CHAPTER 2

REVIEW OF LITERATURE

2.1. INTRODUCTION

Meningitis denotes to an inflammatory process of the CNS covering leptomeninges and CSF inside the subarachnoid space of the brain and spinal cord (Begum et al. 2007). Meningoencephalitis states to soreness of the tissue layer and brain parenchyma (Kumar et al. 2012). It was stated that the infection of the central nervous system (CNS) may be diffuse or focal. Meningitis and encephalitis are cases of diffuse infection. Meningitis suggests prime association of meninges, whereas encephalitis specifies brain parenchymal connection. Since these anatomic boundaries are habitually not differentiating, most of the patients have sign of both meningeal and parenchymal involvement which leads to have meningoencephalitis (Prober 2007). Meningitis could also be chronic, sub-acute and acute, and can have infectious or non-infectious causes (Jacewicz 2009). Etiologic agents of meningitis comprise bacteria, Mtbc, Treponema pallidum, Lyme disease, fungi, viruses and rarely protozoan parasites (Prober 2007; Jacewicz 2009 and Khan et al. 2011) and non-infectious causes of meningitis including Ankylosing Spondylitis, Systemic Lupus Erythematous (SLE), tumour and leukaemia (Prober 2007).
2.2. PYOGENIC MENINGITIS

Pyogenic meningitis is a serious disease and very common among neonates and paediatrics age group. Worldwide more than one million cases come out in every year with 1.35 lacks of mortality (Alam et al. 2007). As per World Health Organization (WHO 2004), the death rate from pyogenic meningitis in South East Asia, Europe, America and Africa were 73, 15, 18 and 20 thousands respectively. In USA, the occurrence of pyogenic meningitis is 0.01% annually (Schueler Beckett and Gettings 2010). The frequency of pyogenic meningitis varies from 0.022% to 0.266% in new borns, more communal in under developed countries (Silva et al. 2007).

2.3. CAUSES

Causes of pyogenic meningitis contrast with age and immune status. Etiologies of neonatal pyogenic meningitis are Group B Streptococci, Escherichia coli and Listeria monocytogenes (Cheesbrough 2000; Llorens and McCracken 2003 and Brooks et al. 2004). Enterococcus and gram negative Flavobacterium meningosepticum might also trigger neonatal meningitis (Cheesbrough 2000). N. meningitidis, S.pneumoniae and H.influenzae type b are the predominant causes of acute pyogenic meningitis. Meningitis by Gram negative bacteria e.g. E.coli, Klebsiella spp, Enterobacter spp and Pseudomonas spp. can stimulates meningitis in immunodeficient patients (Jacewicz 2009). An alternative study done by Ceyhan et al. (2008) stated that 90% of affected cases of pyogenic meningitis in infants and children beyond 1 month of age were by three most communal causative bacteria such as S.pneumoniae, H. influenzae type b and N. meningitidis.
As per CDC, the common bacterial pathogens based on age with bacterial meningitis are: Newborns: Group B *Streptococcus, Streptococcus pneumoniae, Listeria monocytogenes, Escherichia coli*. Babies and children: *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* type b (Hib), group B *Streptococcus*. Teens and young adults: *Neisseria meningitidis, Streptococcus pneumoniae*. Older adults: *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* type b (Hib), group B *Streptococcus, Listeria monocytogenes*.

**2.4. CLINICAL FEATURES**

The clinical features of bacterial meningitis are non-specific, inconstant, or even obscure in infants and children. In new-borns, they may be existing with hypothermia, fever, distress, lethargy, seizures or bulging fontanel’s. In kids, the following clinical manifestations are existing nausea, vomiting, fever, photophobia, headaches, stiff neck, altered mental status (confusion), fatigue, or irritability and seizure. Additional signs of pyogenic meningitis on bodily examination consist of Kernig’s sign, focal neurological findings, Brudzinski’s sign, and increased intracranial pressure (Haslett et al. 2002). Ciphers of meningeal frustration are visible in seventy five percentages of children and it is vague in those below 1 year (Kim 2010). Hart (2009) specified that neonates (17%) with meningitis showed bulging fontanels, 23% with neck stiffness, 33% with opisthotonos and 12% with convulsions. Focal neurological signs, such as oculomotor or abducens nerve palsy, facial or auditory, headache, emesis, bulging fontanels, stupor or coma and cranial neuropathies of the ocular may be present in children (10-20%).
2.5. TRANSMISSION

In general, the pathogens that cause bacterial meningitis spread from one person to another. Beyond the new born period, the most common causes of bacterial meningitis are *Neisseria meningitidis, Streptococcus pneumoniae*, and *Haemophilus influenzae*. All three are respiratory pathogens. They spread by close contact with respiratory droplets. Once acquired, they can inhabit the mucosa of the nasopharynx and oropharynx. From there, they cross the mucosa and enter into the blood. Once reached in the blood, they can enter the meninges, causing meningitis, or other body sites producing other syndromes. Some microbes, such as *Listeria monocytogenes*, can spread through food. Mothers can pass group B *Streptococcus* and *Escherichia coli* to babies during labour and birth. Humans spread *Hib* and *Streptococcus pneumoniae* by coughing or sneezing. *Neisseria meningitidis* spread by sharing respiratory or throat secretions (saliva or spit). People can get *Escherichia coli* and *Listeria monocytogenes* by eating contaminated food prepared by person who did not wash their hands well after using the toilet.

