Meningitis: Etiologic agents and their Antibiotic Susceptibility Pattern in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia

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Abbreviations

AIDS……. Acquired Immunodeficiency Syndrome

ATCC …….. American Type Culture Collection

CLSI..... Clinical Laboratory Standards Institute

CNS..... Central nervous system

CoNS…… Coagulase negative staphylococcus

CSF…… Cerebrospinal fluid

EHNRI …….. Ethiopian Health and Nutrition Research Institute

ESCH……. Ethio-Swedish Children's Hospital

FRPC……. Faculty Research Publications Committee

GBS……... Group B Streptococci

Hib..... *Hemophilus influenzae type b*

HIV……..... Human immunodeficiency virus

IU..... International Unit

IL..... Interleukin

LA..... Latex agglutination

LP ..... Lumbar puncture

MDR……. Multi-Drug Resistance

mg/dL..... Milligram per deciliter

mmol/L..... Millimole per liter

microL..... Microliter

NCCLs…….. National Committee for Clinical Laboratory Standards

PCR ……Polymerase chain reaction

TNF.....Tumor Necrosis Factor

Abstract

Bacterial meningitis remains a common disease worldwide. Its most frequent causes are *Nessieria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae*. In Ethiopia there are a number of previous published reports concerning meningitis. However most of the studies have been conducted during epidemic seasons. Meningitis is a serious emergency, which requires the early identification of the causative bacterium and its antibiogram. However, it is a usual practice to start empirical therapy before the complete laboratory result is available. Such practice requires knowledge of the most frequent etiological agents of meningitis and their antibiotic sensitivity patterns in the local population. This will help to influence the choice of initial therapy before a sensitivity result is available and in areas where laboratory facilities may not be available.

To readdress this situation, this study was undertaken to isolate and identify the bacterial etiologic agents, including their antibiotic susceptibility pattern and serogroup/serotype isolated from patients with meningitis (n=340) visiting Tikur Anbessa University Hospital, Addis Ababa, Ethiopia, between November 2007 and June 2008. Of the patients investigated, 53.5% were males and 46.5% were females resulting in an overall male to female ratio of 1.2:1. Over half of the patients, 51.2% were children and the rest 32.6% and 16.2% were neonates and adults respectively.

A total of 26 bacterial and fungal pathogens were isolated in all age groups. Of which, in neonates (n=2), children (n=16) and adults (n=8). Bacterial isolates accounted for 22 (84.6%) of the total isolates and the others 4 (15.4%) were fungal. In general, *Streptococcus pneumoniae* accounted for (36.4%) followed by *Haemophilus influenzae type* b and *Nessieria meningitidis* (Serogroup A and C) (13.6%) each. *Cryptococcus neoformans* was the only fungal isolate, which accounted for half of the isolates in adults.

All gram positive bacteria were sensitive to Ceftriaxone, Ciprofloxacin, Chloramphenicol, Erythromycin and Rifampicin and Low level of resistance (<60%) were observed to Penicillin, Tetracycline and Trimethoprim-sulphamethoxazole. exceptionally, all isolates of *S.pneumoniae* showed high level of resistance (>80%) to Gentamicin. Whereas, the gram-negatives showed high level of resistance (>80%) to Tetracycline and Trimethoprim-sulphamethoxazole, intermediate level of resistance (60-80%) to Ampicilin and low level of

resistance (<60 %) to Ceftriaxone, Ciprofloxacin, Gentamycin, Chloramphenicol and Rifampicin.

Most of the tested drugs were effective against gram-positive bacteria where as Gram-*negative* bacteria showed high level of resistance. Overall ceftriaxone and ciprofloxacin were the most effective drugs when compared to other drugs tested against the gram-positive and gram-negative bacteria. Multiple resistance (resistance to two or more drugs) was observed in 2/11(18.2%) and 11/11(100%) gram positive and gram-negative bacteria respectively.

In conclusion, in the present study, *S. pneumoniae* was the predominant isolate followed by *H. influenzae type b* and *N. meningitidis*. The susceptibility pattern of isolates from the study showed that ceftriaxone and ciprofloxacin were the most effective drugs. Therefore, efforts should be directed towards early diagnosis and treatment of meningitis and continued re-evaluation of the resistant patterns of organisms to optimize treatments and reduce complications. In addition, vaccination policies should reflect the primary etiologic agents, including pneumococcus and meningococcal serogroups A and C and *H. influenzae* type b.

Key words: Meningitis, Bacterial etiologic agents, Antibiotic susceptibility pattern, Serogroup/serotype, Neonates/children and adults, Ethiopia.

CHAPTER I: INTRODUCTION

1.1. Introduction

Meningitis is the inflammation of the protective membranes covering the central nervous system, known collectively as the meninges. Meningitis may develop in response to a number of causes, including infectious agents, physical injury, cancer, or certain drugs. It is usually caused by viral, bacterial or fungal pathogens (Richard *et al.,* 2000; Ryan and Ray, 2004). Meningitis is among the ten most common infectious causes of death and is responsible for approximately 135,000 deaths throughout the world each year (Thomas *et al.*, 2006). The disease is more common in developing countries (WHO, 1998).

Bacterial meningitis can be quite severe and may result in brain damage, hearing loss, or learning disability and death if not treated early (Richard *et al.,* 2000). World wide bacterial meningitis accounts for approximately 1.2 million cases annually (Thomas *et al.*, 2006). The relative frequency of isolation of various bacterial species as a cause of meningitis varies with age, and among geographical regions. About 80% of all cases of bacterial meningitis are caused by *Nessieria meningitidis*, *Streptococcus pneumoniae, and Hemophilus influenzae* (WHO, 1998; David *et al.*, 2005).

Bacterial meningitis occurs when bacterial virulence factors overcome host defense mechanisms that normally protect against central nervous system infection in the subarachnoid space (Francisco de Assis, 2006). Headache is the most common symptom of meningitis followed by nuchal rigidity. The classic triad of diagnostic signs consists of nuchal rigidity, fever and altered mental status. All three features are present in only 44% of all cases of infectious meningitis. Other signs commonly associated with meningitis are photophobia, phonophobia, irritability and delirium (in small children) and seizures (in 20-40% of cases). In infants (0-6 months), swelling of the fontanelle (soft spot) may be present (van de Beek *et al.*, 2004). In "meningococcal" meningitis, a rapidly spreading petechial rash is typical, and may precede other symptoms (Thomas *et al.*, 2002).

The suspicion of meningitis is generally based on the nature of the symptoms and findings on physical examination. The most important test in identifying or ruling out meningitis is analysis of the CSF through lumbar puncture (LP) (Provan and Andrew, 2005).

Bacterial meningitis is a medical emergency and has a high mortality rate if untreated (Beckham and Tyler, 2006). All suspected cases, however mild, need emergency medical attention. Empiric antibiotics must be started immediately, even before the results of the lumbar puncture and CSF analysis are known. Antibiotics started within 4 hours of lumbar puncture will not significantly affect laboratory results. Adjuvant treatment with corticosteroids reduces rates of mortality, severe hearing loss and neurological sequelae (van de Beek *et al.,* 2007).

In Ethiopia, several studies have been conducted during epidemic seasons (Tsega, 1978; Habte-Gabr *et al.*, 1979; Habte-Gabr *et al.*, 1984; Agzew, 1985; Enaro *et al.*, 1990; Gebre Kirstos and Muhe, 1993; Mengistu *et al.*, 2003; Norheim *et al.*, 2006). Hospital-based studies are few in number and mainly dealt in children (Muhe and Klugman, 1999; Hailu and Muhe, 2001; Amsalu and Assefa, 2005). A retrospective study conducted in Gondar showed that *N. meningitidis and S. pneumoniae* are the commonest pathogens of meningitis patients with isolation rate of 5.6%. Eighty one percent of cases of acute bacterial meningitis were caused by *N. meningitidis, S. pneumoniae* and *H. influenzae* and 70% of meningococcal isolates are from children (Mulu *et al.*, 2005). Therefore, the objective of this study was to determine the status of meningitis interms of etiology and serotype and to assess their antibiotic susceptibility pattern during the period of November 2007 to June 2008 in neonates/children and adults in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

1.2. Literature Review

1.2.1. Etiologies of Meningitis

Most cases of meningitis are caused by microorganisms, such as viruses, bacteria, fungi, or parasites, that spread into the blood and into the cerebrospinal fluid (CSF). Non-infectious causes include cancers, systemic lupus erythematosus and certain drugs (Ryan and Ray, 2004). Unlike viral meningitis, bacterial meningitis can be very serious and is having a high mortality in both adults and children. Even among those who survive the infection, there may remain permanent disability, with sequelae of deafness, difficulty in movement and other neurological disorders (Shah *et al.*, 2008).

a) Bacterial meningitis

The bacterial form of meningitis is an extremely serious illness that requires immediate medical care. If not treated quickly, it can lead to death within hours or to permanent brain damage in about 30% of people. Bacterial meningitis is most commonly caused by one of three types of bacteria: *H. influenzae* type b (Hib), *N. meningitidis*, and *S. pneumoniae* (Shah *et al.*, 2008).

H. influenzae type b (Hib)

Haemophilus influenzae, gram-negative coccobacillus (Figure 1.1), is a bacterial agent that causes disease globally. There are six serological types of of *Haemophilus influenza*, types a, b, c, d, e and f. These separate types are determined based on the antigen structure of the capsular polysaccharides recognized by the host immune system (Kenneth, 2008). *Haemophilus influenzae* type b (Hib) was the most common cause of bacterial meningitis and a leading cause of other serious invasive infections in young children before the introduction of Hib vaccines. About 55% to 65% of affected children had meningitis, the remainder suffering from epiglottitis, bacteremia, cellulitis, pneumonia or septic arthritis. *H. influenzae* is also commonly associated with otitis media, sinusitis, bronchitis and other respiratory tract disorders. New vaccines being given to children as part of their routine immunizations have reduced the occurrence of serious Hib disease (Quagliarello, 2008).

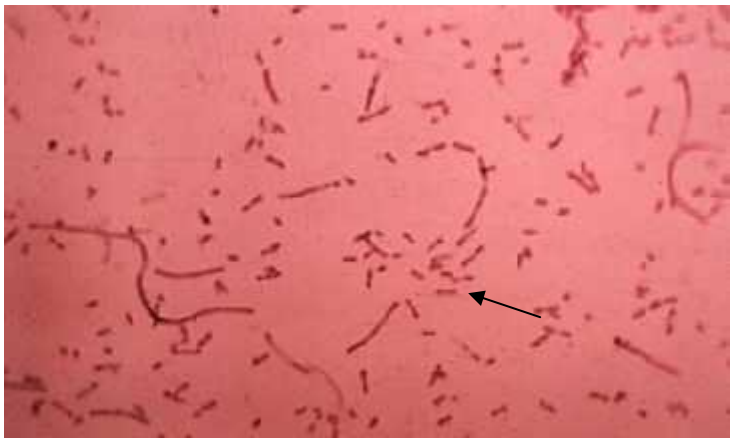

Figure 1.1. Gram stain of *Haemophilus influenzae* (gram negative coccobacilli) (Kenneth, 2008)

N. meningitidis

Neisseria ***meningitidis*** is a Gram-negative coccus, 0.6 to 1.0 µm in diameter, usually seen in pairs with adjacent flattened sides (Figure 1.2). The organism is frequently found intracellularly in polymorphonuclear leukocytes (neutrophils). It possesses a typical Gram-negative outer membrane composed of proteins, phospholipids, and lipopolysaccharide (LPS) and a prominent antiphagocytic polysaccharide capsule. Meningococcal capsular polysaccharides provide the basis for grouping the organism. Twelve serogroups have been identified (A, B, C, H, I, K, L, X, Y, Z, 29E, and W135). The most important serogroups associated with disease in humans are A, B, C, Y, and W135 (Kenneth, 2008).

