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Diagnosis and Differential Diagnosis of Meningitis at Patient's Bed side Using Urine Reagent Strip to Evaluate Cerebro spinal Fluid

A Strategy for Early Diagnosis and Treatment

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Dissertation

Diagnosis and Differential Diagnosis of Meningitis at Patient's Bedside Using Urine Dip Strip to Evaluate Cerebrospinal Fluid.

A Strategy for Early Diagnosis and Treatment

BEST Meningitis:

Bedside Extended Strategies for Bacterial Meningitis

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Key Words

(CSF, Bacterial meningitis, Reagent strip)

Abstract

Background: Bacterial meningitis is a life threatening condition associated with high morbidity and mortality. The prognosis of the disease depends on early diagnosis and management. In low resource countries the major drawback is prompt diagnosis due to poor laboratory quality.

Aims: To study the performance of urine reagent strip in a low resource setting and compare it with standard local results and clinical outcome. To create a clinical algorithms for bed side diagnosis using reagent strip.

Methods: A prospective study among children with suspicion of bacterial meningitis, conducted for 15 months. Bacterial meningitis for analysis purpose was based on confirmed and probable cases based on modified WHO criteria for diagnosis of meningitis based on local available laboratory test.

Results: Among 180 cerebrospinal fluid (CSF) specimen evaluated, 125 were normal and 43(25.5%) were considered as having pleocytosis. The overall performance of urine leucocyte strip for the diagnosis of bacterial meningitis was better with a sensitivity of 75%, specificity of 85.14% a NPV of 94.0% and PPV of 52.1% in comparison to CSF wbc count with 56.7%, specificity of 85.14%, NPV of 89.6% and PPV of 39.53% respectively, The Protein portion of urine strip also performed better than the pandy test with sensitivity of 78.1%, specificity of 70.2%, PPV of 36.2% and NPV of 93.6% in comparison to 43.3%, 93.8%, 61.9% and 87.7% respectively.

Conclusions: Urine reagent strip is a useful test for evaluation of CSF and may aid in bedside diagnosis of meningitis. They can further aid decision making of whether or not to initiate antimicrobial therapy in low resource settings.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AIDS – Acquired Immune Deficiency Syndrome

ARV – Antiretroviral Treatment

BBB – Blood Brain Barrier

BM – Bacterial Meningitis

CNS – Central Nervous System

CSF - Cerebral Spinal Fluid

DNA – Deoxyribonuclease Acid

ED – Emergency Department

Hb – Hemoglobin

LP – Lumbar Puncture

NPV – Negative Predictive Value

PCR – Polymerase Chain Reaction

PPV – Positive Predictive value

TB – Tuberculosis

RBC – Red blood cells

WBC – White blood cell

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1. General Introduction

Community acquired bacterial meningitis is a severe infection of the central nervous system (CNS) which causes inflammation of the leptomeninges and can rapidly progress to multiple organ failure and death (Bloch and Tang., 2011). Despite various accomplishments achieved in the field of infectious diseases, especially in bacterial meningitis, such as, the introduction of new vaccines and antimicrobial drugs. However, there has been very little improvement in the diagnostic methodology. Bacterial meningitis is still associated with high mortality and lifelong sequelae (Schuchat et al., 1997, Scheld et al., 2002, WHO, 2011b). Bacterial meningitis is rated one of the “top ten” causes of death among all infectious diseases (Grimwood et al., 2000, Scheld et al., 2002). The mortality varies from 10 to 20% and among 50% of survivors, severe lifelong disability can ensue (Maria and Bale, 2006, Prober, 2007).

1.1 Historical perspective on meningitis

Infections of the central nervous system are as old as mankind. Anthropological specimens dated back as far as 5000 BC have demonstrated signs of Tuberculous meningitis (Tyler, 2009).

The first description of patients with meningitis was published by Thomas Willis a neuro anatomist, in 1661, who recognized this serious condition and described meningitis as an ‘inflammation of meninges with continual fever’ and correlated the symptoms with the cerebral anatomy (Tyler, 2009). Later, in 1805 the first European descriptions of meningococcal disease and epidemics that occurred in Geneva came from Gaspard Vieusseux and his colleague Andre Matthey. A year later Elisha North described similar outbreak in Massachusetts (Tyler, 2010).

Heinrich Quincke was the first physician to describe and use the technique of lumbar puncture. This provided the opportunity for cerebrospinal fluid (CSF) evaluation and the diagnosis of bacterial meningitis. Portrayal of CSF chemistry variations currently known as the classical CSF reference for meningitis: pleocytosis, hypoglycorrhachia and hyperproteinorrachia. This reference is the fruit of analysis of the largest assembly of CSF samples described in history by Fremont Smith and Merritt (Tyler, 2010). Merrit and Fremont-Smith emphasized that "Changes that occurred in the cerebrospinal fluid were very important essentially, the same regardless of the organism and consist chiefly of an increase in pressure, a pleocytosis, an increase in protein, and a decrease in the sugar and chloride contents" as referred by (Merritt and Fremont-Smith, 1937) from (Tyler, 2010).

During the late 19th century the first causative microorganisms for meningitis, *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae*, were identified and Vladimir Kernig and Josef Brudzinski described the cardinal signs that accompanied meningitis. The first effective treatment for meningococcus using intrathecal equine antiserum was initiated in Germany by Georg Joachmann and in America by Simon Flexner (Tyler, 2009, Tyler, 2010). In the year 1913, this intervention drastically reduced mortality from 75-80% to 20%. The introduction of sulfonamides in 1930 and use of penicillin in 1940 further decreased mortality from 20% to 15% - 10%. (Swartz, 2004).

The first specific measures for the prevention of bacterial meningitis was the development of vaccine against *N.meningitidis* and the implementation of a vaccination program (Tyler, 2010). The introduction of a vaccine against *Haemophilus influenzae* and some serotypes against *Streptococcus pneumoniae* played a key role in reducing morbidity and mortality in countries where these programs were implemented. Unfortunately, many developing countries are not yet covered by efficient vaccination programs and mortality and morbidity is still very high. Despite such long history and advances that have occurred with modern technology, many physicians in developing

countries still face major challenges every day in the diagnosis and management of bacterial meningitis (Tyler, 2010).

1.2 Definition

Meningitis is defined as an inflammation of the protective membranes that surround the brain and spinal column in particular the arachnoid and pia mater (Bloch and Tang., 2011). It can be infectious or noninfectious in origin. The severity of the disease depends on the cause. Meningitis can be caused by bacteria, viruses, mycobacteria, parasites and fungi (CDC, 2012a). The noninfectious causes of meningitis include inflammatory diseases like sarcoidosis, systemic lupus erythematous, cancer like leukaemia, drugs , head injury and brain surgery (Prober, 2007, CDC, 2012c)
Among these, viral meningitis is the most common and bacterial meningitis is the most severe and It is the cause for significant morbidity and mortality worldwide (CDC, 2012a).

Encephalitis refers to the presence of an inflammatory process in the central nervous system and is usually accompanied with clinical evidence of neurological dysfunction. When the condition is affecting the meninges then it is termed as meningoencephalitis. Both diseases are grouped together as they share same epidemiology, etiology and management (Bloch and Tang., 2011).

1.3 Epidemiology

Despite various achievements in the field of bacterial meningitis (BM) with introduction of new vaccines, new antimicrobial therapy and improvement in diagnostic methodology for cerebrospinal fluid evaluation, bacterial meningitis is still associated with high mortality and lifelong disabilities (Schuchat et al., 1997, Scheld et al., 2002). Almost any bacterial pathogen that can affect humans, has the capacity to cause BM (CDC, 2012b). Throughout history, many studies have persistently demonstrated that *S.pneumoniae, H.influenzae and N.meningitidis* are responsible for more than 70% of cases (Carpenter

and Petersdorf, 1962, Hoen et al., 1993). Further confirmations of same pathogens came from a report of surveillance system established by the Centre for Disease Control and Prevention (CDC). This report had gathered information on bacterial meningitis from 27 states in the United States of America (USA) from 1979 to 1981. Which demonstrated that indeed the 3 most important pathogens were streptococcus, meningococcus and hemophilus which accounted for 80% of the cases with an incidence of 3/100.000, from these 76,7/100.000 cases occurred in children below the age of one (Schlech et al., 1985).

Further knowledge of other important etiologic agents came through a laboratory based study in USA involving a population of 34 million in 1986 and 2 more pathogens were detected namely *L.monocytogenes and S.agalactiae*. (Schlech et al., 1985).

According to WHO estimation, 500.000 new infections occur each year worldwide leading to 50.000 deaths every year (Pollard, 2004). It's estimated that the burden of disease is higher in low income countries compared to high income countries. The highest incidence occurring in sub Saharan Africa (WHO, 2011d). While the endemic form is not frequently seen in developed countries (Scheld et al., 2002) though outbreaks have occurred in crowded population (Harrison, 2000, Brooks et al., 2006) but are more frequent in low income countries (Scheld et al., 2002). The most affected area in Africa is the "Meningitis belt". This zone comprises of 22 countries from Senegal to West of Ethiopia (WHO, 2013). This region has had an annual epidemic in past decades. From 1993 to 2012 nearly one million suspected cases were reported by WHO, causing around 100.000 deaths (WHO, 2013).

1.4 Aetiology

Meningitis is an important differential diagnosis in a febrile child with altered mental status or with signs and symptoms that suggest involvement of the brain. Though many pathogens are responsible for meningitis, it's possible to identify specific pathogens

based on the age, immune status of the host and epidemiology of the microorganism (Prober, 2007).

The most important pathogens causing bacterial meningitis are: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitides* , group *B streptococcus* , *Listeria monocytogens* and *Mycobacterium tuberculosis*. though there are other microorganisms causing similar disease in newborns and immunocompromised individuals (Hasbun, 2014). The first 3 are the most common causes. Table 1. Shows different causative organism according to different age groups.

Table 1. Aetiology of Bacterial meningitis according to the age groups

Age groups	**Causes**
Newborn	Group B *Streptococcus, Escherichia coli, Listeria monocytogenes*
Infants and children	*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenazae B*
Adolescents and young adults	*Streptococcus pneumoniae, Neisseria meningitidis*

Source:(CDC, 2012a)

1.4.1 Neisseria meningitidis

Neisseria meningitidis, a gram negative bacterium is the cause of the endemic form of meningitis. The polysaccharide capsule that surrounds the bacteria is important for the classification of *N. meningitidis* into 12 serogroups. Out of these 12 serogroups only 6 are responsible for most of the infections in humans: A, B, C, W135, X and Y (WHO, 2011d)

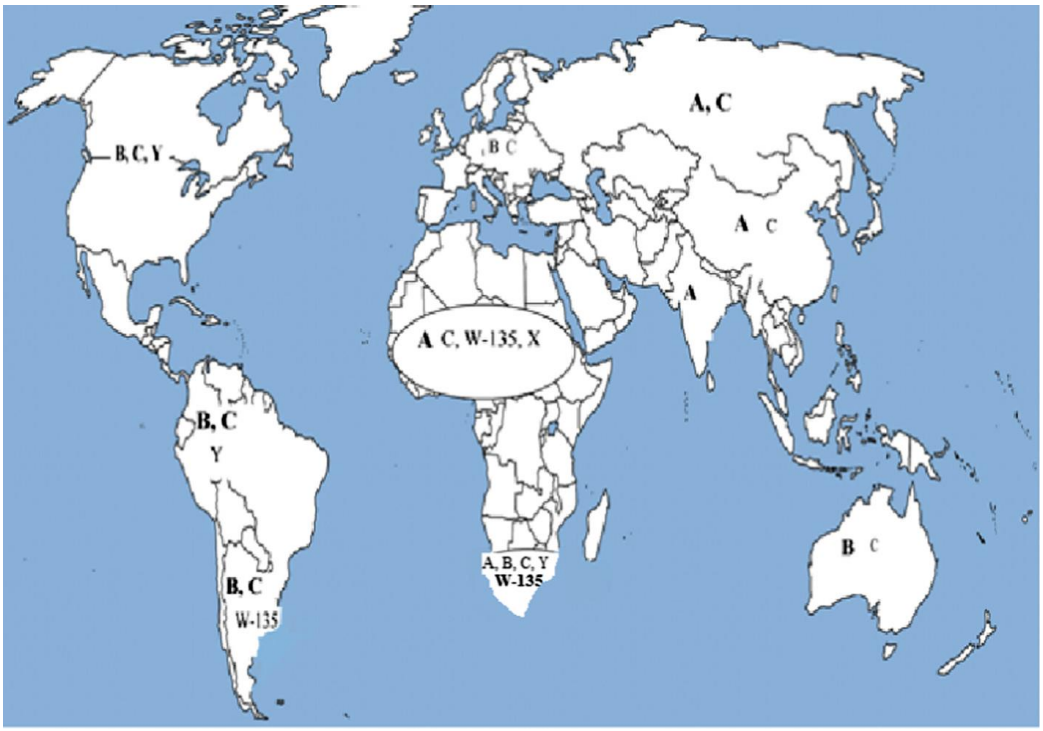
The serogroups are widely distributed around the globe. The serogroups B and C are more frequent in America, Europe, and Australia (Jafri et al., 2013) while A is

responsible for epidemics in Asia and in Africa accounting for 80-85% of outbreaks. Occasionally serogroups can emerge like serogroups C in China or serogroup Y in North America (WHO, 2011d) and new strains may also emerge for example, strain Y and W135 have been observed in 2 new foci (Jafri et al., 2013, WHO, 2013). Figure 1 shows global distribution of *N. meningitidis* serogroups.

(WHO, 2013) Epidemiology bulletin reported that annually 1.2 million cases are estimated to occur of *N. meningitidis* with 135.000 deaths in the region of 'Meningitis Belt' alone. *N. meningitidis* causes severe brain damage and is fatal in 50% of cases if untreated, causing significant mortality within 24 to 48 hours (WHO, 2011b).The survivors are in high risk of developing neurological sequelae with cognitive, motor, visual and hearing impairment (Edmond et al., 2010a).

In 2010 a vaccine against the *N.meningitidis* strain A was introduced in the counties of the 'Meningitis belt', which has reduced incidence in this region significantly (Jafri et al., 2013)

Figure 1. Worldwide distribution of *N.meningitidis* serogroups responsible for meningococcal meningitis. Source (Jafri et al., 2013)



Source (Jafri et al., 2013)

1.4.2 Haemophilus influenzae type b

Haemophilus influenzae is a gram negative coccobacilus, which is aerobic in nature, but may also grow under anaerobic conditions. It is a cause for several severe and life threatening diseases in children below 5 years of age. There are 6 capsular types from (a-f). In 95% of cases, invasive infections including meningitis are caused by subtype b (CDC, 2012b). It was first described in 1892 by Pfeiffer and later named "*Haemophilus*" by Windslow et al in 1920. It is a main cause of bacterial meningitis and other serious infections in children below the age of 5. In the pre vaccine era, most of the affected children were 18 months or younger (CDC, 2012b). Humans are the only known reservoir for Hib. The germ is found in the nasopharynx of 0.5%–3% infants and children who are asymptomatic (WHO, 2014b).

Before the wide spread introduction of the Hib vaccine, *Hemophilus influenzae* type b was responsible for almost 45 to 48% of cases of bacterial meningitis in the United States of America and a significant cause of death (Schlech et al., 1985, Brouwer et al., 2010). One in every two thousand children developed Hib meningitis or invasive disease below the age of 5. After the introduction of a conjugated Hib vaccine in 1980, the incidence of Hib infections decreased by 90%. Currently Hib is responsible for 6.7% of all bacterial meningitis cases (Muller, 2014, Hasbun, 2014).

The conjugated vaccine does not only provide protection to vaccinated children, but also decreases the carriage of Hib and protects unvaccinated children by reducing exposure of Hib from vaccinated children. In developing countries the vaccination program was started in 2007 in 184 countries and has a global coverage with 3 doses of 45 %. Due to vaccine's low coverage for *H. Influenazae*'s vaccine program it remains a major problem in many African countries (WHO, 2014b).

Despite the availability of the conjugate vaccine and appropriate therapy, Hib still is responsible for a case fatality rate of 2 to 5 % in developed countries in comparison to 20 to 30% in tropical countries (Naik and Seyoum, 2006, Ramakrishnan et al., 2009,

CDC, 2012b). Sigauque et al demonstrated in their study from a Manhica district in Mozambique, that the mortality was as high as 55 % among children under 5 years who had *H. influenzae* meningitis (Sigauque et al., 2008). However, due to lack of laboratory material, technicians, diagnostic difficulties, no reliable data on Hib meningitis is available for the whole country.

1.4.3 Streptococcus pneumoniae

S. pneumoniae, is gram positive encapsulated bacteria with a capsular diversity so large that more than 90 serotypes have been identified based on the polysaccharide in bacterium's capsules. Many subtypes have the capacity to cause invasive pneumococcal disease, however only few serotypes cause severe disease worldwide (CDC, 2012b).

Serotype distribution and burden of disease varies worldwide. Serotype 1 and 5 are more frequent in low income countries (CDC, 2012b). *S. pneumoniae* meningitis affects mostly young children below the age of 5 years and the elderly. In developing countries the case fatality rate for meningitis due to *S. pneumoniae* in children under the age of 5 years is as high as 73% (Brouwer et al., 2010).It is the most frequent cause of BM in Europe and USA accounting for 58% of cases (Thigpen et al., 2011).

In order to decrease this burden, efforts have been made to reduce the incidence by introducing vaccines. Various types of vaccines have been introduced. A 23- valent vaccine which contained 74-90% of the serotypes that caused meningitis was initially used for high risk groups and showed decrease in 50 % of cases (Bolan et al., 1986, Butler et al., 1993). Later a Heptavalent vaccine showed some promising results with a decrease in 59% of pneumococcal meningitis in children below the age of 2 years (Whitney et al., 2003). This pneumococcal vaccine contained 7 strains that were frequent in USA in comparison to other countries in Europe (Prevnar- PCV 7 conjugate) (Schuchat et al., 1997). Later the heptavalent vaccine was replaced by a 13 valent vaccine (Prevnar- PCV 13 conjugate) with 6 additional strains to cover other subtypes

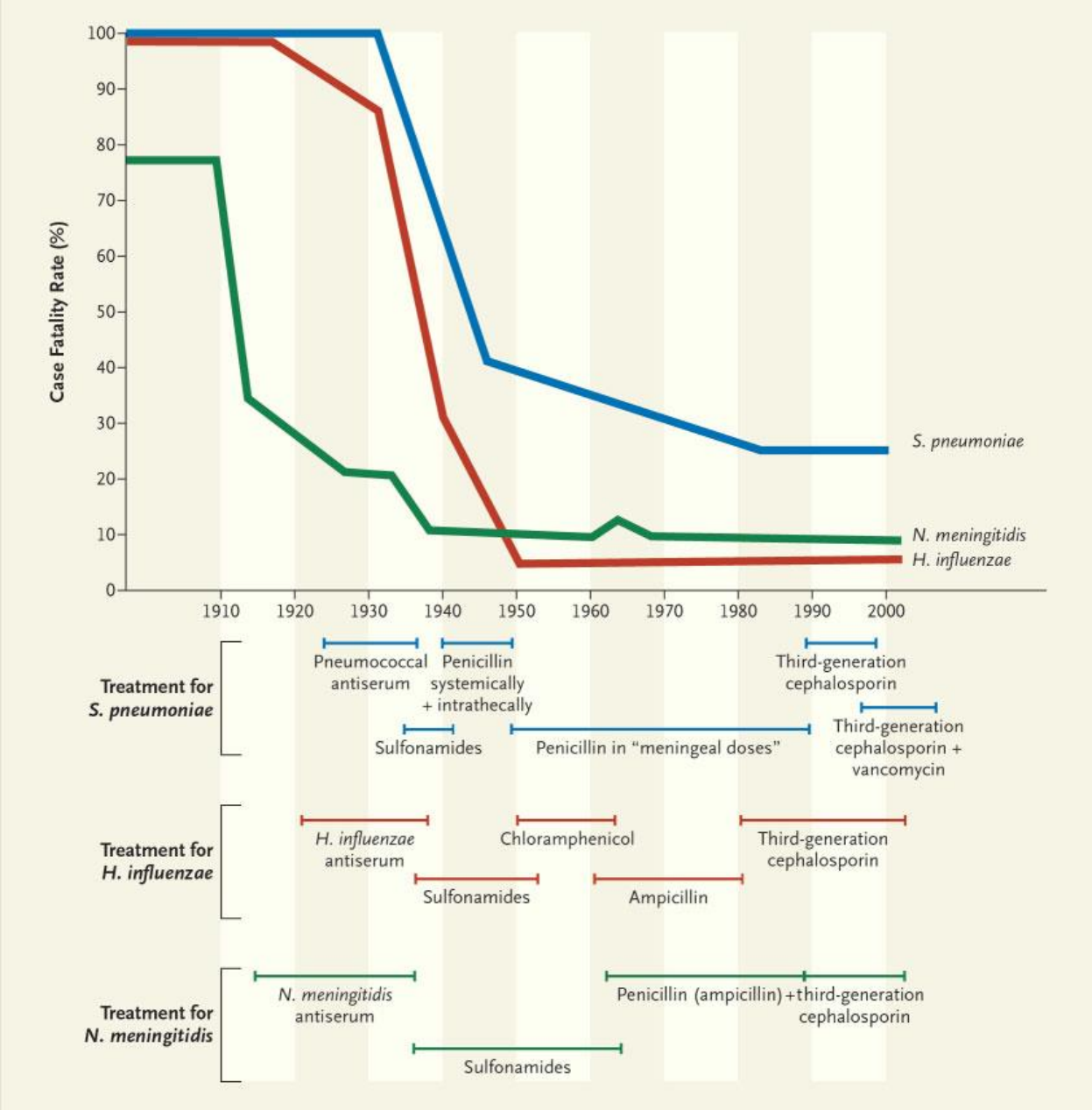
of pneumococci that caused invasive disease worldwide (Brouwer et al., 2010, NNii, 2010).

The CDC reported a significant decline in burden of disease due to invasive pneumococcal infections in the industrialized countries after introduction of heptavalent vaccine, but in non-industrialized countries, is still an important cause of death. The Invasive pneumococcal infections in low resource countries, causes between 700.000 to 1.000.000 deaths every year among children below the age of 5 (Brouwer et al., 2010, CDC, 2012b). To reduce mortality from pneumococcal disease in low income countries, WHO recommended inclusion of the heptavalent vaccine in routine vaccination programs worldwide in 2007 (WHO, 2011c).

By 2008 only 26 countries had managed to introduce the heptavalent vaccine. Out of 26 only 3 were from low income countries and the rest were from high income countries. In 2012 GAVI (Global Alliance for Vaccine and Immunization) funded Pnuemo ADIP plan (Pneumococcal vaccines Accelerated Development Plan) is helping low income countries to introduce the heptavalent vaccine. By the end of 2012, through this alliance, 88 countries had introduced vaccine against pneumococcal infection, with a global coverage estimated at 19%. It is estimated that by 2020 with Global Vaccine Action Plan, vaccination coverage will be more than 80% (WHO, 2011c, WHO, 2014b).

The Figure 1.2 Below shows a graph with the trend of case fatality rate among the three main pathogens responsible for bacterial meningitis over the past 90 years.

Figure 1.2 Demonstrates Trend of three main pathogens responsible for meningitis over the 90 years



Source (Swartz, 2004)

1.4.4 Listeria monocytogenes

Listeria monocytogenes is a gram positive rod shaped bacterium (Fischbach, 2009). In the USA it accounts for 2% of all cases of bacterial meningitis as quoted by ((Brouwer et al., 2010) from (Thigpen, 2005). Discovered during 1980 after an outbreak, is found in soil, water and sewage. Transmission is mainly via contaminated food (CDC, 2013) specially undercooked food or unpasteurized dairy products (Schuchat et al., 1991). Children below the age of 3 months and elderly are most frequently affected. One important group is pregnant women as they are at higher risk for acquiring this disease (CDC, 2013) Symptoms are very similar to other meningitis; mortality ranges from 15-17% in children and among survivors, 25% develop neurological sequelae (CDC, 2013)

1.4.5 Streptococcus agalactiae

Streptococcus agalactiae also known as group B streptococcus is a leading cause of neonatal sepsis and death in USA. However there has been under reporting of Group B infections among infants so the epidemiology is unknown in other parts of the world (Brouwer et al., 2010). The main risk factors for neonates are premature rupture of membranes of the mother, maternal fever and a positive vaginal group b streptococcus culture (CDC, 2012d). Infection occurs either vertically from mother to child or later in first week of life, while caring for the newborn (Brouwer et al., 2010). Mortality in neonates ranges from 7-27% (Brouwer et al., 2010).A third of the surviving children develop neurological sequelae with spastic quadriplegia, profound mental retardation, hemiparesis, deafness and blindness (van de Beek, 2012). According to the guidelines established by the CDC in 2003, every pregnant woman between the 35 to 37 week of gestation should have a vaginal and rectal swab for screening S. agalactiae and an intrapartum antibiotic prophylaxis should be administered to positive cases (CDC, 2012d). This recommendation has reduced the infection rate in the USA and Canada, but in many developing countries these guidelines have not been established. One study done in Malawi demonstrated that 22% of isolates from neonates admitted with

sepsis were culture-positive for S.agalactiae. The manifestation of early onset disease below 1 months of age was mostly as a neonatal sepsis which accounted for 52% of the cases. Late onset infection was mainly as meningitis with 39%, with a case fatality of 49% (early onset disease) and 29% (late onset disease), which is much higher than found in USA. (Gray et al., 2007).

1.5 Risk factors

Many studies throughout the decades have shown various factors associated with increased risk of bacterial meningitis.

1.5.1 Age

Several studies have repeatedly demonstrated that there is a direct relation between the age and meningitis. Affecting very young and the elderly (Takala et al., 1989, Prober, 2007),the association seems to be related to lack of immunity in younger children below the age of 1 and elderly due to combination of other diseases and immunosuppression (Prober, 2007).

1.5.2 Poor Housing and overcrowding

Overcrowding and poor household, facilitate transmission of respiratory droplets from the carrier or symptomatic patients to healthy individuals. In study involving college freshmen it was observed that college students who were residing in college dormitories had a higher risk of acquiring meningococcal disease in comparison to the ones who were not (Froeschle, 1999, Harrison, 2000).

1.5.3 Tobacco

Tobacco seems to increase the of risk bacterial meningitis among both active and passive smokers (Coen et al., 2006, Slama et al., 2007, Murray et al., 2012). It

increases the risk of carriage with more pathogenic bacteria like, H. influenzae, pneumococcus, and meningococcus (Slama et al., 2007) increasing risk of transmission to children and other adults. One case control prospective study done in England demonstrated that the risk of meningitis was higher among teenagers exposed to passive smoking than among a non-exposed group (Coen et al., 2006, Murray et al., 2012).

1.5.4 Indoor air pollution

In numerous studies done in developed countries passive smoking has been linked to the increased risk of developing bacterial meningitis. Lately it has been demonstrated that there is a strong association between cooking with fire wood and bacterial meningitis. As indoor air pollution with fire wood has a similar effect on children as passive smoking, increasing the risk of carriage state among children and predisposing to bacterial meningitis (Staton and Harding, 1998, Hodgson et al., 2001).

1.5.5 Socio- economic factors

Socioeconomic factors have an important role in the increased incidence of bacterial meningitis. These factors include poverty, household crowding, limited access to health care, and lower educational level are important factors (Reis et al., 2008).

