ETIOLOGY OF BACTERIAL MENINGITIS IN ETHIOPIA, 2007 – 2011: A RETROSPECTIVE STUDY

Thesis submitted as a part of the Master of Philosophy Degree in International Community Health.

ARSLAN AHMED

Supervisor: PROF. DOMINIQUE A. CAUGANT, Ph.D

Co-supervisors: DR. ABRAHAM ASEFFA, M.D, Ph.D

DR. GUNNSTEIN NORHEIM, Ph.D

SEPTEMBER, 2012

University of Oslo

Faculty of Medicine

Institute of General Practice and Community Medicine

Section for International Health

Table of contents

Acknowledgements

This study has been an exciting experience for me. As I conclude with the write-up, I realize that this work could not have been possible without the help of my supervisor, co-supervisors and the collaborating staff. As an early stage researcher working on my first research project I was always in need of guidance. In this regard I am grateful to my supervisor, Prof. Dominique A. Caugant for being patient with my mistakes and encouraging me and helping me out at each step.

I would extend my deepest gratitude to my co-supervisors Dr. Abraham Aseffa and Gunnstein Norheim for providing me valuable feedbacks in the light of their experience. I must thank Gunnstein Norheim for his continuous support during the process of study and also accompanying me on my initial visit to Ethiopia. I owe my deepest gratitude to the staff at Norwegian Institute of Public Health particularly Jan Oksnes for the training I received on laboratory detection techniques and Real-Time PCR.

My sincere appreciation goes to the collaborating staff at Armauer Hansen Research Institute, Gondar University Hospital and Awassa Referral Hospital in Ethiopia. The help of Mr. Yarid Merid at Awassa and Mr. Kasim Molla at Gondar made the data collection easier. I am highly grateful to Mrs. Wude Mihret, Tsehaynesh Lema and Melaku Yedenekachew who were always very helpful during my visits to Ethiopia. I would thank the wonderful people of Ethiopia because it was for their love that I never felt away from home.

In the end, I would like to thank all my friends who encouraged me and supported me through the long hours of thesis writing. Finally, it was the continuous support of my parents, my brother, my sister and my grandmother that served as the main motivational and driving force. I could not have done it without your support.

And for all the people of Ethiopia who could not make it in their struggle against meningitis. May your souls always rest in peace!

Arslan Ahmed

30th August' 2012.

List of abbreviations:

AHWO	African Health Workforce Observatory
AHRI	Armauer Hansen Research Institute
BBB	Blood Brain Barrier
CNS	Central nervous system
CFR	Case fatality rate
CSF	Cerebrospinal fluid
EFNS	European Federation of Neurological Societies
GDP	Gross domestic product
GAVI	Global Alliance for Vaccine and Immunization
Hib	*Haemophilus influenzae* type b
HSDP	Health Sector Development Program
LPS	Lipopolysaccharide

LP	Lumbar puncture
MVP	Meningitis Vaccine Project
MLST	Multilocus sequence typing
NRERC	National Health Research Ethics Review Committee
NIPH	Norwegian Institute of Public Health
OMV	Outer membrane vesicle
PCR	polymerase chain reaction
REK	Regional committee for medical and healthcare research ethics
SNNPR	Southern Nations, Nationalities and Peoples' Region
TBM	Tuberculous meningitis
WHO	World health organization

Abstract:

Bacterial meningitis is a serious infection and is associated with considerable mortality and morbidity in various parts of the world. It has a global epidemiology but sub-Saharan Africa bears the highest burden of the disease. Located in the eastern horn of Africa, Ethiopia is also endemic for bacterial meningitis with frequent meningococcal epidemics occurring every few years particularly in the dry season from December to June. Although it is generally considered a disease of the childhood, no age group is exempt from the infection. In the developing countries the fatality rate associated with bacterial meningitis can often be very high. In the absence of proper treatment, bacterial meningitis is known to cause serious neurological complications which may persist throughout the life.

Bacterial meningitis has remained a serious health concern for Ethiopia for the past few decades. Formulation of effective preventive strategies can only be based on the estimates of the recent epidemiological trends of bacterial meningitis. The study conducted focuses primarily on the recent trends of the disease in two major cities of Ethiopia i.e. Gondar and Awassa. The data collected retrospectively from the hospitals' clinical and laboratory records provide an insight into the epidemiology, demographical characteristics such as age-wise and sex-wise distribution of the disease, seasonal variation of the etiological agents and the treatment outcomes of bacterial meningitis in Ethiopia. The bacterial cultures of the cerebrospinal fluid (CSF) and the Gram staining results from the past five years were studied to find out the estimated prevalence of the common agents of bacterial meningitis. The clinical records from the hospital wards provided insight into the various common clinical signs and symptoms associated with bacterial meningitis and the treatment outcomes including the various common complications of the disease.

The study showed a higher prevalence of bacterial meningitis in males with an observed male to female ratio of 1.7:1 at Gondar and 1.9:1 at Awassa. The disease incidence was highest in small children and young adults. Infants were the most commonly affected age group at Gondar University Hospital which formed almost 27% of the cases. Young adults between 15-24 years of age were among the most effected age groups at Awassa Referral Hospital and also accounted

to about 27% of the cases. A marked effect of seasonal variation was observed with more cases occurring in the summer months. Almost 35% of the cases of bacterial meningitis at Gondar were recorded in the months of May and June. Culture specific results show that this variation was most pronounced in meningococcal disease in which almost 2/3 of the cases (67%) occurred in the dry season during the second quarter of the year i.e. April to June. Among the various agents of bacterial meningitidis, *Streptococcus pneumoniae* was the most common organism which was identified in the CSF cultures of 35 patients (35.3%). This was followed by *Neisseria meningitidis* from 27 cases (27.3%) and *Haemophilus influenzae* from 9 cases (9.1%).

In the absence of laboratory facilities the diagnosis of bacterial meningitis largely depends upon the clinical signs and symptoms at the time of presentation. The most common clinical symptoms that were recorded from the clinical records of Awassa Referral Hospital include high grade fever (88.9% of the cases), neck rigidity (74.8%), headache (69.6%) and nausea and vomiting (59.3%). Altered mental state was present in more than half of the patients. Various treatment outcomes were recorded including complete recovery (56.7%), partial recovery with sequelae (9.2%) and death which was recorded in 23.5% of the cases.

The study had been conducted with the aim to provide data that will be useful for formulation and implementation of preventive strategies against bacterial meningitis in Ethiopia. The results represent two major and demographically distinct cities of Ethiopia. These results can be generalized to give estimate of the recent trends and the current prevalence of bacterial meningitis in Ethiopia which may provide basis for future research not only in these study sites but also in other cities of Ethiopia.

Chapter One: INTRODUCTION

Meningitis is the inflammation of membranes covering the brain and spinal cord. Meningitis can be due to both infectious and non-infectious causes. Infectious causes are more common and on the basis of the causative organism, they can be classified as pyogenic or bacterial meningitis, viral meningitis, tuberculous and aseptic meningitis. Bacterial meningitis is a serious condition which demands early diagnosis and prompt treatment.

1.1. Bacterial meningitis:

Bacterial meningitis is the most prevalent type of meningitis. The most common agents of bacterial meningitis are *Haemophilus influenzae* type b, *Neisseria meningitidis*, serogroups A, B, C, W135 and Y, and *Streptococcus pneumoniae*. Globally, bacterial meningitis affects approximately 1.2 million people each year and causes almost 170,000 deaths [(1)]. In the absence of proper treatment, the mortality rate associated with bacterial meningitis can be as high as 50% [(2)]. For this reason bacterial meningitis is among the 10 leading causes of mortality due to infections worldwide [(3)]. Survivors of bacterial meningitis can suffer from serious neurological complications such as deafness, blindness, cognitive and intellectual impairment etc which often persist throughout the life. Although no age group is exempt from acquiring the infection, bacterial or pyogenic meningitis has the highest incidence in the first year after birth. Adolescence also shows a higher incidence between 15-24 years of age which accounts for almost 30% of all the cases of bacterial meningitis [(4)].

1.2. Other causes of meningitis:

Apart from bacterial meningitis many other causes of meningitis exist; these are viral meningitis, tuberculous meningitis (TBM) and other non-infectious causes of aseptic meningitis. Viral meningitis, which is usually less severe than bacterial meningitis, is a result of meningeal infection by various viruses. A virus may only be identified in 50% of the cases; the most common of those identified are some enteroviruses [(5)]. Common childhood infections such as chicken pox and measles have often been implicated in viral meningitis. TBM is caused by

Mycobacterium tuberculosis and is a very severe form of disseminated tuberculosis. Like acute bacterial meningitis, TBM also results in high rates of neurological complications and often lifelong sequelae. Without proper treatment the mortality rate with TBM can be very high [(6)]. Tuberculosis is a disease linked to low socio-economic status; therefore TBM is rare in developed countries. The individuals most at risk of acquiring TBM are the young children already exposed to primary tuberculosis, immunocompromised such as very old age, malnourished or patients with concurrent HIV infection [(7)]. Aseptic meningitis is a term reserved for the meningitis for which initial clinical examination and routine laboratory tests (including Gram staining and CSF culture) fail to reveal a definite cause. The etiology of aseptic meningitis often includes viral, fungal or TBM. Non-infectious causes of aseptic meningitis such as malignancies with brain metastasis or some medications notably sulphamethoxazole and non-steroidal anti-inflammatory drugs have also been identified [(8)].

1.3. Disease definition:

By definition *"bacterial meningitis is an inflammatory response to bacterial infection of the membranes covering the brain and spinal cord"* [(9)]. In literature various practical definitions have been used to set up an inclusion criterion for cases of bacterial meningitis. A study conducted in Mali in 2009 on the persistence and spread of meningococcal meningitis defined a "suspected case" as the one which is only clinically diagnosed. A "probable case" was defined as a suspected case with a cloudy CSF sample. A "case" was confirmed only after the etiology was established biologically [(10)]. Some studies defined cases based on clinical signs and symptoms specific to bacterial meningitis such as neck stiffness, altered consciousness, high grade fever, seizures etc [(11)]. But clinical symptoms are often non-specific and vary from patient to patient. Other studies in literature have defined a "case" based on WHO recommendations which defines a case as a patient with purulent CSF and with a cell count of >100 cells/mm3 [(12)]. But this definition requires readily available laboratory assistance which may not be possible in many hospitals in the developing countries of the world. Due to this reason a more clinical definition of meningitis is used in many studies to formulate an inclusion or exclusion criteria.

1.4. Epidemiology of bacterial meningitis:

The exact incidence of bacterial meningitis worldwide remains difficult to estimate due to the variation in the surveillance mechanisms present in the different parts of the world. While surveillance is well established in the industrialized world, the incidence of bacterial meningitis is underreported in many developing countries. In the past decade a sharp decline in the incidence of bacterial meningitis in the developed countries has been witnessed, where the incidence now lies between 1-3 per 100,000 population [(13)]. This decrease is attributed to the introduction of vaccines against common pathogens of bacterial meningitis. Development of vaccine against *H. influenzae* type b and its routine use in childhood immunization schedules has nearly eradicated the corresponding organism from developed countries. Similarly, a substantial reduction in childhood pneumococcal meningitis has been observed following the introduction of the conjugate vaccine covering seven different serotypes of *S. pneumoniae* [(14)]. But still in some developing countries the incidence may be as high as 800 cases per 100,000 population [(15)].

Although most of the environmentally acquired bacteria have the potential to cause meningitis, the majority of the bacterial meningitis cases are due to *S. pneumoniae, N. meningitidis* and *H. influenzae* type b (Hib). In recent years, due to the decline in the cases of *H. influenzae, S. pneumoniae* and *N. meningitidis* have become the most common causes of bacterial meningitis [(16)]. The etiology of bacterial meningitis varies with the population under study, the geographical conditions and the season of the year. This is easily demonstrated by the sharp increase in the incidence of meningococcal meningitis during the dry season in some parts of the sub-Saharan Africa. This is particularly attributed to the dry climate and harsh winds, thus causing the "ill wind bringing meningitis" effect [(17)]. Different age groups show high susceptibility to certain organisms. Gram negative enteric rods such as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and some other organisms such as *Listeria monocytogenes* are more common during the neonatal period, while *S. pneumoniae* and *N. meningitidis* are more common in the children and young adults [(18)].

1.4.1. *Streptococcus pneumoniae*:

S. pneumoniae is one of the most common causes of bacterial meningitis worldwide. It is a capsulated bacterium which has 93 serotypes based on the different polysaccharide

characteristics of the capsule. Most of the serotypes are capable of causing disease but majority of the infections in the developing countries are caused predominantly by serotypes 1 and 5 [19]. Although no age group is exempt from pneumococcal meningitis, it usually affects small children under the age of 2 years. The other age group with high susceptibility to pneumococcal infection is the old age. Immunocompromised people are also at a higher risk of acquiring pneumococcal meningitis [20]. Like *N. meningitidis* and *H. influenzae*, *S. pneumoniae* spreads as respiratory droplets. The high rates of pneumococcal infections may partly be due to the high carriage rates among the general population. Children under 6 years of age have the highest rates of nasopharyngeal carriage [20]. Due to this reason pneumococcal disease has become the leading cause of vaccine preventable deaths in that age group [21]. The incidence in children under 5 years of age is estimated to be 17 cases per 100,000 population, which is also associated with a high mortality rate often reaching up to 73% in some parts of the developing world [22]. Pneumococcal meningitis incidence may exhibit mild seasonal variations. Although some strains of *S. pneumoniae* have been implicated in large outbreaks, causing widespread epidemics is not considered typical of pneumococcal disease [23] [24].

1.4.2. *Neisseria meningitidis*:

N. meningitidis is an obligate commensal residing in the human nasopharynx. The highest incidence of nasopharyngeal carriage of *N. meningitidis* is in adolescents especially those residing in overcrowded spaces. Particularly prone are school-going children and college students, household contacts of meningococcal patients and also military recruits [25]. Other factors that may predispose to meningococcal carriage include lower socio-economic status and concurrent viral or bacterial respiratory tract infection. In such individuals the carriage rates can be as high as 34% [26] [27]. Recent estimates show that the global incidence of meningococcal disease is 500,000 per annum with a worldwide mortality rate of 10% [28]. *N. meningitidis* can exist with or without a polysaccharide capsule. However, nearly all of the meningococcal meningitis infections are caused by the capsulated form. Based on the polysaccharide characteristics *N. meningitidis* can be divided into at least 12 different serogroups. Serogroups A, B, C, W135, X and Y are isolated in almost 90% of the infections [15]. The serogroup distribution is often related to the age of the patient and more importantly to the geographical location [26]. Serogroup A is frequently isolated from CSF samples of meningitis patients in sub-Saharan

Africa where it causes epidemics. In these epidemics the incidence is very high, often reaching up to 1 case per 100 population. The fatality rates even with treatment can be more than 10% [29] [30]. Serogroups B and C are more common as a cause of meningitis in Europe, America and Australia [31]. Serogroup C has occasionally been the cause of epidemics and outbreaks in these countries [32]. The incidence of serogroup C has been reduced considerably in the recent years due to the development of effective conjugate vaccines [29]. Other serogroups such as serogroups W135, X and Y are prevalent in some parts of Africa and US respectively [2]. Meningococcal disease may develop into a widespread blood infection known as meningococcemia, which is a serious and often fatal form of meningococcal infection.

1.4.3. *Haemophilus influenzae* type b:

H. influenzae is a common respiratory pathogen which can occur either as capsulated or un-capsulated form. The difference in structure of the polysaccharide capsule is the basis for the division of *H. influenzae* into 6 serotypes; a, b, c, d, e and f [33]. Out of these 6 serotypes, serotype b is associated with most of the meningitis infections. Once known as the most common cause of acute bacterial meningitis, the incidence of Hib has been reduced substantially, principally due to the introduction of vaccine. A conjugate protein polysaccharide vaccine that was introduced in early 1990s has been very effective in controlling Hib infections [16]. The infection occurs usually in children less than 5 years of age and is rare in adults. The incidence varies in different parts of the world but it is generally estimated to be higher in Africa where the incidence is around 46 per 100,000 population. In Europe this incidence is much lower and is around 16 cases per 100,000 population [34]. In unimmunized patients, the mortality rate is estimated to be almost 43% [34].

1.4.4. Neonatal meningitis:

Neonates are particularly prone to acquiring bacterial meningitis possibly due to the immaturity of their immune system. Even in the industrialized countries the incidence of neonatal meningitis is about 0.3 cases per 1000 live births [35]. In some parts of Africa and South Asia the incidence in much higher and is estimated to be around 6.1 per 1000 live birth [36]. Although the mortality rates are less than 10% [37], the high incidence of long term neurological complications is the matter of most concern [38]. The organisms causing neonatal meningitis include group B

streptococci which accounts to about half of all the cases of neonatal bacterial meningitis. This is followed by Gram negative enteric rods particularly *E. coli* which is isolated in 20% of the cases. Another 5-10% of the cases are caused by *L. monocytogenes* [39] [40]. In developing countries the incidence of gram negative rods such as *E. coli* and *K. pneumoniae* may be much higher [37]. A recent decrease in the incidence of group B streptococci is attributed to the antibiotic prophylaxis given pre-partum to the neonates at risk [41].

1.4.5. Other organisms of bacterial meningitis:

Other uncommon causes of bacterial meningitis include *Staphylococcus aureus, Pseudomonas aeruginosa* and some other enterococci. They are usually associated with nosocomial infections and may be acquired after trauma or some surgical interventions. However, with advancement of antibiotic therapy, immunization and aseptic techniques during interventions, their incidence is on a rapid decline [42].

1.5. Pathophysiology of bacterial meningitis:

The majority of the symptoms produced as a result of bacterial meningitis are as a result of the inflammatory response to the invading organism [43]. This inflammatory response is a step wise process of acute and chronic humoral immunity directed against the pathogens that produces the effects of meningitis. The events involved in the development of the disease can be summarized as follows:

1.5.1. Bacterial invasion:

High grade bacteremia or the invasion of the blood by bacteria having the potential to cause meningitis is the foremost step in the development of bacterial meningitis. Alternatively, meningitis can occur as a consequence of direct invasion of the central nervous system (CNS) which may result from dural defects or local infection of the CNS. Contagious spread of infection from sinuses and internal ear is also a recognized cause of meningitis in a small portion of patients [44]. But usually the infection of the meninges follows a high grade bacteremia [45]. The exact site at which the transmission of the bacteria from blood to the CNS occurs is uncertain. The choroid plexus is believed to be associated with this transmission. This was demonstrated by Daum *et al.* in 1978, who observed the transmission of *H. influenzae* via the

choroid plexus [46]. Recently, with the advancement in imaging and laboratory techniques, certain other sites that may also serve as a potential point of entry to the CNS have been identified. Studies have documented the presence of meningococci in the meninges in addition to the choroid plexus [47]. Similarly, pneumococcal infiltration of the leptomeningeal vessels has also been documented [48].

The blood brain barrier (BBB) and the sophisticated tight junctions restrict the bacterial entry to the CNS. The breach of the BBB or the blood-CSF barrier is therefore crucial to the entry of the bacteria into the CNS. This is achieved by the presence of certain proteins on the surface of the bacteria which cause a breach in the BBB. The identified proteins include Streptococcal proteins such as CbpA, meningococcal PilC1 adhesin and outer membrane proteins that assist in bacterial adhesion and subsequent endocytosis [49]. Similar adhesive molecules are also identified in GBS [50] and *E. coli*, both of which are a common cause of meningitis in newborns. The opacity proteins expressed on the outer membrane of *N. meningitidis* (Opa and Opc) serve the purpose of bacterial adhesion and endocytosis [51].

1.5.2. Inflammatory response:

With the bacterial invasion occurs the inflammatory response of the endothelial cells. This inflammatory response leads to the leukocyte infiltration which is a multi step process involving the accumulation of leukocytes particularly the granulocytes. The presence of granulocytes in the CSF is therefore important in the diagnosis of bacterial meningitis. The process of bacterial invasion and inflammation seem to occur parallel, with the later assisting the former by increasing the permeability of the BBB. The products of leukocyte activations which include the matrix metalloproteinases, nitric oxide and others affect the BBB and the blood-CSF barrier by causing it to break [52]. This provides bacteria the opportunity to infiltrate the barrier and gain entry into the CNS. Once inside the sub-arachnoid space, the bacteria replicate. The increase in number of bacteria along with their autolysis enhances the process of inflammation which is the basis of pathogenesis of bacterial meningitis.