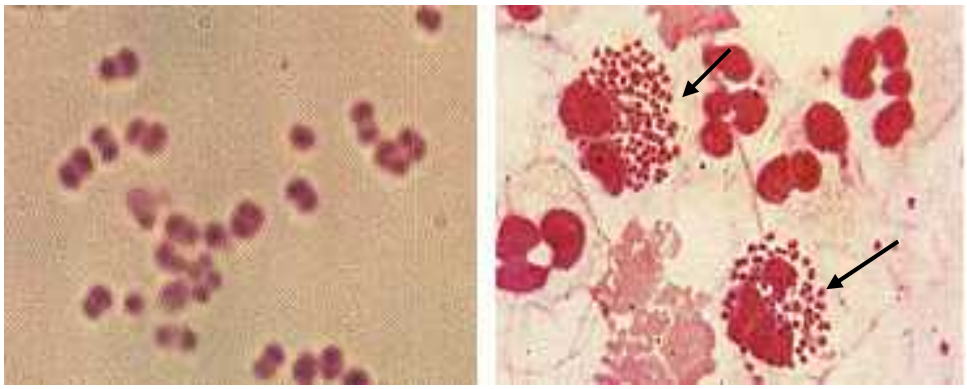

Figure 1.2. Gram stain of *Neisseria meningitidis* (Kenneth, 2008)

S. pneumoniae

Streptococcus pneumoniae are Gram-positive, lancet-shaped cocci (elongated cocci with a slightly pointed outer curvature). Usually, they are seen as pairs of cocci (diplococci) (Figure 1.3), but they may also occur singly and in short chains. When cultured on blood agar, they are alpha hemolytic. Individual cells are between 0.5 and 1.25 micrometers in diameter (Kenneth, 2008).

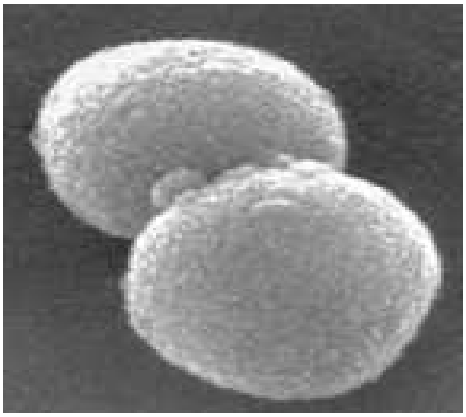

Figure 1.3. *Streptococcus pneumoniae* scanning electron micrograph of a pair of diplococci (Kenneth, 2008)

The etiologic agents of bacterial meningitis differ with age group (Table 1.1) (Ray, 1994). Early onset neonatal meningitis is more likely to be caused by *Streptococcus agalactiae* (GBS), *Escherichia coli and Listeria monocytogens*, while late onset of neonatal meningitis may be caused by other Gram-negative organisms as well as *Staphylococcal* species. Group B Streptococcus (GBS), *E. coli and L. monocytogens* have been the organism isolated most frequently in developed countries; while gram-negative organisms such as *Klebsiella* species *and Serratia marcescence, Pseudomonas* and *Salmonella* species are more common in developing countries (Vergnano *et al.*, 2005; Heath *et al.*, 2003). However, the most common bacterial strain strongly associated with neonatal meningitis are Serotype II GBS, K1 antigen of *E.coli*, and Serotype IVb *L.monocytogens* (Bingen *et al.*, 1997). *S. pneumoniae* cause the majority of cases of adult meningitis (Isabelle *et al.*, 2005). More

over, tuberculous meningitis caused by *Mycobacterium tuberculosis* can appear as acute or chronically progressive disease (Ray, 1994).

Table 1.1. Etiologies of acute bacterial meningitis by age group (Adapted from Ray, 1994)

Age group	Agent
Newborns (<1 month old)	GBS and *E. coli* (most common), *L. monocytogens*, *Klebsiella* species and other enteric Gram-negative bacteria
Infants and children	*H. influenzae* type b (Hib), *N. meningitidis* and *S. pneumoniae*
Adults	*N. meningitidis* and *S. pneumoniae*

b) Fungal Meningitis

This form of meningitis is rare in otherwise healthy people, but is a higher risk in those who have AIDS, other forms of immunodeficiency and immunosuppression. In AIDS, *Cryptococcus neoformans* is the most common cause of fungal meningitis (Gottfredsson and Perfect, 2000). Candida meningitis can occur as a manifestation of disseminated candidiasis, which most often occurs in premature neonates, in the presence of ventricular drainage devices, and as isolated chronic meningitis (Fernandez, 2000)

C. neoformans

Cryptococcus neoformans is an encapsulated fungal organism (Figure 1.4) that can cause disease in apparently immunocompetent, as well as immunocompromised, hosts. Most susceptible to infection are patients with T-cell deficiencies. *C. neoformans* var. *neoformans* causes most cryptococcal infections in humans (Mitchell and Perfect, 1995). It has four serotypes (A to D). *Cryptococcus neoformans* was generally accepted to include two varieties; var. *gattii* and var. *neoformans.* These two varieties were recently separated into

two species, *C. neoformans* and *C. gattii*, based on their genetic background and phylogenetic diversity, as proposed by Kwon-Chung and co-workers (2002).

On cornmeal tween 80 agar, *C. neoformans* produces round, budding yeast cells. No true hyphae are visible. Pseudohyphae are usually absent or rudimentary. The capsule is best visible in India ink preparations (Figure 4). The thickness of the capsule is both strain-related and varies depending on the environmental conditions. Upon growth in 1% peptone solution, production of capsule is enhanced (Larone, 1995).

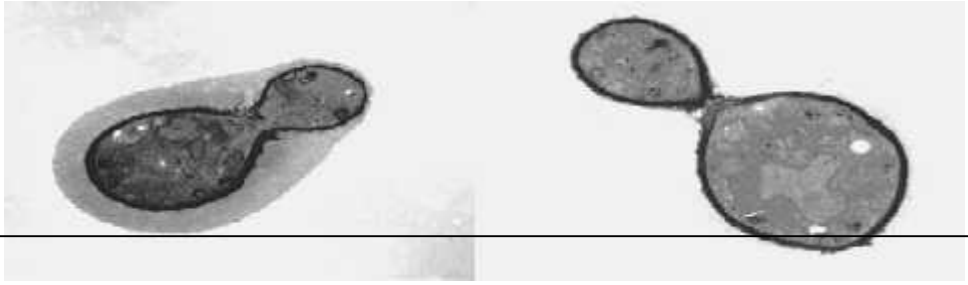

Figure 1.4. Transmission electron micrograph: *C. neoformans* showing the characteristic polysaccharide capsule (Mitchell and Perfect, 1995)

Figure 1.5. India ink preparation of *C. neoformans* (Larone, 1995)

c) Viral Meningitis

Viruses account for over half of all cases of meningitis, generally causing a much less severe form of the disease than bacteria. Viral meningitis usually lasts 7 to 14 days and is associated with headache, a stiff neck, and fever. Most cases occur in late summer and early fall and are caused by one of the many enteroviruses belonging to the Coxsackie or ECHO families. However, many other viruses, including mumps, herpes simplex II, and even HIV, can cause viral meningitis (Bloom *et al.*, 2007).

1.2.2. Epidemiology

Despite advances in vaccine development, chemoprophylaxis and treatment, acute bacterial meningitis remains a significant cause of substantial morbidity and mortality in children worldwide. According to the Pediatric Bacterial Meningitis Surveillance Network (WHO, 1999), an estimated 100,000 to 160,000 child deaths per year is attributed to *H. influenzae* type b; 250,000 to 400,000 deaths per year is caused by *S. pneumoniae.*

Meningococcal meningitis, caused by *N. meningitidis*, commonly designated as cerebrospinal meningitis, is the only form of bacterial meningitis, which causes epidemics. Epidemics can occur in any part of the world. However, the largest epidemics occur mainly in the semi-arid areas of sub-Saharan Africa, designated the African meningitis belt (Figure 1.6). Apart from epidemics, meningococcal meningitis occurs sporadically throughout the world, with seasonal variations, and accounts for a variable proportion of endemic bacterial meningitis. In non-epidemic conditions, only laboratory investigation of cerebrospinal fluid (CSF), obtained by lumbar puncture, can reliably differentiate meningococcal meningitis from other types of bacterial meningitis (WHO, 1998). Of the five common serogroups (A, B, C, Y and W135) responsible for about 90% of infections caused by *N. meningitidis*, serogroups A, B and C account for most cases of meningococcal disease throughout the world, with serogroups A and C predominating throughout Asia and Africa and serogroups B and C responsible for the majority of cases in Europe and the Americas (Manchanda *et al.,* 2006)

Meningitis occurs in people of all age groups, but very young individuals (infants and young children) and elderly individuals (>60 year) are more predisposed to the infection (Francisco de Assis, 2006). Meningitis is a substantial cause of morbidity and mortality in neonates (Garges *et al.,* 2006).

In a study done in Africa, mortality rates associated with pneumococcal, *H. influenzae* type b, and meningococcal meningitis were (45%), (29%), and (8%) respectively (Table 1.2). At 0–4 years of age, the estimated incidences of *H. influenzae* type b meningitis and all classic *H. influenzae* type b diseases were 70 and 100 cases per 100,000 population per year, accounting for approximately 90,000 and 120,000 cases per year, respectively. Including older age groups and, especially, non bacteremic *H. influenzae* type b pneumonia in the estimates of *H. influenzae* type b disease in Africa increased the overall numbers manifold; the numbers of pneumococcal infections were even greater. Studies undertaken in the 1990s (Ethiopia, The Gambia, Malawi, and Swaziland) showed fatality rates that remain astonishingly high: 42% for pneumococcal, 29% for *H. influenzae* type b, and 17% for meningococcal meningitis (Peltola, 2001).

Although cryptococcosis is an established disease, its worldwide prevalence was much lower before the AIDS era; however, over the last three decades, its incidence has been continuously rising for various reasons (Hoang *et al.,* 2004). In patients with AIDS, meningitis occurs in up to 30% of cases in Africa, and 6-10% of those in USA. About 90% of AIDS patients who are infected with *C. neoformans* develop meningitis. According to a study done in India the prevalence of cryptococcal meningitis in HIV-positive patients was between 49 and 100 % (Meena *et al.*, 2006). A study in Ethiopia showed 7% prevalence of meningitis due to *C. neoformans* (Woldeamanuel and Haile, 2001).