1.5.6 Other factors

Alterations of host defense due to anatomic defects or immune deficits also increases the risk of meningitis from less common pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus,* coagulase-negative staphylococci and *Salmonella* spp., (Prober, 2007, Muller, 2014). Factors that cause immunosuppression such as HIV (Mao et al., 1996), congenital asplenia or post-splenectomy (Shutze E et al., 2002) and cochlear implants (Reefhuis et al., 2003) increases the risk of bacterial meningitis caused by *Streptococcus pneumoniae* . The Ig G subclass deficiency, children with

head trauma and other skin infection (Prober, 2007) and ventricular shunt (Meirovitch et al., 1987, Ronan et al., 1995) have also shown to be associated with increased risk of meningitis.

1.6 Pathophysiology of bacterial meningitis

Bacterial meningitis, results from a complex multistep interaction between the host and the pathogen. These sequential steps are important for the development of bacterial meningitis. Despite many studies on the pathogenesis of bacterial meningitis, many features remain unclear. It is crucial to understand this complex process for future development of adjunctive treatment to reduce morbidity and mortality associated with the disease. Important features responsible for high mortality and morbidity are still not clearly understood (Kim, 2003).

Every bacterium that has potential to cause disease in man can also cause meningitis but it's still unclear why only some are more frequent. Listeria monocytogens, group B streptococcus and E are the main cause of meningitis in neonates, but are not frequent in older children and adults. (Kim, 2003)

The first step for development of bacterial meningitis, typically is a colonization of the nasopharynx mucosa followed by bacterial invasion of vascular system, survival and multiplication of bacteria in the blood stream, passage through blood brain barrier (BBB), host inflammatory response and neuronal damage (Kim, 2003).

1.6.1 Transmission and Colonization

Most of the pathogens that cause meningitis are found in nasopharygeal mucosa without affecting the host. This state is referred as "Carriage" (Yazdankhah and Caugant, 2004). This carriage state can be seen in 10% of asymptomatic population in nonepidemic setting (Bolan et al., 1986). Other routes include hematogenous and contiguous spread. Despite other routes available for pathogens to access the

meninges, in most cases the access is via nasopharynx and transmission of the pathogens occurs through close contact and from contaminated nasal or oral droplets (Yazdankhah and Caugant, 2004).

Once a bacterium colonizes the nasopharynx it provokes an immune response from the host providing natural immunity. The capsule subtypes are responsible for different sero subtypes of bacterium. Among 50% of the carriers the bacterium lacks the polysaccharide capsule and therefore is non serogroupable (Claus et al., 2002) These non serogroupable bacteria can switch on and off capsular production. The lack of capsule in the carrier state gives the bacterium opportunity to escape an immune response allowing colonization of the nasal mucosa (Swartley et al., 1997).

The prerequisite for colonization is the capacity of the pathogen to adhere to the nasal mucosa and escape neutralization of the bacterium from an Immune response. Adhesion of the bacteria to mucosa occurs via adhesion molecules called pili or fimbriae, hair like structure that are found on the bacterial surface. In case of *N.meningitidis* the type IV pili interacts with a membrane cofactor protein, CD46 (Pujol et al., 1999, Johansson et al., 2003). This cofactor protein is found in all cells, except erythrocytes (Johansson et al., 2003, Yazdankhah and Caugant, 2004).

Once the colonization has taken place, the bacteria need to survive and grow. For the growth, the bacteria use iron and acquires it from lactoferrin and transferrin. The meningococci acquires iron by binding human transferrin, and lactoferrin with OMP receptor protein, which in turn releases the iron and is uptaken by the bacterium for its growth (Larson et al., 2002, Kim, 2003).

The bacteria may escape immune responses by producing Ig A_1 extracellular protease. These proteases are produced by *H.influenzae, S. pneumonia and N.meningitidis* which cleaves human Ig A_1 found in the nasal mucosa. This Immunoglobulin IgA_1 has an important role in preventing adherence of the bacterium to the mucosa (Yazdankhah and Caugant, 2004, Hasbun, 2014). Any prior damage to the nasal mucosa either by

irritants like smoke exposure, prior respiratory tract infections may promote adhesions of bacteria to nasal mucosa and facilitate transmission (Hasbun, 2014).

After the invasion of the nasal mucosa, pathogens might cross the nasopharyngeal mucosa by different methods. This is done by pinocytosis in the case of N. meningitidis, via surface proteins that binds to platelet activating factor (PDF) in the case of pneumococcus (Huang and Jong, 2001, Kim, 2003, Maria and Bale, 2006). While haemophilus separates the apical tight junction between cells and gains access to blood vessels. Once in the blood stream they survive phagocytosis due to presence of the capsules and are resistant to complement mediated bactericidal activity (Kim, 2003).

Pathogens may also access the meninges by hematogenous spread during bacteremia via passage of infected leucocytes,(Maria and Bale, 2006) through the choroid plexus , via rupture of superficial cortical abscesses, a contiguous spread from adjacent infection or via shunt mechanism in a skull fracture (Kim, 2003).

1.6.2 Survival in the blood streams

Survival of bacteria in the vascular system is essential for multiplication and invasion of the meninges**.** This is possible because these bacteria have developed mechanism to escape the immune response by displaying a wide range of surface antigens and polysaccharide capsules. This capsule protects them from phagocytosis by neutrophils and the classic complement mediated bactericidal activity and aid in multiplication of the bacteria (Maria and Bale, 2006, Idro et al., 2005).

1.6.3 Bacterial invasion

The blood brain barrier which is composed of the arachnoid membrane, the choroid plexus epithelium and brain micro vascular endothelial cells (BMCE) acts as a barrier that strictly regulates passage of molecules and ions into CNS. It is therefore protecting the brain from harmful toxins (Tunkel and Scheld, 1993, Maria and Bale, 2006). The

structure is semi selective and by the mean of the tight junctions formed by the brain micro vascular endothelial cells (BMCE), allows small amount of pinocytosis and active transportation for glucose. This barrier also protects the brain from neurotoxins and microbes circulating in the blood. The meningeal pathogens can also cross the blood brain barrier in form of live bacteria in to the CNS (Kim, 2003). They may cross the barrier "transcellulary, paracellularly or via infected phagocytes (Trojan horse mechanism)" (Kim, 2008).

There seems to be a correlation between the threshold of bacteremia and development of bacterial meningitis, in neonates it has been shown that when an E.coli count $>10^3$ ml^1 is found in the blood then it is more likely the neonate to have meningitis in comparison with lower than 10^3 ml^1 colonies. Similar conditions were seen with other meningeal pathogens confirming the hypothesis that a threshold of bacteremia is a pre determinant factor for development of meningitis (Kim, 2003).

The first site for bacterial invasion in the CNS seems to be the choroid plexus. The choroid plexus is a highly vascular structure and has a very high blood flow of approximately of 200 ml/gr/min in comparison with other CNS structures (Tunkel and Scheld, 1993). It seems that this high blood flow of the plexus is responsible for bringing more bacteria to this site and with the help of the receptors that are found in the plexus's endothelium it promotes entry into the plexus (Tunkel and Scheld, 1993). Huang in his study demonstrated that these proteins are specific for each type of bacteria. *E coli* invades via Ibe A, Ibe B and Omp A, *S.pneumoniae* via protein Cbp and *N. meningitidis* via Opc, Opa, and Pil C.(Huang and Jong, 2001). Once the bacteria has entered the arachnoid space it is capable to further multiply as there are few host defenses due to lack of antibody, complement and opsonic activity in the CNS (Maria and Bale, 2006).

1.6.4 Inflammatory response

Once a live bacterium has crossed the BBB accompanied by leucocytes, these bacteria replicate and produce a stronger immune reaction by liberation of pro inflammatory and toxic substances which cause further migration of leucocytes particularly of neutrophils, across the BBB, causing a condition known as pleocytosis. “This pleocytosis is the hallmark for diagnosis of meningitis” (Hoffman and Weber, 2009).

Once the bacteria reaches the subarachnoid space, they further multiply and undergo autolysis. This self-destruction releases more cell wall components, lipopolysaccharide, teichoic acid, peptidoglycans and bacterial DNA initiating an increased inflammatory response. This activates an inflammatory pathway leading to a production of cytokines like tumor necrosis factor (TNFα), interleukin 1b further activating release of other inflammatory mediators like interferon ɤ (INFɤ), platelet activating factor (PDF), chemokines, prostaglandins and nitric oxide (NO). TNFα production increases within hours and is strongly related with severity of disease (Maria and Bale, 2006). This starts a vicious cycle with further activation of leucocytes which in turn liberates reactive oxidants and matrix metaloproteinases (MMP) causing further tissue damage (Maria and Bale, 2006, Hoffman and Weber, 2009).

In the subarachnoid space there is an increase in exsudate due to predominance of phagocytic polymorphonuclear leucocytes in early phase. In the later phase by lymphocytes histiocytes, fibrinogen and blood proteins further contributing to inflammatory exsudate (Maria and Bale, 2006).

Nitric oxide has a very important role in the pathogenesis. It is a potent vasodilator and causes dilatation of cerebral vessels and increase in blood flow to the brain aggravating cytotoxic damage to the BBB and further increase cerebral edema (Scheld et al., 2002, Kim, 2003). Table 2. Demonstrates the main cytokines that are increased in different types of meningitis.

1.6.5 Cerebral Edema

The cerebral edema in bacterial meningitis is multifactorial in origin. It can be vasogenic, cytotoxic or interstitial in origin (Tunkel and Scheld, 1993). The Vasogenic effect is due to increase in BBB permeability, the cytotoxic effect occurs due to swelling of the cells which is a result of toxic substances induced by leukocytes via inflammatory mediator and due to secretion of antidiuretic hormone (ADH), which further aggravates cytotoxic edema with hypotonic extracellular fluid and increase in the permeability of the BBB and causes cerebral edema. The interstitial edema occurs due to obstruction of subarachnoid structures due to bacterial and tissue debris (Tunkel and Scheld, 1993, Scheld et al., 2002, Hoffman and Weber, 2009).

Table 1.2 Cytokines that increase in response to meningitis

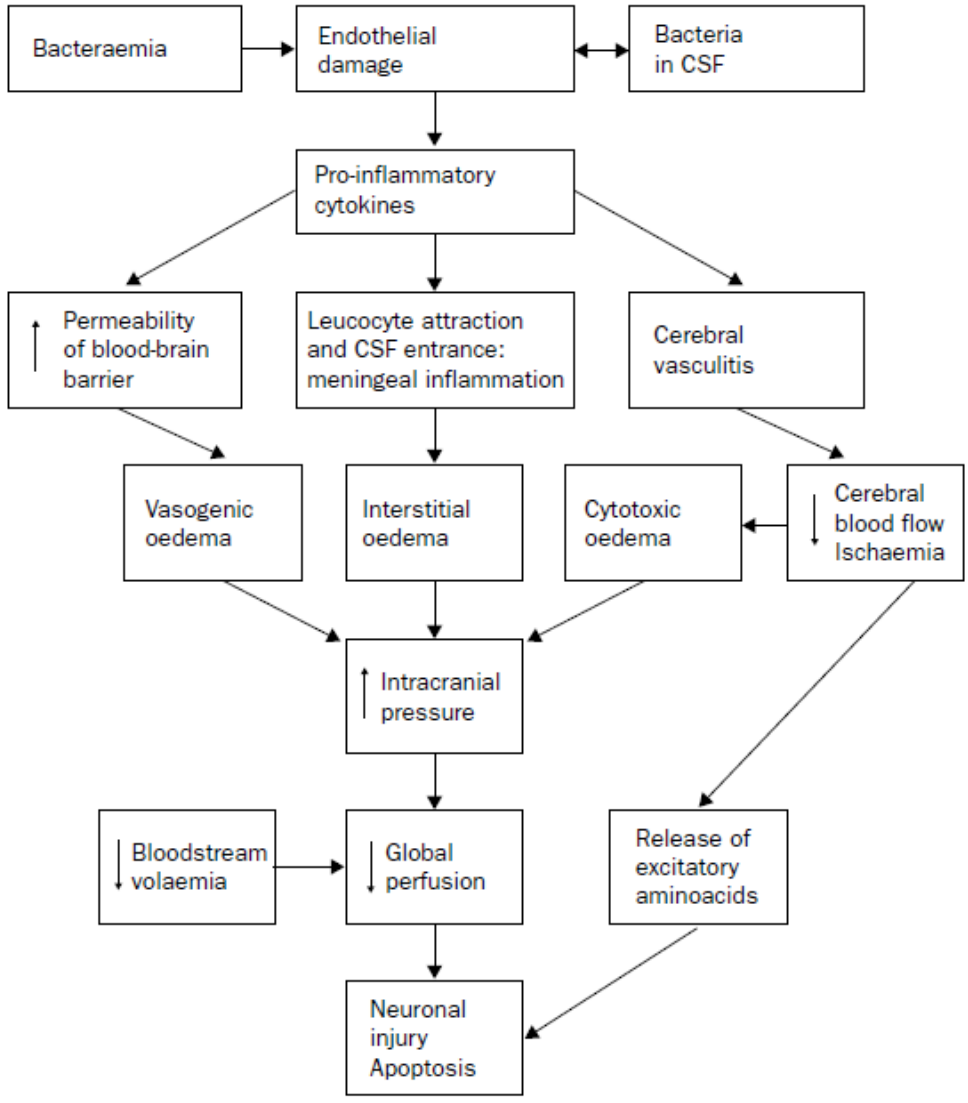
Cytokines	Serum	Viral	Bacterial
IL 1	+	+	++
IL 6	++	++	++
IL 8	-	++	++
IL 9	-		
TNF	+		+
Î²		-	+
PDF	-	-	+
C reactive protein	+	+	++
IL interleukin , Î² tumor growth factor			

Source (Maria and Bale, 2006)

The increase in cerebral oedema plus the obstruction of the villi at the arachnoid matter and in the choroid plexus causes hydrocephalus and interstitial oedema. These conditions further increase intracranial pressure which in turn decreases the cerebral blood flow and causes loss of cerebrovascular auto-regulation. Once the cerebrovascular auto regulation is lost the cerebral blood flow becomes dependent on the mean arterial blood pressure which increases to maintain adequate cerebral perfusion. In some cases if the blood pressure is lower due to shock it may severely

affect cerebral perfusion and increases the risk of neurological injury. In acute intracranial hypertension, brain perfusion is also altered and increased the risk of brain herniation (Maria and Bale, 2006). The figure 3 summarizes the pathophysiological cascade in bacterial meningitis. Source (Sáez-Llorens and McCracken, 2003).

Figure 1.3 Summarizes pathophysiology cascade in bacterial meningitis



Pathophysiological cascade in bacterial meningitis

Source :(Sáez-Llorens and McCracken, 2003)

1.7 Clinical picture

The clinical manifestations of acute bacterial meningitis varies according to the age of the patient. The younger the patient the more subtle and atypical the signs and symptoms are. The classical triad of fever, altered mental status and neck stiffness which is frequently observed in older children and adults is rarely seen in infants and younger children (Sáez-Llorens and McCracken, 2003, Maria and Bale, 2006, Prober, 2007).

Two important forms of presentation have been described by (Prober, 2007) the acute onset form which is rapidly progressive to coma or death within 24 hours which is rare. The commonly encountered presentation is the subacute type with several days of prodromal symptoms before the development of nonspecific signs and symptoms of CNS involvement (Prober, 2007).

In neonates the prodromal signs are low grade fever, poor feeding or refusal to feed, somnolence, behavioural change or irritability followed by vomiting, lethargy and seizures. The physical examination is also nonspecific and can present signs of irritability, hyper reflexia and a bulging fontanel. In severe cases with systemic involvement hypotension and disseminated intravascular coagulopathy (DIC) is observed (Maria and Bale, 2006).

While in older children prodromal signs are: fever accompanied by upper respiratory or gastrointestinal tract symptoms followed by CNS involvement (Prober, 2007). Other nonspecific signs include anorexia, headache, myalgia, arthralgia, hemodynamic alterations with hypotension and tachycardia. In case of meningococcal infection there may be associated dermatological alterations such as petechial purpura or macular erythematous rash accompanied to meningeal signs (Maria and Bale, 2006, Prober, 2007).

The signs that demonstrate that CNS is affected are headache associated with photophobia results of meningeal inflammation and increased intracranial pressure. Other signs include head retraction, neck stiffness, spinal rigidity result of meningeal irritation of the spinal root (Prober, 2007, Ward et al., 2010).

Meningeal signs are comprised of nuchal rigidity, back pain, Kerning sign and Brudzinski sign. In affected children below the age of 18 months Kerning and Brudzinski signs are frequently negative. Earlier studies in adults have shown that nuchal rigidity, headache and fever is only seen in 35 - 40% of adults with bacterial meningitis (Puxty et al., 1983, Prober, 2007). The neck stiffness, spinal rigidity and head retraction reflect irritation of meninges and spinal roots and are mechanism to protect the spinal axis by immobilization and shortening of the roots. The stretching of these roots cause pain and reflex spasm (Maria and Bale, 2006). It is the stretching of the roots and spasm the basis of Kerning and Brudzinski signs (Vincent et al., 1993).

Seizures are frequently observed among children with bacterial meningitis and these can be focal or generalized. Seizures are observed in 44% of *H. influenzae,* 25% of *S. pneumonia*e and 10% in *N. meningitidis* meningitis (Dodge and Swartz, 1965). Focal seizures are result of localized involvement of brain parenchyma either by bacteria or inflammatory lesion (Samson et al., 1969) There is a strong association between seizure (generalized or focal) with diagnosis of bacterial meningitis. About 18% of first seizures in children was caused by meningitis. Therefore any child with first episode of seizure should undergo a lumbar puncture to exclude the diagnosis of bacterial meningitis (Samson et al., 1969, Prober, 2007).

Another important clinical feature common to bacterial meningitis is alteration of mental status. This could be due to increased intracranial pressure, cerebritis or hypotension. May present as irritability, lethargy, stupor, obtundation or coma (Prober, 2007). Intracranial pressure can present with headache, vomiting, bulging fontanel, diastasis of cranial sutures, cranial nerve palsy [oculomotor (anisocoria and ptosis) and abducent], hypertension with bradycardia, apnea or hyperventilation, stupor , coma or decerebrate

posturing (Prober, 2007) However some of the signs associated with bacterial meningitis are not always present at the same time. Due to the non-specificity of clinical picture in younger population it is difficult to make diagnosis of bacterial meningitis based on clinical feature (Curtis et al., 2010).The table below demonstrates frequency of signs and symptom (Valmari et al., 1987).

Table 1.3 Frequency of signs and symptoms that are present in bacterial meningitis

Signs and symptoms	**Frequency**	**References**
Fever	94%	(Valmari et al., 1987)
Irritability	85%	(Valmari et al., 1987)
Emesis	82%	(Valmari et al., 1987)
Impaired consciousness	79%	(Valmari et al., 1987)
Neck stiffness in older children	78%	(Valmari et al., 1987, Maria and Bale, 2006)
Seizures	10- 44%	(Dodge and Swartz, 1965) (Valmari et al., 1987)
Headache in older children	92%	(Valmari et al., 1987)
Focal signs	10- 20%	(Prober, 2007)

Source (Valmari et al., 1987, Maria and Bale, 2006, Prober, 2007)

1.8 Diagnosis and management of bacterial meningitis

1.8.1. Diagnosis of bacterial meningitis

When managing community acquired bacterial meningitis, the efforts to make a diagnosis and to start an effective, treatment should be started immediately to reduce the high mortality and morbidity that accompanies this disease. The task for the pre hospital care are the stabilization and transportation of a critically ill child (Muller, 2014). Patient must be transported to hospital with facilities of Intensive care unit, where patient would be managed appropriately. For management of children, a paediatric ICU (Intensive care unit) care is required (van de Beek et al., 2006, Muller, 2014).

The Initial approach, in managing a child with meningitis starts with evaluation of vital signs followed by proper history and physical examination. Simultaneously determine, based on the findings suspicion for bacterial meningitis and initiate an appropriate

approach for diagnosis and antimicrobial treatment (Tunkel et al., 2004). If the child is stable and with signs indicating meningitis a lumbar puncture should be performed immediately to avoid any delay in diagnosis and the first dose of antibiotic should be given while awaiting laboratory confirmation (Tunkel et al., 2004). However, in case of the critically ill child with hemodynamic instability, GCS < 11, cardiorespiratory compromise, focal neurological signs, persisting focal seizures, signs of intracranial mass and intracranial hypertension (headache, vomiting, and papilledema) lumbar puncture should be delayed to avoid cerebral herniation and immediate death. In these cases first CT of the head should be performed prior lumbar puncture but antimicrobial therapy and management of ICP should be commenced simultaneously without further delay, while awaiting for the CT results (van de Beek et al., 2006, Prober, 2007, Muller, 2014). Figure 4 demonstrates the guideline for approaching community acquired BM by (van de Beek et al., 2006).

Other contraindications for lumbar puncture include patient with coagulopathy as this increases risk of hematoma into the subarachnoid space, skin infection on the site of LP (Hasbun, 2014). Contraindication for immediate lumbar puncture (Hasbun et al., 2001, Joffe, 2007, Hasbun, 2014) includes:

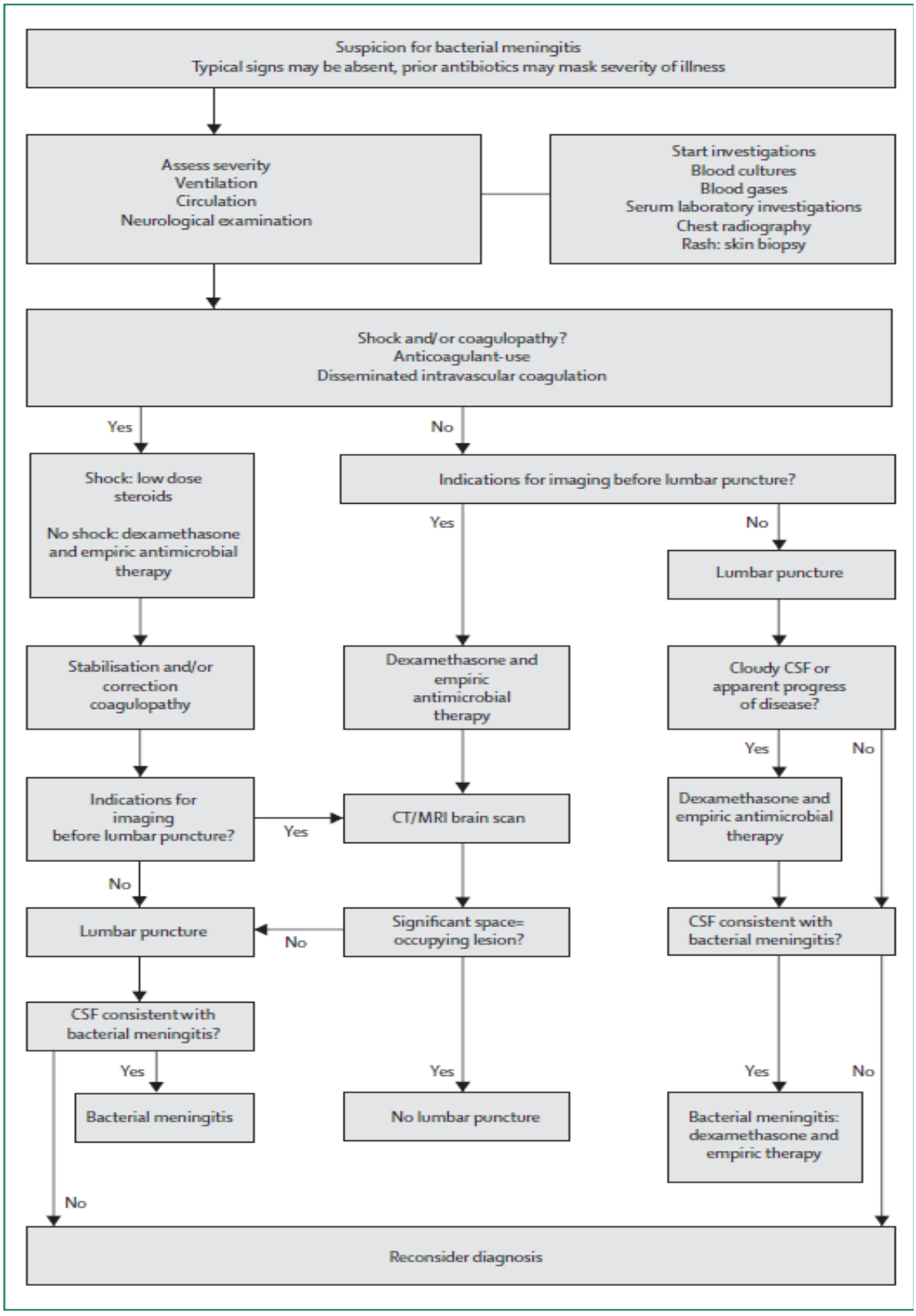
- Intracranial hypertension
- Brain abscess
- Papilloedema
- Focal neurological sign (excluding single or isolated nerve palsy)
- GCS < 11
- Severe immunodeficiency (e.g., AIDS)
- New onset of seizure
- Septic shock
- Patient with known cerebral lesion.

There have been many studies that recommend a CT scan before performing a LP in bacterial meningitis (van de Beek et al., 2006, Fitch and van de Beek, 2007, Kestler et al., 2013). However the data is not very conclusive till the date. According to a study done,

every patient with bacterial meningitis has a raised intracranial pressure, however, death by herniation in BM accounts only for 5% of all cases from LP. Many studies have measured intracranial pressure in children with bacterial meningitis, (Dodge and Swartz, 1965). The mean intracranial pressure can rise up to 307 mm H_2O which is well above the normal value of 70-180 mm H_2O (Bonadio, 2014) reported, but these ICP were still lower than reported in a study done by Minns et al. More importantly, despite higher pressure in BM in (Minns et al., 1989) study no fatalities were reported associated with LP in children with raised ICP. Larsen and Goldstein, 1999 quote in their article that “CT does not measure intracranial pressure, and there may be clinically significant ICP in the absence of any abnormality on a CT scan” (Larsen and Goldstein, 1999, Bailey et al., 2012). This suggests that increased ICP evaluated by CT of the head cannot be conclusive and reduce the risk of herniation of intracranial hypertension in context of bacterial meningitis.

Another important point is that this recommended guideline for the use of head CT before LP to prevent herniation related to increase in ICP cannot entirely be applied to lower income countries. Many low income countries only have CT scan for central or major hospitals and are not even frequently available or the service is only available during certain working hours and hardly ever available during emergencies or there are no specialist for interpretation available (Kestler et al., 2013). These guidelines are not feasible for low income countries but caution needs to be taken when in presence of focal neurological signs (Kestler et al., 2013). Whenever LP is not possible samples for blood culture should be acquired prior antibiotic therapy (van de Beek et al., 2006).

Figure 1.4 Algorithm for Management of patients suspected with community acquired bacterial meningitis. Source (van de Beek et al., 2006)



1.9 Laboratory Diagnosis of Meningitis

The crucial step for the diagnosis of bacterial meningitis is the analysis of the CSF which is essential. The most common procedure to acquire CSF is via lumbar puncture. The procedure is a common practice in the evaluation of a child with fever and / or with signs of CNS involvement. In order to interpret the CSF samples it's important to understand its composition and variation that occur with different ages.

1.9.1 CSF: Formation and Composition

Cerebro-spinal fluid (CSF) is a clear, colourless fluid formed by the choroid plexus of the lateral, third and fourth ventricles of the brain. The choroid plexus produces around 70% of this fluid by ultrafiltration and secretion. The rest of the CSF is produced by the ependymal lining of the ventricles and the cerebral subarachnoid space. On average 500 ml of CSF are formed daily, but at any given moment only 90-150 ml of CSF is present in an adult. Arachnoid villi are responsible for reabsorption of the CSF (Bonadio, 1992, Fischbach, 2009, Sakka et al., 2011). In children between the age of 4 - 13 years at any given time a volume of 65 – 150 ml are found (Bonadio, 1992). In a lumbar puncture a volume of 3-5 ml may be taken depending on the age. The volume removed during the lumbar puncture can be restored within an hour of the procedure (Bonadio, 2014).