The inflammatory response to the bacteria is a complex process involving a variety of inflammatory cells notably endothelial cells, mast cells and perivascular macrophages [53]. Bacterial components capable of inducing host inflammatory response include peptidoglycans, lipoprotein, lipopolysaccharides and lipoteichoic acid. Experiments showed that their potential to

trigger the inflammatory mediators remains unaltered even if heat killed bacteria are inoculated into the host [54] [55].

1.5.3. Neuronal damage:

Bacterial meningitis has a very high incidence of neurological complications with almost 50% of the patients showing neurological deficits to varying degree [56]. The neuropathy results from the inflammation of the subarachnoidal space, vasculitis and edema of the brain tissue. Neuronal injuries to the cerebral cortex, hippocampus and inner ear are also an important cause for most of the complications [57]. The damaged caused to the CNS by the invading bacteria is attributed to multiple factors such as bacterial toxins, the inflammatory response to the invading organism or the cytotoxic elements of the complement system. In addition to these factors, the indirect effect of these intracranial complications on the surrounding structures of the CNS is responsible for the symptoms due to the "space occupying lesion" effect that accompanies meningitis. The bacterial toxins for *S. pneumoniae* include pneumolysin, which is a pore forming cytolysin and hydrogen peroxide [58]. The pneumolysin preferentially affects the mitochondrial membrane causing the damage by virtue of its pore forming activity [59]. The key virulence factors for *N. meningitidis* and other related gram negative bacteria are the lipopolysaccharides and lipooligosaccharides. These endotoxins trigger the release of interleukin-6 and tumor necrosis factor alpha (TNF α) along with other cytokines mediators. These mediators are responsible for the tissue or organ damage and subsequently the symptoms that are characteristic of bacterial meningitis [60].

1.6. Clinical presentation:

Clinical assessment of severity of bacterial meningitis is crucial for identifying the factors that affect the outcome of bacterial meningitis. Most common signs and symptoms include the "classic triad" of fever, headache and neck stiffness. However, these classical symptoms occur in less than half of the cases [61]. Usually, the symptoms are non-specific early in the course of the disease with fever, headache and malaise as the main presenting features. Specific symptoms such as neck stiffness, photophobia and impairment of consciousness represent meningeal irritation and develop later as the disease progresses [62]. Signs of meningeal irritation contribute significantly to the diagnosis of bacterial meningitis, more so in the settings devoid of modern

day laboratory facilities. But these signs may not be present in unconscious patients, very small children or in the immunocompromised patients [63]. Such cases may present as a diagnostic challenge. Other symptoms that may accompany bacterial meningitis include nausea, vomiting, back rigidity, shock, seizures, unconsciousness and bleeding from skin [64]. Petechial rash is a characteristic of infection by *N. meningitidis* and represent meningococcemia.

In the developing countries with minimal laboratory facilities these clinical symptoms form the mainstream for the diagnosis of bacterial meningitis. Owing to the importance of these clinical symptoms many studied were conducted to find out the incidence of the various common signs and symptoms of bacterial meningitis. A study in Gondar University Hospital, Ethiopia on 151 children showed that vomiting was present in almost 80% of the cases. This was followed by fever (75%), stiff neck (70%) and altered mental state (49%) [65].

1.7. Complications of bacterial meningitis:

If left untreated, bacterial meningitis can cause various complications such as hearing defects, speech abnormalities, intellectual impairment, learning difficulties and seizures [66] [67] [68]. The rate of these complications can be as high as 50% [69]. The chances of acquiring these complications increase depending upon the organism involved, age of the patient, the severity of disease and the quality of treatment provided [70]. These complications are more common in small children and can cause serious neurological defects that often tend to be long lasting. Included among these complications are focal neurological deficits such as paralysis of the limbs, developmental disabilities, seizures, cerebral abscesses and hydrocephalus [71] [72]. Most of these complications in children usually resolve within 2-3 years but 10% of the children may develop complications that persist throughout the life [73].

1.8. Diagnosing bacterial meningitis:

Bacterial meningitis is best diagnosed by clinical assessment assisted by laboratory evidence of the causative organism. The presence of bacteria in the CSF forms the basis of the diagnosis of bacterial meningitis. Bacterial detection rate in CSF can be as high as 90%, as compared to a mere 50% detection rate when blood samples are used for the same purpose [45]. A lumbar puncture (LP) is done to draw CSF samples from the patients. LP is a minimally invasive

procedure but not without possible complications. It is therefore, subjected to the decision of the attending physician, especially in the very young, very old, immunocompromised or patients with skin infections.

Various laboratory tests are used for the diagnosis of bacterial meningitis but CSF cultures and polymerase chain reaction (PCR) are considered as "gold standard" [(74)]. Other laboratory techniques include Gram staining, the oxidase test and latex agglutination test. On Gram staining *N. meningitidis* appear as Gram-negative diplococci, which resemble "coffee bean". *S. pneumoniae* are Gram-positive diplococci with lanceolate appearance often occurring in short chains. *H. influenzae* are small Gram-negative pleomorphic rods which depict random arrangements [(75)]. Gram staining of CSF and CSF culture are reliable methods for detecting bacterial meningitis, but in case of prior antibiotic treatment the yield can be low. Studies conducted by the American Academy of Pediatrics on 231 patients during 2001-2004 showed a decrease in CSF culture from 88% to 70% if the patients were pre-treated with antibiotics [(76)].

The biochemistry and cytology of CSF aspirate is very helpful in the overall diagnosis of bacterial meningitis, initiation of antibiotic therapy and accessing the progress of treatment. The CSF characteristics highly suggestive of bacterial meningitis include an elevated CSF cell count (>500 cells/μl), predominantly neutrophils. Increased protein levels in the CSF (>1g/l) is also an important diagnostic factor and indicates disruption of the blood-brain or the blood-CSF barrier. Similarly increased levels of CSF lactate (>0.3g/l) and lowered CSF/blood ratio of glucose (<0.4) is also suggestive of bacterial meningitis [(77)]. Although these CSF values are not highly specific and can also be associated with some other conditions, when combined with other clinical and laboratory investigations they serve as a valuable tool for the diagnosis of bacterial meningitis.

For characterization of various strains of bacterial meningitis into serogroups and serotypes, immunological methods are used. For genetic differentiation, techniques such as PCR and multilocus sequence typing (MLST) are used. A study was conducted by Norwegian Institute of Public Health (NIPH) in Southern Nations, Nationalities and Peoples' Region (SNNPR) and North Gondar zone of Ethiopia in 2002-2003 for characterization of various strains of meningococci. The study relied on similar techniques including MLST for identification of the current meningococcal strains in Ethiopia [(78)]. A study on laboratory based surveillance of

bacterial meningitis was carried out in Khartoum, Sudan in 2004-2005. CSF samples from 1,830 suspected cases of bacterial meningitis were taken. CSF samples were inoculated on Trans-Isolate medium [79] and PCR was carried out on those samples. The study concluded that by using laboratory surveillance at least 30% more cases can be diagnosed which otherwise would remain undiagnosed [11]. In both the studies mentioned above Trans-Isolate medium was used for the transport and storage of CSF samples as it can support the survival of *N. meningitidis, S. pneumoniae* and *H. influenzae* for at least 3 months [79].

1.9. Treatment:

Acute bacterial meningitis is a serious emergency requiring timely and proper treatment. Before the 20th century, acute bacterial meningitis was almost always fatal [80]. The invention of antibiotics has drastically improved the outcome of bacterial meningitis. Initiation of proper treatment within 6 hours of presentations reduces the mortality rates by more than 8 times [81]. Delay in initiation of antibiotic therapy is shown to be the single most important risk factor related to the outcome of bacterial meningitis [82]. The European Federation of Neurological Societies (EFNS) taskforce on bacterial meningitis highly recommends initiation of antibiotic treatment within the first hour of admission [83]. The usual treatment is with a broad spectrum third generation cephalosporin which is usually given empirically while the laboratory results are awaited [84].

1.10. Prevention:

Bacterial meningitis is a vaccine preventable disease and vaccines form a cornerstone in its prevention. Various types of vaccines are currently being used to prevent bacterial meningitis. Hib protein polysaccharide conjugate vaccine which was introduced in early 1990s is widely used in many countries throughout the world as a part of national childhood immunization schemes [16]. It is due to this vaccine that the incidence of Hib has fallen sharply in the past few years to the extent of virtual disappearance in some industrialized countries of the world [85]. Similarly, pneumococcal conjugate vaccine has been used for pneumococcal meningitis prevention with encouraging results. First introduced in 2000 in the United States as a 7-valent conjugate vaccine [86], the efficacy of the vaccine was about 80% for the targeted serotypes [87]. The incidence of pneumococcal disease has also fallen sharply, particularly in the countries

where the vaccine is incorporated into the national immunization schemes. A striking example is the White Apache Mountains area in Eastern Arizona where the incidence of pneumococcal disease due to the seven serotypes was reduced from 275 per 100,000 to almost none within the course of one decade from 1997-2006 [88]. Pneumococcal conjugate vaccine is now being introduced with support from The Global Alliance for Vaccine and Immunisation (GAVI) in developing countries. With the effectiveness of the Hib and the pneumococcal vaccines in view, WHO now recommends the inclusion of Hib [89] and pneumococcal conjugate vaccine [90] into the immunization schedule of all countries.

Currently two types of vaccines are being used against various serotypes of *N. meningitidis*: pure polysaccharide vaccines and conjugate vaccines [91] [26]. The conjugate vaccines are considered superior to the polysaccharide vaccines as the later is known to be less immunogenic in children and provides only a temporary protection ranging from three to five years [18] [91]. Both pure polysaccharide and conjugate vaccines against serogroups A, C, Y and W135 have been developed. Conjugate vaccine against the serogroup C is now regularly used in routine childhood immunization schedules in some European countries [30]. An example is United Kingdom where after the introduction of vaccine in 1999, the incidence of meningitis due to serogroup C has fell more than 94% in immunized people. Some reduction was also noted in un-immunized people supporting the belief that the vaccine also provides herd immunity [92]. The vaccine has also resulted in a significant decline in the nasopharyngeal carriage rates of serogroup C. Development of a vaccine against serogroup B has encountered difficulties due to the poor immunogenic nature of the polysaccharide capsule. This has led to the development of vaccines targeting other structures such as an outer membrane vesicle (OMV) vaccine. Success has been reported with the use of OMV vaccine in New Zealand with an overall efficacy ranging between 70-80% [93]. With the success of OMV vaccine against serogroup B, prospects of developing a similar outer membrane vesicle vaccine against other sergroups such as serogroup A and W135 are also underway. The development of such vaccines may pave the way for preventing most of the meningitis cases in sub-Saharan Africa [94].

Meningococcal serogroup A is more common in sub-Saharan parts of Africa where it is often the cause of widespread epidemics. A conjugate vaccine which is currently believed to be the most effective vaccine against *Neisseria meningitidis* serogroup A is available in some countries of the sub-Saharan Africa under the Meningitis Vaccine Project (MVP). The MVP, which is an

initiative by the WHO and Program for Appropriate Technology in Health (PATH) aims to provide low cost serogroup A conjugate vaccine to a target population of 250 million across 25 African countries [95]. The vaccine has currently been introduced in Burkina Faso, Mali, Niger, parts of Nigeria and Chad, but not yet in Ethiopia [96]. The successful implementation of meningococcal serogroup A conjugate vaccine in Ethiopia requires a detailed information about the prevalence trends of meningococcal meningitis and its current circulating strains in Ethiopia. For the purpose of vaccine design and implementation a study was conducted in 2002-2003 to investigate the prevalence and circulating strains of meningococcal meningitis is Ethiopia. The study was conducted in Southern Nations, Nationalities and Peoples' Region (SNNPR) and North Gondar Zone in Ethiopia. The study found antigenic variation between the meningococcal A strains of 2002-2003 when compared with that of previous strains and recommended further investigation of these potential antigens for implementation of preventive measures and introduction of new vaccines [97].

1.11. Meningitis belt:

The highest burden of bacterial meningitis occurs in an area of sub-Saharan Africa known as the "meningitis belt", described by Léon Lapeyssonnie in 1963 [98]. The area of meningitis belt that stretched from Mali to Sudan in 1963 gradually extended in the past decades. The meningitis belt that we know today stretches from Senegal in the west to Ethiopia in the east and includes 400 million people and 21 nations [99]. This area is characterized by high prevalence of bacterial meningitis. Marked seasonal fluctuations occur in the prevalence of meningococcal meningitis which rises during the dry season from December to June with incidence as high as 1000 cases per 100,000 population during an epidemic [100]. The incidence then falls steeply on the arrival of the rainy season. The mechanism by which the dry season affects the incidence of meningococcal disease may be multifactoral. Although dry climate and harsh winds may have an effect of propagation of meningococcal disease by droplet spread, the more widely accepted view is the effect of the dry climate on the integrity of the mucosal surface of nasopharynx. The breaks in the mucosal barrier lead to the progression of the carrier state to the invasive infection [101] [102]. The nasopharyngeal carriage rates of *N. meningitidis* in the area of meningitis belt are estimated to be as high as 30% [103]. This high carriage rate further increases the chances of

developing or spreading infection during the dry season of the year. Recent studies suggest, however, that the carriage rate might be much lower in absence of outbreak [104].

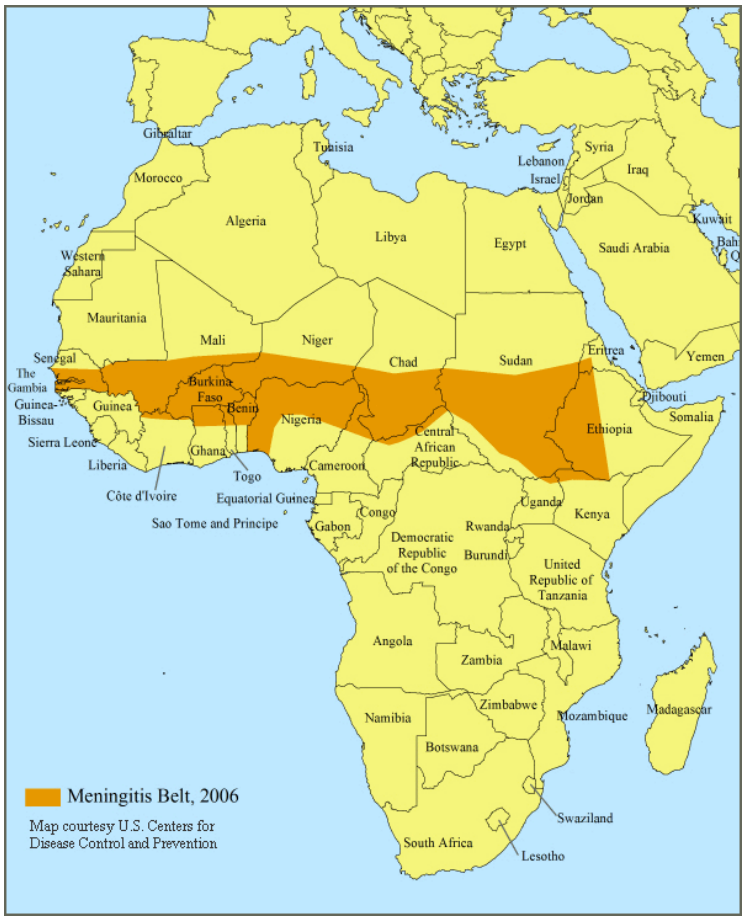


Figure 1: Map of Meningitis Belt

The part of sub-Saharan Africa that constitutes the meningitis belt is characterized by recurrent meningococcal outbreaks. The records show that major outbreaks tend to occur every 8–12 years [97]. After the first reported outbreak in 1840 almost 400 epidemics of bacterial meningitis have been recorded in the meningitis belt. The largest epidemic was reported in 1996 which affected 250,000 people and resulted in almost 25,000 deaths and 50,000 disabilities [105]. In addition to the major epidemics, smaller isolated outbreaks involving only a community also occur frequently. Most of the epidemics have been due to *N. meningitidis* serogroup A but some epidemics due to other serogroups such as serogroup C, X and W135 have also been reported

[106]. The introduction of meningococcal conjugate A vaccine under the MVP may, therefore, cause a considerable reduction in the incidence of meningococcal disease in the years to come.

2.1. Ethiopia: country profile

Located in the Horn of Africa, The Federal Democratic Republic of Ethiopia lies at the crossroads between Middle East and Africa. Ethiopia is bounded by Eritrea to the north and Kenya to the south. The eastern part is bounded by Somalia and to the west lies Sudan and South Sudan. Ethiopia covers a vast land area of 1.1 million square kilometers and is the second most populous country in Africa with a population of more than 84 million [(107)].

2.1.1. Demographics:

Ethiopia's population continues to grow at a rate of 2.5%, and has increased from 33.5 million in 1983 to 84 million in 2012 [(108)]. The capital city of Ethiopia is Addis Ababa which is also the largest city with a population of almost 3 million. Other large cities of Ethiopia include Mekele, Adama, Gondar and Awassa. Ethiopia is one of the least urbanized countries of the world with only 17% of the population residing in the urban areas. But in the recent decade rapid urbanization has occurred with the urban population increasing at a rate of 4.1% [(108)].

Ethiopia is famous for her ethnic diversity. People from at least 80 different ethnic backgrounds reside in Ethiopia. Oromo form the largest ethnic group (34.5%) followed by Amhara (26.9%), Somali (6.20%) and Tigray (6.07%) [(107)]. Due to this reason many native language are spoken in Ethiopia. These include Oromifa, Tigrinya and Somali. Amharic is the main language spoken and understood throughout Ethiopia. English is the most commonly spoken foreign language. The literacy rate still remains low with the adult literacy rate estimated to be about 30%.

2.1.2. Climate:

Most of the area of the country is covered by highlands which make the climate much cooler than the neighboring African countries. Many major cities of Ethiopia, including Addis Ababa and Gondar, are located at an elevation of more than 2000 meters which provides a considerably cooler but much uniform temperature throughout the year. The southern part of Ethiopia including the South Nations and Nationalities Peoples' Region (SNNPR) is located at a lesser elevation as compared to the northern part. The capital city of SNNPR, Awassa is located at an elevation of 1700m (in contrast to 2000m of Addis Ababa and Gondar). Due to this, Awassa has a climate much hotter than the central and northern part of Ethiopia. The seasons can be defined by rainfall into rainy season between June to September and a dry season ranging from October to February.

2.1.3. Economy:

The main domestic product of Ethiopia is agriculture which accounts to about 41% of the total GDP and makes up to 80% of the total exports. Agriculture is also the major profession in Ethiopia with almost 80% of the population associated with agriculture. Although Ethiopia showed highest economical growth within the non-oil dependant African economies in 2007-2008, the per capita GDP still remains one of the lowest in the world. The poverty rates are very high with almost 39% of the population living below the poverty line of earning less than US$ 1.25 per day.

2.1.4. Health profile:

The main health related problems in Ethiopia are due to communicable diseases. The preventable causes of death due to communicable diseases account to about 74% of the total deaths. The high rates of infectious diseases are due to poor sanitation, unavailability of healthcare facilities and lack of trained staff especially in the rural parts of Ethiopia. Another important dilemma for the healthcare system is the very high incidence for nutritional deficiencies. The main healthcare statistics are provided below:

Table 1: An overview of health statistics of Ethiopia	
Life expectancy at birth (Males)	53 years
Life expectancy at birth (Females)	56 years
Under 5 mortality rate (per 1000 live births)	106 (135 rural, 99 urban)
Maternal mortality rate (per 100,000 live births)	350
Extended program on immunization (EPI) coverage	72.6%
Immunization coverage under 1 year of age for Hib	89%
Doctor/Patient ratio (physician per 100,000 population)	2
Nurses or mid-wives (per 100,000 population)	24
Population using improved drinking water sources	38%
Population using improved sanitation	12%
Percentage of death due to communicable disease	74%
Note: All sources are from WHO factsheet [109]	

Ethiopia has about 149 hospitals, at least 67 of which are privately owned. There are 1343 health centers and 1788 heath stations or health clinics. There are a total of 12488 health posts which is the smallest unit of the health care system of Ethiopia. But the attendance at the healthcare services and the health coverage remains low due to far distances from the healthcare centers coupled with lack of proper transportation [110].