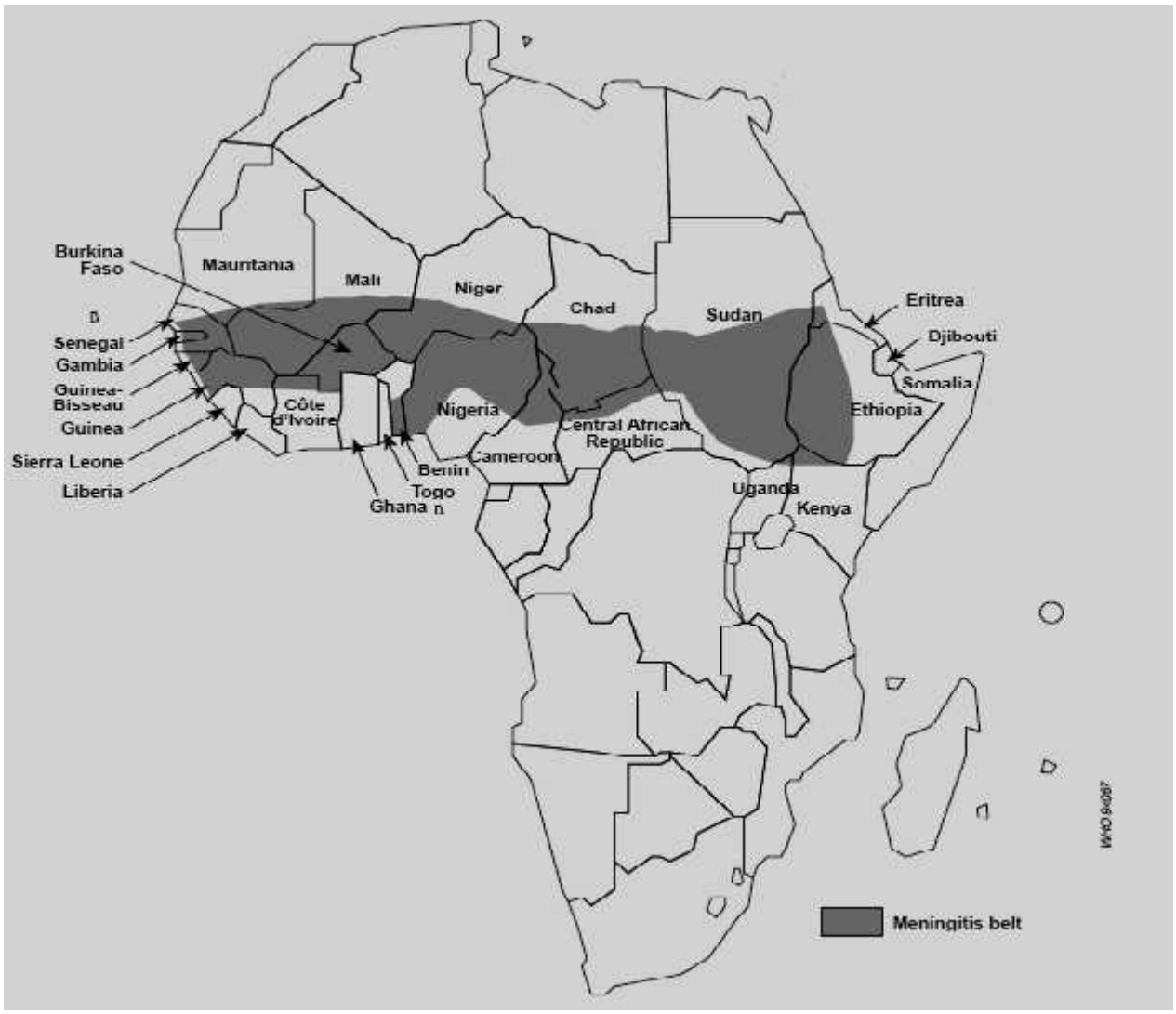

Figure 1.6. The African Meningitis Belt (WHO, 1998)

Table 1.2 Case-fatality rates for bacterial meningitis in Africa (Peltola, 2001)

Country	Year(s) of study	Case-fatality rate, no. (%) of cases			
		***H. influenzae* type b**	**Pneumococcal**	**Meningococcal**	**Other**
Burkina Faso	1986–90[a]	NG/92 (22)	NG (55)	NG (17)	NG
Cameroon	1982–83	13/53 (25)	29/74 (39)	2/6 (20)	5/21 (24)
Congo Dem. Rep. (Zaire)	1959–72	8/45 (18)	14/45 (31)	NG	17/51 (33)
Egypt	1971–75	9/35 (26)	33/105 (31)	35/744 (5)	33/427[b] (8)
Ethiopia	**1975–76**	**NG/50 (19)**	**NG/26 (39)**	**NG/30 (7)**	**NG**
Gambia	1985–87[a]	28/77 (37)	NG	NG	NG
Ghana	1983–84	5/8 (63)	21/32 (66)	5/19 (26)	NG
Ivory Coast	1985–86	15/67 (22)	NG (35)	NG	NG
Kenya	1985–86[a]	9/24 (38)	11/32 (34)	6/15 (40)	32/58 (55)
Malawi	1996–97[a]	18/42 (43)	27/59 (46)	0/7 (0)	17/48 (35)
Mali and Niger	1989–90	48/133 (36)	77/115 (67)	21/161 (13)	11/17 (65)
Nigeria	1976–80[a]	33/127 (26)	73/159 (46)	4/26 (15)	NG
Rwanda	1983–90	22/80 (27)	54/103 (52)	3/29 (10)	20/50 (40)
Senegal	1973–77	82/248 (33)	NG	NG	NG
South Africa	1991–92[a]	4/74 (5)	5/26 (19)	1/101 (1)	NG
Swaziland	1991–92[a]	2/8 (25)	12/25 (48)	4/10 (40)	3/8 (24)
Zambia	1970–71[a]	5/13 (39)	12/24 (50)	0/1 (0)	0 (0)
Cases with full information		**389/1352 (29)**	**549/1211 (45)**	**104/1236 (8)**	**175/803 (22)**

NOTE. Dem Rep., Democratic Republic; ND, not determined; NG, not given.

a Prospective study.

b Applies to purulent meningitis.

1.2.3. Routes of Infection

The etiologic agents of meningitis often live harmlessly in a person's mouth and throat. In rare instances, however, they can break through the body's immune defenses and travel to the fluid surrounding the brain and spinal cord. The bacteria are spread by direct close contact with the discharges from the nose or throat of an infected person. The bacteria that cause meningitis are not very contagious, i.e. not spread by casual contact or by simply breathing the air where a person with meningitis has been (Quagliarello, 2008).

Many infectious agents reach the CNS by hematogenous spread. Pneumococcal infection may also arise from chronic infection of the paranasal sinuses and or middle ear. Furthermore, organisms present in the nasopharyx may reach the CNS directly, if the dura mater is damaged in some way following head injury or surgery (Tunkel and Scheld, 1993).

Bacteria from the maternal genital tract colonize the neonate after rupture of membranes, and specific bacteria, such as group B streptococci (GBS), enteric gram-negative rods, and *Listeria monocytogenes,* can reach the fetus transplacentally and cause infection (Miller, 2008). Cryptococcosis spreads through inhalation of airborne fungi (Mitchell and Perfect 1995).

1.2.4. Pathology and Pathogenesis

Bacterial meningitis occurs when bacterial virulence factors overcome host defense mechanisms that normally protect against central nervous system infection in the subarachnoid space (Francisco de Assis, 2006). The initial step in development is colonization of the nasopharynx by the organism. Many bacteria possess specialized surface structures, called pili (or fimbriae) that allow adherence to receptors on nasopharyngeal mucosal cells. Once colonization has occurred, the organism may locally invade tissues and gain access to the bloodstream. Common meningeal pathogens, such as *S. pneumoniae, N meningitidis,* and *H. influenzae,* possess an outer polysaccharide capsule that acts as a virulence factor by preventing phagocytosis as well as complement-pathway activation (Quagliarello and Scheld, 1992; Francisco de Assis, 2006).

After survival and replication in the bloodstream, the organism may cross the blood-brain barrier and invade the subarachnoid space. Host defense mechanisms are unable to control infection in the cerebrospinal fluid (CSF) because of relatively low levels of local antibody and complement activity. Bacterial replication and accumulation of white blood cells (WBCs) in the CSF enhance a local inflammatory response in the subarachnoid space because of production and release of inflammatory mediators. Among these mediators are cytokines, IL-1, IL-6, TNF, prostaglandins (especially prostaglandin E2), and leukotrienes (especially leukotriene B4) (Quagliarello and Scheld, 1992; Francisco de Assis, 2006).

Inflammation of the meninges and ventricles produces a polymorphonuclear response, an increase in cerebrospinal fluid (CSF) protein content, and utilization of glucose in CSF. Inflammatory changes and tissue destruction in the form of abscesses are more pronounced in gram-negative meningitis (Miller, 2008).

Fungal infection follows inhalation of the yeast cells, which in nature are dry, minimally encapsulated, and easily aerosolized. The primary pulmonary infection may be asymptomatic or may mimic an influenza-like respiratory infection, often resolving spontaneously. In compromised patients, the yeasts may multiply and disseminate to other parts of the body but preferentially to the central nervous system causing cryptococcal meningoencephalitis (Jawetz, 2001).

1.2.5. Clinical Features

In newborns and infants, the typical symptoms of fever, headache, and neck stiffness may be hard to detect. Other signs in infants might be inactivity, irritability, vomiting, and adults and older children with acute meningitis usually have overt signs and symptoms of meningeal inflammation, including fever, headache, neck stiffness, and an altered level of consciousness. As the disease progresses, signs of increased intracranial pressure may appear (hypertension, bradycardia, coma). Signs of meningeal irritation (e.g., Kernig's and Brudzinski's signs) are detected in about 50% to 60% of patients. Focal neurologic signs or seizures occur less often. Patients with meningococcal meningitis and associated

meningococcemia have a diffuse rash, which often becomes petechial or purpuric (Sarah, 2006).

In elderly patients, signs of meningeal inflammation may or may not be present (e.g., high fever may be absent); insidious symptoms (e.g., confusion, obtundation) may be found instead. Infants and young children also may lack obvious signs of meningitis and often present with lethargy, irritability, vomiting, and poor feeding. Nuchal rigidity or a bulging fontanel is present in less than 50% of infants and young children with meningitis (Quagliarello and Scheld, 1992).

1.2.6. Diagnosis

Except in unusual circumstances, in which severe increase in intracranial pressure makes the procedure dangerous, a lumbar puncture is the first step in the workup of a patient with suspected CNS infection to draw cerebrospinal fluid (CSF) (Ray, 1994).