Normal intracranial pressure is the result of well a balanced system between CSF production and reabsorption which is dependent on venous pressure as all the reabsorbed fluid is drained in to venous system. Though continual balance of CSF is maintained there is a significant pooling in the lumbar sac site located between L4 to L5. This location is used for Lumbar puncture for acquisition of CSF for analysis because of the pooling and due to lower risk of damaging the roots (Fischbach, 2009).

The CSF has 4 main functions (Fischbach, 2009, Sakka et al., 2011).

1. Mechanical protection for the spinal cord and the brain by providing buoyance.
2. Homeostasis of interstitial fluid and regulation of neurological function
3. Delivery of nutrients to the brain and removal of waste material.
4. Regulation of the intracranial pressure

The CSF is composed of 99% water, which contains a small amount of glucose, electrolytes, minerals, proteins and other nutrients. (Merritt and Fremont-Smith, 1937).The chemical composition is strictly controlled to maintain chemical balance. The electrolytes like K^+, Ca^{2+}, Mg^2 are regulated by a specific transport system. Glucose, urea, creatinine and proteins enter the CSF by passive diffusion via pinocytosis. The protein concentration in the CSF is strictly related to plasma concentration of the protein. In case of damaged BBB eg. In bacterial meningitis, albumin, which is found in higher concentration in the plasma crosses the BBB causing increase in protein concentration in the CSF (Fischbach, 2009).

Though the CSF is considered as acellular a very small amount of cells can be found. The parameter of cells found in the CSF varies according to the age. A cell count of 0 - 5 WBC and RBC in the CSF are considered normal in adults but in children may vary from 0-20 cells and In neonates it may be even higher due to BBB prematurity and may range from 0-30 cells (Prober, 2007, Fischbach, 2009).

CSF contains a higher concentration of Na^{2+} and Cl^- in comparison with plasma while the levels of K^+, Ca^{2+} and Mg^{2+} are lower. (Bonadio, 1992, Fischbach, 2009). The average concentration of electrolytes is: sodium (Na^2) 140mEq/l; potassium (K); 2mEq/l, chloride 115mEq/l; calcium 2,5 to 3mEq/l; phosphorus 1,6mEq/l and magnesium of 2,2mEq/l (Merritt and Fremont-Smith, 1937). Table 1.4 Demonstrates all the characteristics of the CSF according to the various ages.

Only disease can affect the permeability of BBB and permit the entry of elements that usually have no access to the barrier and allow entry of erythrocytes and leukocytes from blood vessel damage or from meningeal reaction. However, in case of neonates, these have a premature BBB and allow passage of leukocytes and proteins in higher concentration during the first 4 to 6 weeks of life, but once maturation occurs, the number of leukocytes and amount of protein fall to become similar as in infants (Bonadio, 1992). In case of meningitis the BBB is also affected.

Table 1.4 Normal Reference Ranges of Cerebro Spinal Fluid According Different Age groups

CSF Characteristics	**Neonates**	**Children 1-4 years**	**5-18 years**	**Adults**
Opening pressure	50-80 mmH_2O		100-200 mmH_2O	
Leukocytes	0- 30 cells x 10^6/L	0-20 cells x 10^6/L	0-10cells x 10^6/L	0 - 5 cells x 10^6/L
Differential				
Lymphocytes	5-35%	40-80%		
Monocytes	50-90%	15-45%		
Polymorphonucler leukocytes	0-8%	0-6%		
Erythrocytes	0–675 cells x 10^6/L	0-10 cells x 10^6/L		
Proteins	70 mg/dL	20-45 mg/dl		
Glucose	>50% of serum glucose	40-70 mg/dl		
Specific gravity	1005		1000-1006	
Bilirubin	None	None		

Source: (Fischbach, 2009, Muller, 2014)

1.9.2 CSF alterations in bacterial meningitis

CSF appearance, cell count and chemistry

The CSF analysis starts with measurement of CSF pressure followed by physical examination of the CSF, cell count, gram stain and culture. The cell count is essential as the cell morphology can help in differentiating between the different types of meningitis and can be performed within 30 min. Normal CSF is acellular or has few leukocytes,

cells usually less than 10 cell/ml with one polymorphonucleocyte (PMN) only (Rumbaugh and Nath, 2009).
The CSF is usually colourless and clear, but in bacterial meningitis the CSF colour may vary from clear to purulent. Turbidity of the CSF is a result of increased leukocyte, red blood cells, protein concentration, glucose or presence of bacteria (Fischbach, 2009).

The normal CSF pressure is usually between 50 - 200 mm H_20 depending on age. However, in bacterial meningitis, it is usually increased except in the early stages of the disease. But in a later stage of the disease the pressure is typically high. In 90% of cases the pressure may be higher than 200 mm H_20 and in 10 -15% of cases may be as high as, or above 500 mm H_20 in adults (Merritt and Fremont-Smith, 1937, Venkatesan and Griffin, 2009). In children the opening pressure is also increased in bacterial meningitis and the median pressure may be as high as 204 mm H_20 (Minns et al., 1989).

The normal CSf WBC found in the CSF vary between 0-5 cells/mm^3 with one ploymophonucleocyte observed. In case more than 2 polymorphonucleocytes are observed, CSF should be considered abnormal (Rumbaugh and Nath, 2009).

In bacterial meningitis cell count is usually altered, typical finding are presence of polymorphonuclear pleocytosis (Pleocytosis is referred as cell count > 10 cells/µl), hypoglycorrhachia and raised CSF protein level (Spanos et al., 1989, Venkatesan and Griffin, 2009).

The leukocyte count in bacterial meningitis varies. Typically the leukocyte count is greater than 1000 cells/µl but in some cases may be as high as 10,000 cells/µl (Schuchat et al., 1997, Venkatesan and Griffin, 2009). However, in rare cases, cell count may be lower than 100 cells/µl (Venkatesan and Griffin, 2009). When this occurs, it's known as 'normocelular' or 'developing bacterial meningitis' usually observed in immunosuppressed patients and is not very common. However, Fremont Smith in his study observed that at least 1% of meningitis patient had cell count < 100 cells/µl (Merrit

and Fremont-Smith, 1938) as cited by (Venkatesan and Griffin, 2009). But the predominance of PMN in the CSF should suggest the diagnosis of BM, though L. Monocytogenes can cause lymphomonocytosis (Rumbaugh and Nath, 2009, Brouwer et al., 2010).

Viral meningitis usually presents with a cell count, which typically ranges from 10 - 1000 cell/µl, but usually lower than < 300 cell/µl with predominance of monocytes and lymphocytes. In case of some viral infection like herpes, enterovirus and arbovirus there could be predominance in PMN during the first 48 hours (Thomson Jr and Bertram, 2001). In general, there is an overlap between viral and bacterial meningitis therefore microorganism identification is essential for confirming diagnosis (Prober, 2007).

CSF glucose is usually reduced and in 75 % of patients is < 50 mg/dl and only in 25 % is below 10 mg/dl (Merrit and Fremont-Smith, 1938) as cited by (Jurado and Walker, 1990). However, in case systemic glucose level is increased the CSF glucose will also be high in these cases. So, many authors suggest for correction by calculating CSF: serum / glucose ratio = 0.6 (Spanos et al., 1989, Fischbach, 2009). In viral meningitis it is often observed that there is a slight decrease in the level of glucose in the CSF, but usually is normal, but in cases of TB meningitis it is very low (Fischbach, 2009). Hypoglycorrhachia has a strong predictive value if accompanied with pleocytosis and clinical signs of BM (Dubos et al., 2008).

The CSF protein is usually elevated in BM. The typical findings are protein above 45 mg/dl. When the protein is above 80 mg/dl, it has a strong predictive value for BM, as a slight increase in protein may also occur in viral and fungal meningitis. (Dubos et al., 2008, Fischbach, 2009). A higher increase above 500 mg/dl is strongly associated with the development of neurological deficits (Schutte and van der Meyden, 1998).

Out of the 3 parameters, leukocytes and protein count are the least influenced by antimicrobial therapy, glucose starts increasing after first 24-48 hours after antibiotic administration. Protein and leukocyte may still be present until the end of treatment

(Steele et al., 1986). The table below summarizes changes seen in the CSF in different types of meningitis.

Table 1.5 Cerebrospinal fluid findings in central nervous system disorders in children

Conditions	Leukocytes (µl)	Protein (mg/dl)	Glucose (mg/dl)
Normal	<5, ≥75% Lymphocytes	20-45	>50 (or 75% of Serum Glucose)
Acute Bacterial Meningitis	100 - 10,000 300-2,000 PMN or >50%	100-500	<40 (or <50% serum glucose)
Partially treated bacterial meningitis	5-10,000; PMNs usual, but mononucleocytes prevalent when prolonged treatment	100-500	Normal or decreased
Viral meningitis or meningoencephalits	Rarely >1000 cells < 500 cells Early PMN and later lymphocytes	50-200	Generally, normal, but may be decreased < 40 in some viral diseases,
Tuberculous Meningitis	10-500; PMNs early, but lymphocytes predominate through most of the course	100-3,000; or higher in case of obstruction	<50
Brain abscess	5-200 , when ruptures >10.000	75-500	Normal
PMN = polymorphonuclear leukocytes			

Source: (Prober, 2007)

1.9.3. Gram stain

The CSF gram stain is a simple and rapid diagnostic test for the identification of organisms in bacterial meningitis. It is also an accurate test. It is a very simple test than can be easily done in a standard laboratory (Gray and Fedorko, 1992).

The test is positive in 75 to 90 % of culture positive bacterial meningitis. The possibility of detecting bacteria depends on the concentration of bacteria in the specimen which can be increased by the use of centrifugation (Gray and Fedorko, 1992). It has an additional value when in presence of culture negative meningitis. The reliability of the test increases when concentration of bacteria are above 10^5 colonies/ml with sensitivity up to 97%. The sensitivity may decrease to 60% when bacteria concentration is below 10^4 colonies/ml, and to 25% when below 10^3 colonies/ml (La Scolea and Dryja, 1984).

The sensitivity of gram stain varies according to aetiologic agent. According to a review a wide range of sensitivity was observed for *H.influenzae* with 25 to 65%, *S. pneumoniae* ranging from 69 to 93%, *N.meningitidis* from 30 to 89%, *L.monocytogenes* from 10 to 35%, *S.agalactiae* from 80 to 90% and S.aureus with 20-44%. When pretreated with antibiotic the sensitivity was further decreased (Brouwer et al., 2010).

1.9.4. Culture

CSF culture is the gold standard for the diagnosis of BM. It has a lower sensitivity in patients pretreated with antibiotics but has a high specificity (Brouwer et al., 2010).
For community acquired bacterial meningitis it is mandatory to use aerobic media and in case of neonates, post neurosurgery or shunt related meningitis anaerobic media must be used (Brouwer et al., 2010).
A study by Nigrovic and collaborators found in 159 patients with BM without prior antimicrobial therapy a sensitivity of 84% in comparison with those who received antibiotic. For those who had received antibiotic less that than 4 hours, the sensitivity

had dropped to 72%. By the end of 24 h of antibiotic therapy sensitivity had further dropped to 59%. The diagnosis of BM in this study was made with confirmed cases (CSF culture positive or csf pleocytosis with wbc>10 cells/UL plus either a positive csf or blood culture positive and a positive latex agglutination test) and probable diagnosis of bacterial meningitis based on the result of gram stain but no culture confirmation (Nigrovic et al., 2008). This emphasizes the need of LP prior to antimicrobial therapy when possible to improve diagnosis by culture. Another retrospective study from Brazil with 4,100 cases which defined bacterial meningitis either with definite diagnosis that is culture positive plus probable meningitis based on CSF parameter alteration. This study involving children reported that the culture was positive only in 67% of patients with the diagnosis of BM (Bryan et al., 1990). A third study demonstrated that in the case of meningococcus sterility occurred as early as 2 h after antibiotic therapy with 3rd generation cephalosporin and for *S. pneumoniae* after 4 hours of antibiotic therapy (Kanegaye et al., 2001).

1.9.5. Latex agglutination

The latex agglutination is a diagnostic method that is used for the aetiologic diagnosis of BM and provides results within 10 to 15 min. It's is recommended when gram stain and culture of CSF, both are negative, to exclude BM (Tunkel et al., 2004). The test uses a serum that contains bacterial antibodies which reacts to bacterial antigens of the CSF derived from polysaccharide capsule of meningeal pathogens. When antigens are present in the CSF a positive reaction is observed in form of agglutination which lasts between 2 to 10 min (WHO, 2011d).

As various commercial kits are available the use of the kit is dependents on manufacturer's instruction. For optimum result the supernatant obtained though the centrifugation must be tested immediately. When immediate testing is not possible, it should be refrigerated at 2 to 8 ^{o}C. The down side of the kit, is that it needs to be kept refrigerated before use, this can be a challenge, especially in tropical climates where the electricity problem is frequent. As high temperature deteriorates the serum quickly

giving unreliable results (WHO, 2011d). The sensitivity of the test varies according to different etiological agents. For *N.meningitidis* it ranges from 29 to 93%, *S.pneumoniae* 59 to 100% and for *H. influenzae* 78 to 100% (Gray and Fedorko, 1992, Wenger et al., 1990), giving it a limited diagnostic value and is not clinically useful when both gram stain and culture are negative (Nigrovic et al., 2008).

1.9.6. PCR

Broad range polymerase chain reaction using 16S RNA gene has been studied extensively to evaluate its efficacy in detecting bacterial genome in CSF from patients with probable or confirmed bacterial meningitis. Many studies have been conducted using the universal primer 16S RNA, which virtually detects all the bacteria that can be studied in microbiology followed by species specific identification for penicillin resistant pathogens (Saravolatz et al., 2003, Brouwer et al., 2010).

Saravoltz *et al*, in their studies compared the broad range PCR with microbiology (culture and gram stain) results and reported a sensitivity of 100% (95% CI, 81,6% - 100%; 17 of 17 samples were positive) and a specificity of 98.2% (Saravolatz et al., 2003).
Other studies have detected a wide range of sensitivity for the 3 main pathogens that cause meningitis. For *H. influenzae* a sensitivity ranging from 72 to 92% has been observed (Gray and Fedorko, 1992, Saravolatz et al., 2003). For *S.pneumoniae* a sensitivity varying from 61 to 100% was observed (Gray and Fedorko, 1992, Hoen et al., 1993, Arditi et al., 1998). While for *N. meningitis* a sensitivity of 88 to 94% has been reported (Tunkel et al., 2004, Brouwer et al., 2010).

Another study by *Welinder et al*. compared the broad range PCR and culture reports, demonstrated that the PCR had the analytic sensitivity of detecting bacteria concentration as low as 10 CFU /ml for S. pneumoniae. While for *S. aureus* and *E.coli* it has an analytic sensitivity of 10^3 CFU /ml. The overall sensitivity of PCR to detect BM was of 59% in comparison to 43% of CSF culture. The specificity was equal of 97% for

both culture and PCR. However, In patients pretreated with antibiotic PCR showed better results with a sensitivity of 79 % in comparison to culture which only detected 45% of cases among patients with prior antibiotic treatment, which was statistically significant with $p < 0.05$ (Welinder-Olsson et al., 2007).

Therefore, in cases of pretreated patients with negative standard microbiology PCR can exclude or confirm meningitis and guide management decision. In many hospitals PCR of CSF are not performed routinely, and is only used when the gram stain and culture are negative or in case of viral meningitis, as the cost per sample vary from 50 to 90 $ (Brouwer et al., 2010, Wang and Mayer, 2012).

Various diagnostic methods exist for diagnosis of BM, however, all the tests have different sensitivities and specificities, moreover, many of these tests are not even available in low resource countries. In low resource countries point of care tests can help in early diagnosis and management using tests which are cheap, affordable and easy to perform as one person diagnostic test. This approach would definitely benefit the patients and orient adequate patient care. The table 1.6 below compares the different available tests for diagnosis of BM and their costs.

Table 1.6 Diagnostic Method for Identification of Microorganism in Bacterial Meningitis

Methods	Sensitivity	Specificity	Time for detection	Advantage & disadvantages	Estimated cost
CSF Culture ***Gold standard***	Low	High	3 – 7 days	Specific, add information on AB sensitivity Prior AB, transportation, type of media and delay in analysis decrease sensitivity.	8$ for negative 15$ for positive
Gram stain	High Lower in pretreated with AB	High	<1 h	Cheap, quick detection Cannot subgroup and serotype microorganism Need of a trained technician	0.90$/ sample
Latex agglutination	Low	High	< 1 h	Not able to detect all serogroups and low sensitivity	10$ / sample
RT- PCR	High	High	4-5 h	Fast identification, close system for analysis and reduced risk of contamination. Not widely used Commercial kits available	8.5$ for negative 13$ for positive 50-90$
Source, (Brouwer et al., 2010, Wang and Mayer, 2012)					

1.10 Empirical Antimicrobial Therapy

Empirical antimicrobial therapy in BM should be started immediately if a delay in lumbar puncture is foreseen (Tunkel et al., 2004). Studies have demonstrated that a delay of antimicrobial therapy can be deleterious for patient's prognosis. Many guidelines have been established for empiric treatment for bacterial meningitis based on age, type of microorganism according to age group and antibiotic that will reach CNS have been established and can be modified once the results are available and can be tailored according to each patient (Brouwer et al., 2010, Sheldon L Kaplan, 2014).

Neonates have a higher risk for meningitis for the factors that have already been mentioned earlier in this chapter. Early onset bacterial meningitis is most likely due to gram negative bacteria from the genitourinary flora of the mother therefore antibiotic must be guided toward these microorganisms. Empiric treatment suggested for this group is with ampicillin and aminoglycosides or a 3rd generation cephalosporin to cover

group B streptococcus and gram negative bacteria (E. coli, klebsiella, enterobacter). For the late onset due to high risk of staphylococci infections, medications containing antistaphilococci activity are preferred and drugs like vancomycin or Nafcillin plus a 3rd generation cephalosporin (cefotaxime or ceftazidime) is preferred (Sáez-Llorens and McCracken, 2003, Prober, 2007). In general empirical treatment can be initiated without any delay before CSF results are available, (Brouwer et al., 2010) in their review have shown empirical treatment based on subclinical groups which has been demonstrated in table 1.7

Antimicrobial therapy destroys bacteria and temporary aggravates host inflammatory response and increases tissue damage, dexamethasone a corticosteroid if given prior antibiotic administration can reduce the inflammatory response (Tunkel and Scheld, 1993, Roos and Van de Beek, 2010) and reduce the complication associated with *H.influenzae* to reduce days of fever, CSF proteins levels and for reducing sensorineural hearing loss specially in children. However it is only beneficial if given 1 to 2 hour prior antibiotic (Prober, 2007). However the use of steroid in children with pyogenic meningitis in a study done in Malawi did not show any additional benefit neither in reducing mortality or neurological sequelae (Molyneux et al., 2002) and this concept is also supported in South Africa guideline of management of bacterial meningitis(Boyles et al., 2013).

Table 1.7 Empirical antimicrobial therapy for bacterial meningitis based on different age groups

Clinical subgroups	Initial AB therapy and dosing regimen	Predominant microorganisms	References
Neonates Early onset	Ampicillin (100-150mg/kg/d Q 8h plus (gentamicin 5mg/kg/d) Q12h or cefotaxime(100-150mg/kg/d) Q8-12H	S.agalactiae, E.coli , L.monocytogenes, Klebsiela	(Muller, 2014)
Neonates Late onset **For S.aureus:**	-Ampicillin (100-150mg/kg/d Q 8h *plus*(gentamicin 5mg/kg/d) Q12h or Cefotaxime(100-150mg/kg/d) Q8-12H -Nafcillin (150-200mg/kg/d) Q8-12H or vancomycin (30-45mg/kg/d) Q 8h plus cefotaxime(150-200mg/kg/d) Q6-8H	L.monocytogenes, S.agalactiae, gram negative bacilli	(Prober, 2007)
Infants and children **Low resource countries**	Penicillin G (450.000/kg/d) Q4-6h or Ampicillin(300-400mg/kg/d) Q4-6h *plus* gentamycin 7.5mg/kg/d Q8 or cefotaxime (100-150mg/kg/d) Chloramphenicol 100mg/kg/d Q6h *plus* Ampicillin or Penicillin	S.pneumoniae, H.influenzae and N.meningitidis	(Prober, 2007, Muller, 2014) (WHO,2000)
Immunocompromised children	Ampicillin(300-400mg/kg/d) Q4-6h *plus* cefotaxime (100-150mg/kg/d) or Vancomycin (60mg/kg/d) Q6h when staphylococcus suspected. Standard Tuberculosis treatment for meningitis	Above mentioned organism plus L.monocytogenes, Tuberculosis , S.aureus	(Prober, 2007, Muller, 2014) (WHO,2000)
Adults	3rd generation cephalosporin plus vancomycin (30-60 mg/kg/d) Q8-12 h	S.pneumoniae, N.meningitidis	
Elderly	3rd generation cephalosporin plus Ampicillin (12 g/day) Q4 h *plus* vancomycin (30-60 mg/kg/day) Q8 12 h	S.pneumoniae, N.meningitidis, L.monocytogenes	(Prober, 2007, Muller, 2014)
Nosocomial	Vancomycin (35-45mg/kg/d) Q8-12h *plus* ceftazidime (6g/d [Q8h] *or* cefepime(6g/ d[Q8h) *or* meropenem(6g/d[Q8h])	S.aureus, S epidermidis, aerobic, Gram negative bacilli	
Basilar fracture	3rd generation cephalosporin *plus* vancomycin (30-60 mg/kg/day) Q8 12 h	S. pneumonia and S. aureus and gram	

Adapted from (Brouwer et al., 2010)

1.11 Complications

During the course of bacterial meningitis, many complications may arise. These complications may be systemic or neurological in origin and may be acute or gradual onset. Many factors like age, severity of the disease, type of meningeal pathogen, time of presentation to the hospital, associated comorbidities of the patient and late initiation of antimicrobial therapy are some of the factors that influence onset of complications (Sheldon L Kaplan, 2014). Some of these complications may arise post meningitis and are known as long term sequelae (Edmond et al., 2010b).

A systemic review by Edmond *et al* reported, that long term disabling neurological sequelae on average among meningitis survivors is of 20%, but it varies from 9% to 25% across the WHO regions with the likelihood of occurring 3 times higher in Africa and Asia in comparison to Europe and America (Edmond et al., 2010b). Another meta-analysis of 45 report from 1977 to 1991 involving children between the age of 2 months to 19 years, reported that in developed countries the risk of neurological complication was seen in 17.5% while in developing countries it was higher with 26% (Baraff et al., 1993). Two thirds of all death due to bacterial meningitis occurs in developing countries, and among survivors the rate of subtle neurobehavioral disabilities at discharge is estimated to be as high as 50% (Prober, 2007).Table 1.8 summarizes all the complications

The most common complications observed in children are sensorineural deafness, seizure disorder and motor deficit in comparison to adults who are in higher risk of developing hearing loss, motor and cognitive deficit or speech problems. These complications are classified as neurological or systemic while neurological can further be divided into focal or generalized complications. These complications may start as early as the entry of meningeal pathogen to the CNS via BBB and may continue post treatment (Siddiqui, 2012, Sheldon L Kaplan, 2014)

Table 1.8 Shows classification of different complications in bacterial meningitis

Neurological	Systemic
Acute : - Altered mental status - Coma - Seizures - Subdural effusion or empyema - Ventriculitis - Hydrocephalus - Cranial nerve palsies - Sensorineural hearing loss - Cerebral edema and raised intracranial pressure - Hemiparesis or quadriparesis - Blindness	- Prolonged Fever - Septic shock - Disseminated intravascular coagulation - Vasomotor collapse - Reactive arthritis - Respiratory distress syndrome and respiratory failure - Pericarditis or pericardial effusion - Bilateral adrenal hemorrhage (Waterhouse–Friderichsen syndrome) - Fluid and electrolyte imbalance - Hypothalamic and endocrine dysfunction - Death
Chronic : - Epilepsy - Ataxia - Cerebrovascular abnormality - Neuropyschological deficit - Developmental disabilities - Intellectual deficit	

(Siddiqui, 2012, Sheldon L Kaplan, 2014)

Feldman (1997) identified in his study several clinical factors that are associated to adverse outcome in children. These factors include alteration of mental status early in the disease; prolonged seizures with more than 72 hours after admission and severe leucopenia are predictors for neurological complications. While patients with CNS symptoms lasting more than 48 hours, development of focal neurological signs, ataxia, or deterioration of consciousness are at a higher risk of developing adverse outcome despite adequate antimicrobial therapy. Beside clinical features cerebrospinal fluid alterations like hypoglycorrhachia, and increased concentration of bacterial count above 10^7 CFU/ml are associated with adverse outcome (Feldman, 1977).

A significant number of children also have multiple neurological deficits (Prober, 2007, Sheldon L Kaplan, 2014). Complications that arise during treatment include seizures, increase in intracranial pressure, stroke, cranial nerve palsies, cerebellar or cerebral herniation and thrombosis of dural venous sinus, ventriculitis, subdural effusion or empyema and syndrome of inappropriate secretion of antidiuretic hormone. (SIADH) (Prober, 2007).

Table 1.9 Frequencies of most common complications in bacterial meningitis in children

Complications	**Frequency**
Hearing loss	33.6%
Seizures	12.6%
Motor deficit	11.6%
Cognitive impairment	9.1%
Hydrocephalus	7.1%
Visual disturbance	6.3%
Cognitive impairment & hearing loss combined	39.1%
Cognitive deficit & motor deficit combined	21.1
Cognitive & motor deficit plus seizures combined	8.1

Source (Maria and Jr., 2006)

Ventriculitis is one of the most common complications observed among neonates in bacterial meningitis. Ventriculitis is an inflammation of the lining of the ventricles and the CSF found inside the ventricles of the brain. It is associated with CSF obstruction especially aqueduct of Sylvius, where the infection acts as an abscess obstructing CSF outflow. It is a common complication observed in neonates (Prober, 2007, Sheldon L Kaplan, 2014, Maria and Bale, 2006). In one study ventriculitis occurred in 92 % of neonates with bacterial meningitis (Berman and Banker, 1966, Salmon, 1972). This condition in a severe and rapidly progressive case may lead to brain stem herniation and impair the perfusion of the periventricular structures (Salmon, 1972).

Subdural effusion is a collection of fluid in subdural space (Encyclopedia, 2012). In 10 to 30% of cases of bacterial meningitis subdural effusion may develop and is usually asymptomatic in 85% to 90% of cases. Subdural effusion is a common finding in infants. It occurs bilaterally affecting the frontal, parietal region and occasionally the occipital region. This condition may be accompanied by bulging of the fontanelle, distasis of the suture, an increase in head circumference, vomiting, seizures, fever and abnormal findings of cranial trans illumination. The diagnosis is made with CT scan or MRI and in severe cases it may be managed with surgical aspiration (Prober, 2007).

The subdural effusion is the consequence of an efflux of intravascular fluid due to thrombophlebitis of veins in the subdural space, increased vascular permeability and arachnoiditis. One study reported that subdural effusion occurs in 45% of cases of meningitis due to *H. influenzae,* 30% in the cases of *S. pneumoniae* and only 10% in case of *N. meningitis* (Snedeker et al., 1990, Maria and Bale, 2006).

