INCIDENCE AND MANAGEMENT PRACTICES OF BACTERIAL MENINGITIS IN CHILDREN IN MOROGORO REGIONAL REFERRAL HOSPITAL

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

The analysis and evaluation of meningitis situation in Morogoro Region was done by looking at the incidence and management practices of meningitis in children at the Morogoro Regional Referral Hospital (MRRF) for a period of 7 months. A total of 1352 children aged between 7 days and 12 years were admitted at MRRH, of these 72 (5.3%) had meningitis symptoms during the period. Lumbar puncture was done on the 72 children with meningitis symptoms to collect CSF for observation and laboratory analysis. Of the examined CSF samples, 23 (31.9%) were positive for *Streptococcus pneumoniae*, 6 (8.3%) for *Haemophilus influenzae*, 5 (6.9%) for Group B *Streptococcus*, 3 (4.2 %) for *Echerichia coli* and one (1.4%) was AFB positive on Ziehl-Neelsen (ZN) stain. Thirty nine percent (n=72) of the samples showed no bacterial growth in the culture media. Latex agglutination test was used to confirm the bacterial colonies. The incidence of meningitis infection was 5.3% (n=1352) among the admitted patients. Intravenous administration of Ceftriaxone at 80mg/kg body weight was given once a day for 10 days to all the 72 children with symptoms of meningitis. Of the 72 treated children, 32 (44.4%) responded well to the treatment of whom 19 (26.4%) fully recovered, while 13 (18.1%) recovered but with neurological defects. Nevertheless, 39 (54.2%) of the treated children did not respond to the therapy whereas 31 (43.1%) of them died while 8 (11.1%) were referred to the Muhimbili National Referral Hospital. Only one case of Tuberculosis (TB) Meningitis was recorded which responded well to TB medication of rifampicin, isoniazid and pyrazinamide in a combination. This study has shown that *Streptococcus pneumoniae* was the major bacteria associated with meningitis in children admitted at the MRRH.

DECLARATION

I, **Ramadhani Chambuso,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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LIST OF ABBREVIATIONS

ABM	Acute Bacterial Meningitis
AFB	Acid Fast Bacilli
BAP	Blood Agar Plate
B-Lactam	Beta Lactam
BLS 2	Biosafety Level 2
CAP	Chocolate Agar Plate
CDCP	Centre for Disease Control
CHA	Community Health Agents
CI	Confidence Interval
CSF	Cerebral Spinal Fluid
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EXPEC	Extraintestinal pathogenic *Escherichia coli*
G. streptococci	*Group b streptococci*
H. influenzae	*Haemophilus influenzae*
Hib vaccine	*Haemophilus influenza b* vaccine
HIV	Human Immunodeficiency Virus
HUS	Hemolytic Uremic Syndrome
IMCI	Integrated Management of Childhood Illness
LT	Heat labile enterotoxin
MBC	Minimal Bactericidal Concentration
MoHSW	Ministry of Health and Social Welfare
MPS	Malaria Parasites

MRRH	Morogoro Regional Referral Hospital
MNRH	Muhimbili National Referral Hospital
NIMR	National Institute for Medical Research
PCR	Polymerase Chain Reaction
PCV 7	Heptavalent conjugate pneumococcal vaccine
PMCT	Prevention of Mother to Child Transmission of HIV
RBG	Random Blood Glucose
rRNA	ribosomal Ribonucleic Acid
S. pneumoniae	*Streptococci pneumoniae*
SPSS	Statistical Package for the Social Sciences
ST	Heat stable enterotoxin
SUA	Sokoine University of Agriculture
TB	Tuberculosis
T-I	Trans-Isolate medium
TTP	Thrombocytopenic purpura
V-factor	Nicotinamide Adenine Dinucleotide
VTEC	Verotoxin producing *E. coli*
WBC	White blood cells
WHO	World Health Organization
ZN-STAIN	Ziehl-Neelsen Staining Procedure

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Bacterial meningitis remains a serious public health threat globally, accounting for an estimated annual 170 000 deaths worldwide (Dagan, 1994). Every year, bacterial meningitis epidemics affect more than 400 million people living in the 21 countries of the "African meningitis belt" (from Senegal to Ethiopia). In this area over 800 000 cases were reported in the last 15 years from 1996 to 2010 (WHO, 2010).

The most common symptoms of meningitis are severe headache and neck stiffness associated with fever, confusion or altered consciousness, vomiting and an inability to tolerate light (photophobia) or loud noises (phonophobia) (Thwaites *et al.*, 2000). In small children, nonspecific symptoms may be present, such as irritability, excessive crying with high pitched sound and drowsiness. If a rash is present, it may indicate a particular cause of meningitis; for instance, meningitis caused by meningococcal bacteria may be accompanied by a characteristic rash (Theilen *et al.*, 2008).

Initial diagnosis of bacterial meningitis can be made by clinical examination followed by a lumbar puncture showing a purulent spinal fluid. The bacteria can sometimes be seen in microscopic examinations of the spinal fluid (Hsu *et al.*, 2009). The diagnosis is supported or confirmed by growing the bacteria from specimens of spinal fluid or blood, by agglutination tests or by polymerase chain reaction (PCR) (WHO, 2010).

In Tanzania, the last epidemic of meningococcal meningitis occurred in 2001 at the Goma refugee camp which was spread from Burundi and Rwanda (WHO, 2009). Although a

number of studies have been conducted (WHO, 2010), meningitis epidemics still constitute an enormous public health burden. WHO is committed to eliminating meningitis disease as a public health problem (WHO, 2010).

The Centre for Communicable Diseases and Prevention (CDCP) conducted training in 2008 to conduct surveillance of the pediatric bacterial meningitis in African countries located in the African meningitis belt and others with high prevalence of meningitis cases. This surveillance showed that Tanzania is one of the countries grouped as medium performers for the surveillance of pediatric bacterial meningitis compared to other African countries (CDC, 2010) Fig. 1

In Vietnam, the report showed that *Haemosphilus influenza* type b caused 53% of culture-proven bacterial meningitis in hospitalized children. In the northern region of Vietnam, a community-based survey showed that Hib was carried by 39% of the children and that 68% of the Hib isolates were resistant to at least one antibiotic (Dang *et al.*, 2002). These finding suggest that the burden of disease is substantial, but limited information on the incidence of meningitis in general and the consequent neurologic sequelae. Globally, prior to introduction of conjugate vaccines directed against *Haemophilus influenzae* type b (Hib), there were an estimated 300 000–400 000 deaths and 2.2 million cases in children each year due to Hib disease (Dang *et al.*, 2002). After the introduction of conjugate Hib vaccines into national childhood immunization programs, the decrease in incidence of Hib disease in children in several countries has been well documented worldwide (Dang *et al.*, 2002).

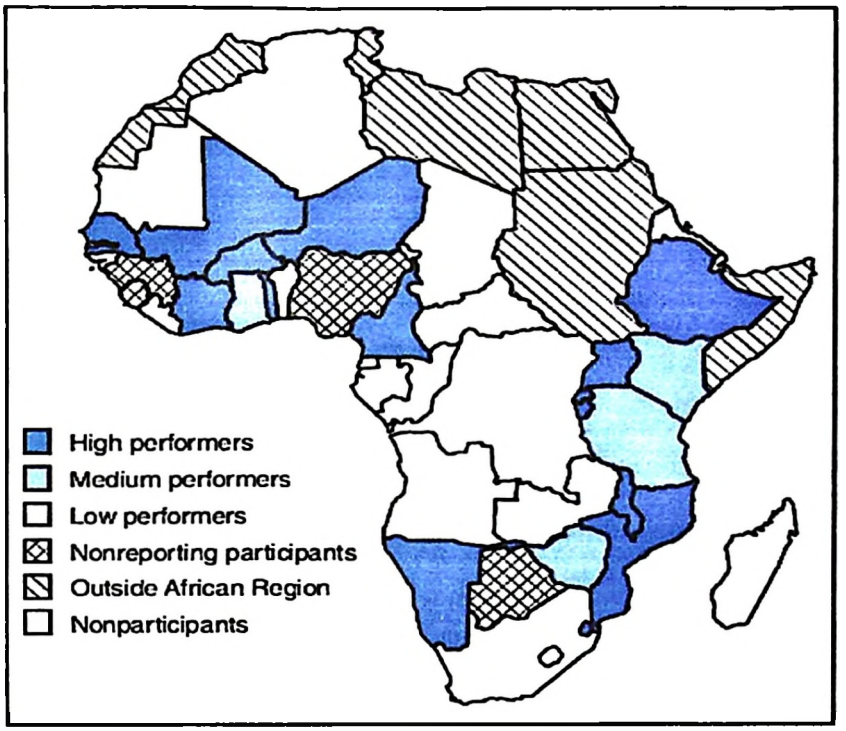

Figure 1: Countries trained to conduct surveillance for the Pediatric Bacterial Meningitis Surveillance Network shown, by performance level, World Health Organization Africa, 2008.

(Source: http://www.cdc.gov/mmwr)

1.2 Problem Statement

Meningitis is still one of the major causes of death, disabilities and mental retardation problems in children in developing countries with significant diagnostic and therapeutical challenges. About 2 to 4% of all paediatric admissions with fever in African hospitals had meningitis and lumbar puncture is rarely performed (Mwangi *et al.*, 2002). Survey of 13 hospitals in Tanzania showed that 38% of health workers failed to recommend lumbar puncture without any specific reasons for children with fever, stiff neck and a positive malaria slide and 30% fail to recommend lumbar puncture to children with fever and atypical convulsions. About 75% bacterial meningitis can be detected only at the bed side by examining the CSF cloudiness' or turbidity (MoHSW, IMCI, 2009).

In MRRH deaths and neurological complications in children due to meningitis are many, however, among them only in few cases lumbar puncture was taken during their admissions and the results were not readily out till their deaths (MRRH, 2009). The high paediatric mortality, disabilities and neurological sequel calls for a detailed survey on the disease in the region in line with the Millennium Development Goal number 04. Additional factors, such as advanced HIV infection, malnutrition, and antibiotic-resistant bacteria, complicate the management of the infected patient. For these reasons, meningitis presents an exceptional challenge to physicians working in resource-poor settings such as MRRH.