I. CSF Analysis

Cerebrospinal fluid (CSF) analysis is a set of laboratory tests that examine a sample of the fluid surrounding the brain and spinal cord. CSF is clear and colorless. It contains glucose, electrolytes, amino acids, and other small molecules found in plasma, but it has very little protein and few cells. CSF analysis includes tests in clinical chemistry, hematology, immunology, and microbiology (Dean *et al.*, 2003; Nathan, 2003).

a) Gross Examination

Color and clarity are important diagnostic characteristics of CSF. Straw, pink, yellow, or amber pigments (xanthochromia) are abnormal and indicate the presence of bilirubin, hemoglobin, red blood cells, or increased protein. Turbidity (suspended particles) indicates an increased number of cells (Dean *et al.*, 2003; Nathan, 2003).

b) Glucose

CSF glucose is normally approximately two-thirds of the fasting plasma glucose (70 to 99 mg/dL). A glucose level below 40 mg/dL is significant and occurs in bacterial and fungal meningitis and in malignancy (Dean *et al.*, 2003; Nathan, 2003).

c) Protein

Total protein levels in CSF are normally very low, and albumin makes up approximately two thirds of the total. High levels are seen in many conditions including bacterial and fungal meningitis, multiple sclerosis, tumors, subarachnoid hemorrhage, and traumatic tap (Nathan, 2003). The findings of CSF differ with different etiologic agents as shown in Table 1.3 (Ray, 1994).

Table 1.3. Findings of CSF analysis: Normal versus Infection (Adapted from Ray, 1994)

Clinical Situation	**Leukocytes/mm^3**	**% Polymorphonuclears**	**Glucose (% of blood)**	**Protein (mg/dL)**
Children & Adults				
Normal	0-5	0	≥60	≤30
Viral infections	2-2000(80)*	≤50	≥60	30-80
Pyogenic bacterial infection	5-5000(800)*	≥60	≤45	>60
Tuberculosis & Mycoses	5-2000(100)*	≤50	≤45	>60
Neonates				
Normal (term)	0-32(8)*	≤60	≥60	20-170(90)*
Normal (preterm)	0-29(9)*	≤60	≥60	65-150(115)*

***** Numbers in parenthesis represent mean value

d) Staining and Culture

The confirmatory procedure which must be performed in all CSF samples in which any infection is suspected includes bacterial cultures and Gram staining. If the CSF is grossly purulent and the patient untreated, a Gram stain of the uncentrifuged CSF or of its

centrifuged sediment will frequently show the infecting organism and indicate the specific diagnosis (Ray, 1994). Bacterial cultures done on 5 % sheep blood agar and enriched chocolate agar remain the gold standards for diagnosing bacterial meningitis (WHO, 1999). According to the clinical indications and results of CSF cytology and chemistry, other microbiological tests may be used including viral cultures, India ink stain for fungi and acid fast bacilli stain for mycobacteria together with culture (Ray, 1994).

e) Non-culture microbiological methods for predicting Meningitis

Immunological methods to detect fungal or bacterial antigens (e.g. Latex agglutination) and PCR may be used during CSF analysis (Ray, 1994).

I) Latex Agglutination

Latex agglutination (LA) allows rapid detection of bacterial antigens in CSF. Sensitivity varies greatly between bacteria. Latex agglutination for *Haemophilus influenzae* has a sensitivity of 60 to 100 percent, but is much lower for other bacteria. The specificity for LA is very low (Dean, 2003). However, LA can be useful in partially treated meningitis cases where cultures may not yield an organism (Pruitt, 1998). Because false positives lead to unnecessary treatment, LA is not routinely used today. Some experts suggest using LA in cases of suspected bacterial meningitis if the initial Gram stain and bacterial culture are negative after 48 hours (Kaplan, 1999). Latex slide agglutination test for cryptococcal antigen is positive in 90% of patients with cryptococcal meningitis (Jawetz, 2001).

II) Polymerase Chain Reaction

Polymerase chain reaction (PCR) has been a great advance in the diagnosis of meningitis to detect viral or bacterial nucleic acids in CSF. PCR has high sensitivity and specificity for many infections of the CNS, is fast, and can be done with small volumes of CSF. Although testing is expensive, there is a potential for cost savings by decreasing overall diagnostic testing and intervention (Cinque *et al.*, 1997).

f) Imaging studies: Neuro-imaging is recommended to detect the complication of meningitis. Complication should be suspected when the clinical course is characterized by shock, respiratory failure, focal neurological deficits, and a positive CSF culture after 48-72 hrs of appropriate antibiotic therapy (Bashir *et al.,* 2003).

1.2.7. Management of Meningitis

a) Antimicrobial therapy

In bacterial, mycobacterial, and fungal infections of the CNS, prompt and aggressive antimicrobial therapy is required. The duration of treatment varies from as little as 10 days for uncomplicated bacterial meningitis to 12 months or longer for tuberculous meningitis and several years for some cases of fungal meningitis (Ray, 1994).

The treatment of bacterial meningitis represents one of the success stories of modern medicine, particularly antibiotics. In the pre-antibiotic era bacterial meningitis was almost always fatal, but the prompt use of appropriate antibiotics together with supportive care can undoubtedly reduce the morbidity and the mortality of this condition substantially (Jonathan, 2003).

The initial treatment approach to the patient with suspected acute bacterial meningitis depends on early recognition of the meningitis syndrome, rapid diagnostic evaluation, and emergent antimicrobial and adjunctive therapy (Allan *et al.*, 2004).

When bacterial meningitis is suspected and results of CSF Gram staining are unavailable or do not demonstrate stainable organisms, empirical therapy should be started with antimicrobial agents that have activity against the most common causative pathogens such as penicillin and chloramphenicol (Allan *et al.*, 2004). Use of a broad-spectrum cephalosporin, such as ceftriaxone sodium (Rocephin) or cefotaxime sodium (Claforan), is recommended (Jonathan, 2003; Allan *et al.*, 2004; Quagliarello and Scheld, 1997). The combination of vancomycin plus either ceftriaxone or cefotaxime is used for infants and children (Allan *et al.*, 2004). Patients less than 3 months or more than 50 years of age, those undergoing long-term corticosteroid therapy, and those with malignant hematologic

conditions should receive ampicillin in addition because of its activity against *L. monocytogenes* (Jonathan, 2003; van de Beek *et al.*, 2002). Once the results of the CSF analysis are known along with the Gram-stain and culture, empiric therapy may be switched to therapy targeted to the specific causative organism and its sensitivities (van de Beek *et al.*, 2002).

For tuberculous meningitis empirical antituberculosis therapy with first line drugs (isoniazid, rifampin, pyrazinamide and ethambutol or streptomycin) must be started as early as possible (Ray, 1994). Patients with cryptococcal meningitis should be hospitalized to start 2 weeks of induction therapy with amphotericin B (0.7 mg/kg/day) given intravenously plus flucytosine (25 mg/kg) given orally every 6 hours. After clinical improvement with 2 weeks of induction therapy (possibly sooner for patients with substantial improvement), the treatment can be switched to oral fluconazole (400 mg once daily to complete 8 weeks of acute treatment). Itraconazole (200 mg orally twice daily) sometimes is used as an alternative for patients who cannot take fluconazole (Saag *et al.*, 2000). In Ethiopia the treatment of choice is Fluconazole 400mg p.o. BID for 10 -12 weeks or Amphotercin B for two weeks then Fluconazole (personal communication).

b. Adjunct therapies

Experimental studies have revealed a correlation between outcome and the severity of the inflammatory process in the subarachnoid space. Animal models of bacterial meningitis have shown decreased inflammation, reduction in cerebral edema and intracranial pressure, and lessening brain damage with use of dexamethasone (van de Beek *et al.*, 2007). Dexamethasone therapy should be started intravenously at the same time as, or slightly before, the first dose of antibiotic (Quagliarello and Scheld, 1997).

The beneficial effects of adjunctive dexamethasone were demonstrated in infants and children with *H influenzae* type b meningitis. Follow-up examination demonstrated a significant decrease in the incidence of neurologic and audiologic sequelae, with evidence of clinical benefit being greatest for overall hearing impairment. Adults with bacterial meningitis showed benefits (lower percentage of unfavorable outcomes including death) in

the subgroup of patients with pneumococcal meningitis but not others (Quagliarello and Scheld, 1997).

However, given the lack of clear benefit favoring dexamethasone use in this setting and the concerns about decreased antibiotic penetration in the CSF with its use, decision to use this agent is considered on a case-by-case basis after weighing the potential risks and benefits. Likewise, data are insufficient to recommend adjunctive steroids in neonates with bacterial meningitis (Miller, 2008).

c) Preventive measures

Bacterial meningitis constitutes a ready source of physical and sensory disabilities in childhood and every effort must be made to ensure its prevention (Urowayino *et al.*, 2004).

I) Immunization

Many cases of meningitis and other invasive diseases caused by *H. influenzae* type b, *S. pneumoniae,* and *N. meningitidis* infection may be preventable with use of currently available vaccines. *H. influenzae* type b conjugate vaccine has been remarkably effective in reducing the incidence of *H. influenzae* type b meningitis and should be administered as part of routine immunization in infancy. Routine use of the currently available quadravalent meningococcal vaccine (Menomune-A/C/Y/W-135) is not recommended (Jodar *et al.*, 2002). In Ethiopia mass vaccination is recommended since selective vaccinations were not effective in controlling the progressing of the epidemic (Mengistu *et al.*, 2003).

Current polysaccharide pneumococcal vaccines (Pneumovax 23, Pnu-Imune 23) have limited efficacy in preventing invasive *S. pneumoniae* infection, especially in infants under 2 years of age and immunocompromised patients. Nevertheless, administration of pneumococcal vaccine is recommended in those at high risk, including patients who are elderly, asplenic, or have significant underlying disease (Quagliarello and Scheld, 1992; Francisco de Assis, 2006). Pentavalent (DPT-HepB-Hib) vaccine is given for *H. influenzae* type b in Ethiopia (Personal communication).

II) Chemoprophylaxis

Chemoprophylaxis for household, day care, and kissing contacts and for medical personnel experiencing intensive contact with oral secretions is recommended in meningococcal epidemics by the health authorities of the United States and several European countries. Rifampin is the drug of choice; ciprofloxacin, ofloxacin, minocycline (not in children), and ceftriaxone are good alternatives (Table 1.4) (Miller, 2008; Marcel *et al.*, 2000).

Table 1.4. Chemoprophylaxis for Contacts of Patients and Index (Case of *H influenzae* type b and contacts of meningococcal disease) (Miller, 2008).

Drug Name	**Age of Contact**	**Dosage**
H influenzae disease		
Rifampin	Adults	< td>600 mg PO qd for 4 d
	≥1 month	20 mg/kg PO qd for 4 d; not to exceed 600 mg/dose
	<1 month	< td>10 mg/kg PO qd for 4 d
N meningitidis disease		
Rifampin	Adults	600 mg PO q12h for 2 d
	>1 month	10 mg/kg PO q12h for 2 d; not to exceed 600 mg/dose
	≤1 month	< td 5 q12h 2 kg>5 mg/kg PO q12h for 2 d
Ceftriaxone	>15 years	250 mg IM once

1.2.8. Significance of the study

Bacterial meningitis is an important serious illness worldwide. Prior to the introduction of antibiotics in the 1940s, case fatality rates for epidemic and endemic bacterial meningitis exceeded 70%. Since then, antibiotic use has reduced case fatality rates for meningitis caused by most bacteria to 25% or less, but no further reduction has been documented in the past 20 years. Despite advances in vaccine development and chemoprophylaxis, bacterial meningitis remains a major cause of death and long-term neurological disabilities, such as mental retardation, convulsions and hydrocephalus. These are best prevented by early diagnosis and appropriate treatment of the disease (Rao *et al.*, 1998).