2.6. EPIDEMIOLOGY OF MENINGITIS

Worldwide, meningitis occurring at irregular intervals and in small outbreaks in most of the world. Acute bacterial meningitis is still a significant cause of morbidity and mortality in the midst of adults, children and infants worldwide (Bhimraj 2012). According to WHO, the incidence of bacterial meningitis is exceeding 1.2 million Cases each year worldwide (World Health Organization 1998). Bacterial meningitis mortality rates varying by area, nation, period, pathogen, and age group. Without treatment, the mortality rate can be as high as 70 %, and one in five survivors of bacterial meningitis may be left with permanent sequelae including loss of hearing, neurologic debility, or
damage of a limb” (CDC 2014). Despite of using new generation antibiotics, adjuvants and vaccination, the disease still has a high toll ranging from 16-32% (Mani et al. 2007).

According to WHO reports, Neisseria meningitidis, Streptococcus pneumoniae and Haemophilus influenzae Type b are the triad responsible of causing community-acquired bacterial meningitis worldwide (Madhumitha and Gupta 2011). Each year, approximately 500,000 cases of meningococcal disease occur around the world, causing about 50,000 deaths (WHO 2010). According to the World Health Organization (WHO 2002), approximately 5-10 percent of people who contract meningococcal disease will die – often within 24-48 hours of the first symptoms – even if they are diagnosed and receive early and appropriate treatment. Annually, 1.6 million cases of deadly pneumococcal disease arisen worldwide, mostly in infants and elderly as per WHO annual reports. Before Hib vaccination, Hib was the leading cause of bacterial meningitis in children below 5 years of age (Ramachandran 2013). The death rate of bacterial meningitis in developing countries is frequently greater (20-40%) than in developed countries. Among those who survive the meningococcal disease, 10-20% experience neurological sequelae (Mani et al. 2007).

Worldwide, the frequency of meningitis due to N. meningitidis is maximum in a region of sub-Saharan African known as the “meningitis belt” (Figure 2.1). This hyper-endemic region lengthens from Senegal to Ethiopia, and is categorised by seasonal epidemics throughout the dry period (incidence rate: 10-100 cases per 100,000 populations).
As per WHO report, it was alarmed as “Meningitis epidemics could affect 34 million people in the next two years and 10 million doses of vaccines would be needed in addition to the stock available in the international emergency stockpile in order to provide a sufficient response” and it may spread to neighboring countries (CDC 2017) (Figure 2.2).
Vaccines are existing for group A, C, Y and, W-135 (Manchanda et al. 2006 and Taha et al. 2010) for the five common serogroups accountable for >90% of meningococcal disease. Meningococcal polysaccharide vaccines (bivalent and quadrivalent) and Meningococcal conjugated polysaccharide vaccine are licenced so far for meningococcal meningitis.
2.7. STUDIES RELATED TO INCIDENCE AND PREVALENCE, CAUSES, DIAGNOSIS, PREVENTION AND MANAGEMENT OF MENINGITIS

A systematic review and meta-analysis was done on Global etiology of bacterial meningitis by Speets et al. (2018). They assessed the etiology of bacterial meningitis in different age groups across global regions. Meta-analyses were directed to determine the frequency (percentages) of seven bacteria known to cause meningitis: *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, group B *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Listeria monocytogenes*, with results being stratified by six geographical regions as mentioned by the World Health Organization, and seven age groups. They analyzed a large number of studies over a five year publication timeframe, giving a broad overview of the current status of the pathogens causing bacterial meningitis worldwide. This review showed that *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* were the major pathogens that produced bacterial meningitis in most ages within the majority of regions in studies published in the last five years. They observed a clear difference in the weighted frequency of bacterial meningitis cases caused by the different etiological agents between age groups and between geographic regions (Figure 2.3).
Figure 2.3. Frequency of seven pathogens that caused bacterial meningitis in all ages by geographic region
(Adopted from Public Health England, United Kingdom)
An update review on bacterial meningitis was conducted by Bekairy et al. (2014) in Saudi Arabia. They provided an update of references on “Bacterial Meningitis diagnosis and treatment” in order to help the health care professionals. They reviewed about the epidemiology and the common bacterial meningitis pathogens in different age groups. They also reviewed about the diagnosis and management of bacterial meningitis with respect to common CSF findings in acute meningitis, Latex Agglutination and PCR based molecular diagnosis along with antimicrobial therapy.

A study was directed on acute bacterial meningitis among children less than 5 years of age by Farag et al. (2005). The study results revealed that there were 344 cases of meningitis due to suspected bacterial etiologies reported in children younger than 5 years of age. *Haemophilus influenzae* 76 (22%) was the most common pathogen identified during the study period; followed by *Streptococcus pneumoniae* 53 (15%) and *Neisseria meningitidis* 37 (11%) respectively. An another study was conducted on acute bacterial meningitis among children by Roca et al. (2008) During the period of study, 475 cerebrospinal-fluid samples were collected from 20,173 children <15 years of age admitted to hospital. Culture results confirmed 71 (15%) cases of ABM. The most prevalent bacterial etiologies were *Streptococcus pneumoniae* (*Pneumococcus, n = 31), *Haemophilus influenzae* (n = 13) and *Neisseria meningitidis* (n = 8). Other important bacteria were *Streptococcus* sp. (n = 7), *Salmonella* sp. (n = 4) and *Staphylococcus aureus* (n = 3). Incidences were more than three times higher in the <1 year age group.

A study was directed on prevalence of newborn bacterial meningitis and sepsis during the pregnancy period for public health care system participants in Salvador, Bahia, Brazil (Silva et al. 2007). In Brazil, the DPH
(Department of Public Health) estimates that the 0.00022 % prevalence of bacterial meningitis. Out of 72 reports of newborns and only 11 (17%) were bacterial meningitis or sepsis newborn cases. Moreover, these cases were related to high intake of ototoxic drugs that can cause oto and nephrotoxicity, and cause serious consequences on the child development.