1.3 Justification

Although there are a number of reference log books and treatment documentation to suspected cases of meningitis in MRRH, the actual incidence of the disease and the outcome of the management practices are not known. In addition, the major causative agents associated with meningitis cases are not well document. This study was designed to determine the incidence and the main organisms associated with suspected cases of meningitis. To add information on the outcome of the treatment, the study also made a follow-up of the cases treated for meningitis in children. The study of cases survey aimed in this study was planned to demonstrate the magnitude of meningitis cases in the study population and also will determine the effect of the outcome of the current meningitis treatment regimen and the importance of lumbar puncture in the diagnosis of meningitis. Given that meningitis can cause a number of early severe complications, the study also recommended further rehabilitation measures of the recovered patients. The rationale of this study lied on the fact that the best way to evaluate the effect of the existing protocols was to perform this incidence study to see if there are changes in the causative organisms and mode of meningitis presentation (CDC, 2010).

1.4 Objectives

1.4.1 Broad objective

The main objective of this study was to determine the prevalence, main bacteria causes and management practices of meningitis in children in Morogoro Regional Referral Hospital.

1.4.2 Specific objectives

(i) To determine the prevalence of meningitis in children in MRRH

(ii) To identify the main bacteria associated with meningitis in children in Morogoro Region.

(iii) To determine the effect of current meningitis management protocol in children in MRRH.

(iv) To recommend the best practices in the diagnosis and management/treatment of meningitis at MRRH

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of Meningitis

2.1.1 Disease distribution and risk areas

The largest burden of meningococcal disease occurs in an area of sub-Saharan Africa known as the meningitis belt, which stretches from Senegal in the west to Ethiopia, Zambia, Kenya, Uganda and Tanzania in the east (Gordon and Zumla, 2009). During the first 10 weeks of the 2006 meningitis season, outbreaks in 32 districts in 7 countries in the African Meningitis Belt occurred (WHO, 2009). In these affected countries a total of 5719 suspected cases, including 580 deaths, have been reported to the WHO (WHO, 2009). Cases have occurred in two foci, one in West Africa, affecting Burkina Faso, Côte d'Ivoire, Mali and Niger characterized by the predominance of *Neisseria meningitidis* (Nm) serogroup A. Outbreaks in the second epidemic foci, in eastern Africa, including Kenya, Sudan and Uganda, *Neisseria meningitidis* (Nm) serogroup W135 was the main pathogen detected (WHO, 2009).

Control of bacterial meningitis is very challenging but recently a vaccine against group A *meningococcal meningitis* has been successfully tested in clinical trials in India and African countries in the meningitis belt (WHO, 2010). The vaccine, MenAfrivac ™ received WHO prequalification in 2010 and progress towards introduction is expected in Burkina Faso, Mali and Niger (WHO, 2010). In a study done in Dar es Salaam, Tanzania 55 of the pathogens identified in Meningitis patients were from children aged less than 15 years. The main organisms isolated included *Streptococcus pneumonia*, *Cryptococcus neoformans* and *Haemophilus influenzae* type b. (Matee *et al.*, 2002). Results of CSF

culture in neonates suspected of having meningitis in northern Tanzania hospital revealed *Salmonella enterica* serotype *Enteritidis* (Vaagland *et al.*, 2004).

In Ghana, 1% of seriously ill children referred to the Korle Bu Teaching Hospital in Accra were admitted with bacterial meningitis and many of these children had been ill for more than 4 days before arrival at the centre. The main causative organisms were *S. pneumoniae* (47.9%), *Neisseria meningitides* (38.4%) and *Haemophilus influenzae* (9.6%). All bacterial isolates were highly sensitive to ceftriaxone (Commey *et al.*, 1994).

In a similar study in Kenya by Mwangi *et al.* (2002), the most common organisms associated with meningitis infants were *Streptococci* and *Enterobacteriaceae.* In Malawi, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib) and *Salmonella typhimurium* were the most common causes of meningitis in neonates and the overall mortality was 40% (Molyneux *et al.*, 2006).

Neuman and Wald (1998), found that in 221 cases of bacterial meningitis in Pittsburgh, USA. *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* were identified. The overall incidence of bacterial meningitis was 40 per 100 000 for children younger than 5 years in Kansas, USA (Walling *et al.*, 1991). No significant excesses of cases were found in male patients or in blacks. Overall, *Haemophilus influenzae* was the most common causative organism and 32 % of cases were due to *Streptococcus pneumoniae* (Walling *et al.*, 1991).

In Yemen, 81.8% out of 77 suspected children were diagnosed as having acute bacterial meningitis (ABM) and the most affected age group was 4 months and 3 years (Sallam, 2002). Based on CSF culture the predominant organism was *Klebsiella* (33.33%), *Haemophilius influenza* (23.80%), *Streptococcal pneumonia* (14.28%), *E. coli* and

Pseudomonas (9.52%) with one case each of *Tuberculosis meningitis* and *Staphylococcus aureus* (Sallam, 2002).

2.1.2 Disease etiology

Meningitis is an inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges. The inflammation may be caused by infection with viruses, bacteria, or fungi, and less commonly by certain drugs (*Geiseler et al.*, 1980). Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore the condition is classified as a medical emergency (WHO, 2009).

Meningitis due to Neisseria meningitides occurs in small clusters throughout the world with seasonal variation and accounts for a variable proportion of epidemic bacterial meningitis (Segal *et al.*, 2004). Meningitis may occur as the result of several non-infectious causes such as spread of cancer to the meninges (*malignant meningitis*) (Chamberlain, 2005) and drugs including non-steroidal anti-inflammatory drugs, antibiotics and intravenous immunoglobulins (Moris *et al.*, 1999). The disease may also be caused by several inflammatory conditions such as sarcoidosis, connective tissue disorders such as systemic lupus erythematosus, and certain forms of vasculitis such as Behçet's disease (Ginsberg, 2004).

Epidermoid cysts and dermoid cysts may cause meningitis by releasing irritant matter into the subarachnoid space (Ginsberg, 2004; Tebruegge *et al.*, 2008). Mollaret's meningitis is a syndrome of recurring episodes of aseptic meningitis; it is thought to be caused by herpes simplex virus type 2 (Ginsberg, 2004). According to WHO (2009), it is a must practice to perform a lumbar puncture in children to exclude bacterial meningitis.

A lumbar puncture is a sterile diagnostic procedure of inserting a needle into a lumbar area of an individual for the purpose of collection of CSF (Straus *et al.*, 2006).

The CSF sample is examined for presence and types of white blood cells, red blood cells, protein content and glucose level. Gram staining of the sample may demonstrate bacteria in bacterial meningitis, but absence of bacteria does not exclude bacterial meningitis. Only 60% of cases may show bacteria in gram staining and this figure is reduced by a further 20% if antibiotics were administered before the sample was taken (Ginsberg, 2004). Gram staining is also less reliable in particular infections such as listeriosis (Mwangi *et al.*, 2002). Microbiological culture of the sample is more sensitive but results can take up to 48 hours to become available. The type of white blood cell predominantly present may indicate whether meningitis is bacterial or viral if lymphocytes are predominant (Matee *et al.*, 2002). However, type of white blood cells present in the CSF may not be a reliable indicator in the beginning of the disease. The less commonly occurring feature is eusinophils-predominant, suggesting presence of parasitic or fungi (Matee and Matre, 2002).

The concentration of glucose in CSF is usually above 40% that of blood (Mwangi *et al.*, 2002). In bacterial meningitis, the glucose level in CSF is typically lower than that in blood (Mwangi *et al.*, 2002). For diagnostic purposes, the CSF glucose levels is divided by the blood glucose level and ratio of ≤ 0.4 or ≤ 0.1 is indicative of bacterial meningitis in infants and newborn babies, respectively. High levels of lactate in CSF indicate a higher likelihood of bacterial meningitis, as is for higher white blood cell count (Mwangi *et al.*, 2002). Advanced bacterial meningitis can lead to brain damage, hydrocephalus, coma, and death. Survivors can suffer long-term complications, including hearing loss, mental retardation, paralysis, seizure and blindness (Gordon and Zumla, 2009).

Of the children with asexual *P. falciparum* parasitaemia under 1 year old, 14% (9/64) had identifiable bacteria in their CSF. Of MP-negative children under 1 year, 37% (14/38) had bacteria isolated from their CSF. Thus, children under 1 year with impaired consciousness are at particularly high risk of having bacterial meningitis whether or not they have malaria parasites seen on a blood film (Barkley *et al.*, 1999).

2.2 *Streptococcius pneumoniae*

Streptococcius pneumoniae may occur intracellularly or extracellularly as gram-positive lanceolate diplococci, but can also occur as single cocci or in short chains *S. pneumoniae* is a fastidious bacterium, growing best at 35-37°C with ~5% CO2 (or in a candle jar) (Commey *et al.*, 1994). It is usually cultured on media that contain blood, but can also grow on a chocolate agar (CA). On a blood agar plate (BAP), colonies of *S. pneumoniae* appear are small, grey, moist (sometimes mucoidal), colonies and characteristically produce a zone of alpha-hemolysis (Commey *et al.*, 1994).

The alpha-hemolytic property differentiates this organism from many species, but not from the commensal alpha-hemolytic (viridans) streptococci. Differentiating pneumococci from viridans streptococci is difficult as young pneumococcal colonies appear raised, similar to viridans streptococci. However, once the pneumococcal culture ages 24-48 hours, the colonies become flattened, and the central portion becomes depressed, which does not occur with viridans streptococci (Ajelo *et al.*, 1984).