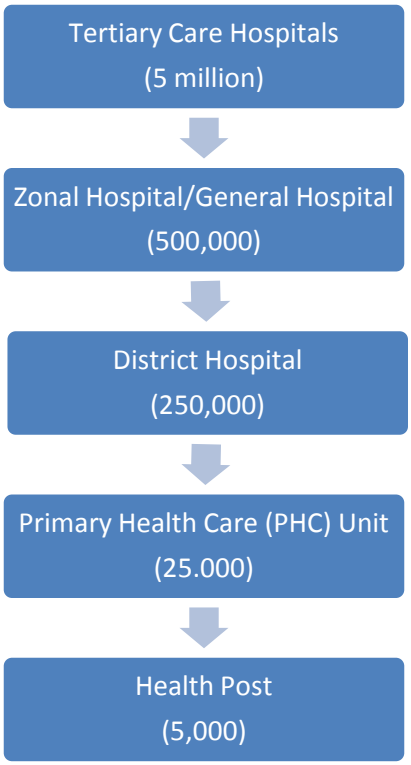


Figure 2: Levels of healthcare system in Ethiopia

(Source: Human Resources for health: country profile Ethiopia. African Health Workforce Observatory (AHWO), June 2010).

In the past there have been no elaborate health policies although a WHO initiated health policy was adopted in 1960s. The emphasis of the policies had been on measures to reduce communicability of infectious diseases with the help of community involvement. As most of the population lives in rural areas, so priority was given to the rural areas. Recently, a government initiative known as Health Sector Development Program (HSDP) has been introduced. It is a 20 years development program which is split into 5 years smaller programs. The program focuses on distribution of health services on equal grounds and encourages the participation of community as well as private sectors such as non-government organization (NGOs). Other aims include capacity building of the healthcare resources and collaboration at the regional and international level with other countries [110].

2.2. Meningitis in Ethiopia:

Located on the eastern part of meningitis belt, Ethiopia is one of the countries which are most affected with bacterial meningitis. The first reported outbreak in Ethiopia dates back to 1901, which was followed by outbreaks in 1935, 1940s, 1950s, 1964 and 1977 [111]. The largest epidemics in Ethiopia were reported in 1981 and 1989, each of which affected almost 50,000 people [112]. Epidemiological studies on the spread on these epidemics in Ethiopia suggest the introduction of meningococcal disease first in western part of Africa. Earliest recorded outbreaks of cerebrospinal meningitis occurred in soldiers stationed in Algiers in 1840 and in labourers working in present day Ghana in 1900 [105]. The epidemic of 1989 that occurred in the eastern part of Africa is believed to be spread by pilgrims returning from Mecca [101]. Since the introduction of meningococcal disease in Ethiopia, the disease has remained endemic with frequent outbreaks. The outbreaks prior to 2001 occurred mostly in the north western, western and south western parts of Ethiopia, the areas that are traditionally included in the meningitis belt. However, outbreaks in 2001 and afterwards have extended to the eastern parts of the country as well [113]. These epidemics were caused mainly by *N. meningitidis* serogroup A, but serogroup C has also been isolated from samples during various outbreaks in 2000 and 2003 [114].

Apart from *N. meningitidis* other agents of bacterial meningitis such as Hib and *S. pneumoniae* are also very common. During the year 1993-1995, A study conducted in a pediatric hospital in Addis Ababa in 1993-1995 showed that almost 5.5% of all hospital admissions were bacterial meningitis. Out of 385 cases diagnosed as bacterial meningitis 74 cases were due to *H. influenzae,* 63 cases were recognized as *M. tuberculosis* and 46 cases were due to *S. pneumoniae.* Meningococcal meningitis was very rare and was identified only in 6 cases. However, in 196 cases out of total 385 cases the exact etiology could not be traced [115] [116]. The study also reported incidence of antibiotic resistance in *S. pneumoniae* and *H. influenzae*. The emerging resistance among the organisms causing bacterial meningitis is a matter of serious concern. Studies have reported increased resistance to the commonly used antibiotics such as penicillin G and chloramphenicol [117] [118].

Bacterial meningitis accounts for about 6-8% of all the hospital admissions in Ethiopia and the case fatality ratio associated with bacterial meningitis is as high as 22-28% [114]. A study conducted at Gondar University Hospital over a span of 5 years from 1998-2003 showed the

prevalence of various common agents of bacterial meningitis in children up to the age of 14 years. *N. meningitidis* was the most common cause of meningitis and accounted to about 28% of the cases. This was followed by *S. pneumoniae* and *H. influenzae* which were identified in 7% and 6.5% of cases, respectively. *S. aureus* and *Salmonella* species were responsible for a small number of cases. Another study conducted in Butajira, a town located in south-central Ethiopia, reported causes of 10,700 deaths during 1987-2008. The results showed that almost 1% of all deaths were due to meningitis [(119)]. A similar study during the same period reported meningitis as a cause of almost 1.9% of all deaths [(120)]. Both these studies lacked laboratory confirmation and relied on "verbal autopsy" to acquire information from the relatives of the deceased.

Laboratory-based clinical studies on bacterial meningitis are essential for predicting accurately the current prevalence of bacterial meningitis and determining the disease causing organisms. Only a few studies in Ethiopia are supported by laboratory surveillance. A WHO led study was conducted in various African countries including Ethiopia to study the prevalence of various agents of bacterial meningitis in infants less than 3 months of age [(121)]. Both blood cultures and CSF cultures were used to identify the causative organism. The study showed *S. pneumoniae* (24%) and *E. coli* (24%) to be the most common organism causing bacterial meningitis in neonates. Other common agents of bacterial meningitis were *Streptococcus pyogenes* (22%), *Salmonella* (12%) and *H. influenzae* (7%).

2.3. Gaps in literature:

A critical review of the literature on bacterial meningitis in Ethiopia highlights the efforts of researchers working on meningitis in this region of the world. The work of the pioneers more than half a century ago and the subsequent continuation of research with addition of more advance laboratory techniques has shaped our knowledge and understanding of meningitis in Ethiopia. This has proven beneficial with respect to the control strategies that are being implemented now, not only in Ethiopia, but other parts of the world as well. Nevertheless, the continuously changing epidemiology, emergence of new strains along with the prospects of newer vaccines and advances in laboratory techniques has led to the demand of new research in this field. The new research needs to be tailor-made to focus on the changing epidemiology to achieve the desired results. With this prospective in mind some of the gaps are identified that

need to be filled in order to achieve better understanding and control of bacterial meningitis in Ethiopia.

1) Most of the studies mentioned in the literature were carried out in the epidemic periods, i.e. from December to June and less emphasis is laid on non-epidemic periods. This implies that most of those studies have focused on meningococcal meningitis and very few studies focused on other types of bacterial meningitis, such as *H. influenzae* and *S. pneumoniae.* These causes bacterial meningitis are also endemic in Ethiopia and are associated with high morbidity and mortality and therefore need to be focused on with priority.

2) A major portion of the studies mentioned have small children as their target group. It is well known that bacterial meningitis also has high incidence in elderly and immunocompromised people. Therefore, for implementing nationwide policies and preventive measures, data on other age groups should also be available.

3) Laboratory surveillance is essential for proper diagnosis of bacterial meningitis and identifying various causative agents. Not all of the studies were assisted with laboratory surveillance. The laboratory techniques used in many studies were outdated and did not meet the criteria of "gold standard" which is considered vital for clinical research.

4) Most of the studies were carried out either in a single hospital or a single city. The results from one specific area cannot be generalized over the whole population. Generalized results are needed to take preventive steps.

5) The WHO website for Multi Disease Surveillance Centre (MDSC) show no or limited data on the etiology of bacterial meningitis in Ethiopia as compared to other countries of meningitis belt [(122)]. Studies are needed that can provide recent data on bacterial meningitis which is crucial for its prevention in Ethiopia.

2.4. Rationale of study:

A study is required to investigate the prevalence of different types of bacterial meningitis in Ethiopia. The current data is limited and does not cover all common types of bacterial

meningitis. Most of the studies mentioned in literature are based on the prevalence of meningitis in the epidemic phases. It is also essential to study the epidemiology of bacterial meningitis in the non-epidemic phases, i.e. from June to December. Meningococcal meningitis is prevalent primarily during the epidemic phases, but pneumococcal meningitis and *H. influenzae* are endemic during the non-epidemic phases as well. Examples from the literature have shown that these non-epidemic strains of bacterial meningitis are also responsible for a vast proportion of cases of bacterial meningitis throughout the year [66]. Therefore the study is planned to investigate the prevalence of these strains in the non-epidemic phases, as their incidence may still be considerably higher than the most other countries of the world.

The incidence of bacterial meningitis varies depending upon the age of the patients, geographical location, climate and time of the year. Previous studies have focused primarily on children and data on other age groups is required to carry out preventive measures for bacterial meningitis. Therefore the study includes all age groups to generate data irrespective of age limitations. Data is also needed that can be generalized over the whole population of Ethiopia. To meet this requirement the study is planned to be carried out in two different cities geographically and climatically different to obtain results that can be generalized. The study has two components, a retrospective component that covers the past 5 years and a prospective component that provides laboratory surveillance of bacterial meningitis for one year. This laboratory surveillance is required to identify the current strains and study their prevalence, with the aim of carrying out preventive measures or implementing new vaccines in Ethiopia.

Several retrospective studies have been conducted in Ethiopia but a study is needed that can correlate the clinical symptoms and the severity of disease with the laboratory results to give a wider view of the factors affecting the severity of bacterial meningitis. Retrospective data from the previous years can help in predicting the current prevalence of various types of bacterial meningitis. As Ethiopia is endemic for bacterial meningitis with cases of bacterial meningitis presenting in the hospitals throughout the year, therefore, the data available in the hospitals' clinical and laboratory record books can be the most accurate indicator of the current status of bacterial meningitis in Ethiopia. These data can also serve as a valuable tool and a reliable indicator of the degree of success of the preventive strategies introduced in the past. Similarly, it

can also be used to highlight the short comings and provide a basis for identifying corrective measures.

Chapter three: METHODOLOGY AND STUDY DESIGN

3.1. Brief description of the project:

"Surveillance of bacterial meningitis and factors affecting meningococcal disease severity" is a project by Norwegian Institute of Public Health (NIPH) in collaboration with Armauer Hansen Research Institute (AHRI) in Addis Ababa, Ethiopia. The project aims to study the current patterns of bacterial meningitis in Ethiopia with emphasis on meningococcal disease. The project has two components: a retrospective study which focuses on the recent trends of bacterial meningitis in Ethiopia during the last 5 years and a prospective study to provide continuous surveillance of bacterial meningitis for a period of one year. The prospective study is under progress with inclusion of new patients and subsequent laboratory analysis on their CSF and blood specimens. To achieve better understanding of the overall trends of bacterial meningitis in Ethiopia, a period of one year surveillance is the minimal duration required. A delay had occurred in the initiation of the prospective study due to the unusually prolonged duration of the ethical clearance process that had been unaccounted for. Due to this reason the one year surveillance period has not concluded yet. Therefore, the thesis will focus on the retrospective component of the study.

3.2. Collaborating institutes:

The collaborating institutes include:

- National Institute of Public Health, Norway (NIPH)
- Armauer Hansen Research Institute, Addis Ababa, Ethiopia (AHRI)
- Gondar University Hospital, Gondar
- Awassa Referral Hospital, Awassa

Retrospective study

3.3. Objectives of study:

3.3.1.Primary objective:

To study retrospectively the various trends of bacterial meningitis in selected Ethiopian hospitals in the previous 5 years from 2007-2011.

3.3.2.Secondary objectives:

- To explore the trends of bacterial meningitis in terms of number of cases over time.
- To find out the prevalence of various etiological factors causing bacterial meningitis.
- To find out the distribution of bacterial meningitis in terms of age and sex.
- To study the effect of seasonal variability on the incidence of bacterial meningitis.
- To describe the most common clinical signs and symptoms associated with bacterial meningitis.

3.4. Study design:

The study involves a retrospective collection of clinical and laboratory data from selected hospitals in Ethiopia, to provide a quantitative assessment of the recent epidemiological trends of bacterial meningitis. The study group constitutes of all consecutive cases of bacterial meningitis that presented in Gondar University Hospital and Awassa Hospital during a defined study period. During a visit to the study sites before the start of the study the extent of the laboratory and clinical data available was assessed with the help of the site co-ordinators of the project. Based on this initial assessment, a wide range of data comprising clinical records of patients as well as data on laboratory-based detection of bacterial meningitis was found. Therefore, a multidimensional approach to study the various clinical, histopathological and biochemical aspects of bacterial meningitis was adopted.

Due to the better laboratory facilities available at Gondar University Hospital, the data on actual causative organisms of bacterial meningitis was used to interpret the prevalence trends of various agents of bacterial meningitis. A high input of patients with bacterial meningitis coupled with the well-maintained clinical records from the in-patients and emergency departments at Awassa Referral Hospital was observed. The data from Awassa were therefore used to calculate the prevalence of bacterial meningitis, the frequency of the most common clinical symptoms, rates of complications and outcome of the disease.

The research design is retrospective. Retrospective studies aim at reasoning the outcome of an effect back to its antecedent cause. This is in contrast to the prospective studies which aim at reasoning from a present antecedent to a future outcome or consequence. Retrospective studies have several advantages. They are less time-consuming, more economical and can provide valuable results in a very short time period. But they are often subjected to recall bias. This effect has been minimized in the current research as the data were recorded directly from the clinical and laboratory records. Another drawback with the retrospective studies is that it is often difficult to eliminate the effect of confounding variables which affect the outcome of study. Possible attempts were made to reduce the effect of confounding variable. Patients who were concomitantly affected by other kind of meningitis, such as tuberculous, viral or aseptic meningitis and other CNS lesions were not included in the study. However, removing the effects of all the confounding variables can be difficult to achieve. Retrospective studies cannot prevent the occurrence of an event which has already occurred, but may be helpful in preventing such events in the future. Retrospective studies can often be useful in the formation of a hypothesis which can then be evaluated in further studies. This forms the basis of the relationship of this retrospective study to the overall project.

The study aimed at providing quantitative assessment of the prevalence of bacterial meningitis in Ethiopia and to provide results that may be generalized over the whole Ethiopian population. The only study design that permits these characteristics of the research is a quantitative study. Quantitative research design can be defined as “the numerical representation and manipulation of observations for the purpose of describing and explaining the phenomena that those observations reflect” [(123)]. Quantitative research provides details of the direct and indirect variables and allows the researcher to measure and quantify the effect. The study is basically a prevalence study and

demands the inclusion of all the patients of bacterial meningitis during the defined study period. Consequently, no randomization was used and all patients that fulfilled the inclusion criteria were included in the study.

3.5. Study sites:

The field work was carried out in two tertiary care hospitals from different cities of Ethiopia i.e. Gondar University Hospital in Gondar and Awassa Referral Hospital in SNNPR region of Ethiopia.

3.5.1. University of Gondar Medical Hospital, Gondar:

Gondar is a city in the North-western part of Ethiopia. Administratively, it is located in the Amhara region. Previously the capital city of Ethiopia, Gondar is now the third largest city in Ethiopia. Famous for its castles, Gondar is a mountainous area with an elevation of 2100 meters above the sea level. Due to this reason the climate is much cooler. The average annual temperature is 19.2^{o}C which ranges from 10^{o}C (December and January) to 29^{o}C (March to May). The total population of Gondar is more than 200,000. Different ethnic groups reside in Gondar, out of which Amhara form the majority (88.9%) followed by Tigrayan (6.7%) and Qemant (2.4%). Amharic is the main language spoken in the Gondar region.

The University of Gondar Medical Hospital is the largest hospital in Gondar. It is a 400-bed teaching hospital associated with a medical college. The medical college was founded in 1954 and is the oldest training institute for health professionals in Ethiopia. The hospital covers a wide range of area and is a referral hospital for four districts hospitals. It has a range of specialities including paediatrics, surgical, gynaecological, psychiatric and an HIV centre. With a staff comprising of 400 healthcare personals, including 50 medical doctors, the hospital covers a population of four million. The hospital has well established laboratory facilities with a staff of at least 25 trained laboratory scientists.

3.5.2. Awassa Referral Hospital, Awassa:

Awassa is a city located in the southern part of Ethiopia close to the lake Awassa of the Great Rift Valley. The city of Awassa lies 270km to the south of the capital city of Addis Ababa.

Awassa belongs to the SNPPR of Ethiopia and is the administrative capital of the region. The total population of Awassa is almost 260,000. It lies at an elevation of 1700 meters and the temperature ranges between 20^{o}C and 30^{o}C.

Figure 3: Map of Ethiopia showing the study sites and the capital city of Addis Ababa

Awassa Hospital is the biggest hospital in the city of Awassa and the Southern Nations, Nationalities and Peoples' Region (SNNPR). Affiliated with the Awassa University, the hospital is a teaching hospital for Awassa Health Science College which was founded in 1996. As a tertiary care teaching hospital it is a 350-bed hospital and includes paediatrics, medical, surgical, ophthalmology and gynaecological/obstetrical departments. The hospital serves for more than 12 million people.

3.6. Study Period:

The study includes all consecutive cases of bacterial meningitis that presented in Gondar University Hospital and Awassa Referral Hospital during a defined study period. The study periods for two study sites differ in length. From Gondar University Hospital retrospective data

were collected for the last 5 years from January 2007 till December 2011 (corresponding to the Ethiopian calendar from 4th month of 1999 to 5th month of 2004). From Awassa Hospital the data was collected for the last 2.5 years from September 2009 till December 2011 (corresponding to the Ethiopian calendar from start of 2002 to the 5th month of 2004). The study periods were selected to permit the maximum utilization of available data as determined on the preliminary visits to the study sites.

3.7. Inclusion criteria for cases:

The patients fulfilling the following criteria were selected for the study:

- Positive CSF culture or Gram staining results suggestive of bacterial meningitis
- Clinically diagnosed cases of bacterial meningitis by a medical specialist in the cases where a CSF culture or Gram staining were not performed.
- All age groups that fulfil the above criteria

3.8. Exclusion criteria:

The patients exhibiting the following characteristics were not included in the study:

- Simultaneous infection with another form of meningitis such as tuberculous, viral or fungal meningitis.
- Presence of another CNS infection that can present with symptoms similar to bacterial meningitis such as cerebral malaria or any other CNS lesion.

3.9. Study population:

All the patients with bacterial meningitis that presented in Gondar University Hospital or Awassa Referral Hospital during the study period and fulfilling the above mentioned inclusion criteria were included in the study and defined as the study population.

3.10. Target population:

The study sites are two different cities with distinct geographical and climatic features. These cities are located in the northern and southern part of Ethiopia. Thus the study is carried out with the intention of providing results that can be generalized over whole of the Ethiopia. Therefore, the target population for this study is the whole Ethiopian population.

3.11. Sample size:

No predefined sample size was set. The study included all the cases of bacterial meningitis that presented in Gondar University Hospital and Awassa Referral Hospital during the defined study period and fulfilled the above mentioned inclusion criteria.

3.12. Data sources:

The clinical and laboratory data were collected from the available hospital clinical and laboratory records. The patient related clinical data were collected from the hospital clinical records. The potential cases were identified by the hospital log books present in the emergency wards, the medical, paediatrics and neonatal in-patient wards and also the out-patient registers. The laboratory log books for CSF cultures and Gram staining results were also searched to find the cases of bacterial meningitis. In addition to the routine log books, separate log books that were used during the off-duty hours such as weekends or public holidays were also searched to avoid missing any potential study participants. Once the cases were identified from any of these sources the original hospital clinical records were accessed with the help of the main record room staff. The records were traced using the patient hospital registration number from the log books. The original laboratory result slips attached inside these clinical record forms were also accessed.

3.13. Data collection:

The information obtained from the above mentioned sources were entered into specialized pre-defined data collection forms (data collection form attached as appendix I). Among the clinical details, the signs and symptoms at presentation such as stiff neck, headache, fever, altered mental

state, seizures, etc and the treatment outcome were recorded. Demographic details such as age, sex and the month of presentation were also recorded. Similarly laboratory findings from the laboratory log book such as results of CSF cultures, Gram staining, CSF cell count, proteins and glucose level, depending upon their availability, were recorded on the data collection forms. On identification of cases, each participant was given a factitious number that was used throughout the research to avoid confidentiality issues. The data were recorded by two researchers to avoid any bias. Any discrepancy in collection and interpretation of data was relieved with the help of a person from the study site who was qualified and familiar with the original data sources (i.e. study coordinators).