Worldwide bacterial meningitis accounts for approximately 1.2 million cases annually and almost 90% of reported cases are caused by *H. influenzae* type b, *S. pneumoniae* and *N. meningitidis* (David *et al*., 2005; Thomas and Vincent, 2006; Tzanakaki and Mastrantonio, 2007). Infections caused by Hib, *S. pneumoniae*, and *N. meningitidis* are responsible for high morbidity and mortality among children in Sub-Saharan Africa. Hib causes an estimated 100,000 to 160,000 child deaths each year in the WHO African Region. *S. pneumoniae* causes 250,000 to 400,000 child deaths per year. *N. meningitidis* is responsible for large epidemics (causing thousands of deaths) in many West and Central African countries (WHO, 1999). The leading cause of acute bacterial meningitis in adults is *Streptococcus pneumoniae*, with a mortality of 20–30% despite highly effective antibiotic therapy and modern intensive care facilities (Durand *et al*., 1993).

Meningococcal infections occur worldwide as endemic disease (Manchanda *et al.*, 2006). The attention given to epidemics of meningococcal meningitis in the sub-Saharan meningitis belt tends to distort the overall picture of serious bacterial diseases during nonepidemic periods in Africa.

Although many studies of meningitis have been done in various parts of Ethiopia, most of them were conducted during epidemic seasons (Tsega, 1978; Habte-Gabr *et al.*, 1979; Habte-Gabr *et al.*, 1984; Agzew, 1985; Enaro *et al.*, 1990; Gebre Kirstos and Muhe, 1993; Mengistu *et al.*, 2003; Norheim *et al.*, 2006) and the others only in children (Muhe and Klugman, 1999; Hailu and Muhe, 2001; Amsalu and Assefa, 2005). Studies on the epidemiology of meningococcal meningitis in adult Ethiopians showed fatality rates for

meningitis and meningococcemia to be 16% and 85%, respectively (Mengistu *et al.*, 2003; Fekade and Zewde, 1992). A retrospective study, which addressed all age groups, conducted in Gondar showed that 5.6 % isolation rate of bacterial pathogens. Of these, *N. meningitidis and S. pneumoniae* are the commonest ones. Pathogen isolation rate was higher in adults (5.8%) than in children (5.5%) (Mulu *et al.*, 2005).

Meningitis is a serious emergency in which the microbiological laboratory plays a critical role in the early identification of the causative bacterium and its antibiogram. However, it is usual practice to start empirical therapy before the complete laboratory result is available. Such blind prescription requires knowledge of the most frequent etiological agents of meningitis in the local population. The antibiotic sensitivity patterns and the seasonal incidence of these isolates will help to influence the choice of initial therapy before a sensitivity result is available and in areas where laboratory facilities may not be available. Therefore, this study was undertaken to isolate and identify the bacterial and fungal etiologic agents, including their serotype/ serogroup and antibiotic susceptibility patterns isolated from patients with meningitis visiting Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. Findings from this study will help to assess changes in the pattern of etiologic agents and their sensitivity pattern through time by comparing the results of the previous studies done in Ethiopia and elsewhere in the world. Findings from this study will also provide update information for appropriate management of meningitis.

1.2.9. Hypothesis

The frequency and the pattern of pathogens isolated from patients with meningitis could be similar to those isolated in other studies.

1.3. OBJECTIVES OF THE STUDY

General Objective

- To determine the etiology of meningitis in patients admitted at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia

Specific Objectives

- To identify the etiologic agents responsible for meningitis in neonates/children and adults at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia
- To study the antibiotic susceptibility patterns of isolates to the commonly used antimicrobial agents in the treatment of meningitis
- To determine the serotype/serogroup of *H. influenzae* and *N. meningitidis* strains

CHAPTER II: MATERIALS AND METHODS

2.1 Study Design and Area

A hospital based cross sectional prospective study was conducted from November 2007 to June 2008 at Tikur Anbessa University Hospital (TAUH), Addis Ababa, Ethiopia. TAUH represents the highest tertiary level of referred patients in the country and located in Lideta sub-city. It receives referred and some directly visiting patients from all parts of the country and provides emergency service. The hospital has five departments including Medical and Pediatric wards where this study has been done.

2.2 Study Population

During the period of November 2007 to June 2008, a total of 340 patients with suspected cases of meningitis were observed at Tikur Anbessa University Hospital as shown Table 3.1. Of the total 340 patients with suspected meningitis, 55 were in the adult group ($\geq$16 yrs), 174 patients were children of 1mth to16 yrs, and the remaining were neonates of <1month. Patients who have started treatment prior to admission and those that were not willing to participate were excluded from the study.

The primary data were obtained from the patients/ attendants through our preformed questionnaire (Appendix I).

The sample size includes all suspected cases of meningitis visiting Tikur Anbessa University Hospital during the study period.

2.3 Sample Collection, Handling and Transport

Cerebrospinal fluid (CSF) was obtained after performing lumbar puncture by the attending physician/pediatrician. Two ml CSF was collected in two sterile test tubes and one of which was transported to the bacteriology laboratory within one hour of collection. The other test tube sent to chemistry laboratory for white blood cell count, glucose and protein determination. Specimens taken during the evening hours were kept in the incubator at 35-37^{0}C until further microbiological analysis.

2.4. Microscopic Examination

Smears were made from fresh uncentrifuged CSF that appeared cloudy or from the sediment of centrifuged sample (centrifuged for 20 minutes at 2000rpm) and stained with Gram's, AFB (Ziehl-Neelsen) and India ink stain and observed for morphology and gram reaction, AFB or capsulated organisms respectively.

2.5. Culture and Identification

The samples were processed following the standard microbiological procedures by inoculating on blood agar, chocolate agar, and MacConkey agar plates (Oxoid Ltd, Basingstoke, Hampshire, UK) prepared as per the manufacturer instruction] and incubated at 37^0C. Sabouraud dextrose agar supplemented with antibiotics was used for fungal culture and incubated at 37^0C and room temperature.

The chocolate and blood agar plates were incubated in a candle jar, which can provide 5-10% CO_2 concentration to create a microaerophillic condition for fastidious bacteria and MacConkey agar plates were incubated aerobically. After 18-24 hours of incubation, the plates were examined for the presence of colonies. Plates, which didn't show any growth, were further incubated for an additional 24 hours. The Sabouraud dextrose agar slant was incubated for about 7 days.

All positive CSF cultures were identified by their characteristic appearance on their respective media, gram-staining reaction and confirmed by the pattern of biochemical reactions using the standard method (Cheesbrough, 2004). Members of the family enterobacteriaceae and non- enterobacteriaceae were identified by indole production, H_2S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests and other tests using API 20E identification kits (Biomerieux, France). For gram-positive cocci coagulase, catalase, bacitracin and optochin susceptibility tests, and other tests were used. In addition, API candida and API NH (Biomerieux, France) were used to identify *C. neoformans* and Neisseria and Hemophilus respectively.

2.6. Serogrouping and Serotyping

Isolated strains of *N. meningitidis*, *and H. influenzae* were serogrouped/ serotyped by using specific antisera (Difco) (Becton, Dickinson and company, USA) and Latex kits (Pastorex Meningitis) (BioRad, UK).

2.7. Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed for all bacterial isolates by disk diffusion method according to the criteria set by the Clinical and Laboratory Standards Institute (CLSI, 2006) (formerly known as the National Committee for Clinical Laboratory Standards / NCCLs).

From a pure culture 3-5 selected colonies of bacteria were taken and transferred to a tube containing 5 ml sterile normal saline and mixed gently a homogenous suspension was formed and incubated at 37^{o}C until the turbidity of the suspension become adjusted to a McFarland 0.5.

A sterile cotton swab was used and the excess suspension was removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar, Sheep blood-Mueller Hinton agar, and Chocolate agar respective to the type of organism isolated.

The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 10 antibiotic discs (Oxoid) were then delivered on the surface of the plate: The drugs for disc diffusion testing were in the following concentrations: Ampicillin (AMP) (10 µg), Ceftriaxone (CRO) (30 µg), Ciprofloxacin (CIP) (5 µg), Chloramphenicol (C) (30 µg), Erythromycin (E) (15 µg), Gentamicin (CN) (10 µg), Penicillin (P) (10 units), Trimethoprim-Sulphamethoxazole (SXT) (25µg), Rifampicin (RD) (25 µg), and Tetracycline (TE) (30 µg). Penicillin and Erythromycin were tested only for Gram-positive bacteria. The plates were then incubated in aerobic and microaerophilic atmosphere at 37^{o}C for 24-48 hours with respect to the organism tested. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a graduated caliper in millimeters, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI (CLSI, 2006). High, intermediate and low level of resistance is defined when the percentage of resistance is >80%, 60-80% and < 60% respectively.

2.8. Reference Strains

P. aeruginosa (ATCC-27853), *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922) were used as a quality control throughout the study for culture and antimicrobial susceptibility testing. All the strains were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

2.9. Statistical Analysis

Data entry and analysis was done using EpiInfo version 6.0 software. The level of significance was set at 0.05 in order to consider a p-value <0.05 as indicator of a statistically significant difference with 95% confidence interval. The demographic, clinical and other relevant data collected was analyzed by descriptive statistics.

2.10. Ethical Considerations

The M.Sc research project proposal was approved by the Department of Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia. It was ethically cleared by the Faculty Research Publications Committee-II (FRPC-II) and endorsed by the Faculty Academic Commission. Official permission from the study sites (Internal Medicine and Pediatrics Departments) was obtained. Written informed consent was obtained from all adult patients and parents/guardians for children and neonates who participated in this study (see Appendix II).

CHAPTER III: RESULTS

3.1. Study Population

The age and sex distribution of patients investigated for meningitis are presented in Table 3.1. A total of 340 patients were included in this study between the periods of November 2007-June 2008. Of the 340 patients, 182 (53.5%) were males and 156 (46.5%) were females ($p > 0.05$) resulting in an overall male to female ratio of 1.2:1. Over half of the 340 patients, 174 (51.2%) were children and the rest 111(32.6%) and 55(16.2%) were neonates and adults respectively.

Table 3.1 Age and sex distribution of 340 patients investigated for bacterial and fungal meningitis at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia (November 2007-June 2008)

Age Group	**Male**	**Female**	**Total**
	No. (%)	**No. (%)**	**No. (%)**
<1month (neonates)	56 (50.5)	55 (49.5)	**111 (100)**
1mth -16yrs (children)	98 (56.3)	76 (43.7)	**174 (100)**
≥16 yrs (adults)	28 (50.9)	27 (49.1)	**55 (100)**
Total	**182 (53.5)**	**158 (46.5)**	**340 (100)**

3.2. Clinical Features

The clinical features of patients investigated for meningitis are presented in Table 3.2. Fever was the commonest feature in all age groups followed by irritability in neonates and neck stiffness in children and adults. Signs of meningeal irritation (Kerning's and Brudzinski's signs) and neck stiffness were not detected in neonates while predominating in children and adults.