Prior to identification and characterization testing procedures, isolates should always be inspected for purity of growth and a single colony should be re-streaked, when necessary, to obtain a pure culture. For the identification and characterization procedures, it is

essential to test alpha-hemolytic colonies that are less than a day old, typically grown overnight at 35-37°C with ~5% CO2 (or in a candle-jar) (Gordon and Zumla, 2009).

The following specialized tests are used to identify colonies on a BAP that resemble *pneumococci. S. pneumoniae* can be identified using Gram stain, catalase, and optochin tests simultaneously, with bile solubility as a confirmatory test (WHO, 2010). If these tests indicate that the isolate is *S. pneumoniae*, serological tests to identify the serotype can be performed (WHO, 2010). This sequence of testing is an efficient way to save costly serotyping reagents and time. Biosafety Level 2 (BSL-2) practices are required for work involving isolates of *S. pneumoniae*, as this organism presents a potential hazard to laboratory personnel and the surrounding working environment.(WHO, 2010).

2.3 *Haemophilus influenzae*

H. influenzae are small, pleomorphic, gram-negative *bacilli* or *coccobacilli* with random arrangements. *H. influenzae* is a fastidious organism which grows best at 35-37°C with ~5% CO2 (or in a candle-jar) and requires hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth (Murray *et al.*, 2007). The standard medium used for growth of *H. influenzae* is a chocolate agar plate (CAP), which can be prepared with heat-lysed horse blood, a good source of both hemin and NAD, although sheep blood can also be used. *Haemophilus influenzae* uses the enrichment growth factors and grow on chocolate agar (Murray *et al.*, 2007).

Growth occurs on a CAP because NAD is released from the blood during the heating process of chocolate agar preparation (the heating process also inactivates growth inhibitors) and hemin is available from non-hemolyzed as well as hemolyzed blood cells (Ajelo *et al.*, 1984). Alternatively, NAD can be included as a component of liquid

H. influenzae growth media supplements which are incorporated into the chocolate agar. *H. influenzae* appear as large, round, smooth, convex, colorless-to-grey, opaque colonies on a CAP (Murray *et al.*, 2007).

Encapsulated strains appeared more mucoidal than non-encapsulated strains, which appeared as smaller, compact grey colonies. No hemolysis or discoloration of the CAP is apparent (Commey *et al.*, 1994). While *H. influenzae* produce a pungent indole smell, plates should not be opened in order to smell the cultures. *H. influenzae* cannot grow on an unsupplemented BAP. Prior to identification and characterization testing procedures, isolates should always be inspected for purity of growth and a single colony should be re-streaked, when necessary, to obtain a pure culture.(Murray *et al.*, 2007). For the identification and characterization procedures, testing should be performed on one hour growth from a CAP at 35-37°C with ~5% CO_2 (or in a candle-jar) (Herbert *et al.*, 2005).

If the oxidase test is positive, hemin and NAD growth factor requirement testing should be performed. If the growth factor requirement test indicates that the isolate may be *H. influenzae*, serological tests to identify the serotype should be performed (Sallam, 2002). This sequence of testing is an efficient way to save costly antisera and time. Biosafety Level 2 (BSL-2) practices are required for work involving isolates of *H. influenzae*, as this organism presents a potential hazard to laboratory personnel and the surrounding working environment (Murray *et al.*, 2007).

2.4 *Group B Streptococcus*

Group B Streptococcus (*Streptococcus agalactiae*) is one of the leading causes of septicemia and meningitis in newborns. The incidence of Group B disease is estimated to be 2-5 infants per 1000 live births (Ajelo *et al.*, 1984 and Herbert *et al.*, 2005). Newborns

are at increased risk for Group B disease if they are born to women who are colonized with *Group B Streptococcus* in the vaginal or ano-rectal areas and who experience prolonged or difficult labor and delivery (Ajelo *et al.*, 1984). As a strategy for prevention of *Group B Streptococcus* disease CDC (2010), has recommended that all pregnant women be screened for ano-gental *Group B Streptococcus* colonization at 35 to 37 weeks gestation, so that intrapartum antimicrobial prophylaxis can be offered to all women identified as carriers.

Group B Streptococcus can also cause serious illness in adults but it is far less common than in newborns (CDC, 2010). Presumptive identification of this organism in this study was made by physiological and biochemical methods (Herbert *et al.*, 2005).

2.5 *Escherichia Coli*

2.5.1 Description of the organism

E. coli is one of several types of bacteria that normally inhabit the intestine of humans and animals. Some strains of *E. coli* are capable of causing disease under certain conditions when the immune system is compromised or disease may result from an environmental exposure. The vast majority of cases of *E. coli* meningitis are caused by a disease-causing serotype known as *E. coli* K1 (Martinho *et al.*, 2008).

E. coli can cause infections in wounds, the urinary tract, biliary tract, and abdominal cavity (peritonitis). This organism may cause septicemia, neonatal meningitis, infantile gastroenteritis, tourist diarrhea, and hemorrhagic diarrhea (Church *et al.*, 2008). An *E. coli* infection may also arise due to environmental exposure. Infections with this type of bacteria pose a serious threat to public health with outbreaks arising from food and water that has been contaminated with human or animal feces or sewage. This type of

bacteria has been used as a biological indicator for safety of drinking water since the 1890s. Exposure may also occur during hospitalization, resulting in pneumonia in immunocompromised patients or those on a ventilator (CDC, 2010).

2.5.2 *Enterohemorrhagic E. coli*

The O157:H7 serotype is the member of the group most often associated with a particularly severe form of diarrhea. (The O indicates the somatic antigen, while the H denotes the flagellar antigen, both of which are found on the cell surface of the bacteria (Chart *et al.*, 2000). The bacterium was discovered in 1977, and first reports of infections followed in 1982. *E. coli* O157:H7, as it is frequently referred to by researchers, causes bloody diarrhea in many infected patients. It accounts for about 2% of all cases of diarrhea in the Western world, and at least one-third of cases of hemorrhagic colitis, or about 20 000 cases per year (Chart *et al.*, 2000).

E. coli O157:H7 is also the most common cause of unique syndromes, known as the Hemolytic-Uremic Syndrome (HUS) and thrombocytopenic purpura (TTP), which causes kidney failure, hemolytic anemia, and thrombocytopenia (Chart *et al.*, 2000). However, about 5% of people who are infected will develop HUS/TTP. This infection also accounts for the majority of episodes of HUS, especially in children (Chart *et al.*, 2000).

This serotype of the bacterium produces a potent toxin called verotoxin, named for toxin's ability to kill green monkey kidney or "vero" cells (Dagan *et al.*, 1994). Bacteria that produce verotoxin are referred to as Verotoxin-producing *E. coli* (VTEC) (Chart *et al.*, 2000). *E. coli* O157:H7; is characterized by the breaking up of red blood cells (hemolysis) and kidney failure (uremia) (Shanson, 1999). The syndrome occurs most often in the very young and very old people *E. coli* O157:H7 is commonly found in cattle and poultry, and

outbreaks of disease have been associated with cattle and bovine products (Shanson, 1999). There are reports of contamination from unpasteurized apple juice, hamburger meat, radish sprouts, lettuce, and potatoes, as well as other food sources. Environmental contamination may occur in water drained from cattle pastures or water containing human sewage used for drinking or swimming. Human to human transmission, through contact with fecal matter, has also been identified in daycare enters (CDC, 2010).

2.5.3 *Enteropathogenic E. coli*

These strains of *E. coli* produce verotoxin, but are serotypes other than O157. There have been as many as one hundred different types implicated in the development of disease (Shanson, 1999). Strain OH111 was found to be involved in outbreaks in Australia, Japan, and Italy. The O128, O103, and O55 groups have also been implicated in diarrhea outbreaks (Shanson, 1999). In Britain, cases of infantile gastroenteritis in maternity hospitals and neonatal units have been attributed to the *E. coli*) non-0157 group. Many of these organisms have been identified in cattle (CDC, 2010).

2.5.4 *Enterotoxigenic E. coli*

Two toxins may be produced by this group, the heat-labile enterotoxin (LT) that can produce enteritis in infants, and a heat stable enterotoxin (ST), the action of which has yet to be determined (Shanson, 1999).

2.5.5 *Enteroinvasive E. coli*

Some strains of the enteroinvasive *E. coli* have been involved in the development of gastroenteritis in infants. The cells of the intestine are affected, with the development of symptoms that are typical of a shigellae infection (Chart *et al.*, 2000).

2.5.6 *E. coli* and acute bacterial meningitis

E. coli account for about 25% in cases of neonatal meningitis worldwide (CDC, 2010). Pregnant women are at a higher risk of colonization with the K1 strain of *E. coli*. This serotype has a protective capsule around the bacterial cell which allows it to survive better in acidic environments like the vagina (Kim, 2001).

The newborn becomes infected as it passes through the vaginal canal during birth. For this reason, the K1 strain is also commonly observed in neonatal sepsis. The mortality rate of neonatal sepsis is 8%, and most survivors have subsequent developmental abnormalities. In adults, *E. coli* meningitis is rare but may occur in surgical procedures or severe trauma to the brain (Kim, 2001).

2.7 Clinical Signs

Meningitis at any age is considered as medical emergency (CDC, 2010). The signs and symptoms of meningitis are not always obvious due to the inability of children to communicate symptoms. Therefore, very close attention to the children overall condition is needed (WHO, 2010). Common early symptoms of meningitis in infants younger than 3 months of age although not specific may include high grade fever, seizure activity, convulsions or twitching, stiff neck, bulging fontanel, increased irritability, rashes, opisthotonos position, decreased liquid intake, vomiting and lethargy (WHO, 2010). For the children older than 1 year of age may include fever, ear discharge, headaches, nausea and vomiting, increased sensitivity to light, altered mental status, lethargy ,seizure activity or convulsions, neck stiffness or neck pain, knees automatically brought up toward the body when the neck is bent forward or pain in the legs when bent called Brudzinski sign, inability to straighten the lower legs after the hips have already been flexed 90 degrees called Kerning sign and rash (CDC, 2010) (Plate 1, 2 and 3).