Yahia and Balach (2014) compared the characteristics of the multiplex PCR and Gram stain with bacterial culture as reference method for detection of five pathogens which multiplex PCR can detect: *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Neisseria meningitidis*, Group B *Streptococcus*, and *Listeria monocytogenes* in cerebrospinal fluid (CSF) samples of patients suspected of acute bacterial meningitis. In this study, Gram stain for any bacteria was positive in 32 cases (29.1%), including the five pathogens in 11 cases (10%). Multiplex PCR was positive in 60 cases (54.5%) and the most bacteria detected was *Streptococcus pneumoniae* in 39/60 cases (65%), followed by *Neisseria meningitidis* in 8/60 cases (13.3%).

Khater and Elabd (2016) concluded in their study as “Meningitis is a serious communicable disease with high morbidity and mortality rates. *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* are endemic disease in Egypt. Some studies were aimed to utilize molecular technology in order to diagnose bacterial meningitis in culture-negative CSF samples. Finally, it was clinched that the use of molecular techniques as real-time PCR can provide a valuable addition to the proportion of diagnosed cases of bacterial meningitis especially in settings with high rates of culture-negative results.
Meningococcal disease is endemic in India (Park 2009). Cases of meningococcal meningitis are reported sporadically or in small clusters. About 8367 cases of meningococcal meningitis were reported in India with about 485 deaths during 2005. Majority of the cases were stated from Andra Pradesh (3734 cases, 36 deaths), Madhya Pradesh (1039 cases, 6 deaths), Uttar Pradesh (659 cases, 124 deaths), West Bengal (702 cases, 64 deaths), Delhi (292 cases, 50 deaths), Maharashtra (394 cases, 100 deaths) and Karnataka (464 cases, 19 deaths).

A study was conducted on incidence of *Haemophilus influenzae* type b meningitis in India by Minz et al. (2008). 97 cases of possible meningitis were reported in this study, an annual incidence of 86 per 100,000 in 0-4 yr old children, and 357 per 100,000 in 0-11 month infants. 18 had proven bacterial meningitis. In infants 0-11 months of age, the incidence of Hib meningitis was 32 per lakh and in the 0-23 month group it was 19. The observed incidence data are similar to the reports before Hib vaccine use, suggest substantial disease before 24 months of age, and provide data useful for policy regarding Hib immunization.

A study was conducted on recent outbreak of meningococcal meningitis in India by Duggal et al. (2007). The study revealed that meningococcal disease presents in various clinical forms, most common being meningitis and meningococcemia. Cases had presented either with the classical features of acute purulent meningitis or as fever with rash. The patients were inspected microbiologically for the causative organism which was identified as *Neisseria meningitidis* in 257 out of 531 cases (48.39%). The classic finding of Gram negative diplococci on Gram stain remained the mainstay of diagnosis.
*N. meningitidis* isolates from culture were sensitive to all commonly used antibiotics.

A study was conducted on bacteriological profile of community acquired acute bacterial meningitis at NIMHANS by Mani et al. (2007). The results showed that the etiological agent identified in 284 (73.8%) of the total 385 cases by culture and/or smear. *Streptococcus pneumoniae* was the predominant pathogen accounting for 238 (61.8%) cases. *Haemophilus influenzae* and *Neisseria meningitidis* accounted for 7 (1.8%) and 4 (1%) cases respectively. Other Gram negative bacilli, *Streptococcus spp.* and *Staphylococcus aureus* were isolated from 19 (4.9%), 9 (2.3%) and 7 (1.8%) cases respectively. Concluded that *Streptococcus pneumoniae* remains the major etiological agent of CAABM both in adults and children in our set-up. No penicillin resistance was detected among the isolates. Further research should focus on preventable aspects of CAABM, especially pneumococcal vaccines, to help reduce the disease burden.

An episodic appraisal of bacterial meningitis globally is essential since the pathogens accountable for the infection differ with time, geography, and patient age. A study was directed on prevalence of acute bacterial meningitis in admitted children less than twelve years of age in a tertiary care teaching hospital in Pune, India by Debnath et al. (2012). In this hospital based cross sectional study, thorough clinical examination of all medically doubted cases of meningitis was done followed by Blood and CSF culture was executed. There were 79 doubted cases of meningitis, majority of cases (74.7%) were under-fives. The case fatality rate (CFR) was 13.9% with a confidence interval extending from 5.9 to 21.5%. Casual isolate was *Klebsiella pneumoniae* as observed in five cases (31.2%). *Neisseria meningitidis*, *H.influenzae*, or
Streptococcus pneumoniae were not identified in any case. Concluded that acute bacterial meningitis is still a significant community health problem with a high case fatality rate.

A study was conducted on fatal group A Streptococcal meningitis in children and adults in India (NIMHANS) by Mani et al. (2007). The study reveals that group A Streptococcal (GAS) infections worldwide, it remains a rare cause of pyogenic meningitis both in children and adults. There is also a need to consider post exposure chemoprophylaxis in close contacts of such cases.

