Analyzed data has been presented to the Department of Paediatrics and Child Health. This study will be published in a peer-reviewed journal and presented in different scientific conferences.

### 4.11. Study Limitations

This study has assessed/tested susceptibility of only six antimicrobials due to unavailability of some discs in the Hospital, which is not exhaustive.

The machine for biochemistry analysis was not functional at the time of data collection that restricted this study from reporting biochemistry analysis of CSF

Research assistants were busy during the night, which made the study unable to enroll eligible neonates at night.

# 5. RESULTS

We prospectively enrolled 115 neonates admitted with suspected neonatal sepsis at Tikur Anbessa Specialized Hospital over the period of December 1st 2013 to March 1st 2014. Among these 115 neonates that satisfied the inclusion criteria, 8 of them were excluded for different reasons (5 had failed lumbar puncture and 3 had clear contraindications to lumbar puncture), therefore, 107 neonates were analyzed and reported in results.

## 5.1. Descriptive Characteristics of the Study Population

Of the 107 neonates enrolled 63.6% were male, and 71 (66.4%) presented to the hospital before or at the age of 7 days and 36 (33.6%) between ages 8-28 days. Forty- two (39.3%) were born with a low birth weight (<2500gm), 68(63.6%) were born at term and 102(95.3%) of the neonates were born in the health facility (Table 1).

As shown in the table 3 most of the mothers were literate 84(78.5%) 40% of them completed secondary level. Maternal fever during pregnancy was observed in 18.7% of mothers and prolonged rupture of membrane was seen in 10(9.3%).

**Table 3: Sociodemographic and Clinical Characteristics of Study Subjects (N=107)**

| Characteristics | | Frequency | Percent |
|---|---|---|---|
| **Maternal Characteristics** | | | |
| Maternal literacy | Illiterate | 23 | 21.5 |
| | Literate | 84 | 78.5 |
| Maternal fever | Yes | 20 | 18.7 |
| | No | 87 | 81.3 |
| ANC follow up | Positive | 103 | 96.3 |
| | Negative | 4 | 3.7 |
| Duration of labor | <12 hrs | 74 | 69.2 |
| | ≥ 12 hrs | 33 | 30.8 |
| Rupture of membrane | <24 hrs | 97 | 90.7 |
| | ≥24 hrs | 10 | 9.3 |
| **Neonate Characteristics** | | | |
| Sex | Male | 68 | 63.6 |
| | Female | 39 | 36.4 |
| Post natal age | ≤7 days | 71 | 66.4 |
| | 8-28 days | 36 | 33.6 |
| Birth weight | <2500gm | 44 | 41.1 |
| | ≥2500gm | 63 | 58.8 |
| Gestational age | <37wks | 39 | 36.4 |
| | ≥37wks | 68 | 63.6 |
| Place of birth | Home/on the way | 5 | 4.7 |
| | Health facility | 102 | 95.3 |

## 5.2. Prevalence of Neonatal Bacterial Meningitis

### i. Clinical Features of the Study Subjects.

Neonates presented with the following non-specific clinical features of neonatal sepsis: feeding intolerance (76.6%), lethargy (49.5%) and respiratory signs (46.7%), temperature instability (39.3%) and skin change (3.7%).

Clinical features specific to meningitis were seen as follows: convulsion in 9(8.4%), neck stiffness/retraction in 6(5.6%) and bulging fontanel in 8 (7.4%).

**Table 4**: **Clinical Features of the Neonates Admitted With Sepsis (N= 107)**

| **Clinical features** | **Frequency** | **Percentage** |
|---|---|---|
| **Features of Neonatal Sepsis** | | |
| Feeding intolerance | 82 | 76.6 |
| Lethargy | 53 | 49.5 |
| Respiratory signs | 50 | 46.7 |
| Temperature Instability | 42 | 39.3 |
| Skin change | 4 | 3.7 |
| **Features specific to meningitis** | | |
| Convulsion | 9 | 8.4 |
| Neck stiffness/retraction | 6 | 5.6 |
| Bulging fontanel | 8 | 7.4 |

### ii. Laboratory Results of Cerebrospinal Fluid of the Study Subjects

One hundred and seven CSF samples were analyzed. The analysis included CSF cell count, microscopy and culture with antimicrobial susceptibility. For microscopy (gram stain) was

not done on 5 samples because they were blood stained. But CSF cell count, culture and antimicrobial susceptibility was performed in all 107 samples.

**a. Cell Counts in Cerebrospinal Fluid**

CSF cell count was performed on 107 CSF samples. High CSF WBC count was defined as > 30 cells/μL. For any blood stained sample high CSF WBC count was defined as presence of WBC/RBC ratio > 1:600. Twelve (11.2%) of 107 neonates had high CSF WBC counts as per the definition.

**b. Bacterial Pathogens Detection Methods**

The table and figure below (see Table 5 and figure 1) shows the methods that were used to detect the pathogens isolated in this study. Six bacteria were detected by CSF culture and 2 were by Gram-stain. The two bacteria isolates that were detected by microscopy were also confirmed by culture. The five samples were excluded from gram stain because of their being blood stained it was difficult to make proper slides of the blood stained samples.