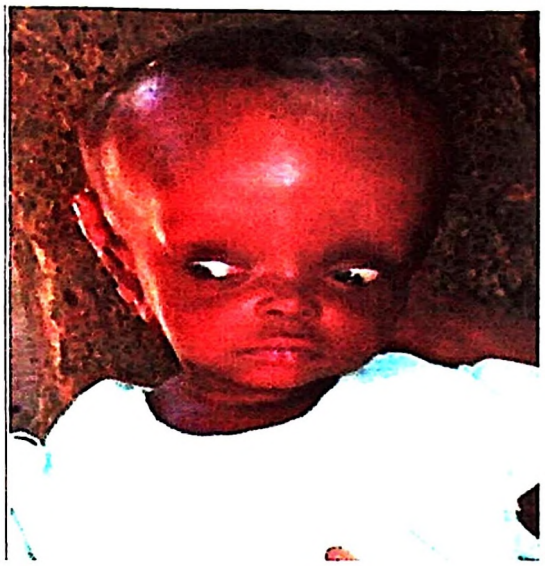

Plate 3: Child with hydrocephalus as a complication of meningitis especially bacterial. (Source: http/www.tanzamed.com/index.php/watoto/degedege)

2.8 Diagnosis

According to the WHO (2010) a probable bacterial meningitis case requires a child to have one or more signs or symptoms of meningitis and either a CSF with turbid appearance or CSF protein > 100 mg/dL or glucose < 40 mg/dL or WBCs > 100 cells/mm3 with > 80% neutrophils and no identifiable bacterial pathogen. Performing lumbar puncture in children is one of the useful in diagnostic procedures for detecting CNS infections or pathology. In some hospitals, lumbar punctures are rarely performed, mainly because of the low rate of isolation of bacterial pathogens even in patients with overt features of acute bacterial meningitis. This may be due to prior antibiotic therapy or poor handling of CSF specimens including delays in processing specimens (Herbert *et al.*, 2005). At Muhimbili National Referral Hospital (MNRH) in Tanzania, indications for lumbar puncture have been adapted from the Integrated Management of Childhood Illnesses (IMCI) guidelines. In neonates these include fever (≥38.5°C) or hypothermia, bulging fontanelle (or acuteincrease in head circumference), high-pitched cry, irritability,

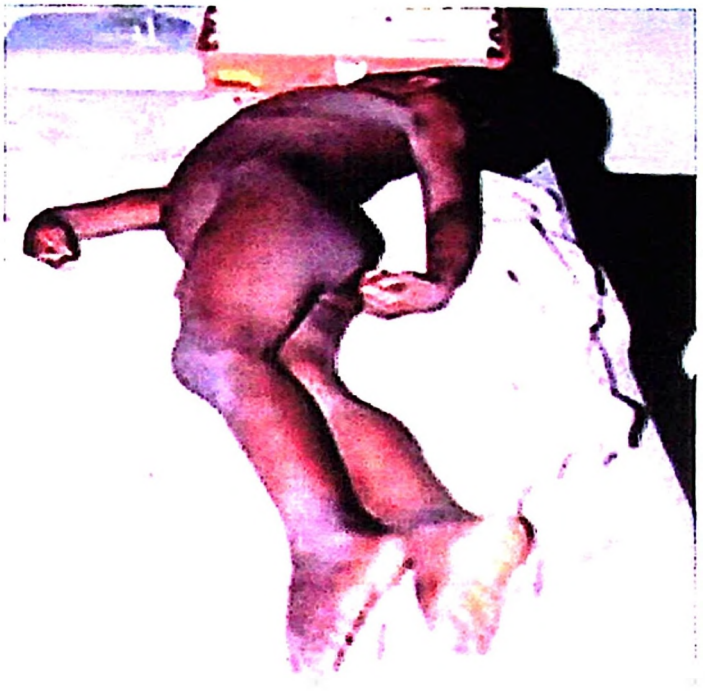

Plate 1: Opisthotonos position for a child with suspected meningitis

(Source: http/www.tanzamed.com/index.php/watoto/degc degc).

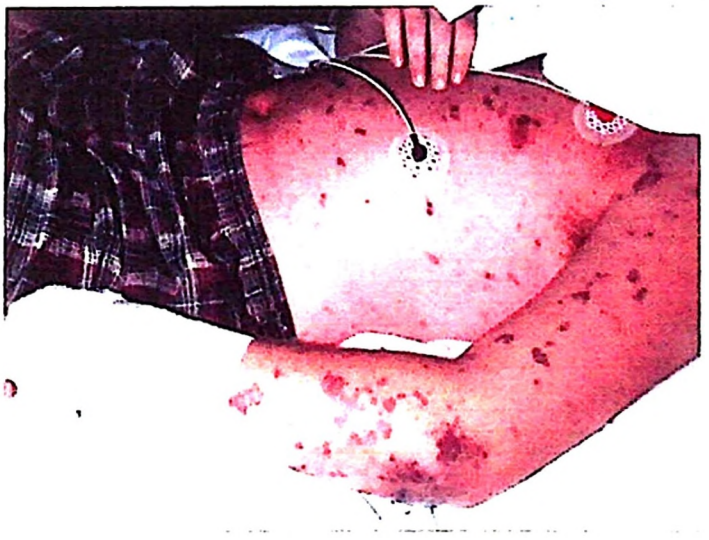

Plate 2: Characteristic rash for meningitis and especially meningitis due to *Neisseria meningitidis*.

(Source; http//www.kidsfriendlynz.com/index.php/meningitis)

lethargy, altered mental state and poor feeding. Bacterial meningitis is usually suspected in a patient (>3 months including adolescents) with a history of fever and headache, photophobia, stiff neck, irritability or lethargy, vomiting and altered state of consciousness or convulsions. In such patients, lumbar puncture is indicated. Patients are usually initiated on treatment with antibiotics and antimalarial targeting suspected bacterial meningitis (Herbert *et al.*, 2005).

Typical findings in the CSF in bacterial meningitis include pleocytosis, usually with a WBC count greater than 1000 cells/mm^3 and predominance of polymorphonuclear leukocytes. In some cases, especially when performed early in the disease, the WBC count can be normal and there may be lymphocyte predominance (Freedman *et al.*, 2001). Glucose concentration usually is decreased with a CSF-to-serum glucose ratio of 0.6 or less in neonates and 0.4 or less in children older than 2 months of age, whereas protein concentration usually is elevated (Saez-Llorens, 2003).

The Gram-stained smear of CSF has a lower limit of detection of about 105 colony-forming units/mL. Of patients with untreated bacterial meningitis, 80% to 90% have a positive CSF Gram stain. Unless unusual pathogens, such as anaerobes, are suspected, agar plate cultures of CSF are preferred to liquid media. Routine inoculation of CSF into broth culture is not recommended because isolates recovered by this technique are frequently contaminants (Meredith *et al.*, 1997; Stugis *et al.*, 1997). With the exception of meningitis caused by gram-negative enteric bacilli, the yield of bacterial CSF cultures decreases soon after antibiotic therapy has been initiated (Kanegaye *et al.*, 2001). The CSF WBC count and glucose and protein concentrations generally remain abnormal for several days, however, after initiating appropriate antibiotic therapy. The use of latex agglutination tests to detect bacterial capsular antigens is recommended in patients with

suspected bacterial meningitis who have been receiving antibiotics at the time the lumbar puncture is performed (Susan *et al.*, 2005).

These tests are not specific, however, and they identify very few cases of bacterial meningitis not already detected by CSF culture (Nigrovic *et al.*, 2004). In the future, more sensitive techniques, such as amplification of the 16S rRNA gene by polymerase chain reaction, may help to diagnose cases of bacterial meningitis in patients pretreated with antibiotics. Broad-range polymerase chain reaction has shown a sensitivity of 86% and specificity of 97% in detecting multiple organisms simultaneously compared with culture (Schurman *et al.*, 2004). Real-time polymerase chain reaction techniques are even more sensitive in the clinical setting (Bryant *et al.*, 2004).

Ziehl–Neelsen stain for AFB

The Ziehl–Neelsen stain, also known as the acid-fast stain, was first described by two German doctors: the bacteriologist Franz Ziehl (1859–1926) and the pathologist Friedrich Neelsen (1854–1898) (Gordon and Alimuddin, 2009). It is a special bacteriological stain used to identify acid-fast organisms, mainly *Mycobacteria*. *Mycobacterium tuberculosis* is the most important of this group because it is responsible for tuberculosis (TB). Acid fast organisms like *Mycobacterium* contain large amounts of lipid substances within their cell walls called mycolic acids (Black *et al.*, 2000). These acids resist staining by ordinary methods such as a Gram stain. It can also be used to stain a few other bacteria, such as Nocardia. The reagents used are Ziehl–Neelsen carbolfuchsin, acid alcohol, and methylene blue. Acid-fast bacilli will be bright red after staining (Kaplan *et al.*, 2004).

A variation on this staining method is used in mycology to differentially stain acid-fast incrustations in the cuticular hyphae of certain species of fungi in the genus *Russula*. It is also useful in the identification of some protozoa, namely *Cryptosporidium* and *Isospora* (Barkley, 1999).

Latex Agglutination Test

The latex agglutination test is a laboratory method to check for certain antibodies or antigens in a variety of bodily fluids including saliva, urine, cerebrospinal fluid, or blood (Martin *et al.*, 2004). After culture is positive the latex agglutination test was done to conclude the presence of suspected bacteria colony in the growth culture where it was mixed with latex beads coated with a specific antibody or antigen. The latex beads clumped together (agglutinate) if the suspected organism was present (David, 2011).

2.8.1 Cerebrospinal Fluid

Examination of CSF is an essential step in the diagnosis of any patient with evidence of meningeal irritation or affected cerebrum and should be collected before starting antimicrobial therapy (CDC, 1998). Physical inspection is recommended immediately after collection and findings should be indicated on a laboratory request forms (Barkley *et al.*, 1999). Clear with Tyndall effect shows high protein content, clear yellowish shows old haemolysis, clear red shows fresh haemolysis, turbid blood-stained shows haemorrhage, turbid white shows high cell or protein content while turbid clot after overnight storage shows fibrin clots (CDC, 1998).