A study was conducted on antimicrobial-resistant Streptococcus pneumoniae: trends and management by Jacobs (2008). The study revealed that management of pneumococcal infections has been challenged by the development of resistance and, more recently, the unexpected spread of resistant clones of serotypes, such as 19A, following the introduction of a conjugate pneumococcal vaccine for use in children in 2000. A retrospective analysis of 7759 clinically suspected cases of meningitis, admitted during a span of 3 years from February 2005 to February 2008 was undertaken in Bombay Hospital, Mumbai by Sonavane et al. (2008). A total 43 bacterial culture isolated from 7759 cases with 0.55% isolation rate Pseudomonas aeruginosa was the highest isolation rate of 23.25% followed by Klebsiella pneumoniae 20.93%, Acinetobacter spp. 20.93% (9/43), and Streptococcus pneumoniae 18.60% (8/43), while other isolates were Neisseria meningitidis 4.65% (2/43), Streptococcus pyogenes 4.65% (2/43), Enterococcus spp. 2.23% (1/43) and other Streptococcus spp. 2.23% (1/43). In conclusion, in recent years Gram negative bacilli are emerging as important pathogens causing acute bacterial meningitis in adults in India.
Chakrabarti et al. (2009) evaluated a multiplex semi nested PCR based method for rapid diagnosis of bacterial meningitis even after initiation of antibiotics and suggested that 16S rDNA PCR can be used in routine clinical practice for diagnosis of meningitis. As high number of morbidity and mortality observed in pyogenic meningitis, speedy and precise finding is necessary to raise the survival mark and decline complications. Hence, interruption in identification and beginning of appropriate antimicrobial therapy can consequence a deprived outcome. Therefore, laboratory diagnosis is mandatory for the rapid diagnosis of meningitis because it cannot constantly be identified on the basis of clinical manifestations (Mani et al. 2007).

2.8. DIAGNOSTIC PROCEDURES

Finding of meningitis is executed by cell cytology, biochemical test, Gram’s stain and culture. Latest improvements in immuno-chemistry have delivered new tactics; Latex agglutination test (LAT), Coagglutination test (COAG) and Counter Immunoelectrophoresis (CIEP) for the speedy recognition of soluble bacterial antigens. To identify the bacteria in Gram’s stain needed $\geq 10^5$ bacteria per mL of CSF. Additional approaches for the finding of bacteria and the element of bacteria in CSF were carried out by Enzyme immunoassay (EIA), Gas liquid chromatography (GLC) and Polymerase Chain Reaction (PCR).

2.8.1. Cell cytology

Typical CSF comprises 0-5 WBCs / cumm$^3$, predominantly lymphocytes, however in newborns cell count is up to 30 cells /mm$^3$ (Collee et al. 1996). CSF Leukocyte count $>2000$/cumm$^3$ and CSF neutrophil count $>1180$ cells/mm$^3$ is extremely important for pyogenic meningitis (Scheld
Anticipated protein level in CSF is 15-45 mg/dL. 100-500 mg/dL of protein level is impressively noteworthy for pyogenic meningitis. Protein level of CSF could be 50-200 mg/dL in case of aseptic Meningitis (Prober 2007). CSF protein is raised in infections, multiple sclerosis, intracranial haemorrhages, Guillain Barri syndrome, some endocrine abnormalities, malignancies, certain medications and a variety of inflammatory conditions (Dean et al. 2003). Average level of glucose in CSF is >50 mg/dL (typically 45-72 mg/dL). In occasion of pyogenic meningitis the level of glucose is decreased, generally <40 mg/dL where as the glucose level is usually normal in aseptic meningitis.

2.8.2. Cerebrospinal Fluid (CSF) findings in several causes of infectious disease
2.8.2.1. Bacterial meningitis

In Meningitis (Bacterial), CSF WBC count is ≥100 cells/mm³ with > 50% neutrophils and or growth of the bacteria in culture. The glucose proportion in CSF to glucose in blood is <0.4 and the protein level in CSF is >200 mg/dL. Pyogenic meningitis was diagnosed as positivity in culture or Gram’s stain or CSF WBC count of 100 to 10,000x10⁹/L (generally polymorphs) and decreased glucose level (<40 mg/dL) with elevated protein (>45 mg/dL) and the fluid is characteristically turbid in pyogenic meningitis (Alamgir et al. 2008).
2.8.2.2. Viral meningitis

In viral meningitis, 50-200 mg/dL of protein is raised and the level of sugar is normal. In tuberculous meningitis, the cell count is moderately raised as 10–500 cells/mm³ and most are lymphocytes. Protein amount is higher (25–500 mg/dL) with reduced level of sugar. In syphilitic meningitis raised cells count predominantly lymphocytes is observed. Level of protein is raised as 50–200 mg/dL with normal sugar level. Parasitic meningitis is seen with raised WBC count (1000 –10,000 cells/ mm³) predominantly polymorphonuclear cells. Protein level is elevated as 50–500 mg/dL and level of sugar is marginal. The following table 2.1 shows the detail of the common CSF findings taken from the review paper by Bekairy et al. (2014).

Table 2.1. Common CSF findings in acute meningitis

<table>
<thead>
<tr>
<th>Finding</th>
<th>Viral</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (cells/mm³)</td>
<td>100-1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>20-40</td>
<td>≥ 80</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>Normal</td>
<td>≤ 40</td>
</tr>
<tr>
<td>Blood/CSF ratio</td>
<td>Normal</td>
<td>≤ 0.4</td>
</tr>
<tr>
<td>Protein (mg/dL)</td>
<td>Normal</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Positive Gram stain</td>
<td>NA</td>
<td>60 % -95 %</td>
</tr>
<tr>
<td>Positive culture</td>
<td>NA</td>
<td>95%</td>
</tr>
<tr>
<td>Lymphocyte predominance</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td>Enterovirus, Herpes virus</td>
<td>Under Investigation for S.pneumoniae, N.meningitidis, H.influenzae</td>
</tr>
</tbody>
</table>

Source: Porto (2012)
2.8.3. Gram’s stain

CSF Gram staining is a cheap and well-validated diagnostic tool to identify the bacteria with inflammatory cells. Identifying the bacteria by Gram’s stain is related with the amount of bacteria in CSF. The yield of CSF Gram staining may be decreased in antibiotic pre-treated patients compared with antibiotic-naïve patients. Several studies have shown the additional value of Gram staining for CSF culture-negative patients. In one Indian study covered 535 suspected meningitis cases, Gram stain identified the bacteria for 36 (65%) of 55 pre-treated patients, while culture was positive for only 5 (9%) patients.