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) may occur in some patients. According to one study SIADH was diagnosed in (22/67) 36.7% of cases among children with bacterial meningitis on admission and additional (6/48) 12.5% developed the condition on the third day of admission and it was strongly correlated with severity of leptomeningeal inflammation. This study also reported that it occurred in 75% of cases of meningitis due to Streptococcus pneumoniae meningitis (Patwari et al., 1995). SIADH is characterized by hyponatremia and reduced serum osmolality and can

aggravate cerebral edema (Prober, 2007). Other blood alteration includes anemia, thrombocytosis and eosinophilia. Anemia occurs due red blood cell hemolysis or bone marrow suppression. Another severe complication is a disseminated intravascular coagulopathy (DIC), which is usually rapidly progressive. It is accompanied by shock, purpura, signs of septicemia, profound hypotension with signs of hemorrhage and thrombosis. The severe hypotension observed in DIC combined with endotoxemia initiate coagulation process which in combination with thrombosis may produce peripheral gangrene which commonly affects lower limbs. This complication most commonly accompanies meningococcal meningitis or meningococcal invasive disease (Prober, 2007).

1.12 Prognosis

The prognosis of patient with bacterial meningitis depends on many factors. The most important factors that influence the outcome of bacterial meningitis are: age, the causative microorganism, CSF parameters like increased level of proteins, the amount of bacteremia and its products, an increased wbc in the CSF, very low level of glucose and delay in diagnosis and treatment of BM (Hodges and Perkins, 1975, Arditi et al., 1998). In the United States of America case fatality rate among children above age of one month ranged from 0 to 15 % depending on the organism and time of survey (Baraff et al., 1993, Schuchat et al., 1997, Thigpen et al., 2011).

2. Rationale of the thesis

In 2010 the annual epidemiologic bulletin reported overall that 677 cases of meningitis were diagnosed in Mozambique, with a lethality rate of 25.55% (MISAU, 2010). This data is an underestimate because it only represents the confirmed cases either with gram stain or culture while all bacterial meningitis that are culture negative were excluded. Delay in analysis is one important determining factor for the prognosis of a patient with bacterial meningitis.

Central Hospital of Beira is the second largest hospital of Mozambique. It is located in the central region, in the second largest city of the country in Sofala province. It is a tertiary hospital that provides service to 8,496,613 people of the central region (WHO, 2014a). Which comprises of 4 provinces namely Sofala Manica, Zambezia and Tete. Out of which 1,715,557 are from Sofala province alone. The hospital has 1028 beds "(extra beds included)" with an annual admission of around 27.000 patients. The Hospital has 1028 staff out which 90 are doctors and 318 are nurses. The hospital also provides teaching ground to 350 medical and nursing students annually (WHO, 2014a).

The hospital has wide range of services available from pediatrics to neurosurgery. It has a central laboratory which does standard blood chemistry and microbiology however the hospital has no microbiologist and can only provide basic microbiology test. The laboratory is run by laboratory technicians with medium level of education and provides services from 7.15 to 2.pm only. The laboratory does not perform CSF, ascetic fluid, pleural and cardiac fluid chemistry. Limiting a lot diagnosis of bacterial meningitis among other diseases (Author's observation).

In the Central Hospital of Beira, CSF analysis of leucocyte including pandy test and gram staining does take a minimum of 90 min. Frequently, the CSF analysis are not available, which delays the diagnosis and treatment of the child .This delay can be catastrophic, because late treatment is associated with high mortality and morbidity. The

availability of a sensitive bedside test would allow the initiation of the treatment without any delay.

Rural hospitals and primary health care centers in sub-saharan Africa frequently lack of suited laboratory, reagents for the tests, and trained technicians to perform the CSF analysis. This leaves no other choice but either start empirical treatment or to transfer the patient to a hospital with more resources which might cause further delay (Petti et al., 2006).

The sub Saharan region has also high prevalence of cerebral malaria which may be indistinguishable from bacterial meningitis. So many children present to the hospital with meningitis like symptoms but do not have bacterial meningitis. The lack of diagnostic facilities further challenges already little resource that is available in the hospitals by treating all patients for bacterial meningitis with antibiotics which are expensive and may already use up the little stock that is usually available, overburdening the health centers and hospitals. Therefore the availability of bedside test for meningitis would lead to a rapid screening and help determine if the patient may require or not intravenous antibiotic at meningitis doses.

Urine dip strips are widely available and determine the semi quantitative measurements of leucocytes, glucose and protein. Therefore, they provide an easily applicable method for the measurement of the relevant chemical properties of the CSF. The aim of the thesis is to evaluate the use of urine dip strip in the setting of meningitis.

2.1 Objectives

2.1.1 Objective of the thesis

1. To evaluate CSF using a standard urine dip strip (glucose, proteins, leucocytes, nitrites and density) and to compare the results with the clinical course and outcome and the local standard methods.

2. Development of a bedside treatment algorithm for meningitis, including the use of urine dip strip results of first objective

3. Paving the way for the development of a test strip specifically aimed at the CSF analysis.

2.1.2 Research questions

1. Is a standard Urine dip strip applicable for CSF analysis at the patient's bedside for the diagnosis of meningitis as a one person procedure?

2. What is the sensitivity and specificity of the dip stick in bacterial meningitis?

3. Is a differentiation between BM, viral meningitis, malaria and TB meningitis using dip strip possible?

3. Methodology

3.1 Study design and study population

A single blinded diagnostical clinical trial (laboratory technicians were not aware of the results of the reagent strip) was conducted in paediatric wards of the Hospital Central do Beira a tertiary hospital in Sofala Province in Mozambique.

We included children from 2 months to 14 years of age, which presented at the pediatric emergency department at the Hospital from November 1st 2012 to February 28th 2014 from 6.30 am to 9.00 pm from Monday to Sunday.

3.1.1 Study location

Mozambique is located in southeast Africa. It is sub divided in to 11 provinces and Sofala is the second largest province of the country. The Central Hospital of Beira is a referral hospital of the central region of Mozambique. It is located in Sofala province in Beira city, which is the capital of the province. The hospital receives patients from all the health centers, district hospitals of the province and from the nearby provinces. It is the second largest hospital at the country level and the biggest hospital at the regional level. It serves 4 provinces namely Manica, Zambezia, Tete and Sofala with a total population of around 8.5 million people. The hospital annually admits 27,068 patients (WHO, 2014a). The province has an area of 67,218 Km^2 and has population of 1,642.920 according to 2007 census (Sofala, 2007).

3.1.2 Inclusion Criteria

Children between 2 months to 14 years of age who had:

- Suspicion of meningitis which included altered mental status, meningeal signs, (bulging fontanel, neck stiffness, kernig or brudzinski sign), neurological deficit, irritability behavioral change or fever without source accompanied by seizures.

- Indication of lumbar puncture.
- Not used antibiotic for more than 48 hours prior lumbar puncture
- No severe immunosuppression eg. terminal AIDS (Acquired Immune deficiency Syndrome) or stage IV AIDS without antiretroviral therapy and cancer patients.
- Parents willing to have their children participate in the study and undergo lumbar puncture and willing to sign informed consent were included.

3.1.3 Exclusion criteria

All the children who did not fulfill inclusion criteria and had used antibiotic more than 48 hours, or were on treatment for active TB (tuberculosis) or on immunosuppressive drugs like anti neoplastic treatment or steroids were excluded from the study.

3.1.4 Patient recruitment

Once a child was observed in the Emergency Department (ED) by the attending physician with suspicion of meningitis and indication for lumbar puncture (LP) the study physician was called for patient enrollment.
Patient recruitment took place after the patient was screened by study physicians and informed consent was sought. Parents or guardians of children were informed on lumbar puncture procedure and its risks, the use of urine strip to analyze CSF and explanation was given that 1 ml of the CSF would be stored and would be later analyzed for PCR and Cytokine in Munich, Germany.

Once all the formalities had taken place, lumbar puncture was performed. Patients were sedated using ketamine prior procedure. For the procedure the skin was disinfected with 2% iodine, a sterile kit was used and a normal standard needle with stylet was inserted at L3–L4, L4–L5, or L5-S1 interspace. When subarachnoid space was reached, the stylet was removed and 2.5 - 3.5 ml of CSF were collected in four different sterile tubes (Bonadio, 2014).

The first tube contained 0.5 ml to 1 ml of CSF to be used for cell count, pandy test and for urine reagent strip testing. The second sterile tube with 1 ml of CSF was used for culture, gram stain, Indian ink for cryptococcal evaluation and Ziehl neelsen stain if asked. The third and fourth sterile tubes contained 0.5 ml each for PCR and cytokines testing.

The first 2 tubes were sent to hematology and microbiology department respectively. The other 2 vials were immediately kept in the fridge at 2 to 8 degrees centigrade and within 2 hours stored at -80 degrees centigrade while patient recruitment was completed.

Once the CSF was obtained 6 drops were used on the reagent strip for leukocyte, glucose, proteins, blood, nitrites and density. The reagent strip used for the study was the Multistix 10 SG from Siemens. The strips are the same that are used for urine examinations. The Multistix 10SG have a small specific areas for enzymatic reaction called the “test pads” (Siemens, 2012). These test pads provide semiquantitative measurements for leucocytes, protein, glucose, nitrite, blood as well as other tests.

3.2 Reagent strip Multistix 10SG

Each Bottle of the Multistix 10 SG from Siemens, contains 100 strips. The recommendations by the manufacturer were followed for the reading of the each test pad. The strips are ready for use upon removing from the bottle and maybe evaluated visually or using CLINITEK instrument. An instrument which can automatically detect colour change and print results. For research purpose the reading was done visually.
The fig. 3.1 shows a picture of the urine strip

Fig. 3.1 Picture of urine strip used in the study from Siemens

Source (Siemens, 2014)

All the recommendations given by manufacturer were followed when examining CSF with reagent strip. The reagent strip's desiccant was kept all the time in the bottle to keep the strip moisture free.

3.2.1 Storage and Handling of strips

To get precise and to avoid false negative or false positive results we followed all the instructions provided in the leaflet by Siemens. The bottle was kept between 15 to 30 degrees centigrade and stored away from the direct sunlight. The desiccant was kept all the time in the bottle to keep the strip protected from moisture.

Monthly quality controls were done with Biorad urine control to check for quality control of the test. When any discoloration or any darkening of the strip was noted the bottle in question was not used for study patients.

3.2.2 Factor affecting the reagents strip results

According to the Siemens leaflet humidity, contaminants in the specimen container, direct sunlight could affect strip reading and every effort was made to avoid these factors (Siemens, 2012). Any substances that could colour the urine could affect the results, but in case of CSF no data were available.

3.2.3 Reagent Test Strip information

Protein

This test is based on protein-error-of-indicators principle. This principle is based on the "ability of protein to alter the color of some acid-base indicator without altering the pH" (MediaLab, 2012). The test pad contains 0.3% tetrabromephenol blue, buffer and a non-reactive ingredient and changes its colour when it is brought into contact with proteins, specifically albumin. The protein test is less sensitive to globulin. So in cases of mucoproteins which are globulin the test may be negative. When the reagents are in contact with albumin it will change its colour from yellow (without albumin) to green or to blue depending on the concentration of albumin in the CSF. The protein test pad for colour change was read at 60 seconds and compared to standard chart provided by the manufacturer and the results were quantified as follow (Siemens, 2012).

Negative	No colour change
Trace	Yellow green
1+ (30 mg/dL)	Green
2++ (100 mg/dL)	Darker green
3+++ (300 mg/dL)	Blue green
4++++ (>2000 mg/dL)	Blue

Blood

The test pad contains the diisopropylbenzene dihydroperoxide, a 3,3,' 5.5,' tetramethylbenizidine, a buffer and a non-reactive ingredient. The blood test is based on the "peroxidase like activity of haemoglobin", which catalyses the reaction of the reagent substances with the chromogen reacting to erythrocyte but also to haemoglobin and myoglobin. The resulting colour ranges from orange to dark blue (Siemens, 2012).

As the CSF normally does not contain erythrocytes the strip for erythrocyte detection can be used to detect blood in cases of intraventricular haemorrhage or subarachnoid haemorrhage and it has been used in a study (Marshall and Hejamanowski, 2011).

This test can be read at 60 seconds for the erythrocytes and for haemoglobin (Hb). The strip has the capacity to detect a minimum of 10 RBC/µl (Red blood cells) and was quantified as (Siemens, 2012):

<u>For non hemolysed erythrocyte:</u>

Negative	Yellow no colour change
Trace (10 RBC/µl)	Yellow with few green spots
2++ (80 RBC/µl)	Yellow with more green spots

<u>For free haemoglobin (Hb) it is quantified as:</u>

Trace (10 Hb)	Green
1+ (25 Hb)	Dark green
2+ + (80 Hb)	Blue green
3+++ (200 Hb)	Dark blue

Leucocytes

The test pad contains a derivative pyrrole amino acid ester; a diazonium salt; a buffer and a non-reactive ingredients. The test is based on the principle that granulocytic leucocytes contains esterase that catalyze the hydrolysis of the pyrrole amino acid ester to liberate a 3-hydroxy-5 phenyl pyrrole, which in turn reacts with diazonium, a salt buffer which in turn changes the color from white to purple. It can detect both lysed and intact leucocyte cells. The leucocyte can be read at 120 seconds and is defined as (Siemens, 2012):

Negative	White (No colour change)
Trace (5-15 cells/ µl)	Light grey
1+ (75 cells/ µl)	Dark grey
2++ (125 cells/ µl)	Light purple
3+++ (500 cells/ µl)	Dark purple

The elevated glucose above 3g /dl or 160mmol/L may decrease the sensitivity of the test results. Other factors that can affect the results are presence of antibiotics like cephalotin, cephalexin, tetracycline and high doses of antibiotics may decrease reactivity and cause false negative results (Siemens, 2012).

Nitrites

The nitrite test pad contains p-arsanilic acid, 1,2,3, 4- tetra hydrobenzoquinolin-3-ol a buffer and a non-reactive ingredients. “The principle of the test is based on the conversion of nitrate from diet metabolites to nitrite by action of gram negative bacteria in the urine” (Siemens, 2012). Any colour change was defined as negative when no colour change occurred and a positive reaction when the test pad changed from white to pink. Many gram negative bacteria give a positive result when the number of bacteria are higher than 10^5 colonies per ml (Siemens, 2012).

Glucose

The glucose test pad contains glucose oxidase, perioxidase, potassium iodide, a buffer and a non reactive ingredient .The principle of the test is based “on double sequential enzyme, reaction utilizing glucose oxidase and peroxidase with a potassium iodide chromagen” and the colour changes from green to brown. It is read at 30 seconds and is specific for glucose. There are no substances that can interfere with the reaction of the glucose test pad to give false positive results, but a high concentration of ketone could give a false negative especially if glucose concentration is low. The glucose result was quantified as (Siemens, 2012) :

Negative	Blue
Trace (100mg/dL)	Green
1+ (250mg/dL)	Green brown
2++(500mg/dL)	Light brown
3+++ (1000mg/dL)	Darker brown
4++++ (>2000mg/dL)	Dark brown

Specific Gravity

The test pad contains bromthymol blue, poly(methyl, vinyl, ether / maleic anhydride) and sodium hydroxide. The principle for the reaction is based on pKa change of of ceratin pretreated polyelectrolytes in relation to ionic concentration” (Siemens, 2012). In the presence of the indicator the colour changes from blue green to darker green and yellow. The test is read at 45 s and increase in osmolality increase can be affected by the higher concentration of protein. The test is quantified as :1.000, 1.005, 1.010, 1.015, 1.020, 1.025, and 1.030 (Siemens, 2012).

3.2.4 CSF Evaluation using urine reagent strip

The technique of using CSF on a reagent strip for evaluation of leucocytes, protein and glucose has been well described in many studies (Parmar et al., 2004, Joshi et al., 2013). However the exact amount of CSF for each test pad and the time required for the reading of colour change specifically for CSF evaluation has not been well established. The reason why the author is following the instruction from the manufacturer for the urine analysis and is applying the instructions for the CSF evaluation.

One drop of CSF was removed from the first tube using pasteurs pipette and was dropped on the specific test pads and visually read after appropriate timing. In some cases the leucocyte reading was prolonged to 150 seconds especially when glucose was very high above 2000 mg/dL which was referred as 4++++.

In cases where the first drop of CSF did not cover adequately the test pad another drop was dripped on the test pad to cover the area. Visual reading was done comparing each pad with corresponding colour block on the bottle. Reading started with the test pad which required the shortest time. In this case it started with glucose at 30 seconds followed by, specific gravity, which was read at 45 seconds. Followed by blood, protein and nitrites all three tests were read at 60 seconds. Finally the leucocyte was evaluated at 120 seconds. All the readings were under natural light a photograph was taken and the results were recorded in patient's case record form.

Once the urine reagent strip test, for CSF evaluation was done the first two tubes were sent to the hospital's laboratory for biochemistry, cell count, gram stain, culture, pandy test and syphilis test if required. The other two tubes were stored at - 80 degrees for control of standard microbiology, PCR and cytokine test in the laboratory of Hauner's Children's Hospital in Munich, Germany. The laboratory technicians were not aware of the reagent strip results and received a laboratory chart sheet which was pseudonymized using a study number.

3.3 Patient evaluation and data recording

Once the patient was recruited a full physical examination was done, vital signs were taken and registered in patient's case report form. The blood glycemia was measured for every patient at the bedside and a rapid malaria test was done. In the case of patients who were already on fluid therapy and had the fluid or glucose bolus prior to lumbar puncture, this information was also recorded.

A full history was taken from patients who could speak or from parents whose children were not able to speak or had impaired consciousness. Signs and symptoms that were important variables for the study were clearly defined in order to avoid false interpretation by the study physician.

The important variables were:

1. Fever prior admission: this was subjective information provided by caregivers as "body being hot". Most of the caregivers do not have access to the thermometer so the exact temperature was not used for defining fever prior to admission.

2. Symptom duration: any symptoms (headache, fever, behavioural change, poor feeding, loss of appetite, feeling sick, vomiting or other) which started first before admission were defined as 0 to 24h, 24 to 48h or more than 72 hours.

3. Irritability: defined as an abnormal reaction to normal stimuli, in context of smaller children it was defined as crying or overreaction to touch or any sensitive stimuli (William C. Lloyd III, 2013).

4. Behavioural change: Any change to the response of the child to a sensorial, motor or painful stimuli which was not appropriate for the age. Possible inappropriate reactions included being overly calm or aggressive or just not wanting to play, feeling sleepy or having a slurred speech (Dictionary, 2009).

5. Coma: Was defined using 2 scales. Glasgow coma scale (GCS) and Blantyre coma scale (BCS). The definition of severe coma included GCS< 8 and BCS< 2. The Blantyre coma scale was used in children below the age of 2 years and for above the age of 2 years Glasgow scale was used. For children with disabilities a modified Glasgow coma scale was used (Chowdhury et al., 2001). In a coma patient, daily evaluation of GCS or BCS score was recorded, beside that every 8 hour evaluation was done either by the study doctor or attending physician and was recorded to find out the duration of coma.

6. Kernig sign: Performed with the patient in supine position, with knees and hip flexed the physician attempts extension of the knee. The sign was interpreted as positive when it was not possible to extend the knee beyond 135 degrees without causing pain (Ward et al., 2010). In cases where the sign was positive it was measured daily until the sign disappeared.

7. Brudzinki sign: There are several signs described by the Polish paediatrician Josef Brudzinski for meningitis. In our study we evaluated Brudzinski's neck sign. This test was performed with the patient in supine position. One hand of the physician is kept behind the head and other on the chest. Then, a flexing of the neck was attempted. The test was considered positive when a patient's lower limbs were flexed (Ward et al., 2010). As with the kerning this sign, was evaluated daily until the disappearance of the sign

8. Nuchal rigidity was defined as impairment or difficulty in flexing the neck (Maria and Bale, 2006).

9. Antibiotic use: Name, number of doses, dates of antibiotics were recorded, in cases, where an antibiotics was changed it was also referred.

3.4. Examination of the Cerebral Spinal Fluid

Laboratory examination of the CSF is usually the first step to confirm the presence of bacterial meningitis. Cytological examination preceded centrifugation. The typical findings associated with bacterial meningitis are following (WHO, 2011a):

- Turbidity
- Increased opening pressure (>180 mm H_20)
  Pleocytosis: PMN leukocytes and WBC count > 10 cells/ µl for older children, for infants it was considered 10-30 WBC/ µl with 50% PMN.
- Decreased glucose concentration (<45 mg/dL)
- Increased protein concentration (>45 mg/dL)

Once the CSF arrived in the Hospital's laboratory. The first tube was taken to hematology department and the second sterile tube was taken to the microbiology laboratory. The volume of the sample was noted for both tubes. In the Hematology department physical examination of the CSF, followed by cell count and Pandy test was done. While in microbiology laboratory gram stain, culture, Indian ink and antibiotic sensitivity test were done.

3.4.1 Physical Examination of Cerebro Spinal Fluid

The physical and cytological examinations were done in the Hematology Department of the Central Hospital of Beira. CSF physical was noted on the patient's laboratory sheet. Normally CSF is crystal clear, but in case of any pathology, it may be turbid or cloudy. The appearance was reported as turbid, clear, xantochromic, purulent or opalescent. The appearance is altered in different conditions and pathologies. For study purposes the turbidity was further divided in slightly turbid and turbid. The table 3.1 shows different colours of the CSF and conditions associated with.

Table 3.1 Cerebro-spinal Fluid Supernatant Colours and Associated conditions

CSF or supernatant color	Conditions or or Causes
Clear	Normal
Xanthochromia	
Yellow	Breakdown of blood products
Orange Pink	Blood breakdown products High carotenoid ingestion
Cloudy or turbid	CSF Leukocytes > 200 wbc/mm^3 RBC > 400 / mm^3
Opalescent slightly yellow	Tuberculous Meningitis
Opalescent to slightly purulent	Bacterial Meningitis
Green	Hyperbilirubinemia Bacterial Meningitis
Brown	Meningeal Melanomatosis

Source: (Seehusen et al., 2003, Fischbach, 2009)

3.4.2 CSF cell count and Pandy test

3.4.2.1 Cell count

The cytological examination based on the cell count was performed in a Neubauer chamber. White blood cells were differentiated into polimorphonuclear cell or lymphocytes and erythrocytes were counted. The chamber has 9 main squares which are further divided into smaller squares. When the CSF was clear the uncentrifuged CSF was diluted with one drop of crystal violet solution 1:1 dilution was preferred and then, it was directly inoculated in the groove. The WBC were counted under microscope (Ochei and Kolhatker, 2008, Cheesbrough, 2009). In case of the turbid CSF it was diluted 1:10 with crystal violet solution and then the cells were counted microscopically (Ochei and Kolhatker, 2008).

The calculation was done using the following formula for Neubauer chamber (Cheesbrough, 2009):

Cells in 1µl = n^o of cells in 4 main square x 25

When cell count was below 5 cell/µl then the cell differentiation was not performed and cell count above 5 cells/ µl were considered abnormal and differential was reported in percentages (Cheesbrough, 2009).

Glucose and protein chemistry was not done as there were no reagents available and the chemistry machine has not been functioning for past 8 years.

3.4.2.2 Pandy's globulin test

This semiquantitative test detects an increase in the level of globulins, which indirectly can be interpreted as an increase in total protein level of CSF by estimation. It is only of use when CSF total protein cannot be measured. However in some connective tissue disorder, multiple sclerosis and neuro syphilis the Pandy test may be positive due to presence of globulins despite the normal CSF levels of proteins (Cheesbrough, 2009). This is exactly what urine strip doesn't measure. By measuring the albumin level in the CSF it gives a better estimate of total protein in the CSF in comparison to Pandy test.

Procedure

First, 1 ml of the saturated phenol (reagent No 45) was pipetted into a glass test tube, then the laboratory technician added one drop of the supernatant of CSF to the phenol containing tube (not to be agitated) and observed for a reaction against a dark background. The procedure of adding the CSF supernatant was done at eye level, so immediate cloudiness that occurred could be observed (Cheesbrough, 2009, MISAU and Beira, 2010)

Reporting of the results were as follows:

A positive Pandy test was defined as any appearance of cloudiness within the CSF the sample. When no change occurred, it was reported as a negative Pandy reaction.
In case of low protein content, the Pandy reaction can be very minute, leading to a false negative. Therefore, it is necessary to maintain optimal circumstances for evaluation. In order to avoid missing cloudiness it was important to do the test at eye level for detection of even a slight reaction (Cheesbrough, 2009)

3.4.3 Cerebro Spinal Fluid Gram stain

The second sterile tube containing CSF was analysed in the microbiology department of the Central Hospital of Beira. Once the CSF sample arrived in the department the volume of the sample was noted. If less than 1 ml of CSF was available, then it was directly plated onto blood agar plate (BAP) and onto a chocolate agar plate (CAP) and the rest used for the Gram stain and Indian ink. When more than > 1 ml of CSF was available, then it was centrifuged according standard operating procedure defined by the Ministry of Health The CSF was centrifuged for three to five minutes at 1500 rpm to sediment bacteria for culture and gram stain (MISAU and Beira, 2010).

In order to guarantee precise results and avoid false negative, the author followed all the recommendation by Ministry of Health of Mozambique standard operating procedure on CSF handling and storage for microbiology as CSF stability varied a lot with environmental changes (MISAU and Beira, 2010).

The samples for microbiology were analyzed within 30 minutes at room temperature. If direct processing of sample was not possible, the samples were kept in an oven at 35 degrees for maximum of 12 to 24 h, especially when the lumbar puncture was done outside the laboratory working hours e.g at night or during the weekend (MISAU and Beira, 2010).

After centrifuging the sample, the supernatant was used for Indian ink for criptococcus detection. In case of tuberculous meningitis suspicion, sediments that remained after the culture was sent to TB diagnostic department for Ziehl Neelson test. When only one tube of CSF was collected, first analysis was done in the microbiology laboratory and what remained later was sent to hematology department for further analysis.

In cases when more than 2 ml of CSF was available, then TB culture and GeneXpert were ordered when tuberculous meningitis were suspected. GeneXpert is an automated technology, which uses a cartridge based amplification assay for detection of Mycobacterium tuberculosis and can also detect the rifampcin resistance, though the technology has been tested in sputum (WHO, 2014c) the local technicians are also using the GeneXpert for CSF analysis at the Hospital.

The sediment was used for gram stain and culture in the first few months till June 2013 after this period centrifugation was not done at the hospital as the centrifuge machine was broken, this could have further reduced the detection of bacteria through gram stain and culture.

3.4.3.1 Gram Stain

The gram stain gives a presumptive diagnosis and sometimes a definitive diagnosis when identification of species is possible (Fischbach, 2009). The procedure was done according to standard operating procedure (SOP) established by the ministry of health (MISAU and Beira, 2010)

Gram stain procedure:

- We centrifuged the clear CSF at about 3 to 5 min at the 1500 RPM rate. A few drops of the sediment were transferred to the slide and were spread uniformly in a thin film for staining procedure. In the case of a purulent CSF, it was not centrifuged and smear was made.
- Once the sediment was fixed to the slide and air dried the staining was started.
- First the sediment was covered by crystal violet stain for 60 s

- After 60 seconds the crystal violet stain was washed with clean running water and drained.
- The next step was to use a second stain iodine, which was also kept for another 60 seconds,
- After 60 seconds it was again washed and drained
- The last stain to cover the smear was safranine which also needed 60 seconds, then washed off again and air dried prior visualization by microscope.
- The first examination was done with 40x objective, to evaluate smear distribution, neutrophils and then observed with objective 100 x oil-immersions.
- Gram positive appeared dark purple (e.g. staphylococci, streptococci, micrococci, pneumococci, enterococci, diphtheria bacilli, anthrax bacilli) and gram negative pink (WHO, 2011a)

Reading the gram stain results:

These were read under the microscope as a gram positive organism when appeared as dark violet or purple and as gram negative when the microorganisms appeared as red or pink. N.meningitidis occurs extracellularly or intracellularly and were described as a gram negative kidney shaped diplococcic, Strepotococcus pneumoniae also occurs extra or intracellularly and were described as gram positive lanceolated diplococcic or gram positive diplococci in short chain, while gram negative organisms were described as gram negative bacilli and gram negative cocci (Cheesbrough, 2009, MISAU and Beira, 2010).