2.9 Treatment

Factors considered when selecting the appropriate antibiotic for treating bacterial meningitis include its activity against the causative pathogen and its ability to penetrate

and attain effective bactericidal concentrations in the CSF with broad spectrum effect. The integrity of the blood-brain barrier is compromised during meningitis, resulting in increased permeability to most antibiotics. Beta Lactam antibiotics achieve concentrations of 5% to 20% of concomitant serum values (Susan and George 2005). Even in the absence of inflammation, penetration of highly lipid-soluble antibiotics, such as rifampin, chloramphenicol, and quinolones, is 30% to 50% of serum concentrations (Susan and George 2005).

Experimental models of bacterial meningitis suggest that prompt bacteriologic cure is predictable if antibiotic concentrations that are10-fold to 30-fold greater than the minimal bactericidal concentration (MBC) for a specific microorganism are attained in CSF (Susan and George 2005).

Intravenous third generation cephalosporin, ceftriaxone sodium was used at 80mg/kg body weight for ten days (Gordon and Alimuddin, 2009). All patients with clinical signs and symptoms of meningitis were treated first with third generation cephalosporin and continued even if the CSF findings were negative. Supportive care included; diazepam in case of convulsions, nasogastric tube for feeding, oxygen therapy, antipyretics for fever, analgesics and urinary catheterization (MoH, IMCI, 2009). Current recommendations support the use of dexamethasone in infants and children with Hib meningitis (Tunkel *et al.*, 2004).

For infants and children 6 weeks old and older with *pneumococcal* meningitis, adjunctive therapy with dexamethasone should be considered after weighing the potential benefits and possible risks. Data are insufficient to recommend dexamethasone therapy in neonates with bacterial meningitis (Tunkel *et al.*, 2004). Recommended dexamethasone dosing

regimens range from 0.6 to 0.8 mg/kg daily in two or three divided doses for 2 days to 1 mg/kg in four divided doses for 2 to 4 days (Saez-Llorens and Mc Cracken, 2003; Tunkel, *et al.,* 2004).For optimal results, the first dose of dexamethasone should be administered before or concomitant with the first parenteral antibiotic dose (Tunkel *et al.*, 2004).

2.10 Control and Prevention

2.10.1 Immunization against meningitis

Immunization with the *Haemophilus, pneumococcal,* and *meningococcal* conjugate vaccines has had a significant impact on the incidence of invasive diseases in children caused by these organisms. The routine use of conjugated Hib vaccines in children has been associated with a reduction of more than 99% of invasive disease, including meningitis, in developed countries. Rates of Hib disease have been affected modestly in other areas of the world where the vaccine is not routinely available (CDC, 2002; Peltola, 2000). However, in the year 2000 the heptavalent conjugate *pneumococcal* vaccine, PCV7, was approved for routine use in infants. Initial clinical trials showed a reduction of more than 90% in invasive *pneumococcal* infections in children (Black *et al.*, 2000). Subsequent clinical studies have confirmed the efficacy of conjugated *pneumococcal* vaccine in children and a concomitant reduction in the incidence of invasive *pneumococcal* disease in adults, attributed to reduced circulation of the bacteria (Black *et al.*, 2000; Kaplan *et al.*, 2004; Whitney *et al.*, 2003).

A major problem of vaccine development for this serogroup is the homology of this bacterium capsular polysaccharide with components of human neural tissue. Current research is on going to improve the immune response to vaccines designed against this serogroup, which is endemic in North America and Europe (Danzig, 2004). Maternal immunization with GBS (*Streptococcus agalactiae*) conjugate vaccine may represent a

future strategy to reduce neonatal GBS *streptococcal* bacterial meningitis disease. Maternal administration of prophylactic antibiotics has an impact on preventing only early-onset GBS disease (Provan and Andrew, 2005).

2.10.2 Chemoprophylaxis

Administration of prophylactic antibiotics to asymptomatic contacts of meningitis index cases is indicated to decrease carriage and prevent spread of the disease (Susan and George, 2005).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area and Duration

This study was conducted at MRRH Paediatrics department. The hospital is situated about 1Km north of Morogoro town, it is the referral hospital for the region. The hospital has five main patients' categories which are outpatient department, paediatrics department, surgery department, internal medicine department and obstetrics and gynecology department. This study was conducted for 7 months involving admitted children in paediatric wards between September, 2011 and March 2012.

3.2 Study Design and Sampling Procedure

A descriptive study was conducted among admitted children in paediatric wards. Non probability convenience sampling method was used to obtain patients to be included in the study. The study population involved children admitted in paediatric wards with the age starting between 7 days old (neonates) and children with the age up to 12 years. A total of 72 children suffering from meningitis were involved in the study according to the non probability sampling method. The sample size was determined according to the number of coming cases during the study duration.

3.3 Ethical Considerations

Ethical clearance was sought from Ethical Clearance Committee of SUA, and permission to conduct the study was requested from Authorities of the MRRH and the National Institute for Medical Research (NIMR) with reference number NIMR/HQ/R.8a/Vol. IX/1240.Respondents were informed on objectives of the investigations and that signed

consent sufficed for one to be included. To ensure confidentiality and improve response no name of respondent or patient was recorded on the data recording book.

3.4 Sample Collections

All patients with fever and either of the following signs; convulsions, neck stiffness, impaired level of consciousness, severe headache, opisthotonos position, irritability and petechial rashes were taken for the study. Lumbar puncture was done and the "opening pressure" of the CSF was assessed (Barkley *et al.*, 1999). Patients with signs of increased intracranial pressure such as bulging of fontanels and parents refusal, lumbar puncture procedure were not performed. Other parameters such as body temperature, level of consciousness, nutritional status assessment and bedside *invitro* antigen-antibody HIV test were done using Tanzanian alogarithm for HIV testing by starting with SD bioline ® and then if positive results, further test were performed using Determine ®. The antibiotic usage information from parents/guardians who sought care of children was asked about prior use of antibiotics for those who came without a referral letter.

The initial appearance of the fluid was assessed. CSF glucose levels were analyzed using electronic glucometer machine model gluco-plus ®. Blood glucose levels were taken using bedside glucometer kit, model gluco-plus ® and the ratio of CSF glucose to serum glucose level was calculated and compared (Barkley *et al.*, 1999). Samples of CSF were kept in sterile red topped bottles which were labeled and sent to the laboratory immediately for analysis.

3.5 Lumbar Puncture Procedure

Only 2 tubes used for chemistry and microbiology because of limited resources. The kit for collection of CSF for children contained: skin disinfectant, sterile gauze, lumbar

puncture needle 23 gauge/2.5 inches, sterile screw-cap tubes, syringe and needle, transport container and Trans-Isolate (T-I) medium was used when CSF could not be analyzed in the microbiological laboratory immediately (CDC, 1998).

The children were lying on the side, with his or her back arched forward so that the head almost touched the knees during the procedure. For conscious children sedation was done to keep the child calm. The skin around the insertion area was dis-infected along the line drawn between the crests of the two ilia with 70% alcohol to clean the surface (CDC, 2010). Povidone-iodine was also applied at the area for further dis-infection. The needle was introduced deeply between Lumbar 3 and 4 or lumbar 4 and 5 and 1-2 mls of CSF depending on the opening pressure were collected into sterile, screw- cap tubes each. The specimens were labeled according to the patient name and file number and were immediately submitted to the laboratory for analysis.

3.6 Laboratory Evaluations

Gram stain was performed on un-centrifuged CSF. One to two drops each of CSF were streaked onto commercially prepared blood and chocolate agar media. Residual CSF was inoculated into 5% Fildes broth. The CSF broth was routinely sub-cultured after 24 hours, 72 hours, and 7 days of incubation (Daly *et al.*, 1992). Blood Agar Plates were applied with a filter paper/disk saturated with X and V factors to the surface of the medium. A bacteriological loop was used to streak the bacteria into single colonies (CDC, 1998). The agar plates were incubated in a 5% carbon dioxide incubator (candle jar). T-I medium was used as transport media, after 24 hours of incubation, with a sterile needle and syringe, 100 mml of the liquid portion of T-I was transferred onto BAP and CAP, and streaked for isolation. Bacterial identification was performed using standard microbiologic criteria according to their colonial morphology and properties (CDC, 1998). Positive

growth cultures were tested for the presence of Hib, *S.pneumoniae*, *E. coli* and *Group B Streptococci* by latex agglutination meningitis test system (Martin *et al.*, 2004).

3.7 Gram Stain Procedure for CSF (Hucker Modification)

The smear was prepared by placing 1 or 2 drops of CSF sample on an alcohol-rinsed and dried slide, allowing drops to form one large drop. The slide was air dried in a biosafety cabinet and passed quickly through a flame three times to fix the smear and it was not flamed until dried. Ammonium oxalate-crystal violet was used to flood the smear and let stand for 1 minute then rinsed gently with tap water and excess water was drained off. The smear was flooded with Gram's iodine solution and let to stand for 1 minute. After that it was gently rinsed with tap water and drained then decolorized with 95% ethyl alcohol 5-10 seconds (CDC, 1998).

Counterstain with carbol-fuchsin for 10-15 seconds was done and then the slide was rinsed with tap water and blot dried. The stained smear was examined microscopically, using a bright-field condenser and an oil-immersion lens (CDC, 1998).

3.8. Performing ZN Staining Procedure

The CSF sample was kept on a glass slide, slightly fixed and then the strong solution of carbol fuchsin was flooded onto the slide. The heat was applied below the slide until the fumes arose and cautions was taken not to ever heat or boil the sample. After cooling for some few seconds the heat was re-applied again until the fumes were seen. The slide was again allowed to cool and washed well with running water for 1 to 2 minutes (Gordon and Alimuddin, 2009).