**Table 5: Cerebrospinal Fluid Microscopy in Neonates with Sepsis**

| **Methods** | **N** | **Characteristics** | **Number (%)** |
|---|---|---|---|
| Microscopy | 102** | Gram positive * | 2(1.9) |
| | | Gram negative | 0 |
| Culture | 107 | Positive | 6 (5.6) |
| | | Negative | 103(96.2) |
| Total Positive | | | 6(5.6) |

* Both these were also positive on culture ** Gram stain unsuccessful in 5 samples

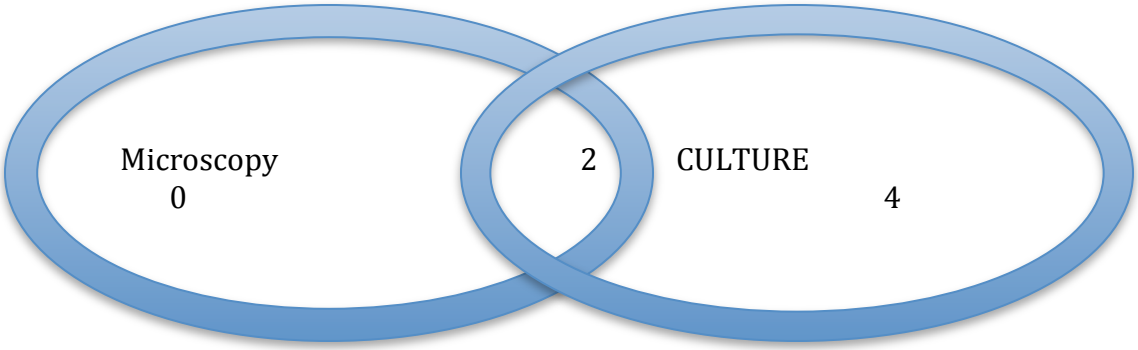


**Fig 1: Cerebrospinal Fluid Microscopy in Neonates with Sepsis.**

### iii. Prevalence of Neonatal Bacterial Meningitis

In this study, bacterial neonatal meningitis was subdivided into probable meningitis and confirmed meningitis.

***Probable Meningitis***

A case of probable neonatal bacterial meningitis was defined as neonates who presented with one or more clinical signs and symptoms of meningitis (convulsions, impaired neonatal reflexes, bulging anterior fontanel, neck retraction) with no culture isolation or microscopic visualization of bacteria in the CSF. As Figure 2, eleven neonates had probable meningitis, which gave a prevalence of probable meningitis of 10.2% [95%CI= 4.5%-15.9%].

***Confirmed meningitis***

Six neonates presented with signs and symptoms of sepsis and also had detectable bacteria from the CSF by culture and/or microscopy; this gave a prevalence of confirmed neonatal bacterial meningitis among neonates with sepsis of 5.6% [95%CI= 1.2% - 9.9%].

17 neonates presented with signs and symptoms specific to meningitis and of these 6 had microbiologic evidence of bacteria in their CSF, giving a prevalence of confirmed meningitis of 35% among those with clinically suspected meningitis.

Combining neonates with probable (11 neonates) and confirmed (6 neonates) meningitis we report an overall prevalence of probable plus confirmed neonatal bacterial neonatal meningitis was 15.8% [95%CI= 8.9 - 22.7%].

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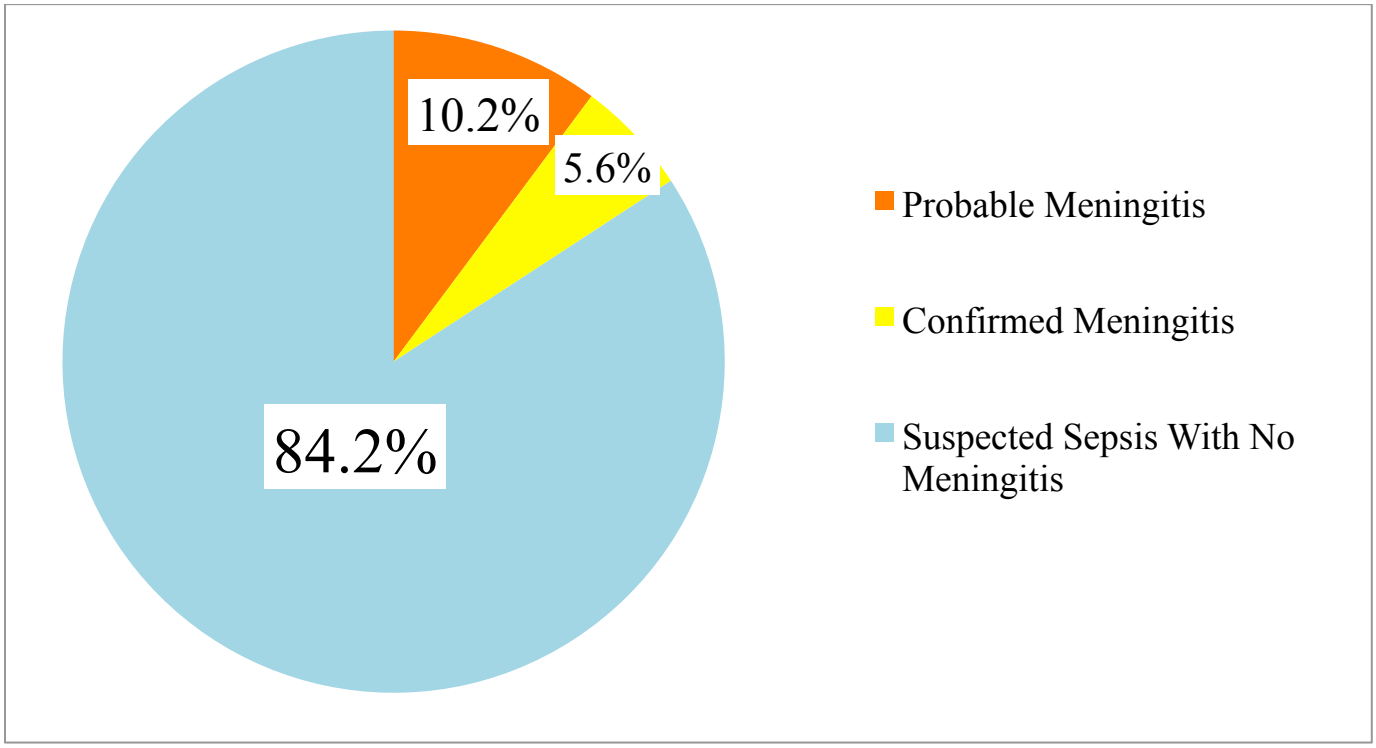