When neutrophils were observed this was an indicative of bacterial meningitis and was reported as polimorphonuclear leukocytes observed and were classified as few, moderate and many or lots (MISAU and Beira, 2010). Occasionally live bacteria were observed, but when CSF culture detection was not possible either because they were very few, in this case we reported as live bacteria observed and probable meningitis was considered if other results of CSF parameters were also altered (Cheesbrough, 2009).

A presumptive diagnosis of bacterial meningitis with gram stain was made when (Cheesbrough, 2009, MISAU and Beira, 2010):

a. If a negative intracellular or extracellular diplococci were observed it was suggestive of N.meningitidis.

b. If a gram positive diplococci or a short chain diplococcic were observed it was suggestive of streptococci which could be *S. pneumoniae*

c. When gram negative rod was observed it was suggestive of possible *H. influenzae* or *E.coli.* As *E.coli* too present as gram negative rod. Further attempt of differentiation was not done as reagents are not available locally.

3.4.4 CSF Culture

3.4.4.1 Storage and stability of the cerebral spinal fluid sample

According to MISAU's standard operating protocol the maximum time allowed for CSF to stay at the room temperature was 30 min without the transport media and 2 hours if the CSF was transported with the transport medium. When conditions did not allow for immediate inoculations of the sediment onto culture broth, it was recommended that the CSF must be kept at 35^0C in an incubator, until the inoculation was done, the next morning. All the samples that were collected after 3 pm were kept in the incubator so the sample could be evaluated the next morning, including during weekends (MISAU and Beira, 2010, WHO, 2011a).

3.4.4.2 Inadequate samples

The CSF samples were considered inadequate when:

1. There was a delay of 24 hours or more for evaluation as the sensitivity of detecting bacteria such as HIB and N. Meningitis both decrease as these do not tolerate low temperatures. Though sensitivity decreases the CSF was still cultured as per protocol of the Hospital (MISAU and Beira, 2010).

2. The quantity for evaluation was less than < 0.5 ml.

3.4.4.3 CSF Culture procedure

The culture procedure was done according to the standard operating procedure approved by the Ministry of Health. Before inoculating the sediment the culture media stored in the fridge at 2 to 8 degrees centigrade were brought to room temperature and were checked for any signs of contamination. If the plates seemed clear then the sediment process was carried out. The sediment from centrifuged specimen was dropped on a chocolate agar plate and a blood agar plate, streaked and incubated for 18 to 24 h at 35 to 37° C with ~5% CO2 in a candle-jar (MISAU and Beira, 2010).

The first evaluation of growth on the culture plate was done in 24 hours. The second reading was done at 48 h and a third at 72 hours. In case of not having observed bacterial growth the culture media plates were still kept till the seventh day. If after a week no growth was observed, then the culture plates were discarded (MISAU and Beira, 2010).

When bacteria grew identification and antibiotic sensitivity was done and noted in the patient's laboratory file.

3.4.4.4 Culture media for meningeal pathogen

There are different culture media for growth of meningeal pathogen. The blood agar plate contains a trypticase soy agar plate which contained 5% sheep blood. This media is ideal for the growth of S. pneumoniae. However occasionally the hospital did not have sheep blood, then human blood was used to make the blood agar plates. Though this is not recommended by WHO, because antibodies in the human blood could negatively impact the growth of *S. pneumoniae* (MISAU and Beira, 2010, WHO, 2011a).

Alternatively, we used chocolate agar plate when human blood was used for blood agar plate preparation for study patients (WHO, 2011a).

The chocolate agar plate is a medium which is supplemented with NAD (nicotinamide-adenine-dinucleotide) and V factor which is ideal for the growth of *H.influenzae* (WHO, 2011a).

While for *N.meningitidis* growth, both chocolate agar and blood agar plate were used and humid atmosphere was provided by adding water plate at the bottom of the incubator to enhance the growth of *N.meningitidis*. The culture media before being inoculated were brought to room temperature and were checked for any sign of contamination prior to inoculation (WHO, 2011a).

We followed the following procedure for inoculation of CSF to primary culture plate (MISAU and Beira, 2010):

- We inoculated 1 to 5 drops of CSF directly onto the blood agar plate and chocolate agar plate,
- When CSF was clear we centrifuged, and one drop of the sediment, was inoculated to the primary culture media.
- Plates then were streaked using a wire loop which was sterilized at each step.
- Blood and chocolate agar plate after inoculation process were incubated at 35-37 ^{0}C in a candle jar.
- Another broth with brain heart infusion (BHI) as a backup media was also prepared for each sample and was inoculated with some of the sediment pellet.

The first evaluation was done at 24 hours, second at 48 h. In case nothing grew plates were still kept till the 7th day. If on the 7th day, no growth of microorganism was observed than the culture plates were discarded (WHO, 2011a).

When bacteria were observed, identification and antibiotic sensitivity were done and recorded in the patient's laboratory file.

A presumptive diagnosis was made based on gram stain and growth of bacteria on culture media. Table 3.2 demonstrates characterization of colonies and identification of microorganism in different culture media used in the Central Hospital of Beira (MISAU and Beira, 2010).

Table 3.2 Summary characteristics of colonies observed and identification of microorganisms

Bacteria	Blood agar	Chocolate agar	Identification scheme
Neisseria meningitidis	< 1-2mm, transparent dew like colony, non hemolytic	< 1-2mm, transparent dew like colony	Neisseria
Streptococcus pneumoniae	< 1-2mm, greyish or green umbilicated or plane, (sometimes mucoid)α-hemolitic	< 1-2mm, 1-2mm, greyish or green umbilicated or plane, (sometimes mucoid)	Streptococcus
Haemophilus influenzae	Normally doesn't grow	1-2mm, greyis humid.	Haemophilus
Coliforms	1-3mm, grey, humid or mucoide, elevated some time show signs of hemolysis	1-3mm, grey, humid or mucoid, elevated, irregular border	Enterobacter
Streptococci β-hemolíticus	< 1-2mm, transparent, elevated ,β-hemolítica	< 1-2mm, white, elevated	Streptococcus

Identification and characterization of N.meningitidis

When N.meningitidis was suspected we performed Kovak's oxidase test.

The reagent of this test tetramethyl-p-phenylenediamine dihydrochloride turns into purple when in contact with organisms that contains the cytochrome c. This color change is the basis for the identification of N.meningitidis. This test is used for determining presence of the cytochrome oxidase which helps identification of N. meningitidis; however other members of the same group also produce a positive reaction (WHO, 2011a).

The preparation of the Kovak reagent was done in the following manner. We used the filter paper method for Kovak's oxidase test (MISAU and Beira, 2010, WHO, 2011a).

Procedure for Kovak Test:

- The filter paper strip was placed in a petri dish with a few drops of oxidase reagent;
- Then the filter paper was left to dry;
- After the paper was dry, the isolate grown on the blood agar plate was picked by a sterilized loop and rubbed into the oxide treated filter paper;
- Then the filter paper was observed for colour change which occurred within 30 seconds.

Reporting Kovak's test results

The colour change of the oxidase paper from white to purple was considered as a positive test (WHO, 2011a).

Identification of S. pneumoniae

Catalase test

Catalase is an enzyme that is found generally in most microorganisms. When catalase produced by microorganism is exposed to oxygen it causes degradation of hydrogen peroxide (H_20_2) into water (H_20) and oxygen (0_2).The production of oxygen is observed as bubbles on the slide (MISAU and Beira, 2010, WHO, 2011a).

The main purpose of this test is to differentiate between gram positive cocci, catalase producing microorganism like staphylococci, micrococci from microorganisms like streptococcus, enterococcus which are catalase negative (WHO, 2011a).

Some organism that have catalase activity are Echerichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus and Shigella dysnteriae (MISAU and Beira, 2010, WHO, 2011a).

However the test can give false negative or false positive results. One major limitation is the age of the culture. If the culture is older than 18 to 24 hours the capacity of the bacteria to produce catalase is reduced giving a false negative reaction. While another

important factor that causes false positive results occurs during the inoculation of a colony from the culture media if blood is also streaked with the colony. This blood from the media plate will produce a false positive reaction (WHO, 2011b).

Procedure for catalase test (MISAU and Beira, 2010, WHO, 2011a):

- The isolate were cultured on the blood agar plate at 35 to 37^{o} C for 18 to 24 hours in a candle jar;
- The next day a colony from the blood agar plate using a sterilized loop was placed on a microscope slide;
- One ml of 2% of H_2O_2 was added to the slide;
- In cases of catalase positive reaction, immediate bubbling was observed

Reporting of the test

Oxidase reaction was considered as positive when bubbling was observed and was considered as negative when no bubbling was observed (WHO, 2011a). Other tests for species identification of *S. pneumoniae* were not available and were not performed at the hospital

Identification of Stahylococcus

Coagulase test

Coagulase testing is a procedure for observation of cultured microorganism that has the capacity to produce coagulase enzyme which coagulates plasma. The conversion of fibrinogen into fibrin causes agglutination and coagulates plasma. The main objective of the test is to differentiate between the coagulase positive staphylococcus aureus from coagulase negative staphylococcus. This differentiation of coagulase positive and negative staphylococci, aids in the distinction between pathogenic or non pathogenic form of staphylococcus. The more pathogenic or virulent strain of staphylococci produced more coagulase (MISAU and Beira, 2010).

The test tube method for coagulase was used in our study to differ microorganism that produces coagulase from those which don't produce this enzyme. Once colonies of microorganism were observed in culture, especially if staphylococcus was observed further analysis was done to see if the staphylococcus species was coagulase positive or not (MISAU and Beira, 2010).

Coagulase procedure

- Three test tubes were used, one for positive control, one for the negative control and one for the sample test;
- Two ml of human plasma was placed in each glass test tube;
- To the negative control test tube a sterile broth was added
- To the positive control tube 0.2 ml of broth with a known colony of staphylococcus was inoculated and observed;
- To the 3rd "test" tube a 0.2 ml of culture broth was added;
- The colony from the culture medium plate was inoculated using sterile inoculation loop into the test tube containing plasma to avoid any contamination;
- The colony with broth was homogenized into plasma, but never agitated
- All the three test tubes were incubated for 2 hours at 37 ^{0}C for 2 hours;
- If no changes were observed than it was again left in the oven for an extra 2 hours and read again. If still no changes was observed it was left at room temperature for a day and then discarded the next day.

Reporting of coagulase results:

The reading was done as positive when plasma coagulation took place and negative when no coagulation occurred. In cases of no reaction it was still kept at room temperature overnight before the final reading, if by then no changes occurred it was considered as negative and the test was discarded. Positive cases were reported as "Coagulase positive staphylococcus aureus" (MISAU and Beira, 2010, WHO, 2011a).

3.4.4.5 Aerobic and nonaerobic differentiation of meningeal pathogen

Thioglycolate

A liquid broth media contains sodium thioglycolate which reacts to oxygen, keeping oxygen level. It's used to differentiate between aerobic and anaerobic pathogens. Once inoculated with the CSF, 24 hours later the reading was done.

When the growth was observed on the top of the medium it was considered as strong aerobic and when growth was observed at the bottom it was considered as strongly anaerobic.

3.5 Case definition for bacterial meningitis according WHO (WHO, 2003):

Clinical description

Bacterial meningitis is characterized by acute onset of fever (usually > 38.5 °C rectal or 38^0 C axillary), headache and one of the following signs: neck stiffness, altered consciousness or other meningeal signs and seizures.

Laboratory criteria for diagnosis

Bacterial meningitis can be confirmed by three methods. (1) Culture method: isolation of a bacterial pathogen from a normally sterile clinical specimen such as CSF or blood. (2) Alterations of CSF glucose, protein and presence of high number of leucocytes. (3) Gram stain results.

3.5.1 Case classification of bacterial meningitis according to WHO (WHO, 2003):

Suspected: Any child with sudden onset of fever (> 38.5 °C rectal or 38.0 °C axillary) and one of the following signs: neck stiffness, altered consciousness or other meningeal signs.

Probable: A suspected case with CSF examination showing at least one of the following:

- Turbid appearance;
- Leucocytosis (> 100 cells/mm3);
- Leucocytosis (10-100 cells/ mm3) and either an elevated protein (> 100 mg/dl) or decreased glucose (< 40 mg/dl).

Confirmed: A case that is laboratory-confirmed by growing (i.e. Culturing) or identifying (i.e. By Gram stain) a bacterial pathogen (Hib, pneumococcus or meningococcus) in the CSF or from the blood in a child with a clinical syndrome consistent with bacterial meningitis

3.5.2 Case definition according to modifies WHO criteria for study purpose

An appropriate history and physical examination accompanied by CSF pleocytosis, negative bacterial culture and gram stain for CSF and negative blood culture

For the study purpose we had to modify the WHO criteria for probable bacterial meningitis based on the available tests locally and these were as follow:

a. Suspicion case plus CSF turbidity plus either CSF leukocytosis or a positive Pandy test with negative gram stain or CSF culture.
b. Suspicion case plus either CSF turbidity or gram stain results with identification of a microbial agent or with the presence of live bacteria or with lots of neutrophils observed as defined by the laboratory but no confirmation was done on the aetiologic microorganism (Cheesbrough, 2009),
c. Suspicion case plus positive Pandy test plus cell count results between 10-100 cells/ mm^3. CSF glucose below < 40 mg/dl and CSF protein > 100 mg/dl were not added to the cell count information as referred by WHO guideline because CSF glycemia were not available in the Central Hospital of Beira, but the positive Pandy reaction was considered instead of CSF total protein.

d. Suspicion case plus CSF cell count above 100/ mm^3 alone were used for diagnosis of probable bacterial meningitis.

However, children who had cell count of > 100 cell with all other CSF laboratory tests negative and did not improve with antibiotic treatment within 72 hours and needed additional antiviral treatment were diagnosed as meningoencephalitis and for statistical analysis purpose they were included in meningoencephalitis group.

The gram stain that contained lots of polimorphonuclear leucocytes and live bacteria's visible were considered gram stain negative as no staining of bacteria were seen, but laboratory guideline (Cheesbrough 2009) clearly defines that in cases when neutrophils are observed in CSF specially more than 5, this is indicative of pyogenic meningitis though the gram stain is negative. Based on this information in our study children with symptoms of meningitis who had neutrophils present in the gram stain were considered as having probable bacterial meningitis.

For analysis purpose both confirmed cases and probable bacterial meningitis were considered as having diagnosis of bacterial meningitis.

3.6 Ethical clearance

The protocol for the study was revised and approved by the National Bio-Ethical Committee of Mozambique, and later approved by the Centre for International Health from Ludwig Maximillians University. Administration approval was acquired from the Central Hospital of Beira for conducting study. Administrative approval form Catholic University of Mozambique was acquired for storage of CSF at -80 degrees.

3.7 Statistical analysis

All manually written data from the patient's case record form was later entered in electronic case report forms in Microsoft Access 2010 and double checked. Later the data was analyzed via SPSS (Predictive Analytic Software and Solution) Version 16

(Texas –USA) for most of the analysis, Graphs and ROC curves were created using Medcal ® Version 13.1.2.0.

For continuous data: mean, standard deviation, interquartile range was calculated. Confidence intervals were estimated under the assumption of a normal distribution. For each laboratory variable; sensitivity, specificity, positive predictive value, negative predictive values were calculated. ROC curve was designed to find the best cutoff value for each parameter of the reagent strip. The Pearson chi square test was used to compare qualitative variables. The student t-test for independent sample was used for quantitative data to see the difference between the age and mean CSF WBC among children with cerebral malaria, bacterial meningitis and meningoencephalitis.

Univariate analysis of diagnostic variables was used and their unadjusted odds ratio were calculated as well as 95% Confidence Interval (95% CI). Variables that were found to have a p value ≤ 0.02 were used for multivariate logistic regressions which were calculated using the regression model for development of bedside diagnostic model which could predict bacterial meningitis based on clinical symptoms and urine reagent strip results. The multivariate logistic regression was calculated in SPSS using conditional logistic regression and Hosmer Lameshow index and Nagel kerke r was considered to evaluate goodness of fit of the model. Hosmer Lameshow above 0.05 was considered.

4. Results

4.1 Baseline characterization of the study population

4.1.1 Demographics

During the period of 15 months from November 2012 to end of January 2014, 250 patients were screened for bacterial meningitis in the Central Hospital of Beira from 6.30 a.m. to 9 p.m from Monday to Sunday.

From 250 patients screened, 200 underwent lumbar puncture, 10 were lost either because of an accident in lumbar puncture or it wasn't possible to get enough CSF for analysis, e.g. less than 0.5 ml

Therefore CSF samples from 190 pediatric patients were included in the study. From those, 10 were later excluded from the study (3 due to antibiotic use more than 48 hours, 3 due to age below 2 months, 2 due to use of steroids and 2 due to terminal AIDS). In the end 180 patients were included in the study.

The study involved 180 participants, out of which 68 (37.8%) were female and the 112 (68.2%) were male. The mean age was 4.09 years, with minimum age of 2 months and maximum of 13.3 years.

The table 4.1 summarizes other baseline characteristics of the study population. It can observed from the table that the most common presenting symptoms at admission were fever in 167/180 (92.7%) cases followed by seizure in 155/180 (86.1%), irritability in 103/180 (57.2%) and behavioural change was observed in 98/180 (54.4%) children.

Table 4.1 General baseline characteristics of study population

Characteristics of study participants	Values
Demographics of patients	
Age (mean, min and max)	4.09 yrs.(2 mo -13.3 years)
Gender	
Male -n^{o} (%)	112/180 (62.2)
Female -n^{o} (%)	68/180 (37.8)
Duration of fever prior admission	
< 24h- n^{o} (%)	41/167 (24.5)
24-48h- n^{o} (%)	38/167 (22.8)
>72h- n^{o} (%)	88/167 (52.7)
Medication prior admission	93/180 (51.7)
Antibiotic prior admission (total) - n^{o} (%)	76/93 (81.7)
One antibiotic prior admission - n^{o} (%)	44/76 (57.9)
Two antibiotics prior admission - n^{o} (%)	32/76 (42.1)
Antimalarial drugs prior admission- n^{o} (%)	34/93 (36.5)
Traditional treatment- n^{o} (%)	10/93 (10.7)
Malnutrition on admission - n^{o} (%) (Mild, moderate and severe combined)	23/180 (12.8)
HIV Status known at admission	10/180 (5.5)
Symptoms and signs at presentation	
Severe coma on admission (GCS< 10 or BCS< 2) - n^{o} (%)	60/180 (33.3)
Seizure on admission	155/180 (86.1)
1 episode of seizure- n^{o} (%)	62/155 (40.0)
2-4 episodes of seizure- n^{o} (%)	61/155 (39.3)
> 5 episodes of seizure - n^{o} (%)	32/155 (20.6)
Signs of hypovolemic shock at presentation	17/180 (9.4)
Other signs and symptoms	
Headache - n^{o} (%) (among the children who could speak)	65/115 (56.5)
Vomiting n^{o} (%)	79/180 (43.9)
Altered mental status - n^{o} (%)	98/180 (54.4)
Irritability- n^{o} (%)	103/180 (57.2)
Unable to feed- n^{o} (%)	81/180 (45)
Hepatosplenomegaly- n^{o} (%)	43/180 (23.9)
Bulging fontanel	36/70 (51.4)
Kernig sign - n^{o} (%)	27/180 (15)
Brudzinski sign- n^{o} (%)	35/180 (19.4)
Neck stiffness - n^{o} (%)	80/180 (44.4)
Opisthotonus	37/180 (20.5)

Among patients admitted 93/180 (51.7%) were already on treatment prior admission with less than 48 hours of treatment. Among those patients that were on treatment 76/93 (81.7%) were using either oral or parenteral antibiotic drug at admission. While 34/93 (36.5%) were on antimalarial drugs and 10/93 (10.7%) were on traditional medicinal prior admission. Among antibiotic users 44/74 (57.9%) were using one antibiotic (oral or parenteral) and the rest 36 (42.1%) were using 2 antibiotic drugs at admission.

Children who had a diagnosis of malaria and were on malaria treatment with coartem a combination of artemether and lumefantrine 34/93 (36.5%) developed seizures with change in mental status and were admitted with either diagnosis of severe malaria or cerebral malaria.

Among children on antibiotic treatment 55/76 (72.3%), almost a third had no diagnosis prior admission and were on empiric treatment for bacterial infection. Only 21 (27.7%) children with antibiotic had a diagnosis of upper respiratory tract infection and piodermatitis during the time of admission.

Most of the children came to the hospital in an advanced stages. Among 60/180 (33.3%) of children had severe coma on admission. While among children with seizures which accounted for 155/180 (86.1%) of cases a 62/155 (40%) came with one episode to the hospital while two third of these children only presented to the hospital when they had more than 2 episodes of seizures. Among those children with more than 2 episodes of seizures a 61/155 (39.3%) of children presented to the hospital when they had between 2-5 episodes of seizures. While a 20.6% (32/155) were brought to the hospital after having more than 5 episodes of seizures at home. This data demonstrates that children often arrive to the hospital in a later stage.

The table 4.2 summarized the baseline characteristic of the laboratory parameters among the study population. A mean peripheral WBC count among children admitted

was 16.44% with a SD of 10.48, and mean neutrophil count was of 62.10% with SD 19.08. Mean Lymphocyte were 26.21% with a SD of 16.1.

Table 4.2 General baseline characteristics of laboratory parameters of the study population

Laboratory parameters	**Values**
Full blood count	
WBC (mean ± SD)	16.44 ± 10.48
IQR	[10.00-19.25]
Neutrophil % (mean ± SD)	62.10 ± 19.08
IQR	[49-77]
Lymphocyte % (mean ± SD)	26.21 ± 16.1
IQR	[13-39]
Platelets 10^3 (mean ± SD) IQR	353.7 ± 211.7 [175-489]
ESR (mean ± SD)	46.6 ± 19.5
CSF parameters	
Pleocytosis (WBC cell > 10 cells) - n^o (%)	43/168 (24.1)
Positive CSF culture- n^o (%)	17/180 (9.4)
Positive gram stain- n^o (%)	17/180 (9.4)
Positive Pandy test- n^o (%)	20/160 (12.5)
Other tests	
HIV was not done- n^o (%)	72/180 (40)
HIV test done n =108	
HIV positive- n^o (%)	26/108 (24.1)
HIV negative- n^o (%)	82/108 (75.9)
Malaria test	
Positive rapid test - n^o (%)	40/180 (22.2)
Positive thick and thin blood film- n^o (%)	29/180 (16.1)
Blood culture n^o (%)	1/10 (10)

FBC was measured in 169 patients, Neutrophil % was present in 152, lymphocytes % in 159, platelets in 169 and ESR was measured in 10 patients

At admission only 5.5 % (10/180) patient's guardian knew their children's HIV status. However during admission HIV test was only done among 108/180 (60%) of cases. Among those who had tested for HIV 26/108 (24.1%) had a positive test and the rest 82/108 (75.9%) had a negative test. The table 4.3 demonstrates discharge information on the patients.

Table 4.3 General baseline characteristics on discharge information of the study population

Most frequent diagnosis at discharge	
Meningoencephalitis- n^{o} (%)	48/180 (26.6)
Severe malaria n^{o} (%)	16/180 (8.9.)
Cerebral malaria n^{o} (%)	21/180 (11.6)
Bacterial meningitis- n^{o} (%)	32/180 (17.7)
Pneumonia - n^{o} (%)	20/180 (11.1)
Tuberculous meningitis	5/180 (2.7)
Suspected meningitis	7/180 (3.9)
Others	31/180 (17.7)
Patients survived- n^{o} (%)	155/180 (86.1)
Patient died- n^{o} (%)	22/180 (12.2)
Patient abandoned- n^{o} (%)	3/108 (1.7)
Complications	67/180 (37.2)
Respiratory failure- n^{o} (%)	16/180 (8.9)
Cerebral abscess- n^{o} (%)	2/180 (1.1)
Cerebral infarction - n^{o} (%)	3/180 (2.8)
Hydrocephalus- n^{o} (%)	8/180 (4.4)
Subdural effusion- n^{o} (%)	2/180 (1.1)
Others- n^{o} (%)	36/180 (20)
Neurological sequelae	32/180 (17.7)
Paralysis (hemiplegic and quadriplegic) - n^{o} (%)	9/180 (5)
Minor and moderate behavior change- n^{o} (%)	6/180 (3.3)
Gait problems- n^{o} (%)	5/180 (2.8)
Hearing loss,	3/180 (1.7)
Vision alteration - n^{o} (%)	2/180 (1.1)
Others	7/180 (3.8)
Paralysis - n^{o} (%)	9/32 (28.8)
Quadriplegia n^{o} (%)	7/9 (77.8)
- Meningoencephalitis - n^{o} (%)	3/7 (42.8.)
- Bacterial meningitis	2/7 (28.5)
- Cerebral malaria	1/7 (14.2)
- Tuberculous meningitis	1/7 (14.2)
Hemiplegia n^{o} (%)	2/9 (22.2)

4.1.2 Patient's discharge information

The most frequent diagnosis observed in our study at discharge were meningoencephalitis 48 (26.6%), followed by malaria 37 (20.5%), bacterial meningitis in 32 patients with (17.7%), tuberculous meningitis 5 (2.7%) and 31 (17.7%) had mixed diagnosis as shown in table 4.3

Out of 37 (20.5%) children with malaria, 21 (11.6%) had the diagnosis of Cerebral malaria and 16 (8.9%) had the diagnosis of severe malaria. Among children with cerebral malaria laboratory confirmation by rapid test was 18/21 (85.7%) and the other 3 (14.2%) were confirmed with malaria slide.
While the 16 cases of severe malaria (8.9%) had the diagnosis either confirmed by rapid test at admission. Either from hospital or a prior rapid malaria test done in health centres within 10 days of admission or a positive malaria slide. Among those with cerebral malaria 11 cases also had comorbidity like: meningonencephalitis, probable bacterial meningitis, HIV and sepsis.

Diagnosis of bacterial meningitis was confirmed by positive culture only in 17 (53.1%) and the rest (46.9%) had a diagnosis of probable bacterial meningitis for analysis purpose final diagnosis of bacterial meningitis included both confirmed cases plus probable cases.

Tuberculous meningitis in 5 (2.7%) were not confirmed by laboratory parameters, but the diagnosis was based on clinical features, positive contact with a TB patient plus thrombocytosis and increased ESR or with predominance of either lymphocytes or monocytes in full blood count. One patient had a diagnosis based on CT scan results (hydrocephalus) while others were diagnosed by excluding other diagnosis like bacterial meningitis, meningoencephalitis, cerebral malaria and possible HIV encephalopathy.

The category of other diagnosis, which comprised of 31 patients, had different diagnosis. 17 (54.8%) with febrile seizures, 6 (19.3%) with sepsis out of which 4

(66.6%) had septic shock at admission. Other diagnoses were: 2 (6.2%) with ischemic stroke due to vasculitis, 2 (6.2%) with pulmonary tuberculosis, 1 (3.2%) cerebral abscess, malformation of vena galena 1 (3.2%) and 1 (3.2%) with cerebral metastasis due to retinoblastoma.

Among the 180 patients admitted to the study, 22 (12.2%) had died. The cause of death among these patients were six meningoencephalitis with either respiratory or multi organ failure (27.3%), five (22.7%) due to cerebral malaria, four (18.1%) due to septic shock, four (18.1%) due to bacterial meningitis, two (9%) due to Tuberculous meningitis and one (4.5%) due to a cerebral abscess.