Then the drops of decolorizer (20% Sulphuric Acid solution) were pored onto the smear and left to act for 1 to 2 minutes. The slide was washed in running tap water and then decolorized with 20% Sulphuric Acid for a few minutes and washing thoroughly until the smear was colorless or faintly. Few drops of the counterstaining Loffler's methylene blue solution was poured on the smear and left to act for few minutes. After that the slide was washed by the running tap water and blot dried ready to be examined under the microscope and for acid fast bacilli, if no AFB it appeared purple and the background was blue in all cases (Gordon and Alimuddin, 2009).

3.9 Performing Latex Agglutination Testing

The isolates to be tested were grown for 18-24 hours on a BAP at 35-37°C in a candle jar. From overnight growth on a BAP, a sterile loop was used to prepare a light to moderate cell suspension (approximately equal to a 0.5 McFarland density standard) in 0.5 ml of 0.85% saline (Murray *et al.*, 2007). On a glass slide or reaction card, 10 µl (1 droplet) of the latex reagent was added then and then 10 µl of the cell suspension added. The two suspensions were mixed together and the latex agglutination reaction was observed at an angle with oblique lighting. A positive reaction was indicated by agglutination (cells clumping together) appearing within 2-10 minutes and a negative reaction was indicated by no agglutination and the suspension appearing homogenous milky and if the reaction time exceeded 30 minutes, it was taken as false positive reaction (Murray *et al.*, 2007).

Each lot of latex suspension was tested for positive agglutination reactions using specific organism's reference strains with known capsular serotypes. For the biosafety reasons, a solution of 5% formalinized physiological saline was used to kill the bacterial isolates after the identification. Antisera was stored in the refrigerator at 4°C and warmed to room

temperature (25°C) before use. It was kept back in the refrigerator as soon as the testing was finished to prevent the loss of binding activity of the antibody (Shanson, 1999). Other analyses included turbidity test done immediately after collection, glucose level, protein level and cell count (Straus *et al.*, 2006).

3.10 Data Management and Statistical Analysis

Laboratory record audits were conducted by comparing logbooks (CSF specimen records) information with study laboratory reports to identify missed meningitis cases. The data obtained were coded and analyzed by SPSS computer program version 12 (SPSS Inc, Chicago, Illinois). Categorical comparisons were performed with the chi-square test. Test statistics were evaluated with a significance level of $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

A total of 1352 children aged between 7 days and 12 years were admitted in paediatric wards from September 2011 to March 2012. Of the admitted children, 72 had signs and symptoms of meningitis and lumbar punctures were performed to collect CSF for observation and laboratory analysis.

Optical observation of the 72 CSF samples revealed turbidity in 33 (45.8%) specimens whereas 39 (54.2%) samples were clear. Only one CSF specimen was ZN stain positive for Tuberculosis. Bacterial culture revealed *Streptococcus pneumoniae* in 23 (31.9%). CSF samples, *Haemophilus influenzae* in 6 (8.3%) samples, group B *Streptococcus* in 5 (6.9%) samples and *E. coli* in 3 (4.2 %) samples. Thirty five (48.6%) samples showed no bacterial growth. A total of 31 (43.1%) children died either before or during treatment where as 19 (26.4 %) children recovered fully and 14 (19.4%) children recovered with signs of neurological deficit. The remaining 8 (11.1%) children were referred to Muhimbili National Referral Hospital. Of the treated patients 34 (47.2%) children responded well to current treatment practice of intravenous ceftriaxone 80mg/kg body weight once a day for 10 days.

Table 1: Bacterial Isolates from CSF culture

Bacteria	Number	Percentage
S. pneumoniae	23	31.9
H. influenzae	6	8.3
Group *B streptococci*	5	6.9
E. coli	3	4.2
No growth	35	48.6
Total	72	100

Table 1 shows that out of 72 children with meningitis symptoms the CSF culture samples yielded 23 (31.9%) *Streptococcus pneumoniae*, 6 (8.3%) *Haemophilus influenzae*, 5 (6.9%) group B *streptococcus*, 3 (4.2%) *Escherichia coli.* and 35(48.6%) with no bacteria growth. However, 35 (48.6%) CSF samples showed no bacterial growth. It shows that *Streptococcus pneumoniae* is the most for quaintly isolated bacteria among children aged between 2 months old and below one year old with meningitis symptoms admitted at the MRRH.

Table 2: Gram stain results

Gram stain	Number	Percentage
Gram positive	30	41.7
Gram negative	7	9.7
No staining	35	48.6
Total	72	100

Table 2 shows that out of 72 children with meningitis symptoms the CSF gram staining yielded 30 (41.7%) gram positive, 7 (9.7%) gram negative and 35 (48.6%) were no staining at all. Gram positive organisms were the most prevalent in meningitis cases in children at MRRH.

Table 3: Culture and Treatment response

Response to therapy	Bacterial growth positive	No bacterial growth	Total
Responded	17	17	34
Not responded	20	18	38
Total	37	35	72

Table 3 shows that out of 72 children with meningitis symptoms 34 (47.2%) responded to treatment where as 38 (52.8%) did not respond to treatment. Of the children who responded treatment 17 (50%) had CSF bacterial culture positive and 17 (50%) had CSF bacteria culture negative.

On the other hand, 17 (48.6%) children with meningitis symptoms and CSF culture negative responded to the treatment regimen. This indicates that the diagnostic methods used were not sensitive enough to identify all the organisms present in the CSF. The table also shows that 18 (51.4%) children were CSF culture negative and also did not respond to the treatment.

Table 4: HIV status and meningitis treatment response

Response to therapy	HIV positive	HIV negative	Total
Responded	10	21	31
Not responded	13	24	37
Total	23	45	68

Table 4 above shows that out of 72 children with meningitis symptoms 68 (94.4%) were tested and HIV status known by *in vitro* antibody antigen diagnosis and only first reading was considered in the conclusion of the HIV status. Out of 68 children, 23 (33.8%) were HIV positive and 45 (66.2%) were negative while 31 (45.6%) responded to the meningitis treatment where as 37 (54.4%) did not respond to treatment. Of the children who responded to treatment 10 (32.3%) were HIV positive and 21 (67.7%) were HIV negative. However, out of 37 (54.4%) of the children who did not respond to the treatment 13 (35.1%) were HIV positive and 24 (64.9%) were HIV negative.

Table 5: Relationship between events CSF Turbidity and bacterial culture

		Culture					Total
		S. neumoniae	*H. influenzae*	Group B Streptococci	*E. coli*	No bacteria growth	
CSF Turbidity	Turbid	7	2	3	1	26	39
	Clear	16	4	2	2	9	33
Total		23	6	5	3	35	72

Table 5 above shows that among 72 CSF sample of the children with meningitis symptoms 39 (54.2%) samples were turbid and 33 (45.8%) samples were clear by optical measures only. Of the turbid CSF samples on culture, 7 (17.9%) showed positive for *Streptococcus pneumoniae,* two (5.1%) for *Haemophylus influenzae*, 3 (7.7%) for group B *Streptococcus*, one (2.6%) for *Escherichia coli* (Plate 18) and 26 (66.7 %) had no bacterial growth. On the other hand, of the 33 (45.8%) clear CSF samples, 24 were bacterial culture positive, 16 (48.5%) showed *Streptococcus pneumonia*, 4 (12.1%) had *Haemophylus influenza*, 2 (6.1%) were group B *Streptococcus*, 2 (6.1%) *Escherichia coli* and 9 (27.3%) did not show any bacterial growth.

4.2 Discussion

The incidence of meningitis among children aged 7days to 12 years was 5.3% (n=1352) which is lower compared to 11% from a similar study done in Kenya (Mwangi *et al.*, 2002). This incidence is also lower compared to another study done in Dar es Salaam at Muhimbili National Hospital by Herbert *et al.* (2005) which was 9%, although the methods of identification with diagnosis were different.This justifies the need for further studies in other regional referral hospitals in order to determine the national incidence and prevalence of bacterial meningitis in children.

The study revealed that *Streptococcus pneumoniae* was the most prevalent bacteria associated with meningitis in children in MRRH. This finding is similar to a retrospective study done in Dar es salaam which reported that *S. pneumoniae* was the most prevalent isolate in the CSF of the children with signs of meningitis (Matee and Matre, 2000). Another study in Malawi found a higher prevalence of *Streptococcus pneumoniae* meningitis cases in children aged between one month and 5 years of age (Molyneux *et al.*, 2006).

The current management of meningitis at MRRH was not optimal since 38 children (52.8 %) did not respond to the therapy of intravenous ceftriaxone (80mg/kg body) weight for 10 days. However, 20 (54.1%) children with CSF bacterial culture positive did not respond to the treatment regimen and might suggest either presence of resistant bacteria or presence of other usual causes of meningitis like fungal, viral or cancer.

Without any doubt, it has been observed that many samples on optical view were turbid but on culture there were no bacterial growth while other samples were clear on optical view but the cultures were positive for bacterial growth. This might be due to the effect of antibiotic used or the sensitivity of the method used to culture the CSF samples. Also the turbidity of the other samples was affected by the contaminants in the red toped bottles used before to keep the CSF samples as started earlier. Therefore, these findings suggest that culture of the CSF sample is mandatory.

According to the literatures, ceftriaxone resistance is still not yet reported hence the need for further studies on the causes of meningitis other than bacterial infections. In a similar study on meningitis in children done in Ghana by Commey *et al.* (1994), all the bacterial isolates were highly sensitive to ceftriaxone treatment. In a randomized controlled trial for the treatment of bacterial meningitis in children, it was shown that ceftriaxone is the best drug for the treatment of acute bacterial meningitis in children done by Schaad *et al.* (1990).

The study also showed that meningitis can affect any child regardless of the HIV status. However, the effect of the HIV status once *in vitro* antibody antigen reading without considering the effect of window period and maternal antibody as discussed above calls

for further studies with the use of DNA-PCR method which is the conclusive diagnosis of HIV virus presence in the body.