Table 2: Data variables collected in the retrospective data

Demographic data	Age, sex, city or region and date of presentation.
Clinical history	Clinical signs and symptoms at presentation such as presence or absence of stiff neck, fever, headache, altered mental state, seizures and petechial rash. Presence of any sequelae.
Treatment outcome	Complete recovery, fatal case and partial recovery with sequelae.

Laboratory results	Gram staining of CSF samples, CSF culture, CSF cell count, proteins and glucose level.

3.14. Data handling:

3.14.1. Data entry:

The data from the filled-in data collection forms were then entered into SPSS version 18.0.0 and later converted to Excel sheets for convenience during discussions with the study co-ordinators.

3.14.2. Statistical analysis:

The results were analyzed using a registered version of SPSS 18.0.0. The statistical analysis was conducted to find out the exact proportion of various etiological factors of bacterial meningitis in the patients that presented in Gondar University Hospital and Awassa Referral Hospital during the defined study period. The various trends of bacterial meningitis over the last 5 years in terms of age and sex distribution were studied. The hospital-wise prevalence of bacterial meningitis in relation to seasonal variation and the time of the year were also studied. The study also focused on the relationship of various clinical signs and symptoms to the overall prognosis and outcome of disease.

3.15. Ethical considerations:

The approval for the main project "Surveillance of bacterial meningitis and factors affecting meningococcal disease severity" was granted by REK "Regionale Komiteer for Medisinsk og Helsefaglig Forskningsetikk" (Regional Committee for medical and healthcare research ethics) in Norway. Following clearance from REK the protocol was submitted to the National Health Research Ethics Review Committee (NRERC) in Ethiopia. Abiding by the regulations of the chief collaborating institute in Ethiopia, the Armauer Hansen Research Institute (AHRI) Addis Ababa, clearance was also sought from the medical research ethics board of AHRI. Once the

ethical clearance was obtained from all of the above mentioned institutes, the institutional review boards (IRBs) of the study sites hospitals were requested to grant ethical approval. Once all these ethical reviews were sought and the approval granted from all of the above mentioned institutes, a final administrative order directing the commencement of the study was issued by the office of the medical director of the respective hospitals.

Figure 4: The process of obtaining ethical clearance for the project

With the commencement of the prospective study and preliminary meeting with the site co-ordinators at the collaborating hospitals, the need to utilize the retrospective data was emphasized. Consequently, the protocol for retrospective study was submitted as an amendment to the main prospective study. The approval for the retrospective study amendment was sought from all the institutes that had earlier granted the ethical clearance. In addition to the above

mentioned approvals, an additional approval from the hospital administration was required to access the hospital main record room and utilize the retrospective data from the clinical records of the patients. As the research is clinical and involves multiple institutions, therefore, the process of acquiring ethical clearance was complicated. Taking into account the clinical nature of the research and the usual application processing times, the whole process of obtaining the ethical clearance took almost 6 months for the main prospective study. The retrospective amendment was approved in a course of 3-4 months.

3.16. Participant confidentiality:

Safeguarding the patients' right to confidentiality should be the foremost responsibility of the researcher. To ensure this right of confidentiality, all the cases were enrolled with complete anonymity. Upon inclusion each participant was provided with an identification number that was used throughout the study and during the data analysis and publication of results. The data record forms were devised as to not permit the recording of any personal details such as name of the patient, the hospital registration number and the address of the patients. The original list of the hospital registration numbers that was used to trace the clinical records of the patients was kept only with the researcher in a place separate from the collected data. The list will be retained with the researcher for a period of 6 months after the publication of the results in order to verify any ambiguity that may arise. The list will be discarded after a period of 6 months from the publication of results.

3.17. Expected benefits of the study:

The study is intended for the host population as being the sole beneficiaries. The retrospective data collected from the two study sites will prove helpful in establishing the current prevalence of bacterial meningitis in those particular settings. The effect of seasonal variability on various types of bacterial meningitis and the age wise and gender wise distribution of disease burden will provide useful estimates on the recent trends of bacterial meningitis. The study sites include two hospitals located in different cities of Ethiopia which imparts generalization to the results. Therefore, the results obtained from the study will also prove helpful for other hospitals in Ethiopia working in similar settings. Special emphasis has been laid to ensure the validity of the

clinical and laboratory data. This 5-year retrospective study component along with the one year prospective study that is in progress may provide valuable data that can be used for further research, formulation of preventive strategies and implementation of new vaccines.

3.18. Funding:

The project is funded by the Norwegian Research Council (PROJECT NO: 192477) under the GLOBVAC fund. The second visit to Ethiopia was funded by Ivar Helles' Foundation, Norway.

3.19. Project timeline:

Table 3: Project timeline for the master's project

Literature search and protocol writing	May 2011 – June 2011
First visit to Ethiopia for setting up prospective study	June 2011 – November 2011
Introductory meetings with collaborating institute regarding retrospective study	June 2011 – July 2011
Setting up study start-up, recruiting study staff, equipment purchase and transfer for the prospective study.	July 2011 – August 2011
Conducting laboratory optimization and training for the recruited staff	August 2011 – September 2011

Ethical clearance for the retrospective study	October 2011- December 2011
Preparing retrospective study start-up and co-ordination with the collaborating institutes.	January 2012 - February 2012
Second visit to Ethiopia and collection of retrospective data	March 2012 – April 2012
Data analysis and thesis write up	May 2012 – August 2012

4.1. Gondar University Hospital:

The laboratory records from the Gondar University Hospital for the 5 years period from January 2007 to December 2011 revealed 128 cases of bacterial meningitis that were confirmed either by CSF culture or CSF Gram staining or by both. These 128 confirmed cases of bacterial meningitis were then analyzed to find out the gender ratios, number of cases in each age group, the most common etiological agents of bacterial meningitis and the seasonal variations in the incidence of bacterial meningitis due to these etiological agents.

4.1.1. Age-wise and sex-wise distribution of bacterial meningitis:

Gender and age were recorded from the laboratory log books in 109 cases. Out of these 109 laboratory confirmed cases of bacterial meningitis in Gondar University Hospital during the 5 years study period, 69 (63.3%) were males and 40 (36.7%) were females. The male to female ratio was 1.7:1.

Table 4: M to F ratio in cases of bacterial meningitis in Gondar University Hospital during 2007-2011

Gender	Number of cases (N)	Percentage (%)
Male	69	(63%)
Female	40	(37%)
TOTAL	**109**	**(100%)**

The ratio of male and female patients followed a similar trend when observed separately for various age groups with the male patients outnumbering the females. The highest incidence of bacterial meningitis was present in infants less than one year of age which accounts to about 29 cases out of 109 (26.6%). This was followed by small children less than 4 years of age which constituted 25 cases (23%). Another peak was observed in the age group 25 to 39 years of age which made up 16.5% of the cases.

Figure 5: Cases of bacterial meningitis in Gondar University Hospital during 2007-2011

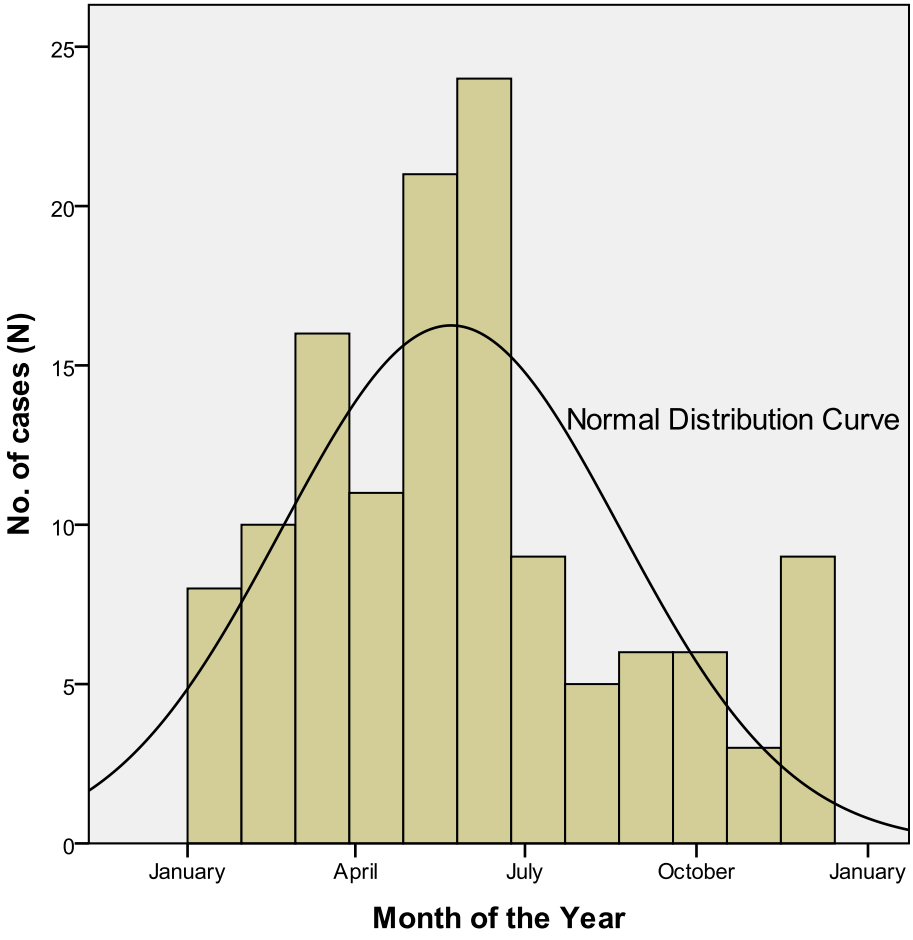
AGE GROUP		GENDER		TOTAL
		MALE	FEMALE	
Infants (0-12 months)	No. of cases (N)	17	12	29
	%	(58.6%)	(41.4%)	(26.6%)
1-->4 years	No. of cases (N)	15	10	25
	%	(60.0%)	(40.0%)	(22.9%)
5-->9 years	No. of cases (N)	9	3	12
	%	(75.0%)	(25.0%)	(11%)
10-->14 years	No. of cases (N)	9	2	11
	%	(81.8%)	(18.2%)	(10%)
15-->24 years	No. of cases (N)	4	2	6
	%	(66.7%)	(33.3%)	(5.5%)
25-->39 years	No. of cases (N)	9	9	18
	%	(50.0%)	(50.0%)	(16.5%)

40-->59 years	No. of cases (N) %	6 (100.0%)	0 (0.0%)	6 (5.5%)
60+ years	No. of cases (N) %	0 (0.0%)	2 (100.0%)	2 (1.8%)
Total	No. of cases (N) %	**69** **(63.3%)**	**40** **(36.7%)**	**109** **(100%)**

4.1.2. Seasonal effect on the incidence of bacterial meningitis:

In Gondar University Hospital among the 128 cases of bacterial meningitis, the highest incidence occurred during the summer months of May and June which had 21 and 28 cases, together forming more than 35% of the cases. The months immediately preceding the summer months also had higher number of cases with March and April together making up 21% of the total cases. The latter half of the year after the summer months had comparatively few cases with only 23% of the cases occurring during the 5 months period from August to December.

Figure 5: Seasonal variation in the cases of bacterial meningitis at Gondar University Hospital during 2007 - 2011

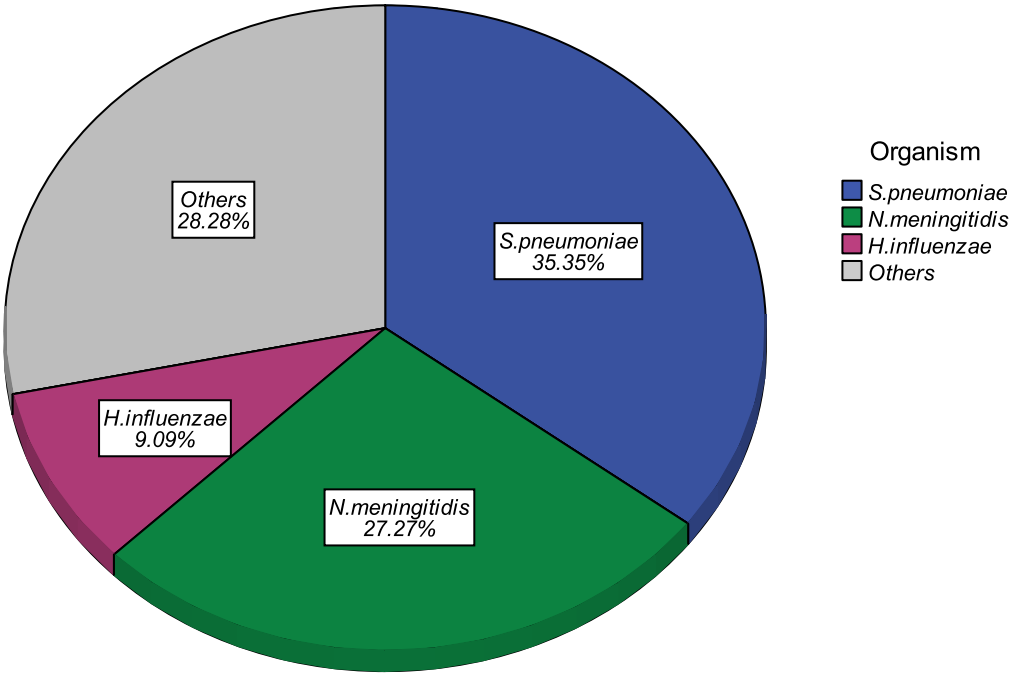


4.1.3. Organisms of bacterial meningitis:

The organisms causing bacterial meningitis was identified in 128 cases in Gondar University Hospital during 2007 to 2011. Bacterial cultures were performed on 99 cases out of these 128 patients and 62 patients were confirmed on the basis of Gram staining results. Both culture results as well as Gram staining results were carried out to diagnosis 33 cases.

Table 6: Laboratory confirmation of cases of bacterial meningitis in Gondar University Hospital during 2007-2011	
Cases confirmed by both by culture and Gram staining	33
Cases confirmed by only by culture	66
Cases confirmed only by Gram staining	29
TOTAL	**128**

Figure 6: Organisms isolated from CSF cultures of patients of bacterial meningitis in Gondar University Hospital during 2007 - 2011



The culture results from Gondar University Hospital showed the highest incidence of *S. pneumoniae* among the common agents of bacterial meningitis. *S. pneumoniae* was isolated from the CSF cultures of 35 patients (35.3%). *N. meningitidis* was isolated from 27 cases (27.3%) and *H. influenzae* from 9 cases (9.1%). A variation in the incidence of these agents of bacterial meningitis was observed in various age groups. Most marked variation was observed with *H. influenzae* and some less common organisms of bacterial meningitis such as *E. coli*, which were more common in infants. All the 9 identified cases of *H. influenzae* were present in children less than 14 years of age, 5 of them in infants. The incidence of *S. pneumoniae* remained almost constant between the various age groups. Both *S. pneumoniae* and *N.*

meningitidis showed comparatively less variations in the various age groups. However, they were most commonly observed in the young children between 1-4 years of age where *N. meningitis* and *S. pneumoniae* were isolated from CSF of 9 and 8 patients, respectively. Variation in the incidence of the various agents of bacterial meningitis according to the various age groups is depicted in figure 7. The median ages for the various culture confirmed cases are presented in table 7.

Figure 7: Age-wise breakdown of causes of bacterial meningitis in Gondar University Hospital during 2007 - 2011

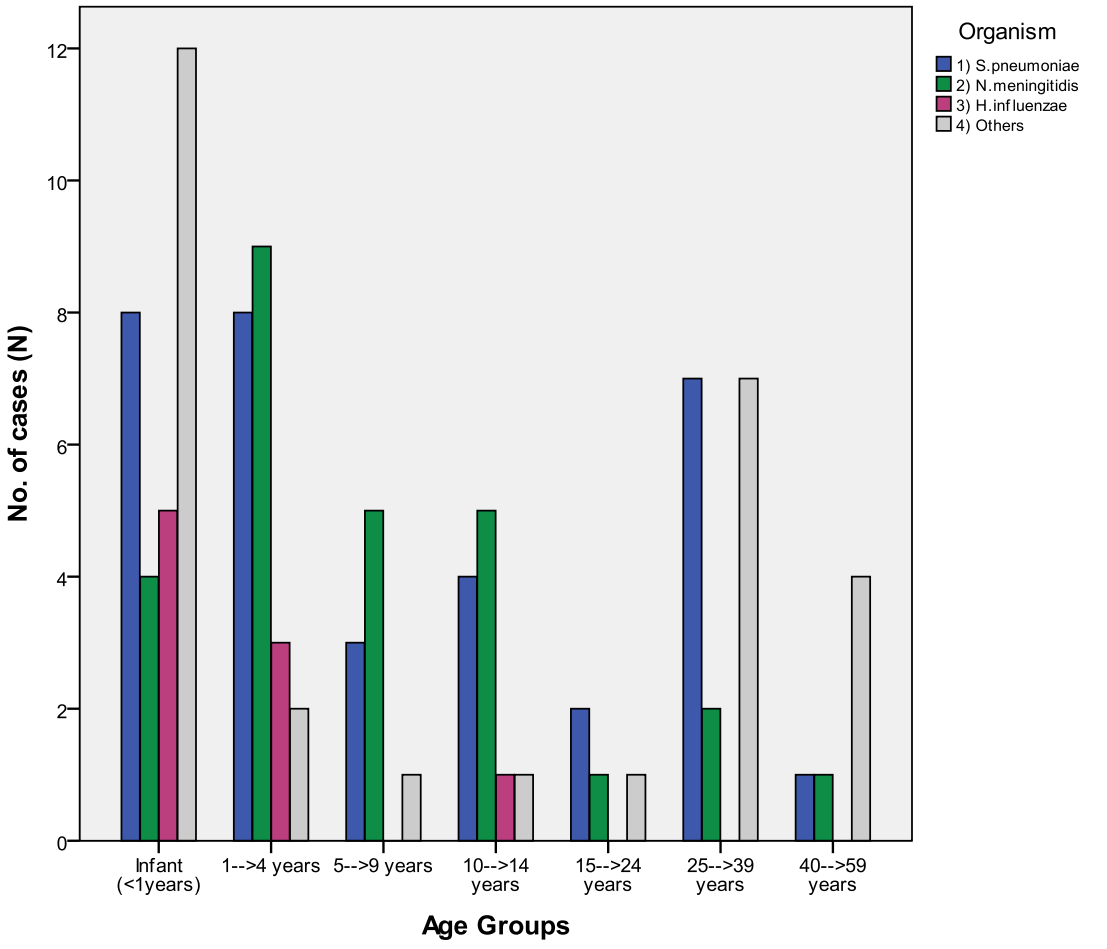


Table 7: Median age for the culture positive cases at Gondar University Hospital during 2007 -2011		
Organism	**Median Age**	**No. of cases (n) (Range)**
N. meningitidis	5 years	*n* = 27 (Range = 0 month – 40 years)
S. pneumoniae	7 years	*n* =35 (Range= 0 month – 48 years)
H. influenzae	1 month	*n* =9 (Range= 0 month – 14 years)
Other organisms	3.5 years	*n* =28 (Range = 0 month – 57 years)

Less common organisms were also isolated from the CSF samples of 28 patients of bacterial meningitis at Gondar University Hospital. Out of these 28 cases *E. coli* was the most common organism and was isolated from the cultures of 8 patients. The next common organisms were *S. aureus* and *Salmonella sp.* that were isolated from 4 and 3 cultures, respectively. The various uncommon agents of bacterial meningitis that were isolated from cultures of patients of bacterial meningitis at Gondar University Hospital are presented table 8.

Table 8: Uncommon causes of bacterial meningitis in patients of Gondar University Hospital during 2009-2011	
Organism	**Number of cases (N)**
E. coli	8

S. aureus	4
Salmonella sp.	3
Acinetobacter	2
S. pyogenes	1
Other *Streptococci*	2
Pseudomonas	1
Providencia sp	1
Morganella morganii	1
K. pneumonia	1
Enterobacter	1
Cryptococcus neoformans	1
Not identified	2
TOTAL	**28**

Positive Gram staining results were used to assist the clinical diagnosis in 62 cases at the Gondar University Hospital. About half of these Gram staining results (33 cases) were also supported by positive bacterial cultures. Gram staining results from the CSF specimens of patients at Gondar University Hospital along with the bacterial morphological features are presented in table 9. The most commonly observed results were Gram positive cocci that were observed in 28 cases (45.2%). Gram negative diplococci were the second most common observed morphology and were seen in 20 cases (32.3%). Gram negative rods were seen only in 14 cases which makes 22.6% of the total cases.