Neurological sequelae were present in 32/180 (17.7%) patients at discharge. Severe paralysis accounted for 5%, minor and moderate behavioural change in 3.3%, gait problem (unable to walk and ataxic gait), 1.7 % presented hearing loss which included hypoacusia and deafness and 1.1 % had some kind of vision deficits: included blurred vision and difficulty in recognizing mother with children who could not speak and blindness in patient who were able to speak. Details with exact numbers of neurological sequelae are presented in Table 4.3.

Among the 32 children with neurological sequelae 9 (28.1%) developed paralysis. Out of which 2 (22.2%) had hemiplegic paralysis; one due to stroke and other due to meningoencephalitis. While the other 7 (77.8%) developed spastic quadriplegia. For whom meningoencephalitis accounted for 3 (42.9%) cases of quadriplegia, bacterial meningitis was responsible for 2 (28.6%) cases and one (14.8%) case due to cerebral malaria and one (14.8%) due to tuberculous meningitis was observed. The table 4.3 summarizes all the neurological sequelae observed.

4. 2 General characteristics and demographics of children with bacterial meningitis

Among 180 cases evaluated 32 (17.7%) were diagnosed as bacterial meningitis. The table 4.21 summarizes CSF characteristics and laboratory parameters of these children. The mean age among children with bacterial meningitis was 42.5 months ± SD 40.6 months range [2 mo - 132 mo], the gender was equally distributed.

Among 32 children with bacterial meningitis a positive HIV test was observed in 6 (26%) children. Among which 3 (50%) were on antiretroviral therapy for 2 to 3 years with no signs of terminal AIDS. While other 3 (50%) were on PMTCT (Preventing Mother –to-Child-Transmission) program with antiretroviral prophylaxis with a negative PCR on admission.

Malnutrition was observed in 6 (18.8%) of children with 2 (33.3%) having severe malnutrition: one with marasmatic kwashiorkor and one with marasmus. Most of the children (28/32) were psychomotorically normally developed. Four (12.5%) had delayed development on admission; none had any neurological disability at admission.

The most frequent presenting signs and symptoms among children with bacterial meningitis were: Seizures 27 (84.4%), severe coma: 14 (43.8%), agitation 12 (37.5%), incoherent speech 8 (25%), signs of shock at admission 4 (12.5%) and fixed gaze 3 (9.3%) was observed.

Seizure was one of the most frequently observed symptoms among the admitted children. From these children 7 (21.9%) had one episode of seizure at admission, 12 (37.5%) had between 2 to 4 seizures while 8 (25%) had more than 5 episodes of seizures. Three children were admitted in status epilepticus.

Headache was observed in 13 (40.6%), behavior change in 28 (87.5%), irritability 25 (78.11%) and 19 (59.4%) were unable to feed or eat at admission. Opisthotonus was present in 12 (37.5%), photophobia was observed in 11 (34.4%) and nuchal rigidity in 27 (84.4%) patients. A bulging fontanel was present in 7 (21.9%) of children with BM.
Symptoms duration varied among children with BM: 9 (28.1%) had symptoms less than 24 hours, 2 (6.2%) between 24 to 48 hours and 21 (65.6%) had symptoms for more than 72 hours prior admission.

On physical examination the main vital signs were as follow: mean systolic arterial pressure was 92 mmHg $\pm$ 14.8 with a range [66 - 137], the mean respiratory rate was 32 $\pm$ 11.6 [22 – 68], the mean heart rate was 123 $\pm$ 24.9 [88 – 160] and the mean temperature in centigrade (^{0}C) at admission was 38.3 $\pm$ 0.8 [37.5 – 40].

Antibiotic treatment prior admission in the study was given in 15 (46.8%) patients, but none had received antibiotic for more than 48 hours. The most frequently used antibiotic was Penicillin G 6/15 (33.3%), 2/15 (13.3%) received metronidazole, 2/15 (13.3%) received ceftriaxone. Five (33.3%) received other antibiotics.

Comorbidities among children with BM

The comorbidities observed in our study among children with bacterial meningitis were:
Pneumonia with 6 / 32 (18.8%)
Malaria 6 / 32 (18.8%)
Sepsis 2/32 (6.2%)
Otitis and cellulitis 2/32 (6.2%)
Femur fracture 1/32 (3.1%)

4.3 Results of Cerebrospinal Fluid Analysis

4.3.1 CSF leucocyte count

Among 180 samples of CSF collected, 125 (69.4%) were considered normal based on a CSF cell count below 10 cell / mm^3. Forty three (23.9%) were considered as having

pleocytosis (wbc >10 cell / mm^3). In twelve (6.7%) cases, samples had missing data on CSF cell count. Among all CSF with normal cell count 7 (5.6%) had positive gram stain and 9 (7.2%) had positive culture

Among the 43 (23.9%) samples with abnormal cell counts, the average cell count was 311.56 ± 421.23 with an (Inter quartile range) IQR of [22-363]. Out of these 43 samples only 7 (41.2%) samples had a positive gram stain results and 6 (35.2%) samples had a positive CSF culture results.

The diagnose of bacterial meningitis for analysis purpose was based on confirmed cases (which included culture positive, or gram stain positive) plus probable bacterial meningitis based on modified WHO criteria for bacterial meningitis. In our study we had 32/180 (17.7%) cases of bacterial meningitis. Out of which 17/32 (53.1%) were gram stain and culture positive. While cell count of above 100 cells were observed 17/32 (53.1%) and positive pandy test among bacterial meningitis was observed in 13/32 (40.6%) of cases

Accuracy of CSF cell count in detection of bacterial meningitis based on a gold standard (positive CSF culture) in comparison with a diagnosis of bacterial meningitis (Confirmed plus probable) was tested.

Table 4.4 Demonstrates the accuracy of cell count in detection of bacterial meningitis based on culture results above 10 cell/ul

CSF cell count	Culture positive BM	Culture negative BM
>10 cells/ul	6	37
<10 cells/ul	9	116

Sensitivity = 40%, specificity = 75.8%, NPV = 92.8%, PPV = 13.9%

The sensitivity of the CSF cell count above 10 cell count for diagnosis of culture positive bacterial meningitis was of 40%, specificity of 75.8%, PPV of 13.9% and a NPV of 92.8%. Among the CSF samples with a normal cell count below 10 cells/ul 9/15 (60%) had a negative culture while more than 10 cell counts only 6/15 (40%) had a positive CSF culture. The table 4.4 demonstrated that most of the culture positive bacterial meningitis 60% of the patients had normo cellular bacterial meningitis.

Based on WHO modified criteria for diagnosis of bacterial meningitis an additional 16 (46.9%) patients were considered to have bacterial meningitis, totaling 32 cases of bacterial meningitis (confirmed plus probable). We tested the accuracy of the CSF wbc cell count for diagnosis of BM at 100 cells/mm^3 cutoff point.

Table 4.5 demonstrates CSF cell count accuracy in detection of bacterial meningitis at > 100 cell/ul

CSF pleocytosis	Bacterial meningitis	No bacterial meningitis
>100 cells	17	26
<100 cells	13	112

Sensitivity = 56.7%, specificity = 81.8%, NPV = 89.6%, PPV = 39.53%

According to the table 4.5 CSF cell count with a cutoff point above 100 cell/ul for diagnosis of bacterial meningitis the sensitivity of cell count was only 56.7% a specificity of 81.8% a NPV of 89.6% and PPV of 39.53%. Overall accuracy of the test was 76.7%.

4.3.2 CSF Pandy test

A semi quantitative Pandy test which measures globulin level and indirectly gives an estimate of total protein in CSF was performed in 160 (88.9%) patients. In twenty cases the data was missing or analysis was not done (11.1%). Out of 160 samples that had Pandy test, 21 (13.1%) had positive results.

Table 4.6 Accuracy of pandy test in the detection of bacterial meningitis (confirmed plus probable)

Pandy test	Bacterial meningitis	No bacterial meningitis
Positive	13	8
Negative	17	122

Sensitivity = 43.3%, specificity = 93.8%, NPV = 87.7 %, PPV = 61.9%

The sensitivity and specificity of pandy test in detection of protein in cases of bacterial meningitis was of 43.3% a specificity of 93.8%, a NPV of 87.7% and a PPV of 61.9% with an overall accuracy of the test 84.3%.

4.4 CSF Evaluation with reagent strip

4.4.1 Leucocytes

The urine reagent strip was tested in 180 samples among these 95 (52.8%) were considered normal or with no alteration and 85 (47.2%) showed alteration for leucocytes. We compared the accuracy of the reagent strip in detecting leucocytes in the CSF and compared to CSF wbc cell count done in the laboratory at different cutoff values. First to test the capacity of urine strip detection of cell count and secondly to test the performance of urine strip and compare with the standard cell count method for diagnosis of bacterial meningitis.

The sensitivity and specificity of the “trace” (defined by value of 0 to 15 cells/ul) reading on the urine dip strip to detect elevated CSF count (defined by value of > 10 cells/ul) was calculated.

Table 4.7 Capacity of the urine reagent strip to accurately detect leucocyte alteration in the CSF at the cutoff point of above 10 cells /ul

Reagent Strip	CSF >10 cells	CSF ≤ 10 cells
Trace positive	37	40
Trace negative	6	85

Sensitivity =86 %, specificity= 68%, NPV=93.4%, PPV= 48%

Table 4.8 Capacity of the reagent strip to accurately detect leucocyte alteration in the CSF at the cutoff point of above 100 cells/ul

Reagent Strip	CSF>100 cells	CSF ≤ 100 cells
2 cross	19	22
Negative	4	122

Sensitivity = 82.6%, specificity = 84.7%, NPV = 96.3%, PPV = 46.3%

When we tested the accuracy of the reagent strip at “trace” and at “2 cross” to detect wbc cell changes in CSF we observed that the sensitivity at “trace” was higher with 86% but a specificity of 68%, and NPV of 93.4%, were lower in comparison to “2 cross”.The “2 cross” cutoff point had a lower sensitivity with 82.6% but a higher specificity with 84.7% and NPV of 96.3%.

We tested correlation among leucocyte count of Multistix urine reagent strip and CSF cell count at 10 cells/ul and 100 cells/ul as cutoff point results using a Pearson chi square and continuity correction for their significance. Table 8 shows the correlation between the 2 tests and their significance levels.

The table 4.9 and 4.10 demonstrates the Pearson chi square correlation between the two types of tests.

Table 4.9 Pearson chi square test for CSF wbc vs urine reagent strip at "trace" as cut off point

	CSF wbc cell count vs Urine reagent strip Value	Asymp Sig(p-value)
Pearson Chi-square	37.644	<0.001
Continuity correction 2x2	35.489	<0.001
Likelihood ratio	40.258	<0.001

Table 4.10 Pearson chi square test for CSF wbc vs urine reagent strip at "2 cross" as cut off point

	CSF wbc cell count vs Urine reagent strip Value	Asymp Sig(p-value)
Pearson Chi-square	48.58	<0.001
Continuity correction 2x2	44.971	<0.001
Likelihood ratio	41.781	<0.001

We found a significant correlation between the urine reagent strip leucocyte evaluation at "trace" with CSF wbc cell count ($p < 0.001$) and at "2 cross" cut off point with a ($p < 0.001$) at 99.9% confidence level

Once the significant correlation was established among the two tests. We further tested urine reagent strip capacity to detect cell alterations in bacterial meningitis (confirmed + probable).

Table 4.11 Results of urine reagent strip leucocyte among children with and without bacterial meningitis

Reagent strip reading	Bacterial meningitis n= 32	No Bacterial meningitis n= 148
Negative	5	90
Trace	0	20
1 cross	3	16
2 cross	4	9
3 cross	20	13

Table 4.12 Accuracy of the reagent strip to detect cellular alteration in bacterial meningitis at different cut off points "trace" and "2 cross"

Reagent Strip	Bacterial meningitis n=32	No bacterial meningitis n= 168
Trace positive[a]	27	58
Trace negative[a]	5	90
2 cross[b]	24	22
negative[b]	8	126

[a]Sensitivity = 84.3%, specificity = 60.8%, NPV = 94.7%, PPV = 31.7%

[b]Sensitivity = 75%, specificity = 85.14%, NPV = 94.0%, PPV = 52.1%

Bacterial meningitis was present in 27/32 (84.3%) of the cases when urine reagent strip showed a "trace" reading. With a sensitivity of 84.3% a specificity of 60.8%, a NPV of 94.7% and a PPV of 31.7%. In comparison with 75% of sensitivity observed at "2 cross" as cut off point, a specificity of 85.14% a NPV of 94.0% and a PPV of 52.1%. However though the sensitivity of "trace" was higher than "2 cross" specificity of "trace" was lower with 60.8% in comparison with 85.14% observed in "2 cross" cut off point.

As per bacterial meningitis diagnostic criteria using cell count of > 100 cells/ul is considered. We used the "2 cross" which represents more than 125 cells /ul for best cut off value for the diagnosis of bacterial meningitis.

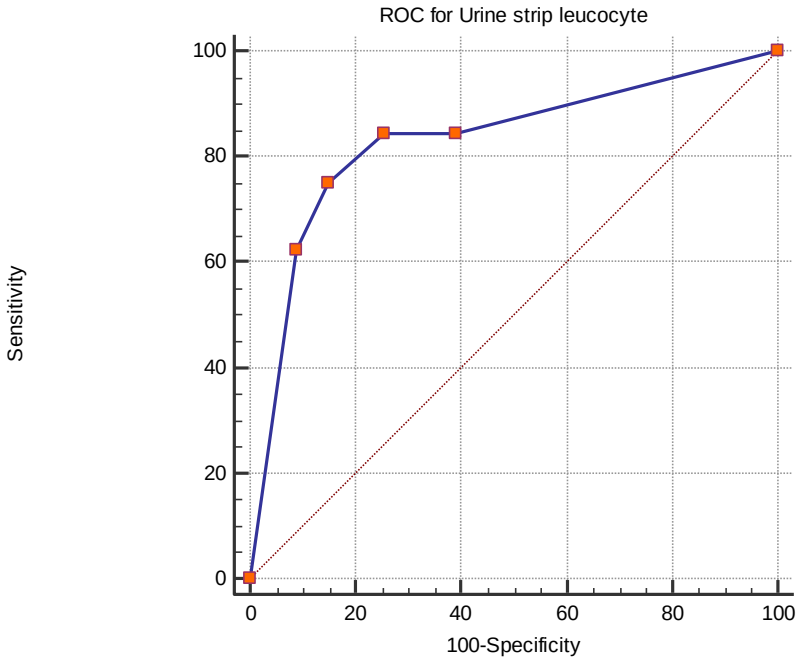
To further determine the best cutoff point for leucocyte for detection of BM we constructed ROC (receiver operating curve).

4.3.2 Construction of ROC (receiver operating curve) to determine cut off point for leucocyte

To conclude a better cut off point for the diagnosis of bacterial meningitis using reagent strips to evaluate leukocytes alteration a ROC curve was drawn to conclude the best possible cutoff point for the reagent strip test.

On the basis of the results of sensitivity and specificity of leukocyte from reagent strip Multistix, ROC was constructed. On the Y axis sensitivity values are shown while in X axis specificity is demonstrated. Along the curve different cut off points were evaluated.

Fig. 4.1 Demonstrates ROC curve for urine strip leucocyte for diagnosis of bacterial meningitis



AUC 0.830, SE 0.074, 95% CI [0.6886-0.974] p<0.0001

The AUC (area under the curve) for urine leucocyte strip was 0.830 AUC 0.830 with a SE 0.074 at 95% CI [0.6886-0.974] p<0.0001.

As bacterial meningitis typically leads to CSF cell count above 100 cells /ul. Therefore a reading of 2 cross of the urine dip strip (which represents leucocyte above 125 cells /ul) was used as the best cutoff point for diagnosis of bacterial meningitis.

The CSF cell count sensitivity for diagnosis of bacterial meningitis at 100 cells as cutoff point in comparison with urine strip at 2 cross for the diagnosis of bacterial meningitis were compared. The table 4.13 summarizes diagnostic performance of both CSF wbc and leucocyte from urine reagent strip from table 4.5 and 4.12.

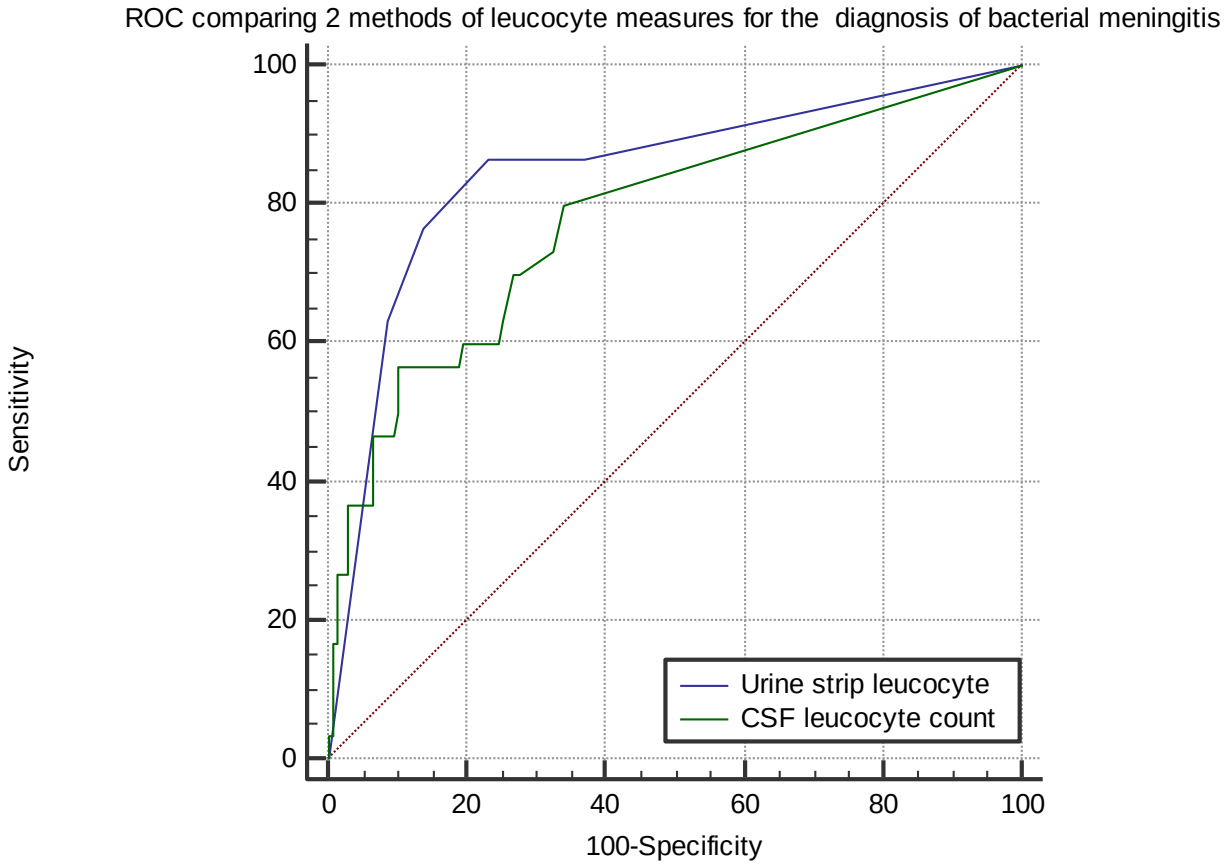
Table 4.13 Comparison of leucocyte measures of urine strip and CSF for diagnosis of bacterial meningitis

Leucocyte	Sensitivity %	Specificity %	NPV %	PPV %
CSF cell count	56.7	81.8	89.6	39.53
Urine strip at 2 cross	75	85.14	94.0	52.1

The urine strip performed better with a sensitivity of 75% in comparison to 56,7% of CSF cell count, a specificity was also higher with 85.14% in comparison to 81,8% of CSF cell count and so were the NPV of 94.0% and PPV 52.1% in comparison to NPV of 89.6% and PPV of 39.53% of standard leucocyte count.

We also compared the performance of cell count and strip leucocyte for the diagnosis of bacterial meningitis with ROC curve. The fig.4.2 demonstrates comparison of two ROC for measurement of leucocytes in diagnosis of bacterial meningitis

Fig 4.2 Pairwise comparison of two ROC for measurement of leucocytes in diagnosis of bacterial meningitis



Comparison of the 2 ROC for the diagnosis of bacterial meningitis

Sample size: 168
BM: 30
No BM: 138

Table 4.14 Pairwise comparison of ROC for 2 methods of leucocyte measures in diagnosis of bacterial meningitis

	AUC	**SE[a]**	**95% CI[b]**
Csf Cell count	0.780	0.0487	0.709 to 0.840
Strip leucocyte	0.848	0.0424	0.784 to 0.898

[a] De long et al, 1998
[b] Binomial exact

Difference between the area 0.0680, SE, 0.0555, 95% CI [0.0408 – 0.177], p = 0.2204
Though the performance of the urine strip for leucocyte count in detection was higher than the CSF cell count the difference was not statistically significant with a p = 0.2204. However though statistically insignificant this difference is clinically significant as the urine dip strip reading takes only 2 minutes and it can be done at patient's bedside as one person procedure. While the CSF cell count may take up to 2 to 3 hours in our hospital, it requires a trained technician and laboratory facilities (Moosa et al., 1995, Cheesbrough, 2009).

This statistical insignificance could be due to smaller sample size of children with bacterial meningitis among compared groups due to missing data 6.6 (12/180) on CSF cell count. Based on the clinical relevance the urine strip results were used for simple logistic regression analysis and for multivariate logistic regression analysis.

4.4.2 Protein

Among the 180 samples of CSF collected, 145 (80.5%) showed a positive reaction (including trace and 1 cross which measure protein level below 30 mg/dl). As bacterial meningitis typically leads to a CSF protein concentration of 100 mg/dL or higher. Therefore, the "2 cross" reading which represents a protein concentration of 100 mg/dL was used. Based on this cut off point at 2 crosses 69 (38.3%) were considered positive for protein and 111 (61.7%) were considered normal.

As there is no information in the literature regarding an equivalent cutoff value for Pandy test's detection capacity, of total protein in the CSF; we attempted to develop a cutoff point for protein detection for Pandy test at various levels to determine which value in the urine strip was the best cutoff point for Pandy test.

Table 4.15 Accuracy of reagent strip for detection of protein in pandy test at "2 cross" and at "3 cross"

Reagent strip	Pandy positive	Pandy negative
2 cross[a]	17	42
Negative	2	99
3 cross[b]	12	15
Negative	10	133

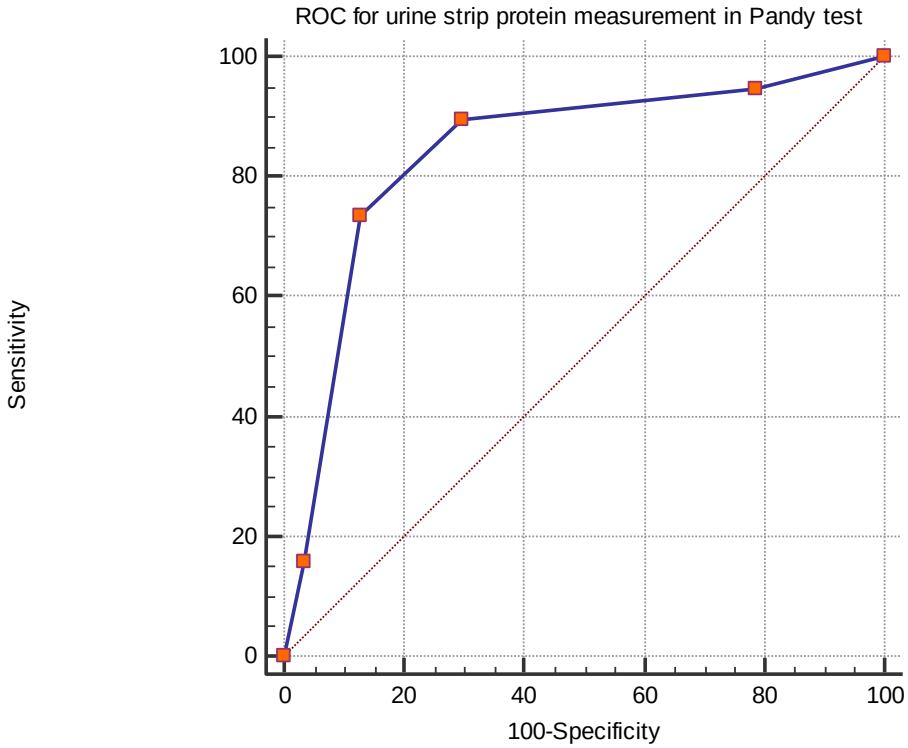
[a] Sensitivity = 89.4%, specificity = 70.2%, NPV = 98%, PPV = 28.8%

[b] Sensitivity = 68.7%, specificity = 89.8%, NPV = 98.2%, PPV = 28.8%

When compared reagent strip accuracy in detecting protein alteration at 2 cross (equivalent to 100 mg /dL) the reagent strip demonstrated a better sensitivity with 89.4 % in comparison with 68.7% at "3 cross" (equivalent to 300 mg /dL), while specificity was higher with 89.9% at "3 cross" in comparison to 70.2% observed in "2 cross". The negative predictive value didn't show alteration with 98.2% and 98% at 3 and 2 crosses respectively.

The best cutoff value with higher sensitivity in detecting proteins in pandy test is at "2 cross" for urine strip. The overall performance of the urine strip to evaluate results of pandy test was demonstrated via ROC. This is demonstrated via Fig. 4.3.

Fig. 4.3 the ROC to demonstrate overall performance of the urine strip to detect protein in pandy test



AUC 0.841, SE 0.0520, 95% CI [0.775 – 0.894], p <0.0001

Sensitivity: 73.68%, specificity 87.23

The urine strip capacity to detect protein in comparison to pandy test which is the standard method at the Central Hospital of Beira, had an overall sensitivity of 73.68% a specificity of 87.23% and an AUC of 0.841 with SE 0.0520 at 95% CI [0.775 – 0.894], p <0.0001.

Urine strip protein alteration in bacterial meningitis

Once it was established that the urine strip has the capacity to detect protein in the CSF and is comparable to local standard method. Sensitivity and specificity of urine strip protein in diagnosis of bacterial meningitis was calculated. A comparison of the two tests (urine strip and pandy) was made for diagnosis of bacterial meningitis. The table

4.13 demonstrated results of protein strip in children with and without bacterial meningitis.

The table 4.16 demonstrates results of protein strip in children with and without bacterial meningitis.

Reagent strip reading	Bacterial meningitis n= 32	No Bacterial meningitis n= 148
Negative	3	32
1 cross	4	72
2 cross	3	29
3 cross	16	10
4 cross	6	5

Table 4.17 Accuracy of the reagent strip to detect protein alteration in bacterial meningitis at different cut off points "2 cross" and at "3 cross"

Reagent Strip	Bacterial meningitis n= 32	No bacterial meningitis n= 148
2 cross positive[a]	27	58
Negative[a]	5	90
3 cross[b]	24	22
negative[b]	8	126

[a]Sensitivity = 78.12%, specificity= 70.27%, NPV= 93.7%, PPV= 36.2%

[b] Sensitivity = 75.0%, specificity= 85.1%, NPV= 94.03%, PPV= 52.1%

When reagent strip accuracy in detecting protein alteration at 2 cross (equivalent to 100 mg /dL) among children with bacterial meningitis was calculated, the urine reagent strip demonstrated a better sensitivity with 78.12 % in comparison to 75% at "3 cross" (equivalent to 300 mg /dL), while specificity was lower with 70.27 % at "2 cross" in comparison with 85.1% observed in "3 cross". The negative predictive and positive predictive value were also lower at "2 cross" with 93.7% and 36.2% in comparison to "3 cross" with 94.03% and 52.1%. However the results of sensitivity were more important

in this case for early detection of bacterial meningitis and “2 cross” was used as best cut off value was used.