Of all the cases with very low CSF pressure, 80 % (N=72) were also turbid. In some clear CSF the culture became positive, hence this strongly suggests for the initiation and continuation of antibiotic irrespective of the turbidity of the CSF. There are no specific places of residence where we grew a certain type of organism only so this rule out the case of epidemics. All of the children who failed to recover were also co-morbid with other terrible diseases like protein-energy mal-nutrition, pneumonia, severe malaria and diarrhea which was not unusual finding. There were few cases which found also to be with measles. So this calls for further studies as to why these children were co-morbid with more than one disease, is it the issue of immunity or presence of one disease influencing the other.

Streptococcal pneumoniae was found to be the most prevalent organism in the culture compared to other bacteria and the age group it most commonly associated was between 2 months old and below one year old. Although this organism is sensitive to ceftriaxone most of the children failed to recover upon administration of the drug so this also needs further evaluation to check if the resistance is present or there is a change in strains of the *S. pneumonia* since this study had a time limit in doing that. Group *B streptococci* were found to be the most isolated organism in culture for the neonates and this correlates with the study done in Sudan (Sallam, 2002).

Also there were 9 cases which had the same infected organisms but it is not known as to why other survived and recovered completely and the other died while all of them were administered with the same drug. There were 5 cases with culture negative and did not

recover from the disease hence call for further investigation to rule out the viral or fungal involvement in the disease (Commey *et al.*, 1994).

There was only one child who was diagnosed to have tuberculous meningitis and had positive ZN stain results and was treated with ant-TB medication, recovered fully. This study proved the presence of TB meningitis in Morogoro. In a hospital based study in Pakistan Qazi *et al.* (1998) reported 11 cases of Tb meningitis over a period of one year. Further studies shall be recommended to be done in Tanzania in order to establish the prevalence of Tb meningitis in children in the country.

The use of *in vitro* antibody antigen test with single reading to test for HIV infection also biased to the study and was source of error since in children below 18 months only early infant diagnosis DNA PCR method is conclusive for HIV infection. This method was not used in this study due to limited budget hence there were possibilities of false positive due to maternal antibodies and false negative due to window period if the HIV infection occurred during labor.

4.3 Study Limitations

First, limited standard clinical evaluations and obtained CSF cultures from children with meningitis and we did not systematically evaluate and enroll children with cellulitis, pneumonia, epiglottitis, septic arthritis, or osteomyelitis which was important to isolate the organisms cause meningitis in children. Delayed reporting for medical services was the main obstacle as many children died before lumbar puncture was performed and thus possibly interfering with the exact number of meningitis cases recorded at the hospital. Also performing the lumbar puncture procedure on conscious children was also a challenge as it was difficult to restraint the children because of the fear of injections.

Second, blood culture were also very important in diagnosis and confirmation of bacterial meningitis but was not done due to the limitations of agar plates and incubators.

Third, although considerable efforts were made to use surveillance and laboratory methods that would enhance detection of meningitis, many of the examined children were diagnosed with meningitis or upper respiratory infection earlier in other hospitals and probably had received antibiotics prior to MRRH admission. In a survey from a community near Morogoro urban and others noted that 95% of children with acute respiratory infection or fever received treatment with antibiotics prior to hospital admissions (MRRH, 2009) Thus, in our study, the antibiotic usage information from parents/guardians who sought care likely underestimates the actual number of children pretreated with antibiotics because parents were instructed to report treatment as unknown if they could not precisely recall the use of an antibiotic.

Finally, in this study, 26 children found with abnormal CSF consistency and turbidity with suspected or probable bacterial meningitis also had negative CSF cultures. These results are consistent with previous studies showing that CSF in patients with antibiotic treatment may show abnormal test results even after cultures and/or antigen test results have become negative (Kanegaye *et al.*, 2001).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

The present study has documented for the first time the presence of bacteria meningitis among children admitted at Morogoro Regional Referral Hospital. The finding of TB meningitis in the study is a very crucial information that calls for an alert to health workers and the public health sector. The positive response to TB therapy implies that early diagnosis of TB meningitis for all meningitis cases is of paramount importance. According to this study, in future, suspected cases of meningitis should be handled as emergency cases, lumbar puncture and start of antibiotic treatment with intravenous ceftriaxone should not be delayed. Also, laboratory results should be out within 24 hours after hospital admission.

Further studies to characterize the organisms in order to rule out the presence of multiple serotypes with varying degrees of resistance are called upon. It is recommended that all samples from meningitis suspected cases, regardless of the turbidity status should be subjected to bacterial culture and sensitivity testing.

The good response to treatment with ceftriaxone in meningitis cases observed recommends for further use of the drug in the treatment. Above all still meningitis vaccination as recommended by WHO (2000) is necessary because of the endemicity of this diseases.

Finally, early diagnosis and treatment might assist in the control of meningitis in children, hence the need for sensitization of the public on the importance of seeking early medical services.

REFFERENCE

Ajelo, G. W., Feeley, J. C., Hayes, P. C., Reingold, A., Bolan, G., Broome, C. and Phillips, C. (1984). Trans-Isolate Medium: A New Medium for Primary Culturing and Transport of *Neisseria meningitidis, Streptococcus* pneumoniae, and *Haemophilus influenzae. Journal of Clinical Microbiology* 20: 55 – 58.

Berkley, J. A., Mwangi, I., Mellington, F., Mwarumba, S. and Marsh, K. (1999). Cerebral malaria versus bacterial meningitis in children with impaired consciousness in Kenya. *QJM International Journal of Medicine* 92(3): 151 – 157.

Beveridge, T. J. and Davies, J. A. (1983). Cellular responses of *Bacillus subtilis* and *Escherichia coli* to the Gram stain. *Journal of bacteriology* 156(2): 846 – 858.

Bicanic, T. and Harrison, T. S. (2004). Cryptococcal meningitis. *British Medical Bulletin* 72: 99 –118.

Black, S., Shinefield, H. and Fireman, B. (2000). Efficacy, safety and immunogenicity of heptavalent *pneumococcal* conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatric Infectious Disease Journal* 19: 187–195.

Bryant, A., Li, Y. and Zaia, A. (2004). Prospective study of a real-time PCR that is highly sensitive, specific, and clinically useful for diagnosis of meningococcal disease in children. *Journal of Clinical Microbiology* 42: 2919 – 2925.

Carroll, C. and Carrol, K. (1993). The epidemiology of bacterial meningitis occurring in a Pacific Island population. *P N G Medicine Journal* 36(3): 234 – 242.

CDCP (1998). *Laboratory Methods for Diagnosing Meningitis Caused. Neisseria meningitidis, Haemophilus influenzae* and *Streptococcus pneumoniae.* Atlanta, USA. 249pp.

CDCP (2002). *Progress Toward Eliminating Haemophilus influenzae.* Type B disease among infants and children United States, 1998–2000.Weekly Report No. 51. New York, USA. 237pp.

CDCP (2009). Meningitis in children. [http://www.cdc./meningitis/about/faq.html] site visited on 3/6/2011.

CDCP (2010). Preventing Foodborne Illness: Escherichia coli 0157:H7. [http://www.cdc. gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm] site visited on 10/9/2012.

Chamberlain, M. C. (2005). Neoplastic meningitis. *Journal of Clinical Oncology* 23(15): 3605 – 3613.

Chart, H., Sussman, M. and Stewart, D. (2000). *E. coli*-Friend or Foe? The society for applied microbiology symposium. *Journal of Applied Microbiology* 20(13); 2059-2061.

Church, D., Baxter, H., Lloyd, T., Miller, B. and Elsayed, S. (2008). *Evaluation of Strep B Carrot Broth Versus Lim broth for Detection of Group B Streptococcus.* Colonization status of near-term pregnant women. Accra, Ghana 2782pp.

Commey, J., Rodrigues, O., Akita, F. and Newman, M. (1994). Bacterial *meningitis* in children in Southern Ghana. *East Africa Medical Journal* 71(2): 113 – 117.

Dagan, R. (1994). *Epidemiology of Pediatric Meningitis Caused by Haemophilus Influenzae B, Streptococcus pneumoniae and Neisseria Meningitidis* Israel. *Journal of Infection Disease* 196(4): 912 – 916.

Daly, J., Seskin, K. and Pezzlo, M. (1992). *Processing and Interpretation of Cerebrospinal Fluid.* Clinical microbiology procedures handbook. American Society for Microbiology Press, Washington, DC. 72pp.

Dang, D., Paul, E., Willium, A., Batmunkh, N., Hoang, T., Long, L., John, D. and Joel, I. (2002). *Haemophilus influenzae* type b meningitis among children in Hanoi, Vietnam. *American Journal of Tropical Medicine* pp. 9 – 515.

Danzig, L. (2004). Meningococcal vaccines. *Pediatric Infectious Disease Journal Section* 285 – 292.

David, C. (2011). Latex agglutination test. Department of Medicine, University of Washington School of Medicine, United States of America. Mediplus website.

Freedman, B., Marrocco, A., Pirie, J. and Dick, T. (2001). *Predictors of Bacterial Meningitis in the era After Haemophilus Influenzae.* Arch Pediatric Adolescent Medicine. 1306pp.

Gordon, C. and Alimuddin, I. (2009). Manson's tropical diseases. *Bacterial Meningitis* 1137: 1141 – 1220.

Geiseler, P., Nelson, K., Levin, S., Reddi, K. and Moses, V. (1980). Review Community-acquired purulent meningitis: A review of 1316 cases during the antibiotic era. *Rev Infection Disease* 2(5): 725 – 745.

Herbert, G., Ndiritu, M., Idro, R, J., Makani, J. and Kitundu, J. (2005). *Analysis of the Indications for Routine Lumbar Puncture and Results of Cerebrospinal Fluid Examination in Children.* Admitted to the paediatric wards of two hospitals in East Africa. pp. 67 – 73.

Hsu, H., Shutt, K. and Moore, M. (2009). Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *England Journal Medicine* 360(3): 244 –256.

Kanegaye, J., Soliemanzadeh, P. and Bradley, J. (2001). Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* 108: 1169–1174.