**Fig 2: Prevalence of Neonatal Bacterial Meningitis Among Neonates With Suspected Sepsis**

### 5.3. Aetiology of Neonatal Bacterial Meningitis

Bacterial culture of CSF was positive in six neonates (5.6%). Four of these isolated bacteria were *E.coli, Pseudomonas, Klebsiella pneumonia, Acinetobacter* while the other two were *Streptococcus Pneumoniae* (Table 7). Among these isolates, *Pseudomonas* and

*E.coli* were isolated from neonates who had early onset meningitis (age 0 - 7 days) while the two *Streptococcus pneumoniae, Klebsiella* and *Acinetobacter* were isolated from newborns who had late onset meningitis (age 8 - 28 days). Seventy- five percent of the Gram negative bacteria (*Klebsiella, E.coli* and *Acinetobacter* were isolated from preterm neonates but the two Gram positive (two *Streptococcus pneumoniae*) cultures were from one preterm and one term neonate respectively.

**Table 6: Aetiology of Neonatal Bacterial Meningitis Among Suspected Neonatal Sepsis**

| Bacteria Type | Bacteria | Number | Number of neonates |
|---|---|---|---|
| Gram- Positive | *Streptococcus Pneumoniae* | 2 | 2 |
| Gram- Negative | *Escherichia Coli* | 1 | 1 |
| | *Pseudomonas* | 1 | 1 |
| | *Klebsiella Pneumoniae* | 1 | 1 |
| | *Acinetobacter* | 1 | 1 |

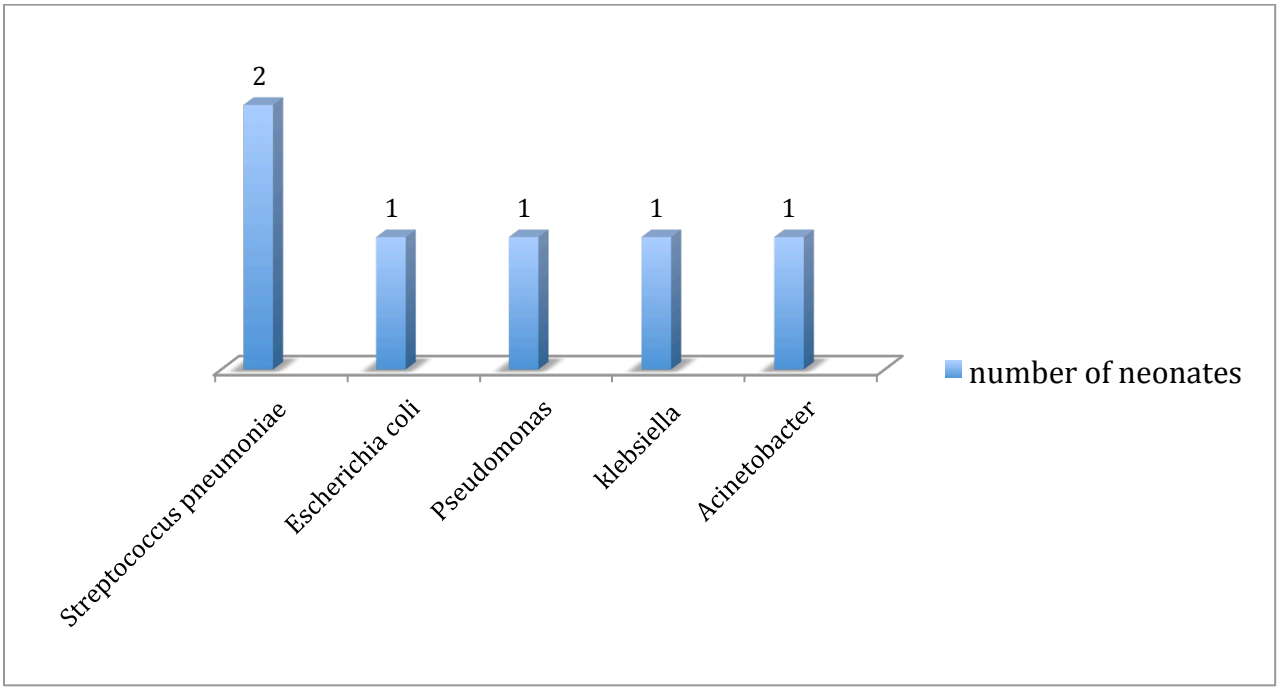


**Fig 3: Bacteria Isolated From Cerebrospinal Fluid of Study Subjects**

### 5.4. Antimicrobial Susceptibility of Isolated Pathogens

Gram Positive bacteria were sensitive to cephalosporins and resistant to ampicillin, gentamycin and chloramphinicol. Gram-negative bacteria were sensitive to cephalosporins and resistant to ampicillin, gentamycin and chloramphenicol (Table 7).