Table 9: Gram Staining Result from CSF samples of patients in Gondar University Hospital during 2007-2011

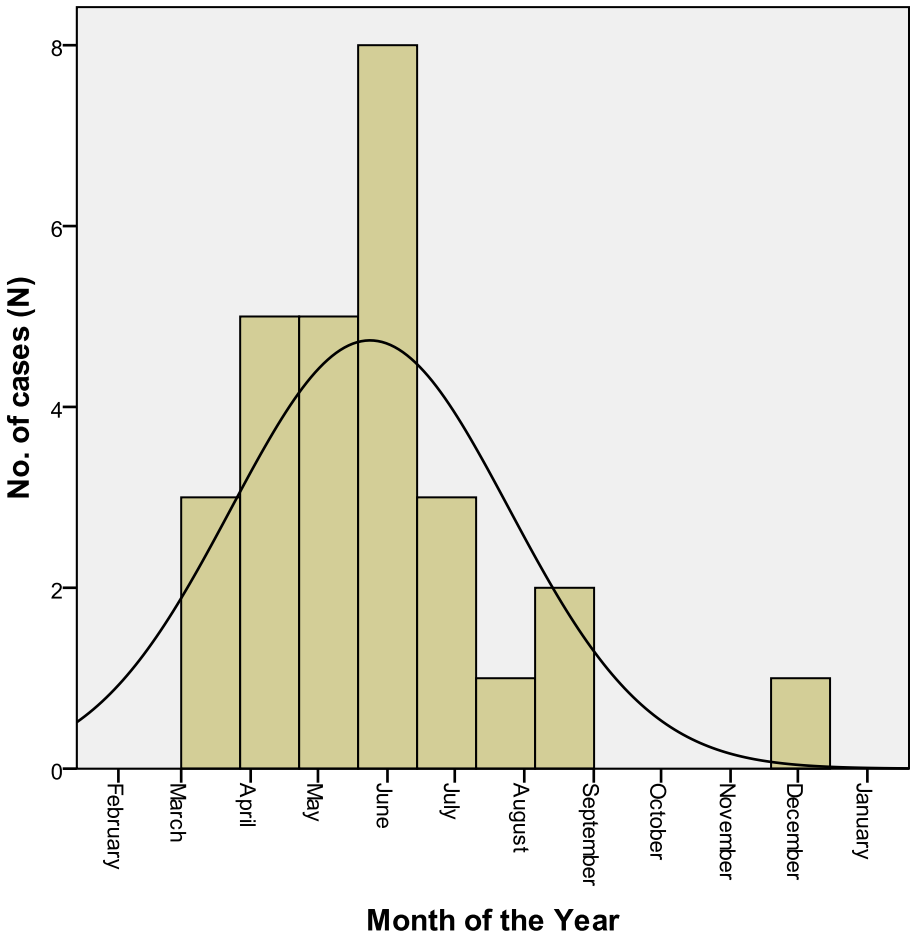
Gram staining result	Number of cases (N)	Percent (%)
Gram positive cocci	28	45.2
Gram negative diplococci	20	32.3
Gram negative rods	14	22.6
Total	**62**	**100.0**

4.1.4. Seasonal variations among the various agents of bacterial meningitis:

The bacterial culture results from Gondar University Hospital were used to interpret the effect of seasonal variations on the prevalence of the organisms causing bacterial meningitis. The whole calendar year was broken down into 4 quarters to assess the effect of change in weather conditions on the common agents of bacterial meningitis. The most marked seasonal variation was observed for *N. meningitidis* which had a high incidence in the 2nd quarter (April to June). During the dry season (April to June) 18 cases were confirmed by culture results as compared to very few cases in other quarters. When the cases were split according to the time of presentation

the highest incidence was observed in the month of June where culture from 8 CSF specimens revealed *N. meningitidis*. The incidence of *N. meningitidis* dropped drastically during the winter months from October to December. The seasonal variation on the incidence of bacterial meningitis is shown in the figure 8.

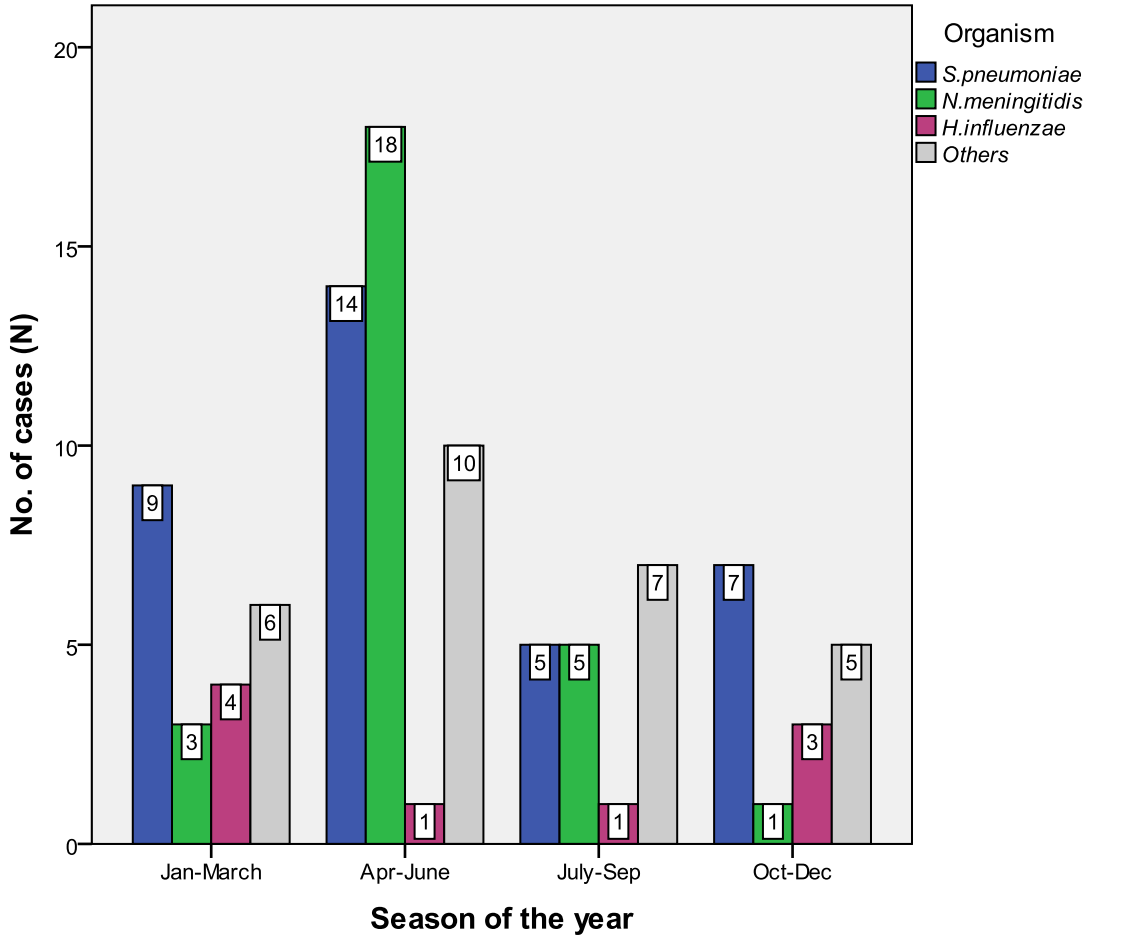
Figure 8: Seasonal variation on the incidence of meningococcal disease in Gondar University Hospital during 2007 - 2011



Apart from *N. meningitidis,* all the other organisms of bacterial meningitis showed little variation during the four quarters of the year. The incidence of *S. pneumoniae* varied between the highest

of 14 cases in the 2nd quarter to lowest of 5 cases in the 3rd quarter. *H. influenza* and other less common organisms did not show considerable variation in the different seasons of the year. Figure 9 presents the incidence of the various agents of bacterial meningitis during the 4 quarters of the year as observed in the culture positive results from Gondar University Hospital during the 5 years study period.

Figure 9: Effect of seasonal variation on agents of bacterial meningitis in Gondar University Hospital during 2007 - 2011



4.2. Awassa Referral Hospital:

The clinical record files from the hospital's in-patient and emergency wards and the laboratory records identified 143 cases of bacterial meningitis at Awassa Referral Hospital from September 2009 to the end of December 2011. These 143 cases include the laboratory diagnosed cases as well as the cases that were clinically confirmed based on the signs and symptoms. The data collected from the clinical record files of these patients were used to study the demographical trends of bacterial meningitis as well as the clinical features and the outcome of treatment.

4.2.1. Age wise and sex wise distribution of bacterial meningitis:

Out of 143 cases, 94 were males and 49 were females making a male to female ratio of 1.9:1. The trend was uniform in all age groups with more case occurring among males. The highest number of cases were in the young adults between the age 15 to 24 years. In this age group 38 cases (27%) of bacterial meningitis were observed. This was followed by adults between the age of 25-39 years which constitutes 29 cases (20%). Only 32 cases (22%) were children less than 4 years of age, half of which were infants less than one year of age. The median age recorded was 20 years (Age range = 0 months – 65 years).

Table 10: Age-wise and sex-wise distribution of cases at Awassa Referral Hospital during September 2009 – December 2011

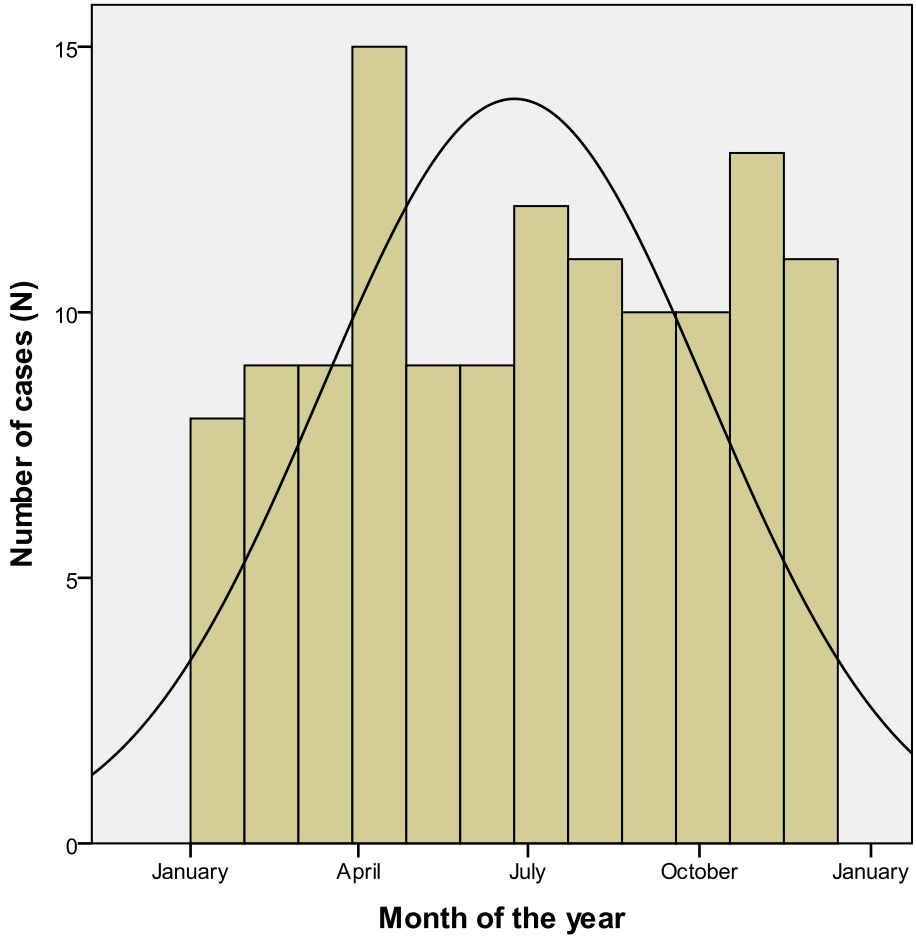
AGE GROUP		GENDER		TOTAL
		MALE	FEMALE	
Infant (0-12 months)	No. of cases (N) Percentage (%)	12 (75.0%)	4 (25.0%)	16 (11.2%)
1-->4 years	No. of cases (N) Percentage (%)	8 (50.0%)	8 (50.0%)	16 (11.2%)
5-->9 years	No. of cases (N) Percentage (%)	9 (75.0%)	3 (25.0%)	12 (8.4%)

10-->14 years	No. of cases (N) Percentage (%)	8 (61.5%)	5 (38.5%)	13 (9.1%)
15-->24 years	No. of cases (N) Percentage (%)	27 (71.1%)	11 (28.9%)	38 (26.6%)
25-->39 years	No. of cases (N) Percentage (%)	16 (55.2%)	13 (44.8%)	29 (20.3%)
40-->59 years	No. of cases (N) Percentage (%)	9 (64.3%)	5 (35.7%)	14 (9.8%)
60+ years	No. of cases (N) Percentage (%)	5 (100.0%)	0 (0.0%)	5 (3.5%)
Total	**No. of cases (N)** **Percentage (%)**	**94** **(65.7%)**	**49** **(34.3%)**	**143** **(100%)**

4.2.2. Effect of seasonal variation of the incidence of bacterial meningitis:

In order to assess the effect of seasonal variation on the incidence of bacterial meningitis in Awassa, two years data from January 2010 to December 2011 were studied (represented in figure 10). A fairly uniform pattern was observed throughout the year: the incidence remained constant during this time. However, the highest number of cases were recorded in April (15 cases) with almost 12% of the cases. This was followed by November which has 13 cases (11%).

Figure 10: Seasonal variation on the incidence of bacterial meningitis in Awassa Referral Hospital during January 2010 - December 2011



4.2.3. Clinical feature of bacterial meningitis:

Patients with bacterial meningitis presenting at Awassa Referral Hospital during the two and a half year study period were studied to find out the incidence of various common signs and symptoms of bacterial meningitis. The relative frequency of each of the common signs and symptoms were recorded for 135 cases. The most common symptom was fever. High grade fever (>38^{o}C) was recorded in 120 cases (88.9%). The second most common sign was neck stiffness which was present in 101 cases (74.8%). Headache and nausea or vomiting were present in 94 and 80 cases, respectively. Altered mental state such as un-consciousness, behavior change or

inability to speak was observed in more than half of the patients. Almost 36% of the cases were reported to have had one or more incidence of seizures before presenting to the hospital. Respiratory symptoms such as cough, wheezing or effort breathing were present in less than a quarter of the patients.

Table 11: Clinical symptoms at presentation in patients of bacterial meningitis at Awassa Referral Hospital during Sep 2009 to Dec 2011

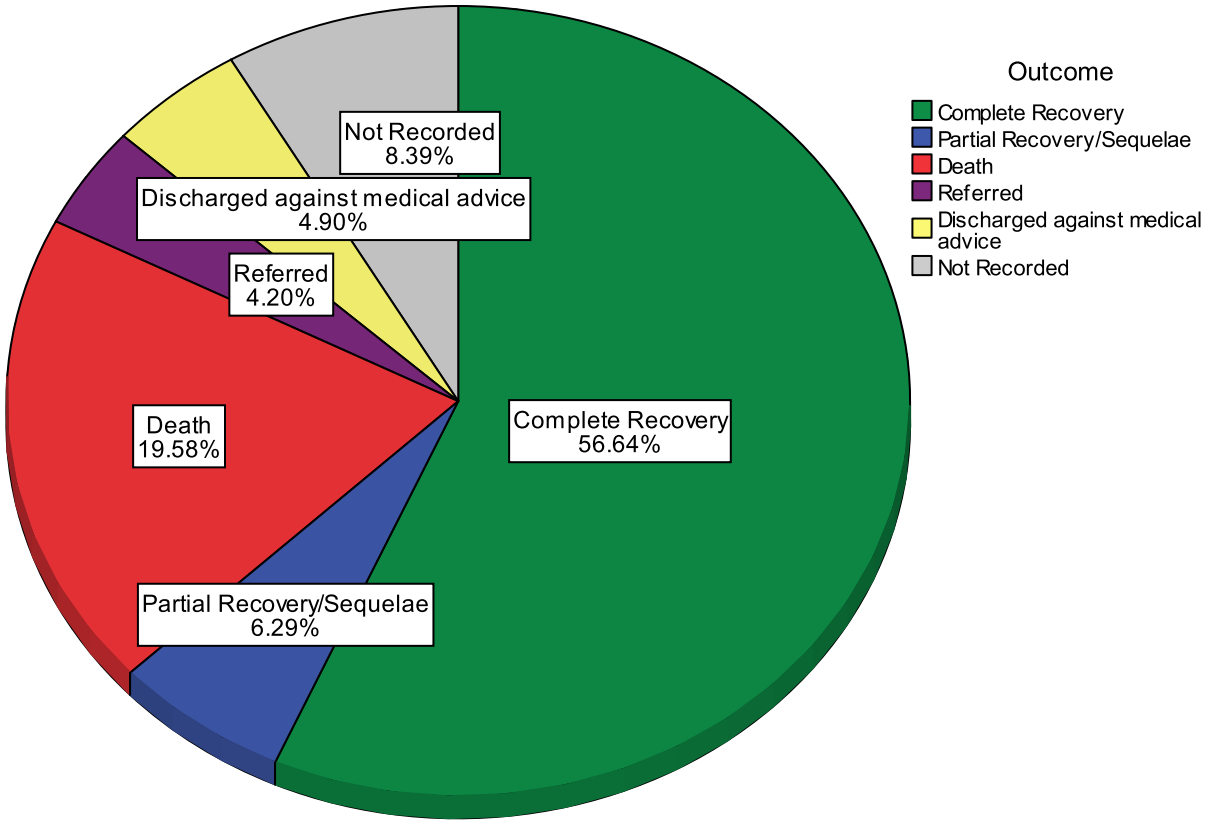
Sign and symptoms	**Frequency**	**Percentage (%)**
Fever $>38^0$C	120/135	88.9
Neck stiffness	101/135	74.8
Headache	94/135	69.6
Nausea and Vomiting	80/135	59.3
Altered mental state	70/135	51.9
Seizures	48/135	35.6
Respiratory symptoms	33/135	24.4
Shock	6/135	4.4

4.2.4. Outcome of bacterial meningitis:

The clinical files of 143 cases of bacterial meningitis at Awassa Referral Hospital were studied to find out the outcome of the treatment. Final treatment outcomes were recorded in 119 cases. The most common outcome of the treatment was complete recovery which was observed in 80 (56.6%) out of 119 cases. Death was the second most common outcome with 28 cases recorded as fatal cases. Neurological or other sequelae were present in 11 cases at the time of discharge from the hospital.

Outcome could not be recorded in the remaining 24 cases. Out of these 24 cases, 6 cases with severe life threatening illness were referred to Addis Ababa for further investigation and management. Treatment outcome could not be recorded in 7 cases which intentionally withdrew from the treatment and were discharged against the advice of the attending physician. In an additional 11 cases the medical records from the clinical files were insufficient to reveal the final outcome of the treatment.

Figure 11: Clinical outcome in patients of bacterial meningitis at Awassa Referral Hospital during September 2009 - December 2011



4.2.5. Adverse outcomes of bacterial meningitis: Death

During the study period 28 deaths were recorded due to bacterial meningitis, thus giving a case fatality rate (CFR) of 23.5%. These fatal cases were then further analyzed by breakup into the various age groups. Most of the fatal cases were in infants or young adults. When age specific CFRs were studied, infants had the highest CFR of 41.6%. A CFR of 33.3% was recorded in older patients above 60 years of age. This was followed by the children between the age groups of 4 to 9 years and 10 to 14 years. A higher CFR of 30% was observed in these age groups. A

gender-wise breakdown of the fatal cases showed that more deaths were recorded in males then females. Results show that 19 out of 94 male patients died due to bacterial meningitis during the two and a half year study period at Awassa Referral Hospital as compared to 9 deaths in a total of 49 females that presented during the same period. This gives a male to female CFR of 1.1:1.