Table 3.2. Symptoms and signs of suspected cases of meningitis investigated for bacterial and fungal infections at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia (November 2007 - June 2008)

Symptoms and Signs	Patient Category			
	Neonates n= 111	**Children n=174**	**Adults n=55**	**Total n=340**
	No. (%)	**No. (%)**	**No. (%)**	**No. (%)**
Neck stiffness	0 (0%)	19 (10.9%)	15 (27.3%)	34 (10%)
Kerning's sign	0 (0%)	4 (2.3%)	11(20%)	15 (4.4%)
Brudzenki's sign	0 (0%)	3 (1.7%)	7 (12.7%)	10 (2.9%)
Bulged fontanel	0 (0%)	5 (2.9%)	0 (0%)	5 (1.5%)
Fever	92 (82.9%)	41(23.6%)	13 (23.6%)	146 (43%)
Irritability	108 (97.3%)	13 (7.5%)	0 (0%)	121(35.6%)
Vomiting	4 (3.6 %)	21(12.1%)	0 (0%)	25 (7.4%)
Seizure	6 (5.4%)	9 (5.2%)	0 (0%)	15 (4.4%)
Lethargy	19 (17.1%)	7 (4%)	0 (0%)	26(7.7%)
Failure to feed	2 (1.8%)	3 (1.7%)	0 (0%)	5(1.5%)
Loss of consciousness	0 (0%)	5 (2.9%)	0 (0%)	5(1.5%)
Skin rash	0 (0%)	2 (1.1)	0 (0%)	2(0.6%)
Headache	0 (0%)	2 (1.1)	6 (10.9)	8(2.4%)

3.3. CSF Findings

a) Appearance

The CSF appearances of patients investigated for meningitis are presented in Table 3.3. Eighty - one percent of the CSF was clear followed by bloody and turbid CSF. Most of bloody 18 (16.2%) and xanthochoromic 6(5.4 %) CSF was collected from neonates. Among the turbid CSF specimens about 14 (70 %) were collected from children.

Table 3.3. CSF appearance of suspected cases of meningitis investigated for bacterial and fungal infections at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia (November 2007 - June 2008)

Age group	**CSF appearance**					**Total**
	Clear	Bloody	Cloudy	Turbid	Xanthochromic	
	No. (%)	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**
<1month	85 (76.6)	18 (16.2)	0(0)	2 (1.8)	6 (5.4)	**111 (100)**
1mth -16yrs	147 (84.5)	11(6.3)	1(0.6)	14 (8)	1(0.6)	**174 (100)**
≥16 yrs	44 (80)	1(1.8)	5(9.1)	4 (7.3)	1(1.8)	**55 (100)**
Total	**276 (81.2)**	**30 (8.8)**	**6(1.8)**	**20 (5.8)**	**8 (2.4)**	**340 (100)**

b) White Blood Cell count, Glucose and Protein

Among the 340 CSF specimens 123 (36.2**%**) had WBC count. Of which, 61(49.6%) and 63 (50.4%) had WBC count ≥ 5 cells/mm^3 and ≤ 5 cells/mm^3 respectively. One hundred fifty two (44.7**%**) CSF specimens did not have WBC count and reported as "No cell". Among the 340 CSF specimens 275 (80.9%) had glucose and protein findings. Of which 246 (89.5 %) and 29 (10.5%) had glucose level ≥ 45 mg/dl and ≤ 45 mg/dl respectively. Thirty-two (11.6%) and 243 (88.4 %) had protein level > 60 mg/dl and < 60 mg/dl respectively. The WBC counts, glucose and protein findings of 65 (19.1%) CSF specimens were incomplete (Data not shown).

c) Direct Microscopy

It was observed that 20/340 (5.9%) gram stained CSF showed evidence for bacteria and the remaining 320 CSF were negative. Similarly only 4 (1.2%) CSF samples were positive by India ink and the remaining 336 were negative. None of the CSF samples examined was found to be positive for AFB. The gram stain findings revealed gram-positive cocci, gram-negative diplococci, gram-negative coccobacilli, gram positive and gram-negative rods. India ink stain revealed encapsulated yeast cells.

d) Culture Findings

I. Overall prevalence

A total of 26 bacterial and fungal pathogens were isolated in all age groups (7.6%). Of which, in neonates (n=2), children (n=16) and adults (n=8) as shown in Table 3.4. Bacterial isolates were 22 (84.6%) of the total isolates and the others 4 (15.4%) were fungal isolates. Of the bacterial isolates, *S .pneumoniae* accounted for (36.4%) followed by *H. influenzae type b* (13.6%), *N. meningitidis* (13.6%), *E.coli* (9.1%), Coagulase negative staphylococcus (CoNS), *S.pyogenes, Listeria monocytogens, Enterobacter spp, Pseudomonas spp,*and *Acinetobacter spp* (4.55%) each. The gram positive and gram-negative bacteria accounted for 50% each ($p>0.05$). *C. neoformans* was the only fungal isolate.

II. Neonates

Among the 111 neonates admitted with suspected cases of meningitis 2 (1.8%) had positive CSF culture for bacteria. Of these two isolates, one was Coagulase negative staphylococcus and the other *L. monocytogens* (Table 3.4).

III. Children

A total of 16 (61.5%) bacterial isolates were found among 174 suspected cases of meningitis (Table 3.4). In children, *S. pneumoniae* was the commonest bacterial pathogen isolated (37.5%) followed by *H. influenzae type b* (18.7%), *N. meningitidis (*group A & C*)* (12.5%), *S. pyogenes, E. coli, Enterobacter spp., Pseudomonas spp.,* and *Acinetobacter spp*. accounted for 6.3% each. There was a mixed infection of *S.pneumoniae, Enterobacter*

spp., and Pseudomonas spp. in one case. So among 174 suspected cases 14 (8%) had positive CSF culture.

IV. Adults

A total of 8 (30.8%), bacterial (n=4) and fungal (n=4) pathogens were isolated among 55 suspected cases of meningitis (Table 3.4). Among bacterial isolates *S. pneumoniae* accounted for 50%, *N. meningitidis (*group A) and *E.coli* 25% each. The other 50% of the isolates in adults were *C. neoformans.* Therefore, 8 of 55 cases (14.5%) had positive CSF culture. Pathogen isolation rate was highest in adults 8(14.5%) than in children 16(9.2%) and neonates 2(1.8%) ($p < 0.05$).

Table 3.4. The various isolates found to be the etiological agents in the suspected meningitis patients who visited Tikur Anbessa University Hospital, Addis Ababa, Ethiopia (November 2007 to June 2008).

S. pneumoniae was predominant among the children group followed by *Hib*. Where as, in the adult group only two isolates of *S. pneumoniae*, followed by *N. meningitidis* and *E. coli* were observed. While only one CoNS and *L. monoccytogenes* were isolated in the neonate group.

Etiologic agents	**Patient Category**			**Total No. (%)**
	Neonates No. (%)	**Children No. (%)**	**Adults No. (%)**	
Streptococcus pneumoniae	- -	6 (75)	2 (25)	8 (100)
Haemophilus influenzae type b (Hib)	- -	3 (100)	- -	3 (100)
Neisseria meningitidis group A	- -	1 (50)	1(50)	2(100)
Neisseria meningitidis group C	- -	1 (100)	- -	1 (100)
Streptococcus pyogenes	- -	1 (100)	- -	1 (100)
Coagulase negative staphylococcus	1 (100)	- -	- -	1 (100)
Escherichia coli	- -	1 (50)	1 (50)	2 (100)
Enterobacter spp.	- -	1 (100)	- -	1 (100)
Pseudomonas spp.	- -	1 (100)	- -	1 (100)
Acinetobacter spp.	- -	1 (100)	- -	1 (100)
Listeria monocytogens	1 (100)	- -	- -	1 (100)
Cryptococcus neoformans	- -	- -	4 (100)	4 (100)
Total	**2 (7.7)**	**16 (61.5)**	**8 (30.8)**	**26 (100)**

3.4. Serogrouping /Serotyping

There were two “Group A” and one “Group C” *N. meningitidis* isolate and all isolates of *H. influenzae* were “type b” (Table 3.4).

3.5. Culture positive CSF findings

The WBC count of culture positive CSF specimens was high (100-57,000 cells/mm^3) with high level of polymorphonuclears (>90%) in bacteria and (200-400 cells/mm^3) with < 50% polymorphonuclears in cryptococcal meningitis. Glucose and protein levels of positive CSF specimens were low (5- 40 mg/dl) and high (60-300 mg/dl), respectively (Data not shown).

3.6. Antimicrobial Susceptibility

a) Gram positive bacteria

The susceptibility patterns of gram-positive bacteria (n=11) isolated from meningitis against 10 antimicrobial agents are presented in Table 3.5. All isolates were sensitive to Ceftriaxone, Ciprofloxacin, Chloramphenicol, Erythromycin and Rifampicin. Low level of resistance (<60%) was observed to Penicillin, Tetracycline and Trimethoprim-sulphamethoxazole. Intermediate level of resistance (60-80%) was observed to Gentamicin.

b) Gram negative bacteria

The susceptibility patterns of gram-negative bacteria (n=11) isolated from meningitis against 8 antimicrobial agents are presented in Table 3.6. All isolates showed low level of resistance (<60 %) to Ceftriaxone, Ciprofloxacin, Gentamycin, Chloramphenicol and Rifampicin. Intermediate level of resistance (60-80%) was observed to Ampicilin. High level of resistance (>80%) was observed to Tetracycline and Trimethoprim-sulphamethoxazole.

c) Multi-Drug Resistance (MDR)

Multiple drug resistance (resistance to two or more drugs) was observed in 2/11(18.2%) and 11/11(100%) gram positive and gram-negative bacteria respectively ($p<0.05$). Among the gram positives, MDR was observed among *S.pyogenes* to Penicillin, Tetracycline and Trimethoprim-sulphamethoxazole and CoNS to Tetracycline and Trimethoprim-sulphamethoxazole, while among the gram negatives, MDR was observed in all isolates. Most of the antibiotics were effective in gram-positive bacteria. In general, ceftriaxone and

ciprofloxacin were the most effective drugs against the tested gram-positive and gram-negative bacteria (Tables 3.5 and 3.6).