Kaplan, L., Mason, O. and Wald, R. (2004). Decrease of invasive *pneumococcal* infections in children among 8 children's hospitals in the United States after the introduction of the 7-valent *pneumococcal* conjugate vaccine. *Pediatrics* 113: 443 – 449.

Kim, K. (2001). *Escherichia coli* translocation at the blood-brain barrier. *Infectious Immunology* 69: 5217 – 5222.

Koneman, W., Washington, C., Stephen, A., William, J., Paul, S., Gary, P. and Gail, W. (1992). *Colour Atlas and Textbook of Diagnostic Microbiology*. Lippincott Williams and Wilkins, Philadelphia. 1736pp.

Logan, S. A. and MacMahon, E. (2008). Viral meningitis. *BMJ Clinical Research* 336(7634): 36 – 40.

Long, K., Vasquez-Garibay, E., Mathewson, J., de la Cabada, R. and DuPont, H. (1999). *The Impact of Infant Feeding Patterns on Infection and Diarrheal.* Disease Due to Enterotoxigenic Escherichia coli, Salud Publica, Mexico. 270pp.

Maconochie, I., Baumer, H. and Stewart, M. E. (2008). Fluid therapy for acute bacterial meningitis. *Cochrane Database System* 1: 47 – 86.

Marais, S., Pepper, D. J., Schutz, C., Wilkinson, R. J. and Meintjes, G. (2011). Presentation and outcome of tuberculous meningitis in a high. *HIV Prevalence Setting* 6(5): 1310 – 1371.

Marie-Christine, F., Muhamed-Kheir, T., Pierre, N., Patrick, C., Jean-Michel, A., Raymond, B., Jeanne, M. and Paul, M. V. M. (2002). Recent increase in meningitis caused by Neisseria meningitides Yaoundé Cameroon. *Serogroups* 8(3): 327 – 329.

Martin, M., Casellas, M., Madhi, S., Urquhart, J., Delport, D., Ferrero, F., Chamany, S., Dayan, H., Rose, E., Levine, S., Klugman, P. and Feikin, R. (2004). Impact of *Haemophilus influenzae* type b conjugate vaccine in South Africa and Argentina. *Pediatr Infectious Disease Journal* 23: 842 – 847.

Martinho, F., Prieto, E., Pinto, D., Castro, R., Morais, M., Salgado, L. and Exposto, F. (2008). Evaluation of liquid biphasic Granada medium and instant liquid biphasic Granada medium for group B *Streptococcus* detection. pp. 69–71.

Matee, M. and Matre, R. (2002). Pathogenic isolates in meningitis patients in Dar es Salaam, Tanzania. *East Africa Medical Journal* 78(9): 458 – 460.

Medilineplus (2011). Latex agglutination test, medilineplus medical encyclopedia. [www.Me dlineplus.com] site visited on 20/09/2012.

Meredith, T., Phillips, K. and Reller, B. (1997). Clinical utility of broth cultures of cerebrospinal fluid from patients at risk for shunt infections. *Journal of Clinical Microbiology* 3: 109 – 111.

MoH (2009). Integrated Management of Childhood Illness Referral Hospital Course. Infectious diseases presentation, Iringa.

Mohapatra, S., Sethy, S., Mohanty, P., Mohapatra, A. and Pradhan, S. (2012). Primary paediatric TH1 immunodeficiency with BCG. *International Journal* 3: 36 – 40.

Molyneux, E., Walsh, A., Phiri, A. and Molyneux, M. (2006). Acute bacterial meningitis in children presenting to the royal Liverpool children's hospital, Liverpool, UK and the Queen Elizabeth Central Hospital in Blantyre, Malawi. *Lancet* 360: 211 – 218.

Moris, G. and Garcia-Monco, J. (1999). The challenge of drug-induced aseptic Meningitis. *Archives of Internal Medicine* 159(11): 1185 – 1194.

MRRH (2009). *Pediatrics Mortality Annual Report for 2009.* Morogoro, Tanzania. 22pp.

Murray, P., Baron, E., Jorgensen, J., Landry, M. and Pfaller, M. (2007). *Manual of Clinical Microbiology.* (9th Edition.), Washington, DC. 1200pp.

Mwangi, I., Berkley, J., Lowe, B., Peshu, N., Marsh, K. and Newton, C. R. (2002). Acute bacterial meningitis in children admitted to a rural Kenyan hospital. *Increasing Antibiotic Resistance and Outcome* 21(11): 1042 – 1048.

Neuman, H. and Wald, E. (1998). Bacterial meningitis in childhood at the children's hospital. *Pittsburgh* 91: 647– 650.

Nigrovic, E., Kuppermann, N., McAdam, J. and Malley, R. (2004). Cerebrospinal latex agglutination fails to contribute to the microbiologic diagnosis of pretreated children with meningitis. *Pediatric Infectious Disease Journal* 23: 786– 788.

Panackel, C. V., Cherian, G., Vijayakumar, K. and Sharma, R. N. (2006). Eosinophilic meningitis due to Angiostrongylus cantonensis. *India Journal Medical Microbiology* 24(3): 220 – 221.

Peltola, H. (2000). Worldwide *Haemophilus influenzae* type b disease at the beginning of the 21st century. Global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. *Clinical Microbiology Reviews* 13(2): 302 – 317.

Provan, D. and Andrew, K. (2005). *Oxford Handbook of Clinical and Laboratory Investigation*. Oxford University Press, Oxford. pp. 622 – 624.

Qazi, A., Saira, K. and Mushtaq, A. (1998). Epidemiology of childhood tuberculosis in a hospital setting Pakistan. *Medical Association* 48: 90 – 93.

Richard, E. B. (2000). *Nelson Textbook of Pediatrics.* (16th Ed.), Bacterial *Meningitis* Saunders, Scheld, W. Michael Publisher, Philadelphia, USA. pp. 757 – 760.

Saez-Llorens, X. (2003). *Acute Bacterial Meningitis Beyond the Neonatal Period.* 2nd Edition. Churchill Livingstone, Philadelphia. 271pp.

Saez-Llorens, X. and McCracken, G. H. (2003). *Bacterial Meningitis in Children.* University of Panama. 937pp.

Sallam, A. (2002). *Etiology and Occurrence of Acute Bacterial Meningitis in Children in Central Referral Hospital.* Yemen. 324pp.

Schuurman, T., de Boer, F., Kooistra-Smid, M. and van Zwet, A. (2004). Prospective study of use of PCR amplification and sequencing of 16S ribosomal DNA from cerebrospinal fluid for diagnosis of bacterial meningitis in a clinical setting. *Journal of Clinical Microbiology* 42: 734– 740.

Segal, S. and Pollard, A. J. (2004). Vaccines against bacterial meningitis. *British Medical Bulletin* 72: 65 – 81.

Shanson, D. (1999). *Microbiology in Clinical Practice.* Butterworth-Heinemann, Woburn. 1450pp.

Straus, S., Thorpe, E. and Holroyd-Leduc, J. (2006). How do I perform a lumbar puncture and analyze the results to diagnose bacterial meningitis? *JAMA The Journal of the American Medical Association* 296(16): 2012 – 2022.

Susan, C. and George, H. (2005). *Bacterial Meningitis in Children.* Pediatric clinics of North America. pp. 795 – 810.

Tebruegge, M. and Curtis, N. (2008). Epidemiology, etiology, pathogenesis, and diagnosis of recurrent bacterial meningitis. *Clinical Microbiology Reviews* 21(3): 519 – 537.

Theilen, U., Wilson, L., Wilson, G., Beattie, J. O., Qureshi, S. and Simpson, D. (2008). Management of invasive meningococcal disease in children and young people. summary of SIGN guidelines. *BMJ Clinical Research* 336: 1367–1370.

Thwaites, G., Chau, T. T., Mai, N. T., Drobniewski, F., McAdam, K. and Farrar, J. (2000). Tuberculous meningitis. *Journal of Neurology, Neurosurgery, and Psychiatry* 68: 289 – 299.

Tunkel, R., Hartman, J. and Kaplan, L. (2004). Practice guidelines for the management of bacterial meningitis. *Clinical Infectious Disease* 39: 1267–1284.

Urs, S., Susanne, S., Athos, G., Juerg, P., Raymond, A., Otto, B., Jean, J. and Joana, W. (1990). A comparison of ceftriaxone for the treatment of bacterial meningitis in children. *England Journal of Medical* 141 – 147.

Vaagland, H., Blomberg, B., Krüger, C., Naman, N., Jureen, R. and Langeland, N. (2004). *Nosocomial Outbreak of Neonatal Salmonella Enterica Serotype Enteritidis.* Meningitis in a Rural Hospital in Northern Tanzania. pp. 155 – 161.

Van de, B. D., de Gans, J., Tunkel, A. R. and Wijdicks, E. F. (2006). Community-Acquired bacterial meningitis in adults. *Microbiological Reviews* 23: 388 – 394.

Walling, A., Kallail, K., Phillips, D. and Rice, R. (1991). The epidemiology of bacterial meningitis. *Journal of the American Board of Family Practice* 4(5): 307 – 311.

Warrell, David. A. (2003). *Oxford Textbook of Medicine Volume One.* Oxford University Press, Oxford. 1129pp.

Weller, P. F. and Liu, L. X. (1993). Eosinophilic meningitis. *Semin Neurol* 13(2): 161–168.

Whitney, G., Farley, M. and Hadler, J. (2003). Decline in invasive *pneumococcal* disease after the introduction of protein-polysaccharide conjugate vaccine. *English Journal of Medicine* 348: 1737– 46.

WHO (2000). Detecting meningococcal meningitis epidemics in highly-endemic African countries (PDF). *Weekly Epidemiological Record* 75(38): 306 – 309.

WHO (2009). *Meningococcal Meningitis in Children.* World Health Organization, Geneva. 319pp.

WHO (2010). *Bacterial Meningitis in Developing Countries.* World Health Organization, Geneva. 210pp.