The experimental efficacy of the Gram’s stain mainly hinge on the bacteria. Microbes are known in 90% of cases affected by S. pneumoniae, 86% by H influenzae, 75% by N. meningitidis, and 50% by Gram negative bacilli and < 50% by Listeria monocytogenes meningitis (Greenlee 1990).

2.8.4. CSF Cultures

CSF culture relics the benchmark for the identification of pyogenic meningitis; aerobic culturing techniques are mandatory for community-acquired pyogenic meningitis. Anaerobic culture may be significant in post-neurosurgical meningitis and CSF shunt meningitis (Trampuz et al. 2007). Nevertheless, it needs 24 hrs or more for culture positivity and may also provide false positives if not appropriately transported, stored and if antibacterial agent is started before the sample is taken (Das et al. 2003). Cerebrospinal fluid cultures are optimistic in >80% of untreated patients of infectious disease (Ross and Tyler 2001). In Brazil, causative agent detection by culture is between 50-60% of the clients of pyogenic meningitis (Camargos et al. 1995). Blood cultures have to be executed in all cases with doubted
meningitis. Blood cultures could disclose the pathogen in 80-90% of circumstances of neonatal meningitis (Prober 2007).

2.8.5. Methods of detecting bacterial antigens

2.8.5.1. Latex Agglutination Test (LAT)

Latex agglutination is an analytic test that has been used for the etiological identification of pyogenic meningitis, giving results in less than 15 min. These tests employ serum comprising bacterial antibodies or commercially obtainable antisera directed against the capsular polysaccharides of meningeal pathogens and have been suggested for patients with doubted pyogenic meningitis with negative Gram staining and CSF cultures. LAT is simple and quick procedures, which have increased sensitivity and specificity, were 93.0% and 100% respectively (Camargos et al. 1995). An additional test for the finding of pyogenic meningitis is mandatory which is trustworthy, less time consuming, user friendly, sensitive and specific. LAT is a significant analytical tool which fulfills the above standards. LAT is sensitive and specific for *Streptococcus pneumoniae*, *Haemophilus influenzae* type B, group B *Streptococcus*, *Neisseria meningitidis* group A, C, Y, W135 and *Neisseria meningitidis* B, *Escherichia coli* k1 (Das et al. 2003 and Tunkel and Scheld 2001). LAT has been established to be valuable but are not completely pertinent because of scanty sensitivity. Trustworthy results are achieved only for samples having more than 105 CFU/ mL. However, nearly 45% of meningitis cases have less than 105 CFU/ mL (Plessis et al. 1998).
2.8.5.2. Quellung procedure

This procedure could be used to detect the existence of pathogens with morphology distinctive of *Streptococcus pneumoniae*, *N. meningitidis*, or *H. influenzæ* type b. In this procedure, antisera particular for capsular polysaccharides of these three bacteria are mixed with separate aliquotes of CSF specimen individually. A drop of CSF, saturated methylene blue and a loopful of exact antisera is mixed on a microscopic slide, concealed and observed under oil objective. The construction of antigen antibody developments on the exteriors of these bacteria brings alterations in their capsules and the capsule seems to be clear and enlarged when observed microscopically (Joklik et al. 1992).

2.8.5.3. Coagglutination Experiment (COAG)

COAG mixtures are made up of materials of *Staphylococcus aureus* that have the cell surface element protein A (12,000 to 43,000 MW protein) that is covalently connected to the peptidoglycan of the bacterium. Immunoglobulin G particles attach to protein A by the Fc end of the immunoglobulin G particle; the immunoreactive Fab end remains free to respond with particular antigen. COAG experiment is simple, precise, low cost, rapid and intense technique for recognition of soluble antigens in the body fluids of numerous infections. No false-positive answers were recognised by this test in body fluids from controls. This test is highly delicate than the counter current immunoelectrophoresis method and can be a useful implement for spotting antigens in body fluids of cases with numerous infections (Suksanong and Dajani 1977).
2.8.5.4. Counter current immuno electrophoresis (CCIEP)

CCIEP has been made known to be a fast procedure for identification (with in 1 hour) of pyogenic meningitis triggered by \textit{H. influenzae} type b; \textit{S. pneumoniae}; \textit{N. meningitidis} group A. C. W 135 and D; and group B \textit{Streptococcus}. It spots antigens from KI strains of \textit{Escherichia coli}, \textit{L. monocytogenes}; \textit{Klebsiella pneumoniae}, and \textit{P. aeruginosa}. The procedure used is sensitive and can identify non-viable pathogen, thus authorising the finding of microbial antigen, even in cases that have been pre-treated with suitable antibacterial agents. The sensitivity and specificity of CCIEP is relatively high (Joklik et al. 1992).

2.8.5.5. Limulus amebocyte lysate (LAL) assay

LAL assay is an appropriate delicate and precise assay for the recognition of endotoxin in CSF. It could identify nearly $10^3$ Gram-negative bacteria / mL of sample. A negative result does not exclude the finding of Gram-positive meningitis (Joklik et al. 1992).