Table 3.5. Susceptibility Patterns of Gram-Positive Bacteria isolated from Suspected cases of Meningitis (November 2007 - June 2008)

Organisms		**Antimicrobial agents (%)**									
		AMP	**CRO**	**CIP**	**C**	**E**	**CN**	**P**	**SXT**	**TE**	**RD**
Streptococcus	S*	100	100	100	100	100	-	100	75	62. 5	100
pneumoniae (n=8)	I*	-	-	-	-	-	-	-	-	-	-
	R*	-	-	-	-	-	100	-	25	37. 5	-
Streptococcus	S*	-	100	-	100	100	100	-	-	-	100
pyogenes(n=1)	I*	100	-	100	-	-	-	-	-	-	-
	R*	-	-	-	-	-	-	100	100	100	-
Coagulase	S*	100	100	100	100	100	100	100	-	-	100
Negative	I*	-	-	-	-	-	-	-	-	-	-
Staphylococcus(n=1)	R*	-	-	-	-	-	-	-	100	100	-
Listeria	S*	100	100	100	100	100	100	100	100	100	100
monocytogenes(n=1)	I*	-	-	-	-	-	-	-	-	-	-
	R*	-	-	-	-	-	-	-	-	-	-
Total (n=11)	**S***	**90. 9**	**100**	**100**	**100**	**100**	**27. 3**	**90. 9**	**63.6**	**54.5**	**100**
	I*	**9.1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**		**-**
	R*	**-**	**-**	**-**	**-**	**-**	**72.7**	**9.1**	**36.4**	**45. 5**	**-**

*S= Sensitive *I=Intermediate *R=Resistant

AMP: Ampicillin; CRO: Ceftriaxone; CIP: Ciprofloxacin; C: Chloramphenicol; E: Erythromycin; CN: Gentamicin; P: Penicillin; SXT: Trimethoprim-sulphamethoxazole; TE: Tetracyclin; RD: Rifampicin

Table 3.6. Susceptibility Patterns of Gram-Negative Bacteria isolated from Suspected cases of Meningitis (November 2007 to June 2008)

Organisms		Antimicrobial agents (%)							
		AMP	**CRO**	**CIP**	**C**	**CN**	**SXT**	**TE**	**RA**
Haemophilus influenzae type b (n=3)	S*	-	100	100	66.7	66.7	-	-	33.3
	I*	-	-	-	-	-	-	-	33.3
	R*	100	-	-	33.3	33.3	100	100	33.4
Neisseria meningitidis gp "A"(n=2)	S*	100	100	100	100	-	-	-	-
	I*	-	-	-	-	100	-	-	100
	R*	-	-	-		-	100	100	-
N. meningitidis gp "C"(n=1)	S*	100	100	100	-	100	-	-	100
	I*	-	-	-	-	-	-	-	-
	R*	-	-	-	100	-	100	100	-
Escherichia coli (n=2)	S*	50	50	50	100	50	50	-	-
	I*	-	-	-	-	-	-	-	-
	R*	50	50	50	-	50	50	100	100
Enterobacter spp. (n=1)	S*	-	100	100	-	100	-	-	-
	I*	-	-	-	-	-	-	-	-
	R*	100	-	-	100	-	100	100	100
Pseudomonas spp. (n=1)	S*	-	100	100	-	100	-	-	-
	I*	-	-	-	-	-	-	-	-
	R*	100	-	-	100		100	100	100
Acinetobacter spp. (n=1)	S*	-	100	100	100	100	-	100	-
	I*	-	-	-	-	-	-	-	-
	R*	100	-	-	-	-	100	-	100
Total (n= 11)	S*	**36. 4**	**90.9**	**90.9**	**63.6**	**54.5**	**9.1**	**9.1**	**18.2**
	I*	**-**	**-**	**-**	**-**	**18.2**	**-**	**-**	**27.3**
	R*	**63.6**	**9.1**	**9.1**	**36.4**	**27.3**	**90.9**	**90.9**	**54.5**

*S= Sensitive *I=Intermediate *R=Resistant ;AMP: Ampicillin; CRO: Ceftriaxone; CIP: Ciprofloxacin; C: Chloramphenicol; CN: Gentamicin; SXT: Trimethoprim-sulphamethoxazole; TE: Tetracyclin; RA: Rifampicin

Chapter IV: DISCUSSION

Meningitis is usually caused by one of a number of bacteria. The most common are *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* type b (Hib) (WHO, 1998; David *et al.*, 2005; www.emedicinehealth.com, 2007). The epidemiology of infectious agent varies with patient age and history of immunization against *H. influenzae* type b, meningococcus and pneumococcus (Jones *et al.,* 2004). In children older than 4 weeks, *S. pneumoniae* and *N. meningitidis* are the most common etiologic agents. *H influenzae* type b has essentially disappeared in countries where the conjugate vaccine is routinely used (Miller, 2008). Pneumococci were the leading causative agents of non-epidemic meningitis and other bacteremic diseases, followed by Hib. Meningococcal diseases were less common (Peltola, 2001).

Neonatal meningitis in developing countries is a serious problem, with a mortality of 33–48%. A multicentre WHO studies on serious infections in young infants involving four centres in the Gambia, Ethiopia, the Philippines, and Papua New Guinea found that the organisms causing meningitis in babies under 1 week were mainly Gram negative. In babies older than 1 week *S. pneumoniae* becomes very common, accounting for 50% of all bacterial meningitis occurring between 7 and 90 days of age, with a case fatality rate of 53%. Of the *S. pneumoniae*, isolated serotype 2 was responsible for 26% of cases (WHO, 1999). Group B Streptococcus, *E. coli, S. pneumoniae*, and *Listeria* account for nearly all cases of neonatal meningitis in developed countries (Isaacs, 2003).

The most common bacterial causes of meningitis in the elderly patients are *S. pneumoniae*, *L. monocytogenes,* gram-negative bacilli (especially *E. coli* and *K. pneumoniae*), and *Streptococcus agalactiae* (group B Streptococcus). *S. pneumoniae* caused the majority of cases of adult meningitis and the majority of deaths due to meningitis and that it had an incidence similar to that of *N. meningitidis,* even during the epidemic season (Isabelle *et al.*, 2005). *N. meningitidis* and *H. influenzae* are not common in adults (Choi, 2001).

Although Cryptococcus is an infrequent pathogen in patients without AIDS, it is known to be a major cause of meningitis in those with the disease. In recent times, the incidence of cryptococcal meningitis in patients infected with HIV has increased worldwide mainly

because of the increased awareness among the physicians and clinical microbiologists (Ashiru and Aleong, 2005).

With the development and widespread use of antibiotics, the types of pathogenic organisms and their resistance to antibiotics have changed. Knowledge of the species, resistance rates and serotypes/serogroups of current pathogens is important for determining the appropriate antimicrobials and vaccination programmes for patients with meningitis. To readdress this situation, this study was undertaken to isolate and identify the bacterial and fungal etiologic agents, including serotype/ serogroup and their antibiotic susceptibility pattern from patients with meningitis visited Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

In the present study, the male to female ratio was 1.2:1 which showed male preponderance than female. This was also noted in other studies done in Ethiopia (Gebre Kirstos and Muhe, 1993; Hailu and Muhe, 2001; Amsalu and Assefa, 2005)

The most common important symptoms of meningitis in infants might be inactivity, irritability, vomiting, and adults and older children with acute meningitis usually have overt signs and symptoms of meningeal inflammation, including fever, headache, neck stiffness, and an altered level of consciousness (Sarah, 2006). The main presenting symptoms in neonates in this study were fever, irritability, and lethargy (Table 3.2). Fever, vomiting, altered mental status and neck stiffness were seen in children and adults. These were the commonest complaints in other studies (Gebre Kirstos and Muhe, 1993; Hailu and Muhe, 2001; Amsalu and Assefa, 2005). The classic meningeal signs were more sensitive in older age groups (children and adults). Therefore the diagnosis of meningitis in neonates requires a higher index of suspicion and must be confirmed by lumbar puncture.

As indicated by Nathan (2003), cloudy CSF could mean there is an infection or a build up of WBC or protein and bloody CSF is a sign of bleeding into the spinal fluid or the result of a traumatic LP. In this study 92.3% of cloudy and turbid CSF samples were positive for culture. A glucose level below 40 mg/dL is significant and occurs in bacterial and fungal meningitis (Dean *et al.*, 2003; Nathan, 2003). Total protein levels in CSF are normally very low, and albumin makes up approximately two thirds of the total. High levels of protein observed in many conditions including bacterial and fungal meningitis. Increased WBC in

the CSF may be a sign of meningitis (Nathan, 2003). The results obtained from the present study agreed with this review.

In this study out of 340 patients with suspected cases of meningitis, 5.9 % and 1.2 % were positive for bacterial and fungal culture respectively. Even though all febrile children and adults without localizing signs have underwent lumbar puncture to rule out meningitis, the bacterial isolation rate was found to be low. The isolation rate of bacteria from CSF culture in the present study is comparable to previous studies conducted in Gondar where isolation rate of 5.2% (Assefa and Yohannes, 1996) and 5.6% (Mulu *et al.*, 2005). In the present study, pathogen isolation rate was highest in adults 8(14.5%) than in children 16(9.2%) and neonates 2(1.8%) ($p< 0.05$).

The type and frequency of pathogens isolated from CSF is shown in Table 3.4. The number of Gram positive and Gram-negative organisms was equal. In this study CoNS and *L.monocytogenes* were the only pathogens isolated from neonates. The three leading microbial agents identified in children were *S. pneumoniae* (37.5%), *H. influenzae type b* (18.75%), and *N. meningitidis* (12.5%) (Table 3.4). Generally the pattern of isolation agrees with previous findings done in Ethiopia (Hailu and Muhe, 2001; Melaku, 2003; Amsalu and Assefa, 2005) and elsewhere e.g. in Brazil (Bryan *et al.*, 1990), North East Thames region (London) (Urwin *et al.*, 1994), Benghazi, Libyan Arab Jamahiriya (Rao *et al.*, 1998), Papua New Guinea (Alpers *et al.*, 1999), Blantyre (Wilson *et al.*, 2003), Saudi Arabia (Al-Mazrou *et al.*, 2003), Romania (Luca *et al.*, 2004), Egypt (Farag *et al.*, 2005), Nigeria (Ogunlesi *et al.*, 2005), Burkina Faso(Isabelle *et al.*, 2005), Yemen (Al-Khorasani and Banajeh , 2006), US (Nigrovic *et al.*, 2007), Oman (Dash *et al.*, 2008), Turkey (Ceyhan *et al.*, 2008), and Southern Mozambique (Sigaúque *et al.*, 2008). However, in some studies *N. meningitidis* was more common than the other bacteria (Dagan *et al.*, 1994; Hailu and Muhe, 2001; Melaku, 2003; Luca *et al.*, 2004; Isabelle *et al.*, 2005; Al-Khorasani and Banajeh , 2006). This is probably due to the endemicity of the organism and to the larger number of cases that may be involved in epidemic situations. In this study, among the isolates of *N. meningitidis* two of them were serogroup A that showed the predominance of Group "A" in Ethiopia that is comparable to other studies done in Ethiopia (Habte-Gabr *et al*, 1979; Mengistu *et al*, 2003; Norheim *et al*, 2006). In the same manner, few reports have shown the predominance

of *H. influenzae type b* (Rao *et al.*, 1998; Kiwanuka and Mwanga, 2001; Amsalu and Assefa, 2005; Farag *et al.*, 2005; Dash *et al.*, 2008; Roca *et al.*, 2009). This might be related to incomplete immunization status of the children. *H. influenzae type* b was the only serotype isolated in the present study which is in agreement with previous study done in Ethiopia (Muhe and Klugman, 1999).

In this study, *S. pneumoniae* (50%) was the most common bacterial cause of meningitis in adults. This is comparable to different studies (Mulu *et al.*, 2005; van de Beek *et al.*, 2004; Isabelle *et al.*, 2005; Schuchat *et al.*, 1997; Wilson *et al.*, 2003). But in other studies *S. pneumoniae* was, the second most common organism following *N. meningitidis* (Sigurdardottir *et al.*, 1997; Apgar, 1997) and may be related to epidemic situations. More over, *C. neoformans* accounted for 50 % of the isolates, which is a little bit lower than a study done in Blantyre, Malawi that accounted for 67% (Wilson *et al.*, 2003). The lower sample size may contribute for this variation. The isolation rate of *C. neoformans* in adults was 7.3 %, which is in agreement with a study done in Ethiopia (Woldeamanuel and Haile, 2001).