Table 12: Fatal cases of bacterial meningitis at Awassa Referral Hospital during September 2009 - December 2011

AGE GROUP	CASES WITH KNOWN OUTCOMES (N)	DEATHS IN EACH AGE GROUP (D)	CASE FATALITY RATE (CFR) NO. OF DEATHS / NO. OF CASES X 100
Infant (0-12 months)	12	5	41.6%
1-->4 years	12	1	8.1%
5-->9 years	10	3	30.0%
10-->14 years	10	3	30.0%
15-->24 years	35	8	22.8%
25-->39 years	25	5	20.0%
40-->59 years	12	2	16.6%
60+ years	3	1	33.3%
Total	**119**	**28**	**23.5%**

4.2.6. Adverse outcomes of bacterial meningitis: Sequelae

Complications of bacterial meningitis were recorded at the time of hospital discharge in 11 cases (7.7%). The most common sequelae were neurological complications such as paralysis or weakness of the limbs (4 cases); facial palsy (4 cases), muscle wasting (2 cases), deafness or hearing impairment (2 cases) and acute psychosis (2 cases).

Chapter five: DISCUSSION AND RECOMMENDATIONS:

5.1. Discussion:

The aim of the study was to explore the various demographical, epidemiological and clinical aspects of the bacterial meningitis in Ethiopia. The results are based on 128 cases from a 5-year period (2007 to 2011) from Gondar University Hospital. These 128 cases include only those cases that had been confirmed by laboratory identification of the causative organism. The data on clinical signs and symptoms and the treatment outcomes represent a two and a half year (September 2009 to December 2011) data of the 143 patients at Awassa Referral Hospital.

The data on 128 laboratory confirmed cases of bacterial meningitis from Gondar University Hospital showed marked male predominance (male to female ratio of 1.7:1). The data from Awassa reveal an even higher incidence in males (male to female ratio of 1.9:1). Throughout the various age groups the trend of male predominance tends to persist. Various large scale studies conducted worldwide show a higher incidence in males. A study on the characteristics of bacterial meningitis was conducted in four major tertiary care hospitals in Bangladesh during 2003 and 2004 [(124)]. The study showed that out of 1841 cases, 1307 were males, giving a male to female ratio of 2.5:1. Similarly, studies from Ethiopia have also shown a higher incidence in males. A 5-year retrospective study conducted in Gondar University Hospital during 1998 to 2003 identified 151 children with bacterial meningitis [(65)]. This study also reported a higher incidence in males with a male to female ratio of 2:1. During the meningococcal epidemic in Gondar in 2001-2002, a total of 1619 probable cases of meningococcal meningitis were reported. Out of these 1619 cases, 960 were males (male to female ratio of 1.5:1) [(125)]. The comparison between this Gondar study and our study shows a much higher number of patients of bacterial meningitis in the former study. The reason for this higher number of cases is due to the inclusion of probable cases which were not laboratory confirmed. The current study focuses on the etiology and has only included the laboratory confirmed cases from Gondar which were confirmed either by CSF culture or Gram staining. This explains the difference in the number of cases in these two studies from Gondar.

What has caused this high male to female ratio at both of the study sites may be linked to various factors. As seen in the literature and from results of the studies mentioned above, the organisms of bacterial meningitis show susceptibility patterns indicating a higher affinity for males. This fact may be exaggerated in the settings where males have more access to the health care facilities, as is presumed to be the cases in this study. The social and cultural factors favor easier access for males to the healthcare facilities as compared to the females which are more dependent on the males for travelling to far-located health care centers. Other barriers that may have affected the female access to the hospitals include fewer financial resources and the additional responsibilities in the family such as caring for children. The data show a greater difference in the number of patients of both genders in the infants and small children. At Awassa 75% of the infants were males. This may also highlight the bias or the preference that may be given by the family to the male children over the female children.

Bacterial meningitis is generally a disease of childhood. In this study the most commonly affected age group was found to be the infants and small children less than 4 years of age. Data from Gondar University Hospital show that almost half of the cases (49.5%) were either infants or children less than 4 years of age. Results from Awassa Referral Hospital show the highest incidence in young adults 15-24 years of age which make 27% of the total cases. Children less than 4 years of age, including the infants, make 22% of the cases. Within the various age groups, a higher incidence in infants has frequently been reported in literature. A 10-year study conducted in United States in California during 1998 to 2007 showed the highest incidence of bacterial meningitis in infants less than 2 months of age [(126)]. For the year 2006-2007 the incidence was 80.7 cases per 100,000 population. Children greater than 2 months and less than 2 years of age showed the second higher incidence (6.9 cases per 100,000 population) during the same year. A large scale retrospective study in Niger showed that in the year 1995-96 [(127)] the incidence of bacterial meningitis was highest in infants less than one year of age (638 cases per 100,000 population), this was followed by children between 1-4 years of age (490 cases per 100,000 population). Older children between 10-14 years and young adults also showed a higher incidence (each having 476 cases per 100,000 population). In contrast to these studies, a study in North Gondar during 2001-2002 showed the highest incidence in young adults between 15-30 years of age (52% of cases) [(125)]. Generally, these results point out at 2 peaks of age that have

high susceptibility for bacterial meningitis i.e. small children and young adults. This also parallels with the findings in this current study.

Certain agents of bacterial meningitis are known to have higher affinity for some age groups, particularly *H. influenzae,* which commonly effects infants. The results of this study from Gondar show a similar trend in which *H. influenzae* was more prevalent in the infancy and younger children (all 9 culture confirmed cases were less than 14 years). Little variation in the various age groups was observed for *N. meningitidis* and *S. pneumoniae,* both of which showed only a slightly higher incidence in small children between 1-4 years of age. This trend in the different age groups for these common organisms has frequently been reported in literature. A study was conducted in Lazio, Italy during the period 2001–2005 to study these variations in age groups. A slight variation in various age groups was reported for *N. meningitidis* and *S. pneumoniae. S. pneumoniae* had a higher incidence at both extremities of age whereas *N. meningitidis* was more prevalent in smaller children less than 4 years. Marked variation was only observed for *H. influenzae* which had a considerably higher incidence in smaller children less than 4 years (25 out of 46 cases) [(128)]. Similar results were observed in a study on the laboratory based surveillance of bacterial meningitis in Sudan during the year 2004-2005. The study showed that out of a total of 24 culture or PCR confirmed cases of *H. influenzae,* 21 (87.5%) had occurred in children less than 5 years of age [(11)].

Seasonal variations are typical of meningococcal meningitis but may occur to a lesser extent with other agents of bacterial meningitis [(100)]. The dry season starting from December and reaching up till June has invariably been implicated in meningococcal epidemics. When the data from the study sites were analyzed the highest incidence for meningitis cases was observed in the summer months. At Gondar University Hospital 35% of the meningococcal meningitis cases presented in the months of May and June and tapered markedly as the winter season approached. The cases peaked in second quarter of the year (April to June), followed by a steep decline in the incidence. This coincides with our knowledge of the seasonal variation in the incidence of meningococcal meningitis in the countries of meningitis belt. However, such sharp decline in the cases of bacterial meningitis was not so apparent in Awassa. This may partly be due to the fact that the data from Awassa represents bacterial meningitis as a whole and the cases of meningococcal disease could not be studied separately due to the very few cultures performed. As all the agents

of bacterial meningitis do not exhibit seasonal variations to similar extent, therefore, when all the agents of bacterial meningitis were studied collectively, the masking of the seasonal effect on the incidence of meningococcal disease can be an important factor.

The agents causing bacterial meningitis may vary considerably depending upon the geographical and climatic conditions, socio-economic status and importantly the immunization schemes in the host community [16]. Culture results from Gondar University hospital reveal the prevalence of various organisms that cause bacterial meningitis. Almost 35% of the cultures were identified as *S. pneumoniae,* followed by *N. meningitidis* and *H. influenzae* which are isolated from 28% and 9% of the cases, respectively. The predominance of these three agents of bacterial meningitis has widely been reported in literature. A large scale WHO study conducted in various countries of meningitis belt including Ethiopia has documented similar patterns with *S. pneumoniae* being the most common organism. It was identified in 26% of the cases. *S. pyogenes* (20%) and *E. coli* (18%) were the other common organisms [121]. A laboratory based study on the surveillance of bacterial meningitis in Egypt during 1998-2004 depicted a high *S. pneumoniae* prevalence (42%) followed by Hib (20%) and *N. meningitidis* (16%) [129]. The etiology of bacterial meningitis may vary with the age of the study group and other characteristics of the population. A 5-year study on children up to the age of 14 years in Gondar during 1998-2003 reported *N. meningitidis* as the leading cause of bacterial meningitis (27.8% of the cases). *S. pneumoniae* and *H. influenzae* were present in 7.1% and 5.6% of the cases [65].

The results from these studies show varying etiological trends subjected to multiple factors, therefore, an estimate of the prevalence of these causative organisms can often be very complex. A study was conducted by Norwegian Institute of Public Health in North Gondar and SNNPR Ethiopia in 2002 and 2003 [78]. A total of 95 cases were included with the aim of characterization of *N. meningitidis* from these isolates. Out of 95 cases 71 cases were identified as *N. meningitidis.* The remaining 24 cases were identified in February-March 2012 by Real-Time PCR as a part of this thesis. (Please refer to appendix II to find a detailed account on the RT-PCR results from those samples).

It is evident from the literature on bacterial meningitis in the meningitis belt countries that in the lack of availability of laboratory facilities the clinical signs and symptoms play a significant role

in the diagnosis of bacterial meningitis. The classic triad of symptoms i.e. fever, headache and neck stiffness are characteristic of bacterial meningitis [61]. These were the most common signs and symptoms that were recorded in the clinical record files of the patients in Awassa Referral Hospital. Fever was present in 90% of the cases and 75% of the patients had neck stiffness. Headache was the presenting complaint in almost 70% of the cases. Petechial rash which is a characteristic of meningococcal sepsis occurred only in a single patient. A very similar study conducted on a 5-year retrospective clinical data in East England during 2005-2010 [130] showed the highest prevalence of fever and headache (82% each), followed by altered mental state (59%), vomiting (51%), neck or back rigidity (33%). The results follow almost a similar trend as observed in the current study. However, in the East England study, 10 out of 39 cases (26%) had purpuric rash, in contrast to only one documented cases of petechial rash in the current study. The darker skin color may offer some hindrance in spotting petechial rash and this may be a contributing factor to the lower incidence of the rash at our study sites. As it has already been mentioned in the literature, that the most common serogroup of *N. meningitidis* in Ethiopia is serogroup A; [29][30] a serogroup which is not usually associated with petechial rash. As the rash is a manifestation of severe meningococcal sepsis and a tertiary care hospital may often be located very far so some of these serious patients may even expire before reaching the hospital. All these reason may explain the reduced incidence of petechial rash observed in this study.

Data on the clinical outcomes of treatment from Awassa Referral Hospital show a fatality rate (CFR) of 23.5%. When compared with different studies conducted around the world the CFR may be highly variable. In the developed world the CFR may be considerably lower than the CFR observed in this study. In a study conducted in the United States during 2003-2007, a CFR of 6.9 was observed in pediatric patients whereas a CFR of 16.4 was recorded in adult patients [126]. But earlier studies from Ethiopia have reported a fatality rate of 22-28% [114]. But even the CFR reported in various studies in Ethiopia varies. In a study on 151 cases of bacterial meningitis in Gondar, 20 patients died, thus giving a CFR of 13.2% [65]. The exact estimation of the fatality rates due to bacterial meningitis may be somewhat difficult as not all the cases present in the hospitals. These rates represent data from a tertiary care hospital with relatively better facilities. In isolated smaller healthcare units with minimal facilities the fatality rates may actually be much higher.

As in the case of CFR, the rate of sequelae may also be much lower in tertiary care hospitals. This may be the reason for a considerably lower rate of sequelae (9.2%) that was observed in this study as compared to the estimated rates that have been mentioned in the literature (up to 50%) [(69)]. The most commonly observed sequelae in the current study were neurological complications such as paralysis or weakness of the limbs (4 cases); facial palsy (4 cases), muscle wasting (2 cases), hearing impairment (2 cases) and acute psychosis (2 cases). The same sequelae, but to a varying degree, have been reported in various studies. A systematic review of 37 studies from 21 African countries has documented sequelae rate as high as 37% [(66)]. The rate was higher with Hib and *S. pneumoniae* (44% and 38%, respectively). Therefore, the chances of sequelae may be highly variable depending upon the treatment facilities, age of the patient (more common in children) and also the organism causing infection as mentioned earlier. Early detection and timely treatment is therefore essential for the prevention of these long-lasting sequelae.

5.2. Limitations of the study:

Throughout the study, the emphasis has particularly been on the efforts to ensure the quality, validity and generalization of the data. Nevertheless, the study is not without its own limitations. Some main limitations that were encountered during the conduct of this study are listed.

1. Due to the lack of availability of laboratory facilities, not all the cases were confirmed by laboratory evidence of the bacterial organism. In such instances the diagnosis was based solely on the clinical diagnosis which may be less accurate then the laboratory assisted diagnosis.

2. The study period was highly dependent on the availability of the clinical and laboratory records. The limited data on the laboratory results along with relatively few CSF cultures and Gram staining confirmed cases were encountered as the major limiting factors, particularly in Awassa. As the laboratory facilities are still developing, therefore, the very recent records had relatively more laboratory confirmed cases as compared to earlier records. Therefore, in order to utilize the data effectively the study period had to be

tailored for each study site. This may have an effect on the uniformity of the study design.

3. Both the study sites that were selected were tertiary care hospitals in larger cities with relatively better healthcare facilities than many other parts of the country. The situation in the periphery of these cities and more so in the remotely located villages may be entirely different. This fact may interfere to some extent with the generalization of the results.

4. Another limitation may be the retrospective nature of the study itself. This implies relying on the quality and quantity of information that had already been recorded in the hospital and laboratory records. Some information loss may have occur at multiple steps starting from the data recorded by attending physician, laboratory staff and finally while recording the data onto the case record forms.

5. The actual data was not computerized and was rather handwritten manuscripts of clinical and laboratory data entered by various physicians and laboratory staff. Reading and interpreting the original data was often difficult. However, to minimize this limitation help was always sought from a person working in the same settings and who was familiar with the data.

6. The laboratory records were distributed in various logbooks belonging to different departments of the laboratory. Often the data were recorded randomly and haphazardly in these log books without a proper uniform pattern. This may increase the chances of missing some data or on the contrary the duplication of the data. The hospital registration numbers and admission dates were used to prevent any possible duplication.

7. The data were entered in predefined data collection forms (attached as appendix I). This may result in loss of some important information that was not accounted for on the data collection forms.

8. As the study was retrospective and follow up was not a problem, but still some of the cases were either referred or were discharged against medical advice. In such patients the final outcome of the treatment could not be recorded.

5.3. Future recommendations:

The research on bacterial meningitis in the meningitis belt countries has evolved persistently in the past years. The new knowledge thus obtained has reshaped our struggle against meningitis and undermines the current and future preventive strategies. This study has been conducted with an aim to pave way for future studies in similar settings. Following are the recommendation from this study:

1. Similar studies in settings with better laboratory facilities may yield more information on the recent incidence of the causative organisms or the current serotypes. Combining the laboratory results with the clinical outcome will help in better understanding of bacterial meningitis.

2. Studies focusing on other hospitals of Ethiopia may provide results specific to other parts of Ethiopia. This will be beneficial not only to the respective hospital but also the Ethiopian population as a whole.

3. Many patients of bacterial meningitis may not present at tertiary care hospitals due to vast distances from hospitals and lack of transport facilities. Any study that has its focus laid primarily on the primary healthcare centers in the peripheries will provide valuable information on a large portion of cases that are easily missed when studies are conducted only in the tertiary care hospitals.

4. During the course of study many patients with co-existent infectious diseases were found. The association of bacterial meningitis with other infectious diseases such as Human Immunodeficiency Virus (HIV) infection or in immunocompromised patients may need further exploration.

5. Standardization of the data entry procedures for hospitals' clinical and laboratory records at the study sites along with a more unified collection and storage of records will certainly prove beneficial for the follow up patients, the hospital staff and even the future researchers working on disease prevention in these settings.

6. Finally, the laboratory techniques to identify the causative organism form the basis of treatment of bacterial meningitis. Laboratory surveillance is essential for the control of meningitis due to continuously changing epidemiology of the disease in the meningitis belt countries. Limitation of resources and lack of properly trained staff form the main reason for the underutilization of these laboratory techniques. A steady and step wise increase in the utilization of these techniques will not only provide prompt and accurate diagnosis for the patients but will also serve as a more reliable indicator of bacterial meningitis trends in the host population.

Chapter six: REFERENCES

1) World Health Organization (WHO). September, 2005. *Wkly. Epidemiol. Rec*. **80**:313-320.

2) World Health Organization (WHO). Meningococcal meningitis [online]. Available from URL: http://www.who.int/mediacentre/factsheets/fs141/en/index.html (accessed 25/07/2012).

3) Gilhus, N. E., Barnes, M. P., and M. Brainin, 2011. Management of community-acquired bacterial meningitis. European handbook of neurological managament. Vol. 2.

4) Holliday, R. A, 2011. An Invitation to Health: Choosing to Change. *J. Nutr. Educ. Behav*. **43**:424.

5) Davey, P. 2010. Infections of the central nervous system. Medicine at a Glance 3rd ed. pg 398-406.

6) Marx, G. E., and E. D. Chan, 2011. Tuberculous Meningitis: Diagnosis and Treatment Overview. *Tuberc. Res. Treat*. 2011.

7) Berenguer, J. R., S. Moreno, F. Laguna, T. Vicente, M. Andrados, A. Ortega, J. González-LaHoz, and E. Bouza, 1992. Tuberculous meningitis in patients infected with the human immunodeficiency virus. *N. Engl. J. Med*. **326**:668–672.

8) Young, V. B., W. A. Kormos, A. H. Goroll, and D. A. Chick, 2010. Blueprints medicine: Meningitis 5th ed.

9) Daroff, R. B., G. M. Fenichel, J. Jankovic, and J. C. Mazziotta, 2004. Diagnosis of neurological disease. Bradley's Neurology in Clinical Practice 6th ed.

10) Philippon, S., H. Broutin, G. Constantin de Magny, K. Toure, C. H. Diakite, N. Fourquet, M. F. Courel, B. Sultan, and J. F. Guégan, 2009. Meningococcal meningitis in Mali: a long-term study of persistence and spread. *Int. J. Infect. Dis*. **13**:103–109.

11) Affifi, S., M. S. Karsany, M. Wasfy, G. Pimentel, A. Marfin, and R. Hajjeh, 2009. Laboratory-based surveillance for patients with acute meningitis in Sudan 2004-2005. *Eur. J. Clin. Microbiol. Infect. Dis*. **28**:429-435.

12) Miranda, J. J., S. Kinra, J. P. Casas, G. D. Smith, and S. Ebrahim, 2008. Non-communicable diseases in low- and middle-income countries: context, determinants and health policy. *Trop. Med. Int. Health*. **13**:1225–1234.

13) Segretti, J., and A. A. Harris, 1996. Acute bacterial meningitis. *Infect. Dis. Clin*. *North Am.* **10**: 707-809.

14) Patel V., A. Woodward, V. L. Feigin, H. K. Heggenhougen, and S. R. Quah, 2010. Mental and Neurological Public Health: A Global Perspective 1st ed.

15) Harrison, L. H., C. L. Trotter, and M. E. Ramsay, 2009. Global epidemiology of meningococcal disease. *Vaccine*. **24**:51-63.

16) Martin, M., J. M. Casellas, S. A. Madhi, T. J. Urquhart, S. D. Delport, F. Ferrero, S. Chamany, G. H. Dayan, C. E. Rose, O. S. Levine, K. P. Klugman, and D. R. Feikin, 2004. Impact of *Haemophilus influenza* type b conjugate vaccine in South Africa and Argentina. *Pediatr. Infect. Dis. J*. **23**: 842-847.

17) Roberts, L., 2008. An Ill Wind, Bringing Meningitis. *Science*. **320**:1710-1715.

18) Agrawal, S., and S. Nadel, 2011. Acute bacterial meningitis in infants and children: epidemiology and management. *Paediatr. Drugs*. **13**:385–400.