2.8.5.6. Gas liquid chromatography (GLC)

Amines, alcohols, carbohydrates, and short-chain fatty acids are examples of microbial metabolites that are formed in body tissues and fluids and that can be detected by GLC. Brice et al. (1979) implemented this technique to start chromatography arrays for the subsequent five bacterial causes of meningitis \textit{S. pneumoniae}, \textit{H. influenzae}, \textit{N. meningitidis}, \textit{S. aureus} and \textit{Escherichia coli}. This technique has not been extensively implemented for the identification of pyogenic meningitis since it needs instrument that is costly and the procedure is precisely challenging than the antigen detection tests.
2.8.5.7. C – Reactive Protein (CRP)

CRP increases quickly within 24 – 48 hours of incidence of pyogenic meningitis (Prasad et al. 2005). Recognition of CRP is trustworthy, lucrative, quick identification tests which could be executed in any normal pathology test centre to aid in the prompt differential identification and controlling of meningitis (Belagavi and Shalini 2011). Some reports endorse that CRP could also be synthesized within the central nervous system, but intrathecal production seems to be least and therefore the common CSF CRP is so obtained from humor (Watson and Scott 1995). Bowers (1985) observed that the intensities of CRP in CSF was <0.2 mg/L in 49.0% cases and 0.2 mg/L or more in 51.0% of cases of pneumococcal meningitis. Philip (2003) stated that the CRP level above 0.4 mg/L as considerable for pyogenic meningitis. Coric et al. (2012) also defined in their study that the lowest concentration for CRP in CSF were 0.40 mg/L in patients with pyogenic meningitis and in normal cases the CRP in CSF were among 0.10 mg/L – 0.55 mg/L. Shameem et al. (2008) were observed in their study, in pre-treated pyogenic meningitis 29.0% were with raised level of CRP and in cases without treatment as 54.66% with raised level of CRP in CSF.

2.8.5.8. Polymerase chain reaction (PCR)

Nucleic acid amplification tests such as PCR assays have been evaluated for their effectiveness in detecting the presence of bacterial DNA in CSF from patients with suspected and proven bacterial meningitis. The technique is confined and easily automated; reaction happens in a single vessel. Sufficient management needs speedy recognition and detection of the bacteria, since long-lasting neurological sequelae may follow in up to 50% of survivors.
Polymerase Chain reaction has been widely employed to detect pyogenic meningitis causing organism in patient’s sample. After the treatment reduces the isolation rates as 50% to <5%, nevertheless it does not disturb PCR outcomes (Saravolatz et al. 2003). PCR currently identify very low number of bacteria in medical samples which does not need the existence of viable pathogens (Plessis et al. 1998).

One study counting 65 patients with culture confirmed community-acquired bacterial meningitis assessed the diagnostic accuracy of a broad-range PCR including primers for *H. influenzae*, *S. pneumoniae* and *N. meningitidis*. The sensitivity for *H. influenzae* was 92%, that for *S. pneumoniae* was 100% and that for *N. meningitidis* was 88%; the specificity was 100% for all organisms.

In another study of 139 bacterial meningitis patients defined by positive CSF culture in 94 cases and positive CSF Gram stain in 12 cases and based on clinical suspicion with negative cultures in 31 cases found sensitivities for *H. influenzae* (88%), *S. pneumoniae* (92%) and *N. meningitidis* (94%) using a multiplex PCR assay, with a specificity of 100% for all three microorganisms.

2.8.5.8.1. Real-time PCR technology

Real-time PCR is also branded as quantitative real-time polymerase chain reaction (Q-PCR/qPCR). It combines amplification and detection in one step through the usage of fluorescent dyes. There are 2 types of detection systems: non-specific and specific. Non-specific detection systems practice a fluorescent dye that intercalates into any double-stranded DNA molecules and emits fluorescence signal. This is comparatively inexpensive but susceptible to
false positivity. Specific detection systems hinge on probes that specifically identify target sequences. It is more costly than non-specific detection systems and requires classy probe designs. Three types of probes are currently in use, including: hydrolysis, hybridization, and hairpin probes (Csako 2006). A fluorescent signal is only produced if the probe binds with its specific target and is consequently hydrolyzed during amplification. The resulting rise in fluorescence is proportionate to the amount of amplicon in the reaction.

2.8.5.8.2. Species-specific real-time PCR assays

PCR finding of \textit{N. meningitidis}, \textit{H. influenzae}, and \textit{S. pneumoniae} can be attained by amplification of several potential gene targets on DNA extracted from clinical specimens (typically, blood and CSF) and bacterial isolates.

\textbf{(a) \textit{N. meningitidis}}

Two genes are targeted in \textit{N. meningitidis} species-specific assays, \textit{ctrA} and \textit{sod C}. The capsule transport to cell surface gene, \textit{ctrA}, is highly preserved among isolates accountable for invasive meningococcal infections and has been used in both real-time and conventional PCR to identify \textit{N. meningitidis} (Mothershed 2004). It is a gene within the capsule locus. However, since at least 16\% of carried meningococci lack \textit{ctrA} (Claus et al. 2002; Dolan-Livengood et al. 2003 and Sadler et al. 2003), a real-time PCR assay to detect all meningococci, irrespective of encapsulation status, was recently developed and validated. The \textit{sod C} assay detects both encapsulated meningococci and nongroupable meningococci that do not contain an intact \textit{ctrA}. For this reason, it is recommended that \textit{sod C} and \textit{ctrA} primers be used for detection of \textit{N. meningitidis}. 
(b) *H. influenzae*

The protein D encoding gene, *hpd*, is more conserved which is present in all *H. influenzae* (Janson 1993). Highly attractive gene target due to its conserved nature of this gene; used for the development of a *H. influenzae* species-specific real-time PCR assay. The *hpd* real-time PCR assay is able to detect all six serotypes and nontypeable *H. influenzae* with high sensitivity and specificity (Theodore et al. 2012).