**Table 7: Antibacterial Susceptibility of Pathogens Isolated in the Study Subjects**

| | **Gram- Positive** | **Gram- Negative** | | | |
|---|---|---|---|---|---|
| **Antimicrobial** | *Streptococcus pneumoniae (n=2)* | *Escherichia coli* | *Pseudomonas* | *Klebsiella pneumoniae* | *Acinetobacter* |
| Ampicillin | R/R | R | R | R | R |
| Gentamicin | R/R | R | R | R | R |
| Chloramphenicol | R/S | R | R | R | S |
| Ceftazidime | S/S | S | S | S | S |
| Ceftriaxone | S/S | S | S | R | S |
| Cefotaxime | S/S | S | S | S | S |

R= Resistant S= Sensitive

# 6. DISCUSSION

## 6.1. Description of the Study Population

Male to female ratio of 1.7:1 seen in this study was similar to the male to female ratio reported in the study on bacterial isolates from cerebrospinal fluid and their antibiotic susceptibility pattern in Gondar University Teaching Hospital, Northwest, Ethiopia and in the study on neonatal bacterial meningitis at the newborn unit of Kenyatta National Hospital, Nairobi (1,2). Four neonates out of the six confirmed meningitis presented as LONS in this present study, a finding which is consistent with the fact that bacterial meningitis occurs in as many as 15% of neonates with bacteremia and among those patients 5-10% present as early onset and 25% of neonates present as late onset meningitis (26). Our finding is also in agreement with the findings in the two and half year prospective study on neonatal bacterial meningitis in north Jordan, yet they used 48 hours as a cut point for Late onset neonatal sepsis (12). Both prematurity and LBW were not found to be significantly associated factors with the presence of meningitis, this finding was similar to what Laving et al found in their study at Kenyatta National Hospital, Kenya (2). Our finding of no association between prematurity and LBW with neonatal meningitis is the opposite of the known fact that low birth weight, preterm delivery and maternal urinary tract infection are among the common risk factors for neonatal bacterial meningitis (17). This difference can be explained by our low sample size and isolates, which made it difficult for the study to show any causal relationship. Maternal fever, which was seen in 20 mothers, was significantly associated with neonatal meningitis (OR=4.14 95%CI 1.33-12.84, P=0.01), it being noted that 7 of the newborns of the 20 mothers with fever had

neonatal meningitis. This finding is in agreement with the known fact that maternal fever is a risk factor for meningitis (17).

### 6.2. Prevalence of Neonatal Bacterial Meningitis

The most common clinical features observed in this study were similar to those found in study done on neonatal bacterial meningitis at Kenyatta National Hospital newborn unit, Nairobi Kenya in year 1999 by Laving et al (2). The reason that made feeding intolerance to be the most common clinical feature in our study could be explained by the fact that we didn't exclude neonates who had prematurity related feeding intolerance or inability to breast feed.

High CSF WBC count was seen in 12(11.2%) of neonates with suspected meningitis and out of these, 11 of them had meningitis. This finding agrees with the known fact about the WBC count in CSF when there is bacterial meningitis (7).

Bacteria were isolated by Gram-stain in only 2 samples. Our rate of bacteremia isolation by gram stain was much lower when it is compared with 68% of gram stain bacteria isolation by Hristeva et al (27). Our rate of isolation by gram stain is the same as the one reported by laving et al in their study neonatal bacterial meningitis at Kenyatta National Hospital in Kenya (2). The rate of bacteremia by culture is the same as the one reported by Laving et al.

The prevalence of overall neonatal bacterial meningitis was 15.8%, which is a bit lower than the previously reported prevalence rate of 16.5% (4) in Melese's study in Ethiopia and also lower than prevalence rate of 17.9% reported in Kenya (2). This difference can be explained by the difference in methods of detecting bacteria and sampling technique. In

this present study, we only analyzed CSF samples and used specific clinical features of meningitis but in the above 2 studies by Laving et al and Melese's study who included blood culture isolates too which eventually increase the overall prevalence. Laving et al also used LPA for detecting bacteria in CSF, an antigen detecting kit.

The prevalence of confirmed neonatal bacterial meningitis of 5.6% in this study was comparable to the prevalence of 5.7% reported in the study on bacterial isolates from cerebrospinal fluid in university of Gondar Teaching Hospital, Northwest Ethiopia (1).

### 6.3. Aetiology of Neonatal Bacterial Meningitis

No *group B streptococci* were isolated in this study; this corresponds to what is known that group B streptococcus appears to be much less frequent cause of neonatal meningitis in developing countries. In this study, Gram- negative enteric bacteria accounted for 50% of organisms isolated. For the confirmed early onset neonatal bacterial meningitis pseudomonas and *Escherichia coli* were the enteric Gram-negative organisms, which were isolated. In two subjects, *streptococcus pneumoniae* was isolated in late onset. These finding are consistent with the study done by Heath et al which have reported that gram negative enteric organisms appeared to account for the majority of early onset bacterial meningitis and *streptococcus pneumoniae* for late onset meningitis in developing countries (14). We isolated both *Escherichia coli* and *Klebsiella pneumoniae* in this prospective study and this finding is in agreement with previous study done in Addis Ababa. Ethiopia (3).