Comparison of urine strip and pandy test for diagnosis of bacterial meningitis

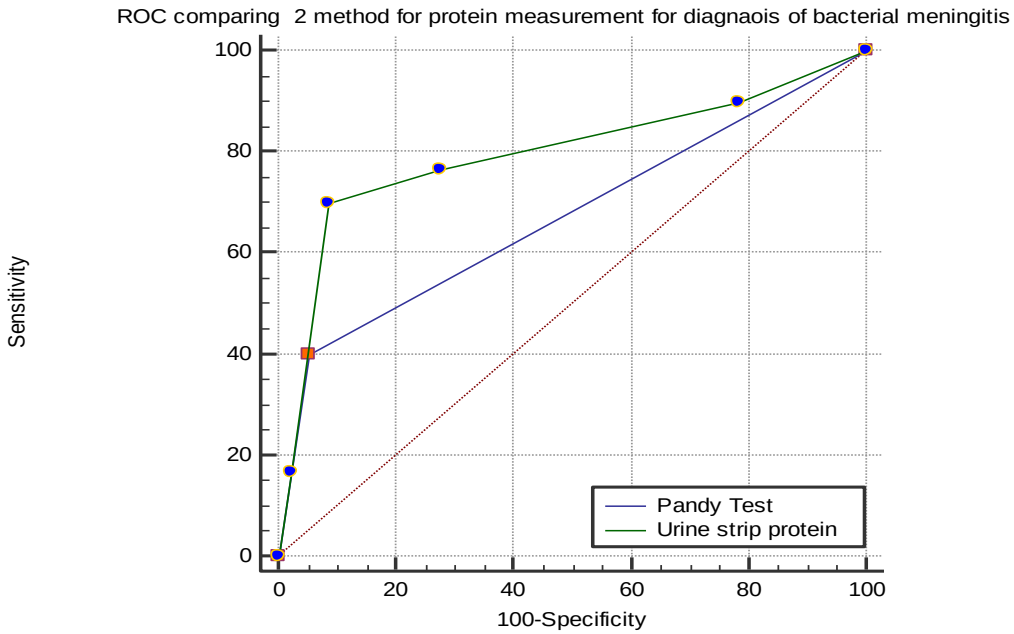
When CSF protein alteration in diagnosis of bacterial meningitis was compared the urine strip demonstrated better sensitivity in comparison to pandy test. The table 4.15 summarizes diagnostic performance results from table 4.5 and 4.14.

Table 4.18 Comparison of protein measures for reagent strip and pandy test for diagnosis of bacterial meningitis

Protein	Sensitivity %	Specificity %	NPV %	PPV %
CSF Pandy test	43.3	93.8	87.7	63.1
Urine strip at 2 cross	78.12	70.27	93.7	36.2

The urine strip performed better with a sensitivity of 78.12% in comparison to 43.3% pandy test, the specificity was lower with 70.27 % in comparison to 93.8% in pandy test. The NPV of 93.7 % in urine strip was higher than of the pandy test 87.7% but PPV 36.2% of urine strip was lower than the 63,1% observed in pandy test.

We also compared the performance of the urine strip protein with pandy test for the diagnosis of bacterial meningitis with ROC curve. The fig. 4.4 demonstrates comparison of the two ROC for measurement of protein in diagnosis of bacterial meningitis



The Fig. 4.4 demonstrates comparison of the two ROC for measurement of protein in diagnosis of bacterial meningitis

Comparison of the 2 ROC for the diagnosis of bacterial meningitis for protein alterations
Sample size: 160
BM: 30
No BM: 130

Table 4.19 Pairwise comparison of ROC for protein alteration in diagnosis of bacterial meningitis

	AUC	**SE[a]**	**95% CI[b]**
Csf pandy test	0.797	0.0544	0.727 to 0.857
Strip protein	0.673	0.0466	0.595 to 0.745

[a] De long et al, 1998
[b] Binomial exact

Difference between the AUC 0.124, SE, 0.0549, 95% CI [0.0167 – 0.232], p = 0.0236

As demonstrated via the pairwise comparison of ROC in fig 4.4 for urine strip protein has better performance in detecting protein alteration in bacterial meningitis in comparison to pandy test with a difference in the area of 0.124 with a SE, 0.0549 at 95% CI [0.0167 – 0.232], p = 0.0236, which is statistically significant.

4.4.3 Glucose

As there were no facilities present to measure the CSF glucose level, it was not possible to compare the urine dip strip glucose field with quantitative measurement. Therefore, only the connection between the dip strip reading and the diagnosis of bacterial meningitis was evaluated.

Table 4.20 Frequency of glucose reagent strip results among cases of bacterial meningitis

Reagent strip for glucose	**With Bacterial meningitis**	**Without bacterial meningitis**
Negative	16	18
Positive	16	130

Sensitivity = 50% specificity = 87.8%, PPV = 47.06%, NPV = 89.04%

Pearson chi square was tested among these categorical variables to test for independence. We observed a significant relationship between negative CSF glucose and bacterial meningitis, X^2 (1, N = 180) = 24.58, p <0.0001

Negative glucose was observed in bacterial meningitis patients and the strip demonstrated a sensitivity of 50% specificity of 87.8%, PPV of 47.06% and a NPV of 89.04%

4.4.4 Nitrate and density

Both variables were tested however no correlation was possible with bacterial meningitis so they were excluded from the study.

4.5 The Cerebrospinal fluid gram stain results

CSF gram stain was done in 180 patients. Among these 16 (8.9%) were considered positive and 144 (91.1%) were considered negative, however in 4 cases polimorphonuclear leucocytes were observed and in one active bacteria were observed. Among the 16 positive gram stain 1 (6.25%) was considered contaminated as CSF leucocyte and culture were both negative in this patient.

Table 4.21 Accuracy of gram stain among culture positive bacterial meningitis

Gram stain	Culture positive bacterial meningitis	Culture negative bacterial meningitis
Positive	14	2
Negative	3	161

Sensitivity = 82.35%, specificity= 98.77%, NPV= 98.17%, PPV= 87.5%

Table 4.22 Accuracy of gram stain among (confirmed and probable) bacterial meningitis

Gram stain	With Bacterial meningitis	Without bacterial meningitis
Positive	16	1
Negative	17	147

Sensitivity = 46.88%, specificity= 99.32%, NPV= 89.63%, PPV= 93.75%

The gram stain in our study demonstrated a sensitivity among the culture positive bacterial meningitis of 82.35% in comparison with 46, 88% bacterial meningitis (Confirmed plus probable). While the specificity was 98.77 % and 99.32 % respectively.

The most frequent microbial agents observed among the positive gram stain were 6 (37.5%) gram positive diplococci, 5 (31.3%) gram negative bacilli, 3 (18.75%) gram positive cocci in clusters and 2 (12.5%) gram negative diplococci.

4.6 Cerebrospinal fluid culture results

The 180 sample of CSF that were cultured only 17 (9.4%) were positive, 3 (1.6%) contaminated CSF was included in culture negative group. The sensitivity of culture among children with diagnosis based on clinical and other laboratorial parameters was 17 (53.1%) specificity of 100% a NPV of 90.8% and PPV of 100%.

Table 4.23 Accuracy of culture among all children with BM (confirmed and probable)

Culture	With Bacterial meningitis	Without bacterial meningitis
Positive	17	0
Negative	15	148

Sensitivity = 53.13%, specificity = 100%, NPV= 90.8%, PPV= 100%

4.7 Other laboratory tests for evaluation of bacterial meningitis

Blood culture were only done in 10 patients and only one person had a positive results who had septicemia. Because of a small sample size, blood culture findings were not considered in the statistical analysis.

For detection of mycobacterium tuberculosis Ziehl Nielsen was performed in 15 cases and indian ink was for detection of Crypytococcus meningitis was performed in all patients. However for both test (Ziehl Nielsen and indian ink) results were negative therefore were not included in statistical analysis.

The table 4.24 summarizes all the cases of bacterial meningitis and CSF characteristic.

Table 4.24 Summarizes the CSF characteristics of patients with bacterial meningitis

PT ID	CSF color	CSF cell count	Strip wbc	Pandy	Gram stain	Culture	Neurological sequelae
M001	Turbid	8	3+	+	Lots of PMN	-	No
M006	Clear	Missing	3+	+	GN bacilli	Positive	No
M013	Turbid	0	3+	-	-	-	No
M015	Clear	0	Neg	-	GN bacilli	Positive	Yes
M022	Clear	125	2+	-	GP diplococci	Positive	No
M043	Clear	322	Neg	-	GN bacilli	Positive	No
M044	Clear	58	Neg	-	GN bacilli	Positive	No
M046	Turbid white	304	3+	-	-	-	No
M048	Clear	3	1 +	-	GP diplococci	Positive	***
M056	Purulent	0	3+	+	-	-	Yes
M062	Clear	1020	3+	+	GP diplococci	Positive	Yes
M063	Purulent	4	3+	-	-	-	No
M071	Turbid white	42	3+	-	-	-	No
M079	Clear	0	Neg	-	GP diplococci	Positive	No
M080	Turbid	103	3+	+	GP diplococci	Positive	No
M086	Turbid yellow	38	3+	+	Bacteria observed	No growth	No
M094	Turbid	323	2+	-	GP diplococci	No growth	***
M103	Turbid	111	3+	-	-	-	Yes
M104	Turbid	missing	3+	+	GP diplococci	Positive	Yes
M105	Turbid	1020	3+	+	-	-	No
M123	Turbid	1841	3+	-	-	-	No
M136	Purulent	575	3+	+	Lots of PMN	No growth	Yes
M141	Clear	3	Neg	+	-	Positive	No
M154	Turbid	816	3+	-	Lots of PMN	-	Yes
M157	Turbid	3	3+	-	GP diplococci	Positive	No
M159	Turbid	595	3+	Missing	-	-	No
M164	Clear	0	1+	-	-	Positive	Yes
M172	Clear	0	2+	-	-	Positive	No
M186	Turbid	125	3+	+	GP diplococci	Positive	***
P01	Turbid	128	3+	+	-	-	***
P03	Clear	1	3+	+	GP diplococci	Positive	No
P05	Purulent	1	3+	+	GN bacilli	Positive	Yes

** Not applicable as patient died

4.8 Laboratory parameters and imaging among children with BM

The reagent strip was positive for leucocyte “trace” in 27/32 patients (84.4%). While the laboratory examination showed pleocytosis in thirteen patients (40.6%) only.
The elevated CSF protein level was detected using the dip strip in 22 patients (68.75%) while pandy test was positive in 13 out of 30 (43.3%) with BM. The reagent strip for protein detection in comparison with pandy test, demonstrated better capacity of protein detection among children with BM.

Culture results

Among 32 children that had diagnosis of BM 17 (53.1%) had positive culture. The identified bacterial were:
Streptococcus pneumoniae 5 /17 (29.4%)
Staphylococcus aureus (2 catalase positive) 3 /17 (17.6%)
Pseudomonas spp 3 /17 (17.6%)
Neisseria meningitidis 2/17 (11.76%)
Morganella morgagani 2/17 (11.76%)
Proteus spp 1/17 (5.9%)
Echerichia coli 1/17 (5.9%)

Culture results and antibiotic use

In order to understand the low detection rate of culture in diagnosis of bacterial meningitis and to observe if prior antibiotic had any effect we further analyzed the data for antibiotic use among culture positive and culture negative bacterial meningitis with a single antibiotic or a combination of 2 antibiotics.

Combination therapy was given to 6/10 (60%):
Ampicillin + ceftriaxone 1/10 (10%)
Ampicillin plus gentamycin 1/10 (10%)
Penicillin plus chloramphenicol in 4/10 (40%),

Single antibiotic was given to 4/10 (40%):

Oral metronidazole 1/10 (10%)

Ceftriaxone 1/10(10%)

Penicillin G 2/10 (20%)

We observed that out of 15 patients who had antibiotic prior admission 10 (66.6%) had culture negative BM. Among those who received antibiotics prior to admission therapy 6/10 (60%) had received two antibiotic drugs.

Among culture positive cases only 5/17 (29.4%) patients had antibiotic therapy. Most had oral therapy. One had received two doses of erythromycin, two patients were on cotrimoxazol prophylaxis and two children received parenteral antibiotic (1 x ampicillin; 1 x ceftriaxone) with a dose each.

Head ultrasound

Ultrasound was done among 7 children with bulging fontanel, of which 4(57.4%) were normal and 3 (42.8%) were abnormal. One child was shown to have involvement of the ventricles and two children had dilated ventricles and hyperechogenity of the meninges.

CT scan

Only one patient with BM had a CT scan done which demonstrated signs of cerebral edema and later after 21 days signs of hydrocephalus. The other 10 CT results belonged to children with TB meningitis, and children who developed neurological sequelae.

4.9 Treatment of bacterial meningitis in Central Hospital of Beira

Most children with bacterial meningitis at admission had combination of antibiotics:

Penicillin G alone was used in children above the age of 5 years and for < 5 years combination therapy was used:

Penicillin G plus gentamycin in 3/32(9.3%)

Penicillin G plus chloramphenicol in 3/32(9.3%)

Ampicillin plus chloramphenicol in 7/32 (21.8%)
Ceftriaxone and ampicillin in 8/32 (25.0%)

The rest of patients had a mono therapy with Penicillin 4/32 (12.5%) and 7/32 (21.8%) with ceftriaxone.

4.10 Logistic regression analysis

In order to create a predictive model for bedside diagnosis we did simple logistic regression analysis of all the signs and symptoms which were predictors of bacterial meningitis in our study. The table 4.22 summarizes all the variables used with unadjusted odds ratio. Only variable with p value < 0.02 were used in multilvariate logistic regression.

Table 4.25 Simple logistic regression analysis for baseline characteristics that are predictors of bacterial meningitis

Predictors	**BM n= 32 (%)**	**No BM n=148 (%)**	**Unadjusted Odds ratio [95%CI]**	**p- value**
Gender				
Male Female	16(50) 16(50)	96(64.8) 52(35.1)	0.54 (0.25-1.70) 1	0.19
Age				
0-2yrs >2 yrs	16(50) 16(50)	49(33.1) 91(61.4)	1.85(0.85 - 4.03) 1	0.1175
Predisposing conditions				
Pneumonia Yes No	 6(18.7) 26(81.2)	 23(15.5) 125(84.4)	 1.25 (0.46 -3.38)	 0.65
Otitis media/ sinusitis/periorbital cellulitis n=8 Yes No	 2(6.2) 30(93.8)	 6(4) 142(96)	 1.57(0.30 - 8.19) 1	 0.58
Undernourished Yes No	 6 (18.7) 26(81.3)	 17(11.5) 131(88.5)	 1.77 (0.64 to 4.93) 1	 0.26

Table 4.26 Simple logistic regression analysis of clinical characteristics predictors of bacterial meningitis

Predictors	BM n= 32 Number (%)	No BM n=148 Number (%)	Unadjusted Odds ratio (95%CI)	p- value
Fever				
Yes No	32(100) 0	133(90) 15 (10)	*******	****
Duration of symptoms				
0-48h >48h	11(34.3) 21(65.6)	76(51) 72(48.3)	0.49 (0.22 - 1.10) 1	0.08
Temperature				
< 38 >38	6(18.8) 26(81.2)	46(31) 102(68.9)	0.5.86 (0.19 – 1.32) 1	0.16
Behavior change				
Yes No	28(87.5) 7(21.8)	70(47.3) 78(52.7)	7.80 (2.60 - 23.34) 1	0.0002
Vomiting				
Yes No	21(65.6) 11(34.4)	58(39.1) 90(60.8)	2.96 (1.33 - 6.59) 1	0.0079
Irritability				
Yes No	25(78.1) 7(21.9)	122(82.4) 26(17.6)	0.76(0.29 -1.94)	0.56
Hepatosplenomegaly				
Yes No	6(18.8) 26(81.2)	37(25) 111(75)	0.69 (0.26 -1.81) 1	0.45
Bulging fontanel n=70				
Yes No	6(19.3) 25(80.6)	30(79) 8(21)	0.62(0.17 - 2.18) 1	0.45
Seizure				
Yes No	27(84.3) 5(15.6)	128(86.4) 20(13.5)	0.84(0.29 to 2.44) 1	0.75
Severe coma				
Yes No	10(31.2) 22(68.8)	44(29.7) 104(70.3)	1.07(0.47- 2.45) 1	0.86
Signs of shock				
Yes No	4(12.5) 28(87.5)	13(8.8) 135(91.2)	1.48(0.45 - 4.88) 1	0.51
Photophobia				
Yes No	11(34.4) 21(65.6)	30(20.3) 118(79.7)	2.06 (0.89 to 4.73) 1	0.08
Headache n=115				
Yes No	14(43.8) 18(56.2)	51(61.4) 32(38.6)	0.48 (0.21 - 1.11) 1	0.08
Unable to feed				
Yes No	19(59.4) 13(40.6)	62(41.8) 86(58.1)	2.02(0.93 - 4.41)	0.07

*Unable to calculate odds ratio as denominator is 0

Table 4.27 Simple logistic regression analysis of clinical characteristics predictors of bacterial meningitis cont.

Predictors	**BM n= 32 Number (%)**	**No BM n=148 Number (%)**	**Odds ratio [95%CI]**	**p- value**
Kernig sign				
Yes No	12(37.5) 20(62.5)	15(10.1) 133(89.9)	5.32(2.17 -12.99) 1	0.0002
Brudzinski sign				
Yes No	14(43.8) 18(56.2)	21(14.2) 127(85.8)	4.07(2.03- 10.86)	0.0003
Nuchal rigidity				
Yes No	22(68.8) 10(31.2)	56(37.8) 92(62.2)	3.61(1.59 - 8.18)	0.0021
Opistotonus				
Yes No	12(37.5) 20(62.5)	25(16.8) 123(83.2)	2.95(1.28 - 6.80)	0.011
HIV status n=108				
Yes No	6(26) 17(74)	20(23.5) 65(76.5)	1.14(0.39 - 3.30)	0.45

The most important predictors for bacterial meningitis in our study were behavior change with an OR 7.80(2.60 - 23.34) P = 0.0002, vomiting was also a strong predictor with an OR 2.96 (1.33 - 6.59) P = 0.0079, Kernig sign OR 5.32 (2.17 -12.99) P= 0.0003 for clinical features and for laboratory parameters specially urine reagent strip were: leucocyte from reagent strip at 10 cell OR 8.37(3.05 - 22.99) P < 0.0000, leucocyte from reagent strip at 100 cell OR (17.18(6.85 - 43.09) P < 0.0001, protein of reagent strip at 100 mg/dl 8.40(3.40 - 20.95) P < 0.0001 and glucose from reagent strip had OR 7.22 (3.08-16.90) P <0.0001

Table 4.28 Simple logistic regression analysis of laboratory parameters predictors of bacterial meningitis

Predictors	BM n= 32 Number (%)	No BM n=148 Number (%)	Odds ratio [95%CI]	p- value
Full blood count				
WBC n=169				
>10.000	20(69)	99(70.7)	0.92(0.38 - 2.18)	0.85
<10.000	9(31)	41(29.3)	1	
Thrombocytosis n=169				
Yes	13(44.9)	38(27.1)	2.18(0.95 - 4.95)	0.06
No	16(55.2)	102(72.9)	1	
CSF analysis				
CSF pleocytosis n=168				
Yes	17(56.6)	26(18.8)	5.63(2.43 -13.03)	<0.0001
No	13(43.4)	112(81.2)	1	
CSF leucocytes at 100 cellls n=168				
Yes	14(48.3)	9(6.5)	13.48 (4.99 - 36.40)	< 0.0001
No	15(51.7)	130(93.5)	1	
CSF pandy test n=160				
Yes	12(40)	7(5.4)	11.71 (4.07-33.65)	< 0.0001
No	18(60)	123(94.6)	1	
CSF Gram stain				
Yes	15(50)	1	129.70(16.11 - 1044.07)	< 0.0001
No	16(50)	148(100)	1	
CSF Culture				
Yes	17(53.1)	0	***	**
No	15(46.8)	148(100)		
Reagent strip analysis				
Strip leucocytes at "Trace"				
Yes	27(84.4)	58(39.2)	8.37(3.05 - 22.99)	< 0.0001
No	5(15.6)	90(60.8)	1	
Strip leucocytes at 2 cross				
Yes	24(75)	22(14.8)	17.18(6.85 - 43.09)	< 0.0001
No	8(25)	126(85.2)	1	
Reagent strip protein at 2 cross				
Positive	25(81.2)	44(29.7)	8.40(3.40 - 20.95)	< 0.0001
Negative	7(18.8)	104(70.3)		
Reagent strip glucose				
Negative	16(50)	18(12.2)	7.22(3.08-16.90)	<0.0001
Positive	16(50)	130(87.8)		

*** Unable to calculate odds ratio as nominator is 0

Table 4.29 Multivariate logistic regression analysis for predictors of bacterial meningitis in children admitted in Central hospital of Beira

Predictors	Odds ratio 95%CI	p- value
Behavior change	7.80(2.60 - 23.34)	0.0002
Vomiting	2.96(1.33 - 6.59)	0.0079
Kernig sign	5.32(2.17 - 12.99)	0.0002
Brudzinski sign	4.07(2.03 - 10.86)	0.0003
Nuchal rigidity	3.61(1.59 - 8.18)	0.0021
CSF pleocytosis	5.63(2.43 -13.03)	0.0001
Strip leucocyte at “Trace”	8.37(3.05 - 22.99)	<0.0001
Strip leucocyte at “2 cross”	17.18(6.85 - 43.09)	<0.0001
Strip protein at “2 cross”	8.40(3.40 - 20.95)	<0.0001
Strip glucose negative	7.22(3.08 - 16.90)	<0.0001

Table 4.30 Possible clinical strategy for bed side diagnosis of bacterial meningitis using different clinical and laboratory predictors of urine reagent strip

Clinical model	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	Overall prediction value	AUC 95% CI
Model 1 Fever Behavioural change Nuchal rigidity Strip leucocyte "Trace"	58.62 (38.9 - 76.48)	90.07 (84.15 - 94.3)	53.1 (34.7 - 70.9)	91.89 (86.27 - 95.7)	85%	0.857 (0.797 - 0.905)
Model 2 Fever Kernig sign Brudzinski signs strip leucocyte 2 ++ Strip protein 2 ++ strip glucose negative	72 (50.6 - 87.9)	90.9 (80.3 - 94.9)	56.2 (37.6 - 73.6)	95.2 (90.5 - 98.08)	88.3	0.854 (0.793 - 0.902)
Model 3 Fever Altered mental status nuchal rigidity strip leucocyte 2 ++ strip protein 2 ++ strip glucose negative	72 (50.6 - 87.9)	90.9 (80.3 - 94.9	56.2 (37.6 - 73.6	95.2 (90.5 - 98.08)	88.3	0.854 (0.793 - 0.902)
Model 4 Fever Strip leucocyte at trace Strip protein 2 ++ Strip glucose negative	67.86 (47.6 - 84.12)	91.4 (85.8 - 95.37)	59.3 (40.4 - 76.3)	93.9 (88.7 - 97.18)	87.8	0.835 (0.77 - 0.86)
Model 5 Fever Strip leucocyte at 2 ++ Strip protein 2 + Strip glucose negative	73.91 (51.9 - 89.77)	90.45 (84.7 - 94.55)	53.1 (34.7 - 70.9)	95.9 (91.39 - 98.5)	88.3	0.874 (0.816 - 0.918

Based on the most important predictors for BM we did a multivariate logistic regression analysis with variable including clinical and urine reagent strip results.

Model 5 based on fever, strip leucocyte at 2 cross, protein at 2 cross and strip glucose as negative tailored for early diagnosis is the best model which had a sensitivity of 73.91 % which is far from ideal. However we were most interested in specificity and which was 90.45% and the overall prediction values which gives percentage of cases correctly identified. In the 5th model the overall prediction value was of 88.3% and it is the simplest model and is the best model for the early diagnosis.

4.11 Cerebral malaria and menigoencephalitis

In order to differentiate between cerebral malaria, meningoencephalitis and bacterial meningitis we compared the mean of CFS wbc and age using t test for independent sample.

Table 4.31 T test for mean of age, csf wbc, csf neutrophil and csf lymphocyte for independent sample in cerebral malaria and meningoencephalitis

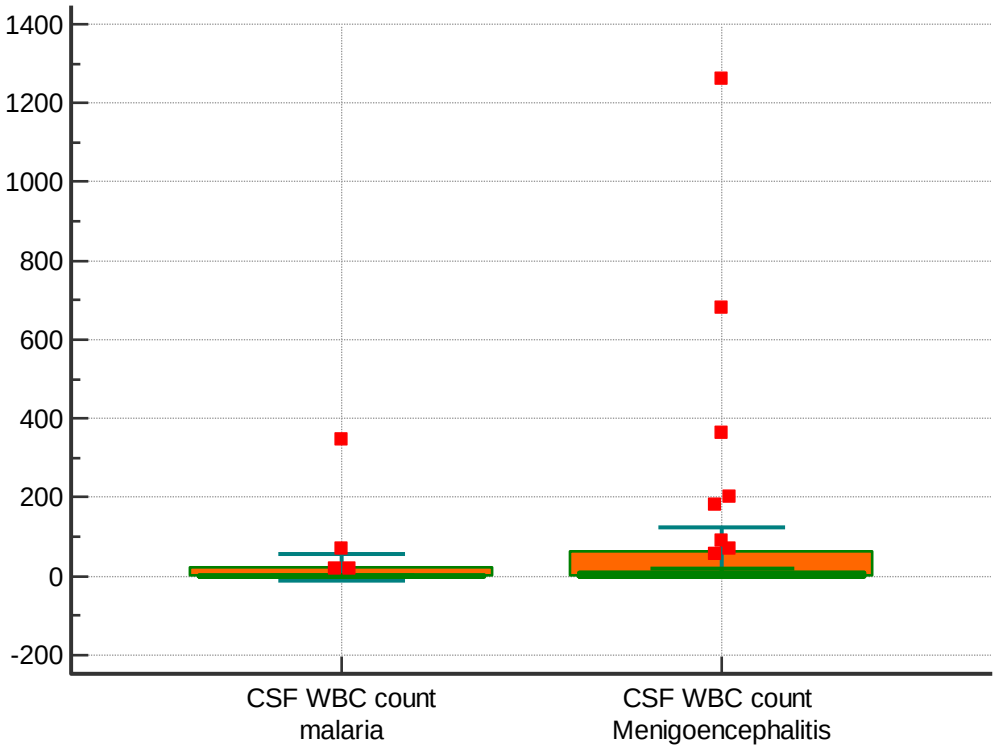
	Cerebral malaria n = 21				Meningoencephalitis n = 48			
	Mean	**SD**	**95% CI**	**P value**	**Mean**	**SD**	**95% CI**	**P value**
Age months	47.8	39.8	29 - 65.9	<0.001	61	50	46.5-75.7	0.04
CSF WBC/ul	22	75.8	12.5-56.5	<0.001	66	224	3.3 -136	<0.001
CSF Neu %	13.7	31.6	0.6 - 28.1	<0.001	12	27	3.3 - 20.6	<0.001
CSF Lym %	5.2	16.6	2.3 - 12.8	<0.001	9.4	24	1.9 - 16.9	<0.001

When mean of age, CSF WBC, CSF neutrophils and lymphocytes were evaluated using t test for independent sample. A difference in CSF cell count and age is observed among the two groups (cerebral malaria and meningoenecephalitis). However, this

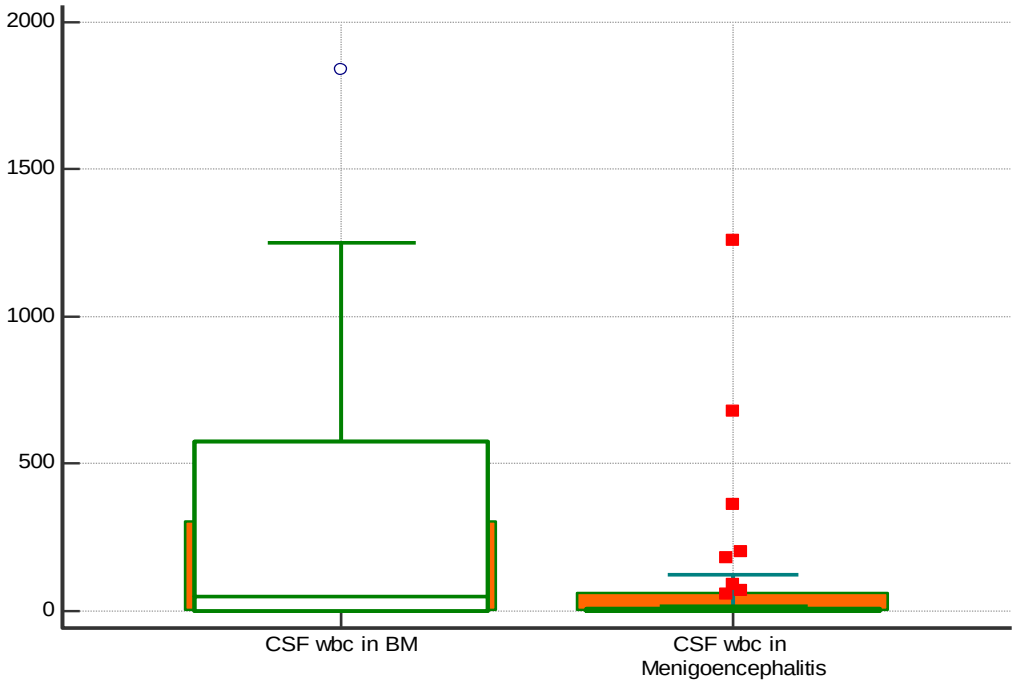
difference in the mean of CSF cell count observed between the 2 sample of 40.13, 95% CI [54.5317 to 134.8026] when tested for statistical significance, the cell count in malaria though was lower than menigoencephalitis it was statistically insignificant t(67) = 0.846, p=0.40 , so they were not included in the study and a differentiation between these 2 group was not possible.