(c) *S. pneumoniae*

The PCR detection assay for *S. pneumoniae* by a specific segment of the autolysin gene (*lytA*) is suggested because it is highly preserved within the species and it has been shown that this assay best separates *S. pneumoniae* from the genotypically similar species *S. mitis, S. oralis*, and *S. pseudopneumoniae* (Messmer et al. 2004). The *lytA*-encoded main autolysin (N-acetylmuramoyl-L-alanine amidase) of *Streptococcus pneumoniae* is a member of a broadly dispersed group of cell wall-degrading enzymes situated in the cell envelope and display functions in cell wall development and cell division. The pneumococcal autolysin has an associated group. Finding of *LytA* gene by PCR is the substitute technique to identify pneumococcal meningitis (Saha et al. 2005). This is highly subtle and quick technique for the analysis of pneumococcal meningitis by PCR currently since it could screen low amount of bacteria in CSF (Plessis et.al, 1998).

A study was conducted on the diagnostic accuracy of a broad-range PCR including primers for *H. influenzae, S. pneumoniae*, and *N.meningitidis* with culture-confirmed community-acquired bacterial meningitis cases of 65. The sensitivity for *H. influenzae* was 92%, that for *S. pneumoniae* was 100%,
and that for *N. meningitidis* was 88%; the specificity was 100% for all organisms (Corless et al. 2001). In another study of 139 bacterial meningitis patients defined by positive CSF culture in 94 cases and positive CSF Gram stain in 12 cases and based on clinical suspicion with cultures negative 31 cases were found sensitivities for *H. influenzae* (88%), *S.pneumoniae* (92%), and *N. meningitidis* (94%) using a multiplex PCR assay, with a specificity of 100% for all three microorganisms (Tzanakaki et al. 2005).

**2.8.5.8.3. Melting curve analysis for identification of PCR products**

The curves produced by melting PCR amplicons are a powerful tool for identifying reaction products e.g. identifying pathogen species following broad range PCR. Melting analysis measures the temperature (Tm) at which the two strands of DNA separate resulting in a rapid decrease in signal when the DNA is labeled with fluorescent probes. The illustrative melting curve revealed in Figure 2.4 represents the rate of changes of relative fluorescence (RFU) with the time on the Y axis against the temperature on the X axis showing fluorescence peaks at the melting temperature (Tm). These curves are characteristic of the amplified product and can be used to identify them when a mixture of products is present. Melting curve analysis also allows exclusion of any non-specific products including primer dimers (Reed et al. 2007 and Wilhelm and Pingoud 2003).
Figure 2.4. Dye saturation model

Source: NHS Technology Assessment by National Genetics Reference Laboratory, Wessex

Figure displays the reallocation of dye molecules (‘dye jumping’) from melted areas back into the dsDNA amplicons of non saturating dsDNA binding dyes such as SYBR Green producing no alteration in fluorescent signal even in the existence of heteroduplexes. Adapted from “Mutation scanning by high resolution melt analysis”, NHS Technology Assessment by National Genetics Reference Laboratory, Wessex (White and Potts 2006).

2.8.5.8.4. High Resolution Melting Analysis (HRMA)

Gene scanning or mutation scanning techniques detect the presence of sequence variation in the target gene derived PCR amplicons. It is based on high HRMA, a novel, closed-tube post PCR method that has enabled genomic researchers to analyse genetic variations in PCR amplicons as an alternative to sequencing. It provides high specificity, sensitivity and convenience at higher
speed and low cost than any other conventional method. HRMA is a relatively new advance that dramatically increases the information that can be gained from melting analysis of PCR products. HRMA depend on a unique highly saturated new generation of dsDNA binding dyes (e. g. LC Green) that can identify the existence of heteroduplexes formed during PCR and measure the changes in the fluorescence intensity during melting at higher resolution than conventional melting analysis. The result is that minor differences in the sequence of PCR amplicons can be detected as slight changes in the melt curve analysis using dedicated gene-scanning software (Erali et al. 2008). This feature is not common with other traditional dyes used in real-time PCR such as SYBR Green I. With highly saturated dye, high concentrations of the dye do not affect the PCR. Instead, it completely saturates the dsDNA in the sample, which remains dye-saturated during the subsequent melting experiment. Under these conditions, even small changes in the melting behaviour result in delicate, but reproducible changes in dye fluorescence.

According to Wittwer et al. (2003), this takes place because the dye cannot redistribute itself from denatured to non-denatured regions of the DNA during melting. In a melt curve assay, fluorescence is initially high due to the sample starts with dsDNA, but fluorescence reduces as the temperature is increase and DNA dissociates into single strands. The detected “melting” behaviour is characteristic of a specific DNA sample. For example, for detection of sequence variations in PCR amplicons, difference plots derived in the melting curve analysis are investigated. Double strand mutant DNA begins to separate into single strands at a lower temperature and with a different curve shape than wild type DNA. In gene scanning experiments, DNA samples are first amplified by real-time PCR in the presence of high resolution melting dye on a conventional instrument such as the LightCycler480. Subsequent high resolution melting analysis of the amplicons is achieved using dedicated gene
scanning software. Analysis is a 3 step procedure as exemplified in Figure 2.5. Step 1 comprises normalization of the raw melting curve data by setting pre-melt (initial fluorescence) and post-melt (final fluorescence) signals of all samples to uniform values. Step 2 includes changing the normalized curves beside a temperature axis by tightly controlled incremental changes in temperature to level the point at which the dsDNA in every sample becomes fully denatured. Finally, in Step 3 the shifted, normalized melting curve for a sample is subtracted from a reference DNA data. The shape of the difference curve formed will be reproducible and characteristic of that specific DNA sequence (Figure 2.5).