### 6.4. Antimicrobial Susceptibility of Isolated Pathogens

All bacterial isolates in this study were susceptible to ceftriaxone, ceftazidime and cefatoxime except for one gram-negative bacteria isolate which was resistant to ceftriaxone, this is consistent with the finding reported by Laving et al in their study done in Kenya where by the majority of gram negative isolates were highly resistant to the first line antibiotics, ampicillin and gentamycin (2) however in Tikur Anbessa Specialized Hospital, ampicillin and gentamycin were prescribed as a treatment for majority of neonatal bacterial meningitis cases as the report by the previously done local retrospective study (4) and still now the practice is the same. Our findings also agrees with those reported by Andargachew et al in their study in Gondar University, Northwest part of Ethiopia, which reported resistance to commonly prescribed antibiotics ampicillin and gentamycin for bacterial isolates from CSF, even though this study included all age group of patients (1).

The limitations of this study included unavailability of some discs in the hospital for different antimicrobial susceptibility testing, which has restricted the number of drugs tested. Biochemistry analysis could not be performed in this study, as the machine was not working during data collection period. We were unable to enroll eligible neonates at night. Our having studied neonates with birth asphyxia in this study this might affect the frequency of clinical feature.

The strength of this study included standardized quality of sample collection with handling and transport to the laboratory. In addition, since the study was carried out prospectively no missed data of eligible neonates encountered.

In general, the prevalence of neonatal meningitis among neonates with sepsis at TASH was 15.8% of which 5.6% was bacteriologically confirmed. Bacterial isolation revealed gram-negative predominance. Both gram-positive and gram- negative isolates were susceptible to cephalosporin and resistant to penicillins and aminoglycosides tested. From this we recommend that neonates in Addis Ababa and its environment, presenting with specific signs/symptoms of meningitis should be empirically treated with cephalosporins as first line therapy as laboratory tests are undertaken.

# 7. CONCLUSIONS AND RECOMMENDATION

## Conclusion

1. The prevalence of meningitis among neonates hospitalized with clinically diagnosed sepsis in TASH, Ethiopia, was 15.8% of which 5.6% was bacteriologically confirmed.
2. Bacteria were detectable in 35.3% of neonates with clinical meningitis, and from 5.6% of all neonates with sepsis.
3. Isolated bacteria were predominantly gram negative.
4. Both gram-positive and gram- negative isolates were susceptible to cephalosporin and resistant to penicillins and aminoglycosides tested.

## Recommendation

- We recommend that neonates in Addis Ababa and its environment presenting with specific signs/symptoms of meningitis should be empirically treated with cephalosporins as first line therapy as confirmatory microbiological tests are undertaken.

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## 9. APPENDICES

### APPENDIX I: DATA COLLECTION QUESTIONNAIRE

**Study Title**: Prevalence, etiology and antimicrobial susceptibility of bacterial neonatal meningitis at Tikur Anbessa Specialized Hospital.

**Principal Investigator**: **Dr Abenet Tassew Zewdie**; University of Nairobi, Department of Paediatrics and Child Health; Telephone: *+251911938409 or +254774210858*

**Co-Investigators:**

**Prof. E. Obimbo** (Professor and Department Head of Paediatrics and Child Health, University of Nairobi)

**Prof. C. JOWI** (Associate Professor, Department of Paediatrics and Child Health, University of Nairobi)

**Patient's unique identification number**:_____

**Data collection date**:_____

**I. Demographic Characteristics of the Neonate**

1. Sex - Male☐ Female ☐

2. Gestational Age _____weeks

3. Birth weight : _____gms

4. Post natal age : _____days

5.Clinical features seen:

Feeding problem/intolerance☐ Bulging fontanel ☐

Lethargy ☐ Neck retraction/stiffness ☐

Convulsion ☐ Temperature instability ☐

Skin change ☐ (Hypothermia/ Hyperthermia )

Respiratory signs ☐ Skin change ☐

**II. Characteristics of the Mother and Labour Condition**

1. Maternal Age:-_____ years

2. Maternal literacy

Illiterate:-☐

Literate

Can read and write only ☐ Graduate☐

Primary level (1st -8th grade) ☐ Postgraduate☐

Secondary level (9th-12th grade) ☐

3. The family income

From :- Father _____

mother_____

other (specify)_____

Total income in birr per month_____

4. Was there maternal fever during pregnancy and/or labour? Yes ☐No ☐

If yes, was the fever

At delivery☐

Week before delivery☐

Earlier in pregnancy☐

Any identified cause of the fever _____

5. ANC follow up? Yes ☐No☐

Any Antenatal illness? Yes ☐No☐

If yes, specify _____

6. Duration of labour: _____hrs

Normal (< 12hrs) ☐Prolonged ( > 12hrs) ☐

7. Rupture of membrane _____hrs

Foul smelling of the liquor? Yes ☐No ☐

8. Place of delivery

At home ☐At health facility ☐ on the way ☐

9. Mode of delivery

Spontaneous vaginal delivery ☐

Cesarean section ☐

Breech☐

Assisted vaginal delivery (vacuum/forceps) ☐

Other (specify) _____

IV. Laboratory results

1. Macroscopic examination/ CSF appearance _____

2. CSF biochemistry parameters

Protein (g/dl) _____

Increased ☐ normal☐

Glucose (mmol/L)_____

Decreased ☐ Normal ☐

3. CSF cytology

WBC count (cells/ml)_____

RBC count (cells/ml)_____

4. Gram stain, bacteria isolated? Yes ☐ No☐

If yes, which organism(s) Gram + _____ Gram –ve _____

Sensitivity of the isolated organism(s) to different antibiotics

| | **Susceptible** | | | **Susceptible** |
|---|---|---|---|---|
| Ampicillin | Yes ☐ | No ☐ | Ceftazidime | Yes ☐No☐ |
| Gentamycin | Yes ☐ | No ☐ | Ceftriaxone | Yes ☐No☐ |
| Chloramphenicol | Yes ☐ | No ☐ | Cefotaxime | Yes ☐No☐ |