In addition, *E. coli, Enterobacter spp., Pseudomonas spp.,* and *Acinetobacter spp*. accounted for 22.7% of bacterial isolates. These organisms were also isolated from meningitis patients in different studies (Kiwanuka and Mwanga, 2001; Hailu and Muhe, 2001; Jones *et al.*, 2004; Mulu *et al.*, 2005).

Infections of the central nervous system (CNS) are potentially life threatening, requiring rapid diagnosis and immediate parenteral treatment (Jones *et al.*, 2004). The need to treat infections of the CNS immediately requires empiric choice of an antibacterial agent. For many years β-lactams have comprised the cornerstone of therapy and parenteral third-generation cephalosporins such as ceftriaxone or cefotaxime are most commonly used (Aronin, 2000). Despite clinical success, antimicrobial resistance caused by β -lactamase and other mechanisms are an emerging problem (Peltola, 2001). For the treatment of the most important pathogens associated with acute community-acquired meningitis including *S. pneumoniae* and *H. influenzae*, resistance to penicillin derivatives is relatively common; resistance to third-generation cephalosporins remains uncommon (Jones *et al.*, 2004). In Malawi, 20% and 50% of Hib meningitis strains were resistant to chloramphenicol and

ampicillin, respectively (Molyneux *et al.,* 1998) and 3% of *H. influenzae* invasive isolates were already resistant to cefotaxime in Mali (Peltola, 2001).

Pneumococcal meningitis remains a potentially devastating disease that carries high mortality and morbidity rates. Until the last decade, antibiotic therapy regimens were simple because all isolates of *S. pneumoniae* were susceptible to penicillin. Thus, amoxicillins were the antibiotics of choice for the treatment of pneumococcal meningitis. The dramatic increase in the prevalence of strains with reduced susceptibility or even resistant to penicillin, amoxicillin, or to third-generation cephalosporins led to the publication of new guidelines for the treatment of *S. pneumoniae* meningitis. According to these guidelines, patients with suspected pneumococcal meningitis should receive a combination of cefotaxime or ceftriaxone plus vancomycin at least until the results of susceptibility testing are available (Tunkel and Scheld, 1995). Since clinicians will likely initiate antimicrobial therapy prior to the microbiological characterization of the infecting agent, with the reduction in incidence of some species and increased resistance in others, resistance surveillance plays an important role in helping to understand trends in predominant pathogens and the impact of resistance on empiric choice (Hoffman *et al.*, 2003).

The present study also provides insights into the susceptibility profile of bacteria isolated from meningitis patients. The susceptibility pattern of gram positive and gram-negative organisms to the most relevant antibiotics is depicted in Tables 3.5 and 3.6. Gram-positive bacteria showed low-level resistance to Penicillin, Tetracycline and Trimethoprim-sulphamethoxazole and intermediate level of resistance to Gentamicin. *S.pneumoniae* showed high level of resistance to Gentamycin. This is in agreement with previous study done in Ethiopia (Mulu *et al.*, 2005). In the present study, gram-negative bacteria showed high-level of resistance to Tetracycline and Trimethoprim-sulphamethoxazole and intermediate level of resistance to Ampicilin. All isolates showed low level of resistance to Ceftriaxone, Ciprofloxacin, Gentamycin, Chloramphenicol and Rifampicin which is comparable to other findings (Mulu *et al.*, 2005).

H. influenzae showed high level of resistance against Ampicilin, Trimethoprim-sulphamethoxazole and Tetracycline. This observation is comparable to that of other researchers (Muhe and Klugman, 1999; Skoczyńska *et al.*, 2005; Roca *et al.*, 2008). *N.*

meningitidis was found to be highly resistant to Trimethoprim-sulphamethoxazole and Tetracycline. This observation is comparable to that of other findings (Mulu *et al.*, 2005; Ozumba, 2005; Norheim *et al.*, 2006).

E. coli isolated from adult found to be resistant to all antibiotics except for Chloramphenicol where as the one which was isolated from child patient was found to be resistant only to Tetracycline and Rifampicin. Multiple drug resistance (resistance to two or more drugs) was observed in 18.2% and 100% gram positive and gram-negative bacteria respectively ($p<0.05$) in the present study. Among the gram positives, MDR was observed among *S. pyogenes* and CoNS, while among the gram negatives, MDR was observed in all isolates. The multiple drug resistance pattern of gram negatives is comparable to findings in other studies (Kiwanuka and Mwanga, 2001; Hailu and Muhe, 2001; Jones *et al.*, 2004; Mulu *et al.*, 2005). In the present study, ceftriaxone and ciprofloxacin were the most effective drugs when compared to other drugs tested against the gram-positive and gram-negative bacteria (Tables 3.5 and 3.6). This is comparable with other studies (Kiwanuka and Mwanga, 2001; Mulu *et al.*, 2005).

LIMITATIONS OF THE STUDY

- ☞ Serotyping of *S. pneumoniae* and biotyping of cryptococcal isolates were not done due to absence of antisera.

CONCLUSION AND RECOMMENDATIONS

In conclusion, the isolation rate of bacteria causing meningitis is low. This may be probably due to the fastidious nature of the organisms, or over clinical diagnosis of meningitis, especially in neonates and children. The relative ratio of gram stain and culture in isolated organisms was similar. *Streptococcus pneumoniae* was the most common organism causing bacterial meningitis in children followed by, *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis*. Bacterial meningitis in adults was mainly due to *Streptococcus pneumoniae*. *C. neoformans* was also the predominant isolate in AIDS patients. All isolates of *Streptococcus pneumoniae* were resistant to Gentamicin. Most of the tested drugs were effective against gram-positive bacteria where as gram-negative bacteria showed high level of resistance. Ceftriaxone and ciprofloxacin were the most effective drugs when compared to other drugs tested against the gram-positive and gram-negative bacteria. MDR was observed among *S. pyogenes,* CoNS and in all isolates of gram negative bacteria.

Based on these findings the following recommendations are made: -

- ☞ Gram stain can serve as a useful means of reaching a presumptive diagnosis.
- ☞ There is a need for a continuous surveillance for resistant bacteria to produce updated information on local pathogens and their sensitivity patterns, which can provide the basis of alternative treatment.
- ☞ Since ceftriaxone and ciprofloxacin were the most effective drugs, these antibiotics may be used for the immediate empirical therapy of meningitis before culture and sensitivity report is available.
- ☞ Alternative laboratory methods such as antigen detection and nucleic acid amplification should be integrated into laboratory procedures especially in researches to improve the detection of microorganisms in CSF.
- ☞ An accurate laboratory confirmation of the etiology in acute bacterial meningitis is essential to provide optimal patient therapy, appropriate case contact management, and reasoned public health actions. Prospectively, it also provides information upon which to base decisions regarding immunization programs.
- ☞ Vaccination and treatment policies should reflect the primary etiologic agents, including pneumococcus and meningococcal serogroups A and C and, among children, *H.*

influenzae type b. Moreover, policies should be responsive to ongoing changes in the epidemiology of meningitis.

- ☞ Further study is required to investigate the outcome of bacterial meningitis including case fatality rate and sequelae.

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APPENDIX I. Questionnaire

Questionnaire for investigation of the Etiologies of meningitis in neonates/ children and adults attending Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia.

I. Sociodemographic data Date _____

Code number _____

Hospital No._____

Address_____

Age _____

Sex_____

II. Clinical Diagnosis

Meningeal Signs

Neck stiffness _____

Kernig's sign_____

Brudzinski's sign _____

Bulge of fontanel _____

III. Laboratory Data

Gross appearance of CSF: Clear and colorless____ Cloudy_____ Pusy_____

Red colored_____ Yellow colored _____

WBC and Differential count _____

Protein _____

Sugar _____

Microscopic Examination:

Gram stain_____ AFB stain_____ India ink stain_____

Culture and Identification: _____

Serogrouping/ Serotyping result_____

Antimicrobial Susceptibility Testing (AST)

Drugs	**Sensitive**	**Resistant**	**Intermediate**
Ampicillin(10 µg)			
Ceftriaxone (30µg)			
Chloramphenicol(30 µg)			
Ciprofloxacin(1 µg)			
Cotrimoxazole(25 µg)			
Erythromycin(15 µg)			
Gentamicin(10 µg)			
Penicillin(10 µg)			
Rifampin (25 µg)			
Tetracycline (30 µg)			

Comment:

_____

_____

Date and signature of principal investigator _____

APPENDIX II. CONSENT FORM

(To be translated in to the patient's language)

Name……..…….. Card no…..……. Ward……..…… Serial no…….

I have been informed that the objective of this study is to assess the bacterial profile and pattern of antimicrobial resistance of meningitis. Because the type of organisms and pattern of antimicrobial resistance in meningitis are different, the results of this study are believed to be important to treat patients appropriately. I have also been informed about the confidentiality of the questionnaires. Therefore, with full understanding of the importance of the study, I agreed voluntarily to give the requested samples in the above for clinical investigation in the study. Results will be reported to the requesting physician/pediatrician for appropriate treatment and management.

Adults

I_____ here by give my consent for giving of the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Neonates/ Children

I_____ parent/guardian here by give my consent for giving of the requested information and specimens from my child as the doctors find best for her/him.

Signature: _____ Date_____

ቅጽ 2

<u>የስምምነት መግለጫ</u>

(ትርጉም በአማርኛ)

ተራ ቁጥር..... ካርድ ቁጥር.....

የመ ከምያ ክፍል.....

የዚህ ምርምር ዋናው አላማ የማንጅራት ገትር በሽታ አምጪ የሆኑ ተህዋስያንንና የፀረ ተህዋስያኑን መድሃኒት የመቋቋም ባህርያቸወን ለማጥናት በመሆኑ፤የጥናት ውጠቱ ደግሞ ለኔ/ ለልጄና ለሌሎች ከሚዎች ህክምና ጥቅም ንድሚውልና ንደሚያገለግል ተረድቻለሁ፡፡ በተጨማሪም በጥናቱ ውስጥ ለእኔ/ ለልጄ የቃል መረጃ በመስጠትና ናሙና ንድሰጥ በአጥኚው ባለሙያ ፈቃደኝነቴን ተጠይቄያለሁ፡፡

በመሆኑም የጥናቱ ዓላማና ጥቅም በሚገባ ስለተገነዘብኩ ፤ ከጥናቱ የሚገኘው ውጤትም ለህክምና ጠቃሚ መሆኑን አውቄ ከላይ የተጠቀሱትን ለጥናቱ የሚያስፈልጉትን ሁሉ ለመስጠት በሙሉ ፈቃደኝነት መስማማቴን በፊርማዬ አረጋግጣለሁ፡፡

ስም.....

ቀን.....

ፊርማ .....

DECLARATION

I, the under signed, declare that this M.Sc thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for this thesis have been duly acknowledged.

M.Sc candidate: **Mebratenesh Mengistu Amare**

Signature _____

Date and Place of submission _____

Addis Ababa, Ethiopia

Supervisor: **Daniel Asrat, MD, M.Sc, PhD**

Signature _____

Date and Place of submission _____

Addis Ababa, Ethiopia

Supervisor **Yimtubezinash W/Amanuel, MD, M.Sc, PhD**

Signature _____

Date and Place of Submission _____

Addis Ababa, Ethiopia