19) Johnson, H. L., M. Deloria-Knol, O. S. Levine, S. K. Stoszek, L. Freimanis Hance, R. Reithinger, L. R. Muenz, and K. L. O'Brien, 2010. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. *PLoS Med*. **7**:10.

20) Lynch, J. P., and G. G. Zhanel, 2010. *Streptococcus pneumoniae*: epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines. *Curr. Opin. Pulm. Med*. **16**: 217-225.

21) Centers for Disease Control and Prevention (CDC), 2006. Vaccine preventable deaths and the global immunization vision and strategy, 2006–2015. MMWR *Morb. Mortal. Wkly. Rep*. **55**:511–515.

22) O'Brien, K. L., L. J. Wolfson, J. P. Watt, E. Henkle, M. Deloria-Knoll, N. McCall, E. Lee, K. Mulholland, O. S. Levine, T. Cherian, and Hib and Pneumococcal Global Burden of Disease Study Team, 2009. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet*. **374**:893-902.

23) Antonio, M., I. Hakeem, T. Awine, O. Secka, K. Sankareh, D. Nsekpong, G. Lahai, A. Akisanya, U. Egere, G. Enwere, S. M. Zaman, P. C. Hill, T. Corrah, F. Cutts, B. M. Greenwood, and R. A. Adegbola, 2008. Seasonality and outbreak of a predominant *Streptococcus pneumoniae* serotype 1 clone from The Gambia: expansion of ST217 hypervirulent clonal complex in West Africa. *BMC Microbiol*. **8**:198.

24) Leimkugel, J., A. AdamsForgor, S. Gagneux, V. Pfluger, C. Flierl, E. Awine, M.Naegeli, J. P. Dangy, T. Smith, A. Hodgson, and G. Pluschke 2005. An outbreak of serotype 1 *Streptococcus pneumoniae* meningitis in northern Ghana with features that are characteristic of *Neisseria meningitidis* meningitis epidemics. *J. Infect. Dis*. **192**:192-199.

25) Yazdankhah, S. P., and D. A. Caugant, 2004. Neisseria meningitidis: an overview of the carriage state. *J. Med. Microbiol*. **53**:821-832.

26) Stephens, D. S., B. Greenwood, and P. Brandtzaeg, 2007. Epidemic meningitis, meningococcaemia and *Neisseria meningitidis*. *Lancet*. **369**:2196-2210.

27) Rosenstein, N. E., B. A. Perkins, D. S. Stephens, T. Popovic, and J. M. Hughes, 2001. Meningococcal disease. *N. Engl. J. Med*. **344**:1378-1388.

28) Centers for Diseases Control and Prevention (CDC). Meningococcal disease: technical and clinical information [online]. Available from website. URL: http://www.cdc.gov/meningitis/clinical-info.html (Accessed 24/07/2012).

29) Centers for Diseases Control and Prevention (CDC), 2006. Active Bacterial Core surveillance (ABCs) report: emerging infections program network. *Neisseria meningitidis* [online]. Available from website URL: http://www.cdc.gov/abcs/ reports-findings/survreports/mening06.pdf. (Accessed 26/07/2012).

30) Stephens, D. S., 2007. Conquering the meningococcus. *FEMS Microbiol. Rev*. **31**:3-14.

31) The European Union Invasive Bacterial Infections Surveillance Network. Invasive meningococcal disease [Online]. Available from website URL: http://www.ecdc.europa.eu/en/publications/Publications/1011_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf#page= 149. (Accessed 01/08/2012).

32) Harrison, L. H., 2010. Epidemiological profile of meningococcal disease in the United States. *Clin. Infect. Dis*. **50**:37-44.

33) LaClaire, L. L., M. L. Tondella, D. S. Beall, C. A. Noble, P. L. Raghunathan, N. E. Rosenstein, and T. Popovic, 2003. Identification of *Haemophilus influenzae* serotypes by standard slide agglutination serotyping and PCR-based capsule typing. *J. Clin. Microbiol*. **41**:393-396.

34) Watt, J. P., L. J. Wolfson, K. L. O'Brien, E. Henkle, M. Deloria-Knoll, N. McCal, E. Lee, O. S. Levine, R. Hajjeh, K. Mulholland, T. Cherian, and Hib and Pneumococcal Global Burden of Disease Study Team, 2009. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *Lancet*. **374**:903-911.

35) Harvey, D., D. Holt, and H. Bedford, 2009. Bacterial meningitis in the newborn: a prospective study of mortality and morbidity. *Semin. Perin*. **23**:218-225.

36) Thaver, D., and A. K. Zaidi, 2009. Burden of neonatal infections in developing countries: a review of evidence from community-based studies. *Pediatr. Infect. Dis. J*. **28**:3-9.

37) Puopolo, K. M., L. C. Madoff, and E. C. Eichenwald, 2005. Early onset group B streptococcal disease in the era of maternal screening. *Pediatrics*. **115**:1240-1246.

38) Bedford, H., J. de Louvois, S. Halket, C. Peckham, R. Hurley, and D. Harvey, 2001. Meningitis in infancy in England and Wales: follow up at age 5 years. *BMJ*. **323**:533-536.

39) Zaidi, A. K., D. Thaver, S. A. Ali, and T. A. Khan, 2009. Pathogens associated with sepsis in newborns and young infants in developing countries. *Pediatr. Infect. Dis. J*. **28**:10-18.

40) Klinger, G., C. N. Chin, J. Beyene, and M. Perlman, 2000. Predicting the outcome of neonatal bacterial meningitis. *Pediatrics*. **106**:477-482.

41) Centers of Disease Control and Prevention (CDC), 2009. Trends in perinatal group B streptococcal disease: United States 2000-2006. *Morb. Mortal. Wkly. Rep*. **58**:109-112.

42) Tunkel, A. R., B. J. Hartman, S. L. Kaplan, B. A. Kaufman, K. L. Roos, W. M. Scheld, and R. J. Whitley, 2004. Practice guidelines for the management of bacterial meningitis. *Clin. Infect. Dis*. **39**:1267-1284.

43) Johnson, A. J., J. H. Ayvazian, and W. S. Tillett, 1959. Crystalline pancreatic desoxyribonuclease as an adjunct to the treatment of pneumococcal menignitis. *N. Engl. J. Med*. **260**:893–900.

44) Kim, K. S., 2003. Pathogenesis of bacterial meningitis: from bacteremia to neuronal injury. *Nat. Rev. Neurosci*. **4**:376-385.

45) Hoffman, O., and J. R. Weber, 2009. Pathophysiology and treatment of bacterial meningitis. *Ther. Adv. Neurol. Disord*. **2**:401-412.

46) Daum, R. S., D. W. Scheifele, V. P. Syriopoulou, D. Averill, and A. L. Smith, 1978. Ventricular involvement in experimental *Hemophilus influenzae* meningitis. *J. Pediatr*. **93**:927-930.

47) Pron, B., M. K. Taha, C. Rambaud, J. C. Fournet, N. Pattey, J. P. Monnet, M. Musilek, J. L. Beretti, and X. Nassif, 1997. Interaction of *Neisseria meningitidis* with the components of the blood-brain barrier correlates with an increased expression of PilC. *J. Infect. Dis*. **176**:1285-1292.

48) Zwijnenburg, P. J., T. van der Poll, S. Florquin, S. J. van Deventer, J. J. Roord, and A. M. van Furth, 2001. Experimental pneumococcal meningitis in mice: a model of intranasal infection. *J. Infect. Dis*. **183**:1143-146.

49) Unkmeir, A., K. Latsch, G. Dietrich, E. Wintermeyer, B. Schinke, S. Schwender, K. S. Kim, M. Eigenthaler, and M. Frosch, 2002. Fibronectin mediates Opc-dependent internalization of *Neisseria meningitidis* in human brain microvascular endothelial cells. *Mol. Microbiol*. **46**:933-946.

50) Maisey, H. C., M. Hensler, V. Nizet, and K. S. Doran, 2007. Group B streptococcal pili proteins contribute to adherence and invasion of brain microvascular endothelial cells. *J Bacteriol*. **189**:1464-1467.

51) Livorsi, D. J., E. Stenehjem, and D. S. Stephens, 2011. Virulence Factors of Gram-Negative Bacteria in Sepsis with a Focus on *Neisseria meningitidis*. *Contrib. Microbiol*. **17**:31–47.

52) Kieseier, B. C., R. Paul, U. Koedel, T. Seifert, J. M. Clements, A. J. Gearing, H. W. Pfister, and H. P. Hartung, 1999. Differential expression of matrix metalloproteinases in bacterial meningitis. *Brain*. **122**:1579-1587.

53) Polfliet, M. M., P. J. Zwijnenburg, A. M. van Furth, T. van der Poll, E. A. Döpp, C. Renardel de Lavalette, E. M. van Kesteren-Hendrikx, N. van Rooijen, C. D. Dijkstra, and T. K. van den Berg, 2001. Meningeal and perivascular macrophages of the central nervous system play a protective role during bacterial meningitis. *J. Immunol*. **167**:4644-50.

54) Hoffmann, O., J. Zweigner, S. H. Smith, D. Freyer, C. Mahrhofer, E. Dagand, E. I. Tuomanen, and J. R. Weber, 2006. Interplay of pneumococcal hydrogen peroxide and host-derived nitric oxide. *Infect. Immun*. **74**:5058-5066.

55) Ivey, N. S., E. N. Martin Jr, W. M. Scheld, and B. R. Nathan, 2005. A new method for measuring BBB permeability demonstrated with Europium bound albumin during experimental (LPS) induced meningitis in the rat. *J. Neurosci. Methods*. **142**:91-95.

56) van de Beek, D., B. Schmand, J. de Gans, M. Weisfelt, H. Vaessen, J. Dankert, and M. Vermeulen, 2002. Cognitive impairment in adults with good recovery after bacterial meningitis. *J. Infect. Dis*. **186**:1047-1052.

57) Roos, K. L., 2010. Handbook of clinical neurology: Bacterial infections of the central nervous system. Elsevier. vol.18

58) Braun, J. S., J. E. Sublett, D. Freyer, T. J. Mitchell, J. L. Cleveland, E. I. Tuomanen, and J. R. Weber, 2002. Pneumococcal pneumolysin and H_2O_2 mediate brain cell apoptosis during meningitis. *J. Clin. Invest*. **109**:19-27.

59) Braun, J. S., O. Hoffmann, M. Schickhaus, D. Freyer, E. Dagand, D. Bermpohl, T. J. Mitchell, I. Bechmann, and J. R. Weber, 2007. Pneumolysin causes neuronal cell death through mitochondrial damage. *Infect. Immun*. **75**:4245-4254.

60) Brandtzaeg, P, P. Kierulf, P. Gaustad, A. Skulberg, J. N. Bruun, S. Halvorsen, and E. Sorensen, 1989. Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. *J. Infect. Dis*. **159**:195–204.

61) Heckenberg, S. G., J. de Gans, M. C. Brouwer, M. Weisfelt, J. R. Piet, L. Spanjaard, A. van der Ende, and D. van de Beek, 2008. Clinical features, outcome, and meningococcal genotype in 258 adults with meningococcal meningitis: a prospective cohort study. *Medicine* **87**:185-192.

62) van de Beek, D., J. de Gans, L. Spanjaard, M. Weisfelt, J. B. Reitsma, and M. Vermeulen, 2004. Clinical features and prognostic factors in adults with bacterial meningitis. *N. Engl. J. Med*. **351**:1849-1859.

63) Cabellos, C., P. F. Viladrich, J. Ariza, J. M. Maiques, R. Verdaguer, and F. Gudiol, 2008. Community acquired bacterial meningitis in cirrhotic patients. *Clin. Microbiol. Infect*. **14**: 35-40.

64) Royal College of Paedeatrics and Child Health, 2010. NICE Clinical Guideline: Bacterial meningitis and meningococcal septicemia in children. Website URL: guidance.nice.org.uk.

65) Amsalu, S., and A. Assefa. 2005. Meningitis in children beyond the neonatal period in Gondar University Hospital. Ethiop. *Med. J*. **43**:175-180.

66) Ramakrishnan, M., A. J. Ulland, L. C. Steinhardt, J. C. Moisi, F. Were, and O. S. Levine. 2009. Sequelae due to bacterial meningitis among African children: a systematic literature review. *BMC Medicine*. **7**:47.

67) Koomen, I., D. E. Grobbee, A. Jennekens-Schinkel, J. J. Roord, and A. M. van Vurth, 2003. Parental perception of educational, behavioural and general health problems in school-age survivors of bacterial meningitis. *Acta Paediatr*. **92**:177-185.

68) Scheld, W. M., U. Koedel, B. Nathan, and H. W. Pfister, 2002. Pathophysiology of bacterial meningitis: mechanism of neuronal injury. *J. Infect. Dis*. **186**:225-233.

69) Grimwood K., P. Anderson, V. Anderson, L. Tan, and T. Nolan, 2000. Twelve year outcomes following bacterial meningitis: further evidence for persisting effects. *Arch. Dis. Child*. **83**:111-116.

70) Kaplan, S. L., and C. R. Woods, 1992. Neurologic complications of bacterial meningitis in children. *Curr. Clin. Trop. Infect. Dis*. **12**:37-55.

71) Aronin, S. I., P. Peduzzi, and V. J. Quagliarell, 1998. Community-aquired bacterial meningitis; risk stratification for adverse clinical outcome and effect of antibiotic timing. *Ann. Inter. Med*. **129**:862-869.

72) Pfister, H. W., W. Feiden, and K. M. Einhaupl, 1993. Spectrum of complications during bacterial meningitis in adults. Results of a prospective clinical study. *Arch. Neurol*. **50**: 575–581.

73) Namani, S., E. Kuchar, R. Koci, K. Dedushi, M. Mehmeti, and V. Krasniqi, 2011. Acute neurologic complications and long term sequelae of bacterial meningitis in children. *The Internet Journal of Infectious Diseases*. **9**:2.

74) Brouwer, M., A. Tunkel, and D. van de Beek, 2010. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. *Clin. Microbiol. Rev*. **23**:467-492.

75) World Health Organization (WHO), 1999. Laboratory methods for the diagnosis of meningitis caused by *Neisseria meningitidis, Streptococcus pneumoniae*, and *Haemophilus influenza* 1st ed.

76) Nigrovic, L., R. Malley, G. Macias, J. T. Kanegaye, D. M. Moro-Sutherland, R. D. Schremmer, S. H. Schwab, D. Agarwal, K. M. Mansoor, and J. E. Bennett, 2008. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. *Pediatrics*. **122**:726:730.

77) Straus, S. E., K. E. Thorpe, and J. Holroyd-Leduc, 2006. How do I perform a lumbar puncture and analyze the results to diagnose bacterial meningitis? *JAMA*. **296**:2012-2022.

78) Norheim, G., E. Rosenqvist, A. Assefa, M. A. Yassin, G. Mengistu, A. Kassu, D. Fikremariam, W. Tamire, E. A. Høiby, T. Alebel, D. Berhanu, Y. Merid, M. Harboe, and D. A. Caugant, 2006. Characterization of *Neisseria meningitidis* isolates from recent outbreaks in Ethiopia and comparison with those recovered during the epidemic of 1988 to 1989. *J Clin. Microbiol*. **44**:861-871.

79) Ajello, G., J. C. Feeley, P. S. Hayes, A. L. Reingold, G. Bolan, C. V Broome, and C. J. Phillips, 1984. Trans-isolate medium: a new medium for primary culturing and transport of *Neisseria meningitidis, Streptococcus pneumoniae*, and *Haemophilus influenzae*. *J. Clin. Microbiol*. **20**:55-58.

80) Hausdorff, W. P., D. R. Feikin, and K. P. Klugman, 2005. Epidemiological differences among pneumococcal serotypes. *Lancet Infect. Dis*. **5**:83-93.

81) Proulx, N., D. Frechette, B. Toye, J. Chan, and S. Kravcik, 2005. Delays in the administration of antibiotics are associated with mortality from adult acute bacterial meningitis. *QJM*. **98**:291-298.

82) Auburtin, M., M. Wolff, J. Charpentier, E. Varon, Y. Le Tulzo, C. Girault, I. Mohammedi, B. Renard, B. Mourvillier, F. Bruneel, J. D. Ricard, and J. F. Timsit, 2006. Detrimental role of delayed antibiotic administration and penicillin-nonsusceptible strains in adult intensive care unit patients with pneumococcal meningitis: the PNEUMOREA prospective multicenter study. *Crit. Care Med*. **34**:2758-2765.

83) Chaudhuri, A., P. Martinez-Martin, P. G. Kennedy, R. Andrew Seaton, P. Portegies, M. Bojar, I. Steiner; EFNS Task Force, 2008. EFNS guideline on the management of community-acquired bacterial meningitis: report of an EFNS Task Force on acute bacterial meningitis in older children and adults. *Eur. J. Neurol*. **15**:649–659.

84) Woehrl, B., M. Klein, D. Grandgirard, U. Koedel, and S. Leib, 2011. Bacterial meningitis: current therapy and possible future treatment options. *Expert Rev. Anti Infect. Ther*. **9**:1053-1065.

85) Adegbola, R. A., O. Secka, G. Lahai, N. Lloyd-Evans, A. Njie, S. Usen, C. Oluwalana, S. Obaro, M. Weber, T. Corrah, K. Mulholland, K. McAdam, B. Greenwood, and P. J. Milligan, 2005. Elimination of *Haemophilus influenza* type b (Hib) disease from The Gambia after introduction of routine immunization with a Hib conjugate vaccine: a prospective study. *Lancet*. **366**:144-150.

86) Lexau, C. A., R. Lynfield, R. Danila, T. Pilishvili, R. Facklam, M. M. Farley, L. H. Harrison, W. Schaffner, A. Reingold, N. M. Bennett, J. Hadler, P. R. Cieslak, C. G. Whitney; Active Bacterial Core Surveillance Team, 2005. Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. *JAMA*. **294**:2043-2051.

87) Lucero, M. G., V. E. Dulalia, L. T. Nillos, G. Williams, R. A. Parreño, H. Nohynek, I. D. Riley, and H. Makela, 2009. Pneumococcal conjugate vaccines for preventing vaccine-type invasive pneumococcal disease and X-ray defined pneumonia in children less than two years of age. *Cochrane Database Syst. Rev*. **4**:CD004977.

88) Lacapa, R., S. J. Bliss, F. Larzelere-Hinton, K. J. Eagle, D. J. McGinty, A. J. Parkinson, M. Santosham, M. J. Craig, and K. L. O'Brien, 2008. Changing epidemiology of invasive pneumococcal disease among White Mountain Apache persons in the era of the pneumococcal conjugate vaccine. *Clin. Infect. Dis*. **47**:476-84.

89) World Health Organization (WHO), 2008. Progress introducing *Haemophilus influenza* type b vaccine in low-income countries, 2004-2008. *Wkly. Epidemiol. Rec*. **83**:61-68.

90) World Health Organization (WHO), 2007. Pneumococcal conjugate vaccine for childhood immunization: WHO position paper. *Wkly. Epidemiol. Rec*. **82**:93-104.

91) Girard, M. P., M. P. Preziosi, M. T. Aguado, and M. P. Kieny, 2006. A review of vaccine research and development: meningococcal disease. *Vaccine*. **24**:4692-4700.

92) Ramsay, M. E., N. J. Andrews, C. L. Trotter, E. B. Kaczmarski, and E. Miller, 2003. Herd immunity from meningococcal serogroup C conjugate vaccination in England. *BMJ*. **326**:365-366.

93) Kelly, C., R. Arnold, Y. Galloway, and J. O'Hallahan, 2007. A prospective study of the effectiveness of the New Zealand meningococcal B vaccine. *Am. J. Epidemiol*. **166**:817-823.

94) Norheim, G., G. Tunheim, L. M. Næss, P. A. Kristiansen, D. A. Caugant, and E. Rosenqvist, 2011. An outer membrane vesicle vaccine for prevention of serogroup A and W-135 meningococcal disease in the African meningitis belt. *Scand. J. Immunol*. **76**:99-107.

95) World Health Organization (WHO), 2011. Meningitis A conjugate vaccine. *Wkly. Epidemiol. Rec*. **86**:42-43.