5. CSF culture

Growth obtained ☐

No growth obtained ☐

If growth obtained, which organism(s) _____

Sensitivity of the obtained organism(s) to different antibiotics

| | **Susceptible** | | | **Susceptible** |
|---|---|---|---|---|
| Ampicillin | Yes ☐ | No ☐ | Ceftazidime | Yes ☐No☐ |
| Gentamycin | Yes ☐ | No ☐ | Ceftriaxone | Yes ☐No☐ |
| Chloramphenicol | Yes ☐ | No ☐ | Cefotaxime | Yes ☐No☐ |

**APPENDIX II**

**CLIENT INFORMED CONSENT FORM (ENGLISH)**

**Study Title** -Prevalence, etiology and antimicrobial susceptibility of bacterial neonatal meningitis at Tikur Anbessa Specialized Hospital.

**Principal Investigator- Dr. Abenet Tassew Zewdie;** University of Nairobi, Department of Paediatrics and Child Health; Telephone: *+251911938409 or +254774210858*

**Co-Investigators:**

**Prof. E. Obimbo** (Professor and Department Head of Paediatrics and Child Health, University of Nairobi)

**Prof. C. Jowi** (Associate Professor, Department of Paediatrics and Child Health, University of Nairobi)

**Investigator's statement:**

Thank you for your willingness to read this form. It offers information about this study, which will help you decide if you will take part in the study or not. Appropriate translation will be carried out in the language you are comfortable with.

**Introduction**

Meningitis is inflammation of the protective membranes covering the brain and spinal cord, known collectively as meninges. The inflammation may be caused by infection with viruses, bacteria or other organisms. Studies have shown that this infection is more commonly occur in neonates (<28days of life), called neonatal meningitis. The most common symptoms of neonatal meningitis include body temperature changes (fever/hypothermia), breathing problems (resp. distress), diarrhea, low blood sugar,

reduced movements, reduced sucking, seizures, slow heart rate, swollen belly area, vomiting yellow skin and whites of the eyes (jaundice). So routinely if a neonate present with one of those signs, lumbar puncture will be performed. Lumbar puncture is a procedure where needle is inserted into the spinal cord canal to extract a sample of cerebrospinal fluid (CSF) that envelops the brain and spinal cord.

This procedure will be performed in a sterile environment. For this, the principal investigator shall wear gown and sterile gloves then the skin at the back where to insert needle will be cleaned after putting a sterile towel to cover other part of the body to avoid contamination. Then CSF samples will be taken to the laboratory with in thirty minutes where it will be examined in a medical laboratory to diagnose or exclude meningitis. Meningitis can be life threatening, can lead to serious long term consequences such as deafness, epilepsy and other neurological squeal if not diagnosed and treated early.

**Benefits**

This study will help the doctors to know more about bacterial neonatal meningitis and to act accordingly specially, in terms of knowing the causative agents, common risk factors, antibiotic preference. This of course will improve the quality of care provided to all neonates with this specific disease so that we can reduce the rate of neonatal morbidity and mortality. Specifically for your baby I will be following his/her laboratory results. For those diagnosed with neonatal bacterial meningitis antibiotics will be started according to the sensitivity to the specific organism so these neonates will be spared from unnecessary antibiotics exposure.

**Risks**

The study has no major risks except creating some discomfort since we will position the baby to perform lumbar puncture to take the CSF specimen. During this procedure since we monitor cardio respiratory condition of the baby, it will not cause harm to your baby. Refusal to participate will not jeopardize the treatment of your baby in any way. Your participation is voluntary. There will be no financial rewards to you for participating in the study.

**Statement about Confidentiality**

The information will be obtained using coded questionnaires and will be kept in strict confidence. No specific information will be released to any person or agency without your written consent. We will discuss the finding in public and publish this study but not anything specific that could identify your baby. There will be no penalty if you wish to withdraw from the study at any stage. You are free to ask me any questions or seek clarifications on the study procedure or on your role as a participant. I will try to answer you as best as I can.

**Participant's Statement**

I _____ having been adequately explained to the study procedure, the risks and benefits, here by agree to participate in the study. I understand that my participation is voluntary and I am free to withdraw from the study at any time. I have been given the opportunity to ask questions and seek clarifications, and these have been answered satisfactorily.

SIGNATURE _____ DATE _____

**Investigator's Statement**

I _____ declare that I have adequately explained to the above participant the study procedure, risks and benefits, and given her/ him to ask questions and clarifications. I have also tried to answer the question to the best of my knowledge and ability.

SIGNATURE _____ DATE _____

In case you have any more issues related to this study, you can contact me on mobile phone +251911938409.