Due to lack of gold standard for diagnosis of meningoencephalitis the reagent strip performance in meningoencepahlitis was not calculated only difference among CSF WBC was evaluated among children with bacterial meningitis and meningoencephalitis.

The figure 4.5 below demonstrated the difference between the 2 groups (Cerebral malaria and meningoencephalitis)



The figure 4.6 below demonstrated the difference between the 2 groups in CSF wbc (Bacterial meningitis and meningoencephalitis)



5. Discussion

Bacterial meningitis is a life threatening disease. Prompt diagnosis and treatment are important predictors of patient outcome. Many studies have been done in attempt to diagnose bacterial meningitis using simple methodology based on clinical signs and symptoms to very complex and expensive tests such as latex agglutination tests, real time PCR and cytokine levels for evaluation of CSF to aid diagnosis of bacterial meningitis.

In resource limited areas most of these tests are not available and diagnosis can be very difficult. Often the doctor finds themselves in dilemma when dealing with unconscious child with no laboratory resources and with high burden of malaria in most African countries. Clinically it is almost impossible to distinguish bacterial meningitis from cerebral malaria or meningoencephalitis, thus clouding the decision to start antibiotic or not. In these cases physicians feel pressured to start empirical antibiotic therapy thus using already little resource that is available in these areas.

Many studies have attempted differential diagnosis between bacterial and viral meningitis based on CSF parameters like leucocyte count, protein and glucose concentration cutoff points and have come up with various models (Bonsu and Harper, 2004, Karanika et al., 2009, Águeda et al., 2013). However many of these models have been created using laboratory analysis and patients from developed countries where prevalence of bacterial meningitis is lower (Thigpen et al., 2011).

Bonsu and Harper in their study predicted a model based on CSF pleocytosis which used age, CSF WBC count , CSF protein concentration and CSF neutrophil percentage and with those results came up with a very accurate model that predicted bacterial meningitis in 80 % of cases (Bonsu and Harper, 2004). But Bonsu and Harper in their model, excluded CSF with low WBC. The normocellular bacterial meningitis is observed among very ill children. It is commonly observed among children with some

immunosuppression and systemic complications (Brouwer et al., 2010). Freemont Smith also presented in his study that 1 % of patients with bacterial meningitis had CSF cell lower than 100 cell count (Merrit and Fremont-Smith, 1938).

The ideal test for diagnosis of bacterial meningitis should have a 100% sensitivity and 100 % specificity to determine diagnosis of BM and not to miss any case. However no single test till today has shown to be 100% sensitive and 100% specific, warranting that clinical and laboratory parameters need to be evaluated together for the diagnosis.

Many studies have used urine reagent strip for diagnosis of meningitis (Moosa et al., 1995, Parmar et al., 2004) with different sensitivities and specificities, however, our study seems to be the first that has attempted bedside diagnosis algorithm for diagnosis of BM using urine strip.

The main objective of our study was to test sensitivity and specificity of the reagent strip among children that were admitted in Central Hospital of Beira and based on the results try to establish a bed side diagnostic algorithm using reagent strip to differentiate among children with meningitis or not and those who require antibiotic treatment from those who do not require.

Findings of the study

In our study the prevalence of 17.7 % bacterial meningitis was observed among children admitted in the study. The diagnosis of bacterial meningitis in the study included both confirmed and probable cases. When we compared the prevalence among culture positive cases our study demonstrated a lower prevalence with 9.4%, which was very low in comparison to 15% that was observed in a study done in Manhica district in Mozambique (Sigauque et al., 2008). When the prevalence was compared to the results from Maputo Hospital our study had a slight lower prevalence for culture positive cases with 9.4% in comparison to 13.8% of culture positivity observed in Maputo (Zimba et al., 2009). The study from Maputo included patients of different age groups, neonates to adults, including HIV and AIDS patients, which were excluded in our study this could

partially explain the lower prevalence. There was no gender difference observed in our study as referred by (Sigauque et al., 2008) in his study.

The lower culture positivity in our setting could be explained by lower detection rate of microbes in our minimally equipped laboratory. The laboratory's working hours are one major drawback. It has been known that the delay in analysis decreases the survival of bacteria like N. meningitis and lowers the detection rate (WHO,2011). The other important factor that could explain lower sensitivity is the use of antibiotic prior lumbar puncture (Brouwer, 2010). Among children admitted with diagnosis of BM. A 46.8% were already on treatment prior admission which could explain the lower detection rate. In his review Brouwer referred sensitivity of culture in developed countries is higher and ranges from 75%-85% when compared to developing countries like Brazil with 67%. Antibiotic use as an added effect could lower detection to below 50% (Sáez-Llorens and McCracken, 2003). Especially in meningococcal meningitis the CSF sterilization can occur as early as 2 hours after administration of a single dose of third generation cephalosporin's (Kanegaye et al., 2001).

When we further analyzed our data we observed that among culture negative BM 10/15 (66.6%) had parenteral therapy, of these 10 (66.6%) who had antibiotic 6 had combined therapy which explains why turbid and purulent CSF were culture negative and the lower sensitivity of the gold standard .

The most common pathogens observed in our study were Streptococcus pneumoniae with 29.4%, Neisseria meningitidis 11.76%. This is significantly different than observed in other studies done in Mozambique. Both studies from Mozambique were done in Maputo and demonstrated very different results. The study from Manhica district demonstrated that overall the three main pathogens S.pneumoniae, H.influenazae and N.meningitidis all together accounted for 73% of all cases (Sigauque et al., 2008). While our study from Hospital patient's demonstrated very low frequencies of S.pneumoniae with 4.8%, and N.meningitidis in 5.4%.

We did not find any Haemophilus Influenzae in our study. This fact could be due to the vaccination program that has been established in the country since 2010. The study done in manhica district which compared Haemophilus Influenzae disease in children before and after vaccine introduction demonstrated significant reduction from 91% - 85% in invasive cases of hemophilus (Sigauque et al., 2013) However, we also had 8/20 (40%) of very turbid CSF where no growth of bacteria was observed. Further analysis with PCR may give more information.

Staphylococcus aureus in CSF culture may be contaminant. It was observed in 2 cases in our study. One patient had severe malnutrition and suppurated otitis media at admission with altered mental status and the other developed meningeal symptoms post femur fracture. The first patient later developed S.aureus from pus from the ear. Both cases in the study were more likely due to infection outside CNS and one post trauma, both conditions have been also documented by (Pedersen et al., 2006) and due to clinical features that were compatible with meningitis it was less likely to be contaminant.

Other very interesting pathogen in our study was Morganella morganii seen in 2 patients M 043 and M 044. Both patients were admitted at more or less the same time. One patient with 36 months of age showed slight CSF wbc count elevation with 322 with 98% lymphocytosis and a negative pandy test. He was admitted initially with diagnosis of cerebral malaria due to opisthotonus, lumbar puncture was done to exclude BM.

The second patient was 11 years old with a positive HIV test. He was also initially admitted with diagnosis of cerebral malaria with high parasitemia and developed hemoglobinuria and respiratory distress due to non cardiogenic pulmonary edema. This is a severe complication of malaria which is frequent among adults but has also been observed in immunosuppressed children with HIV (Hendriksen et al., 2012).
Both morganella cases were observed in children with cerebral malaria who were already admitted in the ICU and lumbar puncture in both cases was done at 40 h and at 48 h, both patients were on artesunate therapy and antibiotic was later added due to

their worsening condition. We suspect that both cases may have been acquired nosocomiallly or were contaminants. However definitive conclusion cannot be made.

Morganella morganii is caused by gram negative rod is known as an opportunistic infection. There is very little in literature on morganella infections most of information is found in isolated cases (O'Hara et al., 2000). There has been reports of a neonatal sepsis (Dutta and Narang, 2004) case with fatal sepsis. A case of meningitis in an adult patient with stage IV Hodgkin disease which developed the meningitis via hematogenous from urinary tract (Samonis et al., 2001) and a case of nosocomially acquired meningitis with morganella in a cardiosurgurgical centre (Williams et al., 1983). In a report by William et al most of the patients that had morganella infection the source of detection was blood, wound, urine, feces and only 2 cases were found in nasopharyngeal secretion and sputum (Williams et al., 1983).

Most frequent presenting signs and symptoms among children with bacterial meningitis were: Severe coma: 14/32 (43.8%), seizures 27/32 (84.4%) which was slightly higher than reported in other studies (Berkley et al., 2001, Sigauque et al., 2008). This could be explained by the fact that (51.7%) of the patients came very late to the hospital with more severe signs and symptoms. In fact 20 children who were later admitted in the hospital had already one episode of seizure that was reported by one of the parents at home and sought medical treatment in nearby health center who were discharged with diagnosis of febrile seizure prior admission with either antibiotic or antimalarial treatment and came rather later to the hospital when patient's condition became worse.

Broad spectrum antibiotic use was found in 43% of admitted children. This is significantly higher than 11% reported by Roca (Roca et al., 2009). But this information is not surprising, antibiotics are easily available in local pharmacies and prescription is usually not required. An additional effect to this point is that almost 48% of the children in our study already had been seen by a health worker with complaint of fever who were treated with either antimalarial in case of positive malaria test or antibiotic in case of negative malaria test.

Other clinical features observed were signs of shock at admission (12.5%) agitation (37,5%), nuchal rigidity (68.8%), Brudzinski sign (43.8%), Kernig (37.5%) and opisthotonus (37.5%). Similar findings were observed for nuchal rigidity (Berkley et al., 2001) for coma (Berkley et al., 2001), agitation was slightly higher in report of Manhica study (Sigauque et al., 2008), brudzinski sign and kernig as meningeal sign was slightly lower as reported in study by (Karanika et al., 2009).

Headache was observed in 13 (40.6%), behavior change in 28 (87.5%), irritability 25 (78.11%) and 19 (59.4%) were unable to feed or eat and symptoms duration varied among children with BM: 9 (28.1%) had symptoms less than 24 hours, 2 (6.2%) between 24-48 hours and 21 (65.6%) had symptoms for more than 72 hours prior admission. Other sign tested among children with BM was photophobia which was observed in 11 (34.4%).

Overall sequelae among children with BM was 28.1%. Which is significantly high and similar findings were observed in many low income countries (Edmond et al., 2010b). The most common type of sequelae found was seizure (12.5%), minor motor deficit (9.1%), and cognitive impairment (9.1%). These findings were similar as reported in systematic review and meta-analysis by Edmond et al. Major motor deficit like quadriplegia and hemiplegia was observed all together in (12,5%). Hearing loss was not frequently observed. These findings differ slightly from (Edmond et al., 2010b), but our sample with BM was rather small and besides we had very heterogeneous population of microorganism.

Use of reagent strip for BM

When we compared the accuracy of reagent strip leucocyte's capacity to detect CSF wbc alterations we noted that at "trace" (>15 cells/ul) and at 2 cross (>125cells/ul) the accuracy was similar with 0.72, however the sensitivity, specificity, NPV and PPV were different with higher sensitivity for 10 cell cut off point. We found sensitivity of 86 %, specificity of 68%, NPV 93.4%, PPV of 48% Sensitivity in our study was higher than

observed in study done in Brasil , but they had better specificity and NPV (Sudbrack et al., 2004). However, Mossa et al achieved a sensitivity of 97 % for diagnosis of bacterial meningitis based on use of urine strip and a specificity of 100 %. This could be explained that the standard laboratory facilities were of high standard and were immediately analyzed reducing false negative (Moosa et al., 1995).

When we used “2 cross” as cutoff point results were as follow: sensitivity of 82.6%, specificity of 84.7%, NPV of 96.3%, PPV of 46.3% which were rather similar with those presented by Simone et al. using “trace” as cutoff point for the CSF alteration had rather good accuracy. However in bacterial meningitis the CSF cell count usually is above 100 cell (Prober, 2007) because using a lower cut would increase false positives a cut off of “2 cross” was used .

We further tested the accuracy of reagent strip leucocyte in detecting BM and compared with CSF wbc and we observed that the reagent strip performed better than standard CSF wbc. Sensitivity of the reagent strip in detecting BM was higher with 73.3% in comparison with CSF wbc of 56.7% and so were the specificity at 85.14%, NPV at 94.3% and PPV of 50.89% in comparison to CSF wbc at 81.8%, 89.6% and 39.53% respectively. This information reflects the poor quality of laboratory results which are common in developing countries, (Petti et al., 2006) due to various factors delay in analysis can reduce CSF wbc by 32%, not centrifuging accordingly (Steele et al., 1986) and inexperienced laboratory technicians (Petti et al., 2006).

We compared the protein from reagent strip and compared with pandy test a semi quantitative test that measures globulins (Cheesbrough, 2009). When we calculated the best cutoff point for protein detection and for detection of BM we found that 100 mg /dl in reagent strip was the best cutoff point. However we did not find any literature that made similar comparison with pandy test.

Many studies have demonstrated comparison of reagent strip and CSF total protein (Moosa et al., 1995, Sudbrack et al., 2004). As this test was not available we compared with local available results.

We found a sensitivity of 89.4%, a specificity of 70.2%, a NPV of 98%, a PPV of 28.8%. When we compared the pandy test reaction against urine reagent strip protein with overall accuracy of 0.69 which was rather good in comparison with 300mg/dl. Using 300mg/dl cutoff point many cases with protein above 100mg/dl would be missed.

When compared the reagent strip protein alteration among children with BM against the pandy test detection for alterations in BM. The strip performed better with a sensitivity of 78.12%, specificity of 70.27%, NPV of 93.7%, and PPV of 36.2% in comparison with 43.3%, 94.62%, 87.2 %, and 63.1% respectively.
This results can be explained because a positive pandy test is slight or minimum when the concentration of CSF protein is below 200 mg/dl giving a slight turbidity which disappears within seconds and may give false negative results (Cheesbrough, 2009).

Bedside clinical model for prediction of bacterial meningitis

After analysis with different variable observed in our study that were significant in univariate anlaysis we choose: behavior change with an OR 7.80 (2.60 - 23.34) P = 0.0002, vomiting which had an OR 2.96 (1.33 - 6.59) P = 0.0079, Kernig sign though not often seen among younger children was a strong predictor with an OR 5.32 (2.17 - 12.99) P= 0.0003 for clinical features. For laboratory parameters based purely on urine reagent strip results which were: leucocyte from reagent strip at “Trace” OR 8.37(3.05 - 22.99) P < 0.0000, leucocyte from reagent strip at “2 cross” OR (17.18(6.85 - 43.09) P < 0.0001, protein of reagent strip at “2 cross” 8.40 (3.40 - 20.95) P < 0.0001 and glucose negative from reagent strip had OR 7.22(3.08-16.90) P <0.0001.

When we predicted clinical model via multiple logistic regression for patients bedside diagnosis using reagent strip we observed that the simplest model using 4 variables : fever with positive leucocyte reaction on the reagent strip at “2 cross” a positive protein reaction on the reagent strip at “2 cross” and a negative glucose reaction had a predicted sensitivity of 73,91%, a specificity of 90.45% a NPV 95.9% a PPV of 53.1% with an overall prediction of 88.3% in accurately predicting bacterial meningitis.

Though many clinical algorithms have been published like Spano's model, Hoen's model and one most recent publication from Angola (Lussiana et al., 2011) who in her work has used simplified predictor for low resource limited contexts, but none could be applied to our patients in Beira, simply because all of the models mentioned above have used laboratory parameter like CSF cell count, CSF total protein count and glucose concentration. The last two are not analyzed in our setting which is a Central Hospital. One can imagine how difficult or it is almost impossible to apply in health centers in districts where laboratories are minimally equipped. Moreover all these models heavily rely on accuracy of laboratory technician work and prompt analysis of CSF.

Most health center and hospital's laboratory only work till afternoon and lumbar punctures done after these hours either are analyzed in emergency laboratory (rural district and central hospital have these laboratories) which only performs pandy test and CSF wbc and results may only be available after 3-4 hours depending on workload (authors' observation and informal interview with colleagues from districts).

To overcome this major drawback we had to use alternatives that could be applied to our context so we tested urine reagent strip applicability in BM. Our predictive model though has lower sensitivity with 73.91% for diagnosis of BM it has a very high specificity and NPV which in our context is very useful.

Sensitivity which defines proportion of people who have positive test that have the disease and specificity which defines proportion of people without disease will test negative (Akobeng, 2007) Which is more useful in our context? We already know that between 100-500 wbc cell count in CSF can also be observed among children with meningoencephalitis, (Prober, 2007) overlapping among this range is common and based on cell count alone for this particular range it is almost impossible to differentiate between these 2 diseases. However, when the test is negative (specificity) which in our case is 95.3%, can rule out the disease in 95.3% of controls thus avoiding unnecessary treatment. In decision making, on when to introduce antibiotics and stimulate decision of other diagnosis. In context of children with cerebral malaria it is almost impossible to

distinguish from bacterial meningitis a simple test at bed side can help in doing differential diagnosis.

Negative predictive value is also another important result in our model because its NPV of 95.9%. NPV defines the proportion of people with negative test that do not have the disease (Akobeng, 2007), in our model could predict that 91.6% would be disease free when all the predictors in model are negative or in other words it would miss 9.4% of cases of BM alone. However NPV is dependent on population prevalence (Akobeng, 2007), as this study was performed in Beira for a period of 15 months one can estimate that this prevalence is more or less accurate and could be applied to our context in Beira.

6. Conclusion

Our results confirm that reagent strip has good sensitivity and specificity in differentiating whether a child has meningitis or not. It can aid physician in decision making. Though many studies have used reagent strip and have shown different test performance and some authors have also recommended (Parmar et al., 2004) as a screening test, in areas where other diagnostic possibilities are difficult. It can be used as the urine strip has a good sensitivity (Romanelli et al., 2001). To conclude reagent strip has shown better performance in diagnosis of BM comparing the results with standard laboratory analysis available in our setting. It is necessary to use this test where no laboratory is available to guide decision making. However the test also has its limitation and can miss cases of normocellular BM or reading can be altered if necessary condition of storage are not followed. In cases of bloody or accident in lumbar puncture the color change in test pad can be altered giving false positive results in some cases. In these cases caution needs to be taken when interpreting results, most important is the clinical judgment that will dictated final decision making.

7. Limitation

Limitation of the study

The main limitation of the study was the minimally equipped laboratory in the Central Hospital of Beira which lack of good “Gold standard” the CSF WBC and CSF culture. Another limitation was some missing data in CSF wbc and pandy test results from the hospital which affected the results of the study.

Limitation of the urine reagent strip

One major limitation is that the urine reagent strip's semi quantitative test pads are not standardized for CSF parameters and this makes it slightly challenging. For example the minimum glucose detection capacity of reagent strip is at 100mg/dl and is negative when at 30 mg/dl . These values are very different from standard CSF glucose values and thus difficult diagnosis. The same condition is applied on protein. A reagent strip which would have a better cut off values standardized for CSF parameters could potentially benefit and could show different results.

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Curriculum vitae

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Ocuppational field General practitioner working at the Central Hospital of Beira, in Pediatric department, lecturer, and assistant teacher at the Catholic University, Faculty of Health Science in Mozambique

Work experience held

2011-2014- Phd student at CIH LMU
2007- 2011 General practitioner working in the pediatric ward at the Central Hospital of Beira
2007- 2011 Part time Assistant Teacher at the Catholic University of Mozambique Faculty of Health Science (Medicine)
2008-2010 local co-cordinator for the multicentre study - AQUAMAT

Occupation or position held

General practitioner working at the paediatric wards at the Central Hospital of Beira for past 7 Years
Tutor for the 5^{th} and 6^{th} year students in paediatric rotations at the Central Hospital of Beira
Local Co-coordinator for the multicentre centre study of malaria AQUAMAT With WELCOME TRUST-MAHIDOL UNIVERSITY OXFORD TROPICAL MEDICINE research programme
Block coordinator, tutor and lecturer at the faculty of Medicine at Universidade Catolica de Mozambique, Faculty of Health Science
Member of PBL (Problem based Learning) department responsible for research and tutoring method to the student

List of Publications

Kajal Chhaganlal, Inge Van Jaarsveld, Kristina Hoffmann, Maria Isabel Ramos, Monique Krober, Dirk De Hoop, Cutaneous disorders in the “bairro Inhamudima” of Beira, Mozambique, International Journal of Dermatology, Volume 46 , p 35-38 , 2007

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Appendice 1

Ethical consent form

Study Number _____

Consent form

Name of the Principal Investigator : Dr. Kajal Dhirajlal Chhaganlal

Name of the Orgnization : Ministry of Health , Central Hospital of Beira

Names of Parteners : Catholic University of Mozambique , Faculty of Health Sciences- Beira

Ludwig Maximillians University – Munich

Information:

We are working for the Ministry of Health of Mozambique in Central Hospital of Beira. We are studying a new method for the diagnosis of meningitis at patient's bedside.

You / your child (dependent) has a suspicion of a disease called Meningitis, and your condition is such as to confirm the diagnosis , it needs a test which is called lumbar puncture , which extracts cerebrospinal fluid from the lumbar region for analysis for meningitis .

The study aims to use urine reagent strip to look for changes of cerebrospinal fluid chemistry and compare with the conventional method which uses biochemistry machines and culture of cerebrospinal fluid for analysis, methods that usually takes 30 min to 48-72 hours, respectively, compared with reagent strip that usually takes less than 2 minutes to obtain results . However the aim of the study is to evaluate the performance (sensitivity and specificity) of the urine reagent strip for a rapid diagnosis of meningitis at the patient's bedside. To find the answer to this important question, we're doing this study in 202 children in this hospital, to better respond to our question. The result of the study will improve our knowledge about the use of reagent strip as a quick means of diagnosis and help develop an appropriate strip for spinal fluid that could be used in Mozambique and other countries and may benefit society in general.

You / your child will perform all tests and receive all the supportive treatment for meningitis according to the guideline of diagnosis and treatment of meningitis in our hospital. In the case of not accepting to participate in the study if there is an indication for lumbar puncture by attending doctor it will be done for diagnostic purpose independent of participating in the study.

Participation in the study signifies permission for the use of cerebrospinal fluid for further analysis. 3ml of cerebrospinal fluid will be drawn from patient. Out of which 5 drops for the examination of urine reagent strip and 0.5- 1ml will be stored for PCR and Cytokines analysis, which will be held in Ludwig Maximillians University in Munich, for better study of the type of microorganisms, especially in cases of encephalitis for which we have no means of diagnosis

in our Hospital. The rest of liquor will be sent to the central laboratory of the Hospital for routine analysis of meningitis. Besides the cerebrospinal fluid, the attending physician may order other exams such as full blood count, HIV Test, X-ray , CT and other tests .These are not part of the study, but the results will be recorded in the patient's file and kept in confidential.

The expected duration of the study for the participant is from now until the time of discharge. Starting now with an interview and examination of the child which takes less than 30 minutes and child's evaluation at regular intervals until discharged. There will be no financial cost to participate in this study and no payments for yourself or your child. We will keep all records in a locked place and use the information solely for the purpose of the study.

If you or your child does not wish to participate in the study, their refusal will not affect your /(your child/ dependent) diagnosis and treatment decision. You will benefit of all facilities available in this center.

You (or your child) may stop participating in this research at any time you want, without losing your rights as a patient.

If you have any question, You may do it now or in later stage or if you want to ask more questions in a later stage you can contact Dr. Kajal D Chhaganlal: 825643910 or Dr. Ermelinda Gomes: 825010098

This proposal was reviewed and approved by the National Ethics Committee of Mozambique. The committee has the task of ensuring that research participants are protected from harm.

Certificate of Consent

For this particular test the patient _____ or guardian (Mr/Mrs) _____, hereby, for all legal purposes,declares giving full permission to the attending physician, to proceed with necessary investigations for the diagnosis of meningitis, and execute the designated exam **"lumbar puncture"** and use of cerebrospinal fluid for the diagnosis of meningitis.

The above procedure was explained to me and hereby I authorize.

Lumbar puncture is performed to examine the cerebrospinal fluid (CSF), especially in suspected meningitis (to confirm or rule out this hypothesis).

COMPLICATIONS and GUIDELINES:

1. The pain that accompanies the lumbar puncture procedure is similar to that of blood collection for examination.
2. After undergoing lumbar puncture the child should remain at rest on his stomach in supine position for a few hours. Despite this care approximately 10% of these people, may experience headache. If the child has this headache, he /she should have absolute rest for 48 hours straight, lying, preferably in the facedown position, taking plenty of water and other hydrating beverages according to medical indication.
3. After collection of cerebrospinal fluid in the lumbar region it may occur, in addition to headache at the puncture site slight pain but rarely infection.

I declare that I have received all the necessary and clear information from the study doctor on study methodology, risks and benefits. I had the opportunity to ask questions and they were answered satisfactorily. I have access to the full text and of all the terms of ethical consent form shown by the study doctor.

I declare that i have thoroughly been informed of the present risks that are posed due to procedure and of any complication that may arise with the disease. I also confirm that i have understood well all the information provided to me.

After all the clarifications and having read and understood all the information on this document, of the procedure and the use of cerebrospinal fluid for use on reagent strip and other Laboratory analysis, I give my, **CONSENT** for performing the above mentioned procedure which was indicated by attending physician and extend the same consent to his other team members.

Beira, _____ of _____ of _____

_____

Signature of patient's guardian Degree of Kinship

If you do not know how to read or write:
Type the name of literate witness (selected by patient or responsible child)

Name of the witness Signature of the witness

_____ _____

Date ___/_____/_____ Date ___/____/_____

Full name of the doctor .....Date ___/___/_____
Signature of the doctor .....Date ___/___/_____
Full name of principal investigator.....Date ___/___/_____
Signature of the principal investigator .....Date ___/___/_____