96) Meningitis Vaccine Project (MVP). Eliminating epidemic meningitis as a public health problem in sub-Saharan Africa. [online] Website: http://www.meningvax.org/ (accessed on 22/07/2012)

97) Norheim, G., A. Assefa, M. Yassin, G. Mengistu, A. Kassu, D. Fikremariam, W. Tamire, Y. Merid, E. A. Høiby, D. A. Caugant, E. Fritzsønn, T. Tangen, B. Melak, D. Berhanu, M. Harboe, J. Kolberg, and E. Rosenqvist, 2008. Specificity of subcapsular antibody responses in Ethiopian patients following disease caused by serogroup a meningococci. *Clin. Vaccine Immunol*. **15**:863-871.

98) Lapeyssonnie, L., 1963. La me´ ningite ce´ re´ brospinale en Afrique. *Bull World Health Organ*. **28**:1-114.

99) Molesworth, A. M., M. C. Thomson, S. J. Connor, M. P. Cresswell, A. P. Morse, and L. E. Cuevas, 2002. Where is the meningitis belt? Defining an area at risk of epidemic meningitis in Africa. *Trans. R. Soc. Trop. Med. Hyg*. **96**:242-249.

100) Mueller, J. E., and B. D. Gessner, 2009. A hypothetical explanatory model for meningococcal meningitis in the African meningitis belt. *Int. J. Infect. Dis*. **14**:553-559.

101) Moore, P. S., 1992. Meningococcal meningitis in sub-Saharan Africa: a model for the epidemic process. *Clin. Infect. Dis*. **14**:515-525.

102) Irving, T. J., K. B. Blyuss, C. Colijn, and C. L. Trotter, 2011. Modelling meningococcal meningitis in the African meningitis belt. *Epidemiol Infect*. **140**:897-905.

103) Trotter, C. L., and B. M. Greenwood, 2007. Meningococcal carriage in the African meningitis belt. *Lancet Infect. Dis*. **7**:797-803.

104) Kristiansen, P. A., F. Diomandé, S. C. Wei, R. Ouédraogo, L. Sangaré, I. Sanou, D. Kandolo, P. Kaboré, T. A. Clark, A. S. Ouédraogo, K. B. Absatou, C. D. Ouédraogo, M. Hassan-King, J. D. Thomas, C. Hatcher, M. Djingarey, N. Messonnier, M. P. Préziosi, M. LaForce, and D. A. Caugant, 2011. Baseline meningococcal carriage in Burkina Faso before the introduction of a meningococcal serogroup A conjugate vaccine. *Clin. Vaccine. Immunol*. **18**:435-443.

105) Greenwood, B., 1999. Society Meeting at Manson House, London, 10 December 1998. Meningococcal meningitis in Africa. *Trans. R. Soc. Trop. Med. Hyg*. **3**:341-353.

106) Greenwood, B., 2007. The changing face of meningococcal disease in West Africa. *Epidemiol. Infect*. **135**:703-705.

107) Central Statistical Agency of Ethiopia. [online]. Website: http://www.csa.gov.et. (Accessed 28/07/2012)

108) United Nations Children's Fund (UNICEF) statistics Ethiopia. [online]. Website: http://www.unicef.org/infobycountry/ethiopia_statistics.html. (Accessed 3/08/2012)

109) World Health Organization (WHO) statistics Ethiopia. [Online]. Website: http://www.who.int/countries/eth/en/. (Accessed 3/08/2012)

110) Central Statistical Agency and ORC Macro. Ethiopia Demographic and Health Survey 2005. Addis Ababa and Calverton, MD: Central Statistical Agency and ORC Macro, 2006.

111) Tadele, T., 1993. Meningococcal meningitis. In: Helmut Kloos and Zein Ahmed Zein (eds.), the ecology of health and disease in Ethiopia. pg 285-293.

112) Habte-Gabr, E., E. Tekle, and M. Mamo, 1984. Meningococcal meningitis in Ethiopia 1974-1983 and strategies of control. *Ethiop. J. Health Dev*. **1**:47-63.

113) United Nations Country Team in Ethiopia (UNCT) April, 2001. Ethiopia Special Alert: Shortage of supplies and funds as meningitis outbreak spreads. [online] website URL: http://reliefweb.int/report/ethiopia/ethiopia-special-alert-shortage-supplies-and-funds-meningitis-outbreak-spreads (accessed 26/07/2012).

114) Hailu, M., and L. Muhe, 2001. Childhood meningitis in a tertiary hospital in Addis Ababa: clinical and epidemiological features. *Ethiop. Med. J*. **39**:29-38.

115) Muhe, L., and K. P. Klugman, 1999. Pneumococcal and *Haemophilus influenzae* meningitis in a children's hospital in Ethiopia: serotypes and susceptibility patterns. *Trop. Med. Int. Health*. **4**:421-427.

116) Mohammed, E., L. Muhe, A. Geyid, A. Dejene, Y. Mekonnen, K. Mammo, A. Afework and R. Muzein, 2000. Prevalence of bacterial pathogens in children with acute respiratory infection in Addis Ababa. *Ethiop. Med. J*. **38**:165-174.

117) Klugman, K. P., 1990. Pneumococcal resistance to antibiotics. *Clin. Microbiol. Rev*. **3**:171-196.

118) Pallares, R., F. Gudiol, J. Liñares, J. Ariza, G. Rufi, L. Murgui, J. Dorca, and P. F. Viladrich, 1987. Risk factors and response to antibiotic therapy in adults with bacteremic pneumonia caused by penicillin-resistant pneumococci. *N. Engl. J. Med*. **317**:18-22.

119) Byass, P., M. Fantahun, A. Emmelin, M. Molla, and Y. Berhane, 2010. Spatio-temporal clustering of mortality in Butajira HDSS, Ethiopia, from 1987 to 2008. *Glob. Health. Action*. **30**:3.

120) Burton, J. L., and J. Underwood, 2007. Clinical, educational, and epidemiological value of autopsy. *The Lancet*. **369**:1471-1480.

121) The WHO Young Infant Study Group, 1999. Bacterial etiology of serious infections in young infants in developing countries: Results of a multicenter study. *Pediatr. Infect. Dis. J*. **18**:17-22.

122) WHO Surveillance Bulletins from Meningitis Vaccine Project (MVP). [Online]. Website: http://www.meningvax.org/epidemic-updates.php. (Accessed: 1/08/2012)

123) Casebeer, A. L., and M. J. Verhoef, 1997. Combining Qualitative and Quantitative Research Methods: Considering the Possibilities for Enhancing the Study of Chronic Diseases. *Chronic. Dis. Can*. **18**:130-135.

124) Gurley, E, S., M. J. Hossain, S. P. Montgomery, L. R. Petersen, J. J. Sejvar, L. W. Mayer, A. Whitney, P. Dull, N. Nahar, A. K. Uddin, M. E. Rahman, A. R. Ekram, S. P. Luby, and R. F. Breiman, 2009. Etiologies of bacterial meningitis in Bangladesh: results from a hospital-based study. *Am. J. Trop. Med. Hyg*. **81**:475-483.

125) Mengistu, G., K. Mitiku, and W. Teferi, 2003. Analysis and reporting of meningococcal meningitis epidemic in north Gondar 2001-2002. *Ethiop. Med. J*. **41**:319-331.

126) Thigpen, M. C., C. G. Whitney, N. E. Messonnier, E. R. Zell, R. Lynfield, J. L. Hadler, L. H. Harrison, M. M. Farley, A. Reingold, N. M. Bennett, A. S. Craig, W. Schaffner, A. Thomas, M. M. Lewis, E. Scallan, and A. Schuchat; Emerging Infections Programs Network, 2011. Bacterial meningitis in the United States, 1998-2007. *N. Engl. J. Med*. **364**:2016-2025.

127) Campagne, G., A. Schuchat, S. Djibo, A. Ousséini, L. Cissé, and J. P. Chippaux, 1999. Epidemiology of bacterial meningitis in Niamey, Niger, 1981-96. *Bull. World Health Organ*. **77**:499-508.

128) Giorgi Rossi, P., J. Mantovani, E. Ferroni, A. Forcina, E. Stanghellini, F. Curtale, and P. Borgia, 2009. Incidence of bacterial meningitis (2001-2005) in Lazio, Italy: the results of an integrated surveillance system. *BMC Infect. Dis*. **9**:13.

129) Afifi, S., M. O. Wasfy, M. A. Azab, F. G. Youssef, G. Pimentel, T. W. Graham, H. Mansour, N. Elsayed, K. Earhart, R. Hajjeh, and F. Mahoney, 2007. Laboratory-based surveillance of patients with bacterial meningitis in Egypt (1998-2004). *Eur. J. Clin. Microbiol. Infect. Dis*. **26**:331-340.

130) Stockdale, A. J., M. P. Weekes, and S. H. Aliyu, 2011. An audit of acute bacterial meningitis in a large teaching hospital 2005-10. *Q.J.M*. **104**:1055-1063.

Appendix I: Retrospective data collection form:

Study title: "Surveillance of bacterial meningitis in Ethiopia and examining factors affecting meningococcal disease severity."

RETROSPECTIVE DATA COLLECTION FORM

PATIENT DATA				
HOSPITAL NAME:		AGE:	SEX:	PATIENT ID:
DATE OF ADMISSION:		CITY/REGION:		
CLINICAL SYMPTOMS AT THE TIME OF ADMISSION				
FEVER (TEMP > 38^0C)	❑	ALTERED MENTAL STATE		❑
HEADACHE	❑	SEIZURES		❑
NAUSEA/VOMITING	❑	SHOCK		❑
STIFF NECK/BACK STIFFNESS	❑	PETECHIAL RASH		❑
CLINICAL DIAGNOSIS:				
TREATMENT OUTCOME				
COMPLETE RECOVERY: ❑		PARTIAL RECOVERY WITH SEQUELAE: ❑ (SPECIFY SEQUELAE)		
FATAL CASE DATE:				
LABORATORY FINDINGS				
TEST	DONE	RESULT		
CSF GRAM STAINING:	❑	POSITIVE ❑ NEGATIVE ❑	MORPHOLOGY:	
CSF CULTURE:	❑	*N. meningitidis* ❑ *S. pneumoniae* ❑	*H. influenzae* ❑ Others ❑	
CSF CELL COUNT PROTEINS AND GLUCOSE LEVEL	❑	CSF CELL COUNT: _____ /mm^3 PROTEINS CONC: _____ g/liter GLUCOSE CONC: _____ g/liter		
OTHER LABORATORY FINDINGS:				

DATA COLLECTED BY: **CROSS CHECKED BY**

Appendix II: Characterization of etiological agent from Ethiopian CSF samples from 2002-2003 by RT-PCR:

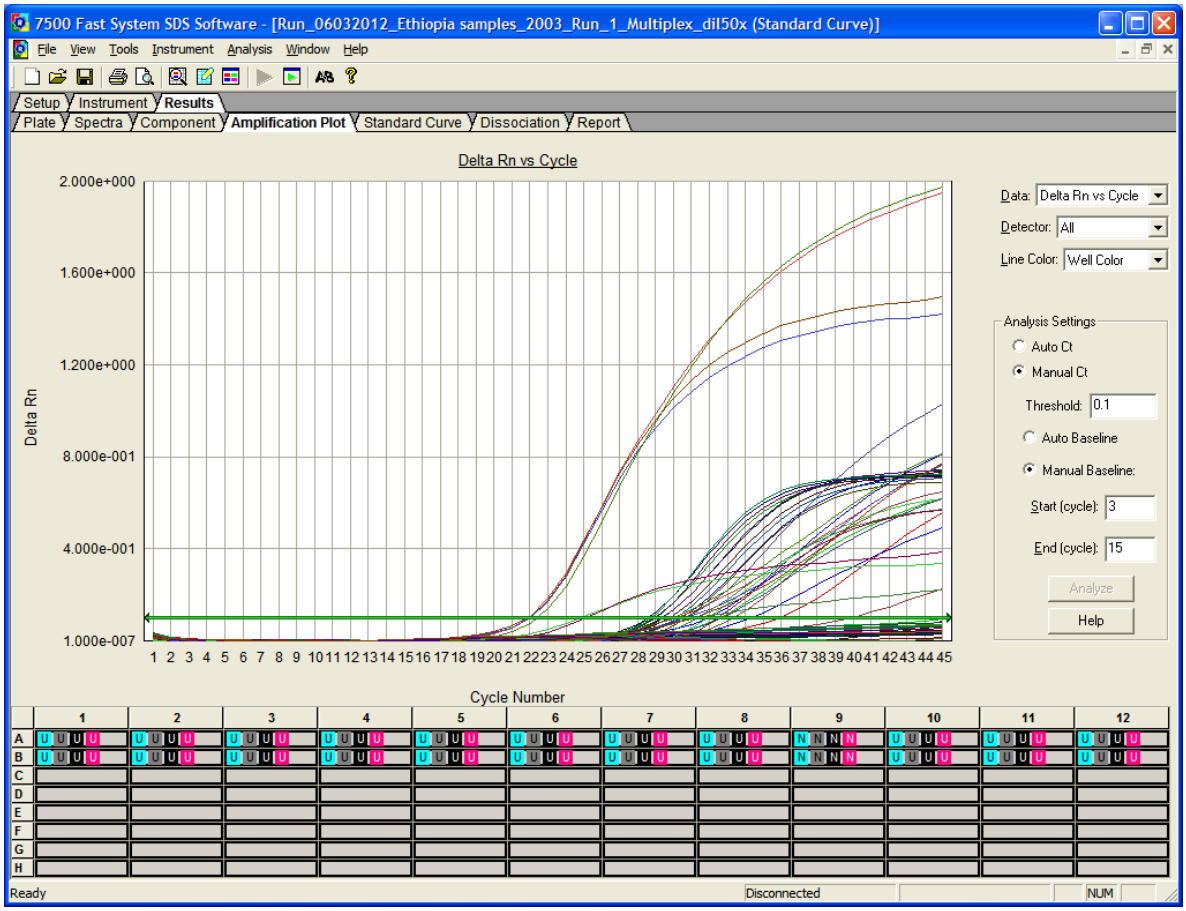
Real time PCR is a modification to the ordinary PCR that allows continuous observation of the increase in the amount of DNA during its amplification. After its introduction in late 1990's, RT-PCR has revolutionized the field of clinical microbiology. Mentioned hereunder are the results from Real-Time PCR that was applied to the Ethiopian samples from 2002 – 2003. The analysis was conducted with the help of the laboratory staff at Norwegian Institute of Public Health (NIPH). This work is seen as an important achievement not only in terms of the characterization or defining of etiology of bacterial meningitis in Ethiopia but more importantly, as an acquaintance with the modern laboratory techniques. The training received and the successful implementation of this training onto the samples is regarded as a significant byproduct of the project and an important additional learning of the laboratory techniques that form an important component of the modern day diagnostic procedures for the diagnosis of bacterial meningitis.

The project co-ordinators from NIPH including Gunnstein Norheim and the principal investigator for the current project Prof. Dominique A. Caugant had conducted a study in 2002 and 2003 in North Gondar and Southern Nations, Nationalities and Peoples' Region (SNNPR) of Ethiopia [(78)]. A total of 95 cases were included between April 2002 and December 2003 with the aim of characterization of *N. meningitidis* from these isolates. Bacterial cultures on the CSF and PCR were used to identify *N. meningitis* and to further study the serotypes. Out of 95 samples 71 were confirmed as *N. meningitidis*. The remaining 24 samples were undetermined except one sample that was identified as *H. influenzae* type b on the basis of culture results. The etiology of the remaining 23 samples remained undetermined.

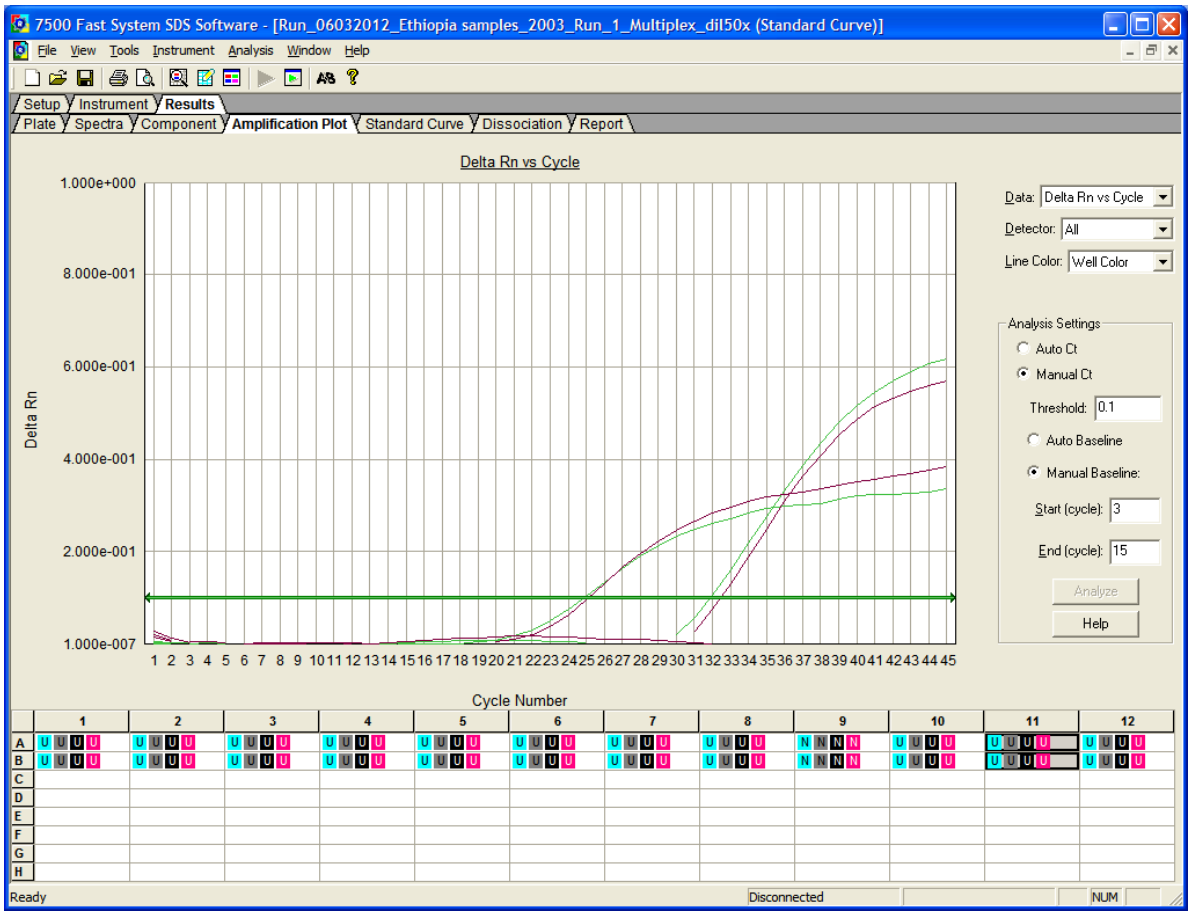
The remaining 23 samples from 2002 and 2003 were analyzed in February-March 2012 by Real-Time PCR as part of this Master's degree. Originally, these 23 samples had tested negative for *N. meningitidis* when run on standard PCR in 2003 and were not tested by PCR for the presence of other etiological agents. To find the presence of other possible etiological agents, these samples were now purified using QIAGEN kits and were then run on the 7500 Fast System. Multiple runs were conducted initially using singleplex and later on by multiplex PCR. The genes amplified

were *ctrA* for *N. meningitidis*, *lytA* for *S. pneumoniae* and *bexA* and *omp* for *H. influenzae*. The real-time results were analyzed using the 7500 Fast System SDS Software. Positive and negative controls were used along with an internal positive control in each run of PCR. Out of 23 samples analyzed, the etiological agent was identified for 8 samples. Out of these 8 cases, *S. pneumoniae* was confirmed in 5 samples and *H. influenzae* in the remaining 3 samples. These results when combined with the original results from the study in 2002 and 2003 give 71 cases of *N. meningitidis* followed by 5 cases of *S. pneumoniae* and 4 cases of *H. influenzae*. The remaining 15 cases tested negative for these organisms.

Although the results provide information on the etiology of bacterial meningitis, the main aim was to familiarize with the steps involved in real-time PCR. The opportunity to work with the highly trained staff and the practical utilization of the theoretical knowledge may prove helpful in conducting research in future.



Realtime PCR results showing DNA amplification in Ethiopian samples along with positive controls.



Real time PCR results showing higher amplification for omp gene in one of the Ethiopian samples in comparison to a positive *H. influenzae* control.