**CLIENT INFORMED CONSENT FORM (AMHARIC)**

**የተሳታፊ የፈቃድና የመረጃ ቅፅ**

**የጥናቱ ርዕስ** :- ማጅራት ገትር በህፃናት ላይ ያለው ስርጭት ፣ የበሽታው መንስኤዎችና ለመድሃኒቶች የመሸነፍ/የማሸነፍ ሁኔታ በጥቁር አንበሳ ሆስፒታል; አዲስ አበባ; ኢትዮጵያ

**ዋና ተመራማሪ:**

ዶ/ር አብነት ጣሠው ዘውዴ; ናይሮቢ ዩኒቨርስቲ የህፃናት/የልጆች ጤና የትምህርት ክፍል; ስልክቁጥር +251911938409 ወይም +254774210858

**አጋዥ ተመራማሪዎች** :-

ፕሮፌሰር:ኤልዛቤት:ኦቢምቦ (ፕሮፌሰር ;ናይሮቢ ዩኒቨርስቲ የህፃናት/የልጆች ጤና የትምህርት ክፍል ሀላፊ)

ፕሮፌሰር ክርስቲን ጆዊ (አሶሼት ፕሮፌሰር;ናይሮቢ ዩኒቨርስቲ የህፃናት/የልጆች ጤና የትምህርት ክፍል)

**የተመራማሪው ማስታወሻ** :

ይህንን የፈቃድ ፎርም ለማንበብ ሰለፈቀዱ አመናመሰግናለን። ይህ ፎርም ስለጥናቱ ምንነት መረጃ በመስጠት በጥናቱ ላይ ለመሳተፍ ወይም ላለመሳተፍ አንዲወስኑ ይረዳዎታል። ይህ መረጃም በሚመቾት ቋአንቁዓ ይተረጎምሎታል።

**መግቢያ**

ማጅራት ገትር የአንጎል ወይም የአይምሮ ኢንፌክሽን ሲሆን መንስኤውም የተለያዩ ባክቴሪያ ፣ ቫይረስ እንዲሁም ፈንገስ ናቸው ። ይህ በሽታም እድምያቸው ከ 28 ቀን በታች የሆኑ ህፃናቶች ላይ በስፋት ይከሰታል ። የበሽታው ምልክቶችም ብዙ ግልጽ ያልሆኑና ከሌላ በሽታጋ የሚምታቱ ናቸው። ለምሳሌ ያህል እንደ ትኩሳት፣ማስመለስ፣ድካም

ያተነፋፈስ ስርአት መዛባት፣ያንገት መገተር እና ሌሎችም ይገኙበታል። ስለዚህ ማንኛውም ህፃን ከላይ ከተገልፀት አንዱን ወይም ከዚያ በላይ ምልክት ካሳየ የአይምሮ /የአንጎል ፈሳሽ ከጀርባው ተወስዶ ምርመራ መደረግ አለበት። አርሶም በጥናቱ ለመሳተፍ ከተስማሙ ስለህፃኑ በሽታ ሁኔታ አንዳንድ መረጃ ከጠየቅን በሁአላ ፈሳሹን በንፅህና አንወስዳለን። በሽታው በረዜ ተመርምሮ ከታወቀ ይድናል። አለበለዚያ ግን ለከባድ የአይምሮ ችግርና ሲከፋም ሞት ያስከትላል።

## የጥናቱ ጥቅም

ይህ ጥናት ሐኪሞች ስለ መንጃይተስ በሽታ የበለጠ አንድያውቁና አንዲመራመሩ ያደርጋል። ይህ ደግሞ ለበሽታው ሚሰጠው የህክምና ጥራት ላይ አስተዋፅኦ ያደርጋል። በጥናቱ ለመሳተፍ ከተስማሙ የህፃኑን የላቦራቶሪ ውጤት ከመጀመርያ ሐኪሙ በተጭማሪ እኔ አከታተላለሁ።

## አደጋ

ይህ ጥናት ከባድ የሚባል አደጋ የማያስከትል ሲሆን በመጠኑ ለምርመራ ሚፈለገው ፈሳሽ ከጀርባ ሲወሰድ ትንሽ ያለመመቸትና መጭናነቅ ለፈጠር ይችላል ለዚህም ፈሳሽ በመውሰድ ሂደቱ ረዜ የህፃኑን ያተነፋፈስና የልብ ምት አንቆጣጠራለን። በዚህ ጥናት ላይ ለመሳተፍ ካልፈለጉ ጥናቱ በፈቃደኝነት ላይ የተመሰረተ ስለሆነ የህፃኑ ሕክምና ላይ ምንም ችግር አይፈጥርም።

## ሚስጥራውነት

ከርሶ ያገኘነውንም ሆነ ከላቦራቶሪ የምናገኝውን ማንኛውንም መረጃ በምስጢር በጥንቃቄ የሚያዝ መሆኑን ልነግሮ አንወዳለን። መረጃዎቹም ለጥናቱ አላማ ብቻ ይውላሉ።

## የተሳታፊ ቃል

እኔ _____ ስለጥናቱ ሂደት ጥቅምና ጉዳት በደንብ ከተረዳሁ በኃላ በጥናቱላይ፡ለመሳተፍ፡ተስማምቻለሁ።ተሳትፎዬም፡በፈቃደኝት፡መሆኑንና፡በፈለኩ፡ጊዜ ከጥናቱ፡መውጣት፡እንደምችል፡ተረድቻለሁ።ተጨማሪ፡ማብራሪም፡ሆነ፡ጥያቄ፡ለመጠየቅ፡እድል፡ተሰጥቶኛል።ጥያቄዎቼም፡በበቂ፡ሁኔታ፡ተመልሰውልኛል።

ፊርማ _____ ቀን _____

## የተመራማሪቃል

እኔ_____ስለጥናቱ፡ሂደት፣ጥቅምና፡ጉዳት፡ከላይ፡ስሙ፡ለተገለፀው፡ተሳታፊ፡ያስረዳሁ፡ሲሆን፡ተጨማሪ፡ማብራሪያም፡ሆነ፡ጥያቄ፡ለመጠየቅ፡እድል፡ሰጥቻለሁ ጥያቄዎችንም፡በአግባቡ፡በተቻለኝ፡አቅምና እውቀት፡መልሻለሁ።

ፊርማ _____ ቀን _____

ጥናቱን፡በተመለከተ፡ተጨማሪ፡ማብራሪያም፡ሆነ፡ጥያቄ፡ለመጠየቅ፡ከፈለጉ፡በዚህ፡ስልክ ቁጥር፡መደወል፡ይችላሉ። +251911938409

## APPENDIX III: Laboratory Procedures For Cell Count And Microscopy of Cerebrospinal Fluid

***Cell Count Procedure:***

- Cell count has to be done within the first hour after collection of the specimen.
- Clean the chamber and leave it to dry.
- Dilute the CSF with 2% acetic acid in a ratio of 1:10 (dilution factor =10). For a clear/ colorless CSF no need to dilute.
- Mix the diluted CSF in a plastic tube, and then fill it into the chamber using a pipette.
- Position the cover slide so that the Newton's ring are visible, which makes the space between the cover glass and the chamber ground amounts to be0.2 μm.
- Count the cells after 2-3 minutes of sedimentation. The chamber has a depth of 0.1mm and an area of $9mm^2$. Each square has an area of $1mm^2$ therefore the cell count is done in each squares.
- Calculate WBC and RBC counts using the formula.

$$\frac{\text{(Number of cells counted X dilution factor)}}{\text{Volume of counted squares}}$$

***Gram Stain Procedures:***

- Centrifuge the CSF at 1000 revolutions for 10-15 minutes
- Divide the glass slide into two sections using a marker. Use one side for the unknown CSF and the other section for a known organism for quality control.
- Prepare a smear by placing 1-2 drops of the well-mixed CSF sediment on the slide; allow the drop(s) to form one large slightly turbid, uniform suspension.

- Leave the suspension to air-dry.
- Fix the smear by the flooding the slide with 95% methanol for a minimum of 2 minutes (3). Then rinse with distilled water and Shake off with excess water.
- Flood the slide with crystal violet ammonium oxalate for 1 minute to stain then was rinse it with distilled water.
- Flood the slide with Gram's iodine for 1 minute. The iodine acts as a mordant as it binds the alkaline crystal violet dye to the cell wall then rinse with distilled water and Shake off excess water.
- Decolorize with 95% ethanol until no more stain washes off (5-10 seconds may be enough).
- Counter stain with safran in for 30 seconds or with carbol-fuchsin for 10-15 seconds then rinse with distilled water and Shake off excess water.
- Gently blot the slide using bibulous paper or a clean paper towel and leave toair-dry.
- When dry, examine the stained smear under a microscope with 100X oil immersion objective.

Gram-positive organisms appear dark violet or purple. Gram-negative organisms appear red or pink (from the counter stain).

## APPENDIX IV: Laboratory Procedures For Bacterial Culture And Antimicrobial Testing of Cerebrospinal Fluid

***Culture Procedure:***

- Centrifuge the CSF for 10 minutes at 3000 revolutions per minute to get the sediment of centrifuged CSF.
- Inoculate at least 20-50µL of the sediment with a sterile pipette on to chocolate, blood agar.
- Inoculate the solid culture media for at least 72 hours at 35-37$^0$C in candle extinction jars to provide 5-8% carbon dioxide.
- Check for growth every 24 hours for 3days.

***Antimicrobial Susceptibility Testing Procedure:*** *Modified Kirby-Bauer technique*

- Prepare Müeller-Hinton agar from a commercially available dehydrated base, immediately after autoclaving allow it to cool in 45-50$^0$C water baths.
- Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
- Store the agar medium in a refrigerator (2-8$^0$C) unless used on the same day.
- Then prepare the inoculum by direct colony suspension method by making a direct broth or saline suspension of isolated colonies from 18-24 hour agar plate.
- Adjust the suspension to match the 0.5McFarland turbidity standard, using saline and vortex mixer.

- 15 minutes after adjusting the turbidity of the inoculum suspension, dip a sterile cotton swab into the adjusted suspension. Rotate the swab several times and press firmly on the inside wall of the tube above the fluid level. This removes excess inoculum from the swab.
- Inoculate the dried surface of Müeller-Hinton agar plate by streaking the swab over the entire sterile agar surface. Repeat this procedure by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, swab the rim of the agar.
- Apply the discs to inoculated Agar plate. Press down each disc to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they are distributed evenly so that they are no closer than 24 mm from center to center.
- Invert and place the plates in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of Haemophilus species, streptococci and N. gonorrhea, the plates are incubated in an increased $CO_2$ atmosphere, because $CO_2$ significantly alter the size of the inhibitory zones of some agents.
- Finally, after 16 to 18 hours of incubation, examine each plate. If the plate is satisfactorily streaked, and the inoculum is correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum is too light and repeats the test. The diameters of the zones of complete inhibition (as judged by the unaided eye will be measured, including the diameter of the disc. Measure the zones to the nearest whole millimeter, using sliding calipers or a ruler.