Figure 2.5. Stages of High Resolution Melting Analysis (HRMA) Adapted from LightCycler® 480 Real-Time PCR Systems, Roche, Technical Note No. 1

The analytical sensitivity of HRMA means that it can detect differences down to the single base level making it a potentially superior and more cost-effective technique for applications where small differences in DNA sequence need to be reproducibly detected e.g. single nucleotide polymorphisms (White and Potts 2006).
Melting analysis was verified in inter-species differentiation of the *M. chelonae-abscessus* group that consists of *M. chelonae*, *M. abscessus* and *M. immunogenum* that are hard to distinguish in the clinical laboratory (Odell et al. 2005). Broad range PCR of the 16S rRNA gene followed by high resolution melting devoid of using any probe was evaluated for the identification of 25 bacterial species (Cheng et al. 2006). Examination of difference plots paved the way for the direct identification of bacterial species including *P. mirabilis*, *K. pneumoniae*, and *S. marcescens*, *A. baumannii* and *H. influenzae*, *S. pneumoniae*, *E. faecalis*, *Bacillus* spp., and *S. pyogenes*, *S. agalactiae*, *E. coli*, *S. typhimurium*, *S. enteritidis*, and *S. flexneri*.

The flexible power of HRMA in genotyping in the case of MRSA typing for epidemiological purposes by Reischl et al. (2009) was served as a hot topic in medical microbiology. Price et al. (2007) first developed a HRMA assay for interrogating the hypervariable CRISPR (cluster, regularly interspaced short palindromic repeats) locus of *Campylobacter jejuni*. In the field of antibiotic treatment and resistance, the extended-spectrum beta-lactamases (ESBLs) represent a significant challenge. For this purpose, several HRM assays were established to detect mutations or alleles responsible for this phenotype. Chia et al. (2005) developed a multiplex PCR to identify blaSHV, blaCTX-M-3–like and blaCTX-M-14–like genes.

### 2.9. TUBERCULOUS MENINGITIS

Tuberculous meningitis (TBM) is that the most severe type of infection caused by mycobacteria, inflicting death or illness in half those affected. Tuberculous meningitis (TBM) is the most occurring disease of central nervous system (CNS) tuberculosis (Hopewell et al. 2005). CNS disease arising for only 5% of all cases of extra-pulmonary tuberculosis and high
frequency is in children below 4 years of age (Farer et al. 1979). However, the amount of adults suffering with TBM has increased as a result of the HIV epidemic. The presenting clinical manifestations and CSF abnormalities of TBM have been extensively described (Davis et al. 1993; Kent et al. 1993; Verdon et al. 1996; Kilpatrick et al. 1986; Hosoglu et al. 1998; Farinha et al. 2000 and Thwaites et al. 2005) and are summarized in Table 2.2. The classic exhibition is with an acute meningitic sickness, which can be difficult to discriminate from other causes of Meningoencephalitis. Once the neurologic symptoms of advanced infection are established (e.g. coma, seizures, raised intracranial pressure and hemiparesis), the diagnosis is apparent but the prognosis is poor. The detection of TBM remains tough as its presentation is non-specific and will mimic different causes of chronic cerebromeningitis. Unusual neurological presentations of TBM may result in diagnostic difficulty (Kocen et al. 1970 and Udani et al. 1971). Rapid recognition of TBM is crucial, however, as delays in initiating treatment are associated with poor outcome. The laboratory designation of TBM is hampered by the low sensitivity of AFB smear and also the slow growth of *M. tuberculosis* in conventional culture systems. Laboratory ways to enhance the fast detection of TBM desperately needed.
<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Clinical Findings</th>
<th>CSF findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children</strong></td>
<td>Early symptoms are non-specific and include fever, cough, vomiting, malaise and weight loss.</td>
<td>Apathy, irritability, meningitis, reduced level of consciousness, bulging anterior fontanelle (infants), VI cranial nerve palsy, optic atrophy, abnormal movements and focal neurological signs, e.g. hemiplegia</td>
</tr>
<tr>
<td></td>
<td>Duration of symptoms &gt; 6 days Seizures more common in children than in adults</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prodromal period with low-grade fever, malaise, weight loss followed by gradual onset of headache (1-2 weeks)</td>
<td>Neck stiffness, confusion, coma, Cranial nerve palsies-VI,III,IV</td>
</tr>
<tr>
<td></td>
<td>Worsening headache, vomiting confusion, coma.</td>
<td>Focal neurological signs, e.g. monoplegia, paraplegia</td>
</tr>
<tr>
<td></td>
<td>Duration of symptoms ≥ 6 days</td>
<td>Urinary retention</td>
</tr>
</tbody>
</table>

Tuberculous meningitis (TBM) is the most frequent form of central nervous system tuberculosis and prompt diagnosis holds the key for its successful management. (Torok 2015). Approximately one third of patients die soon after hospitalization and many who survive are left with severe neurological sequelae (Brancusi et al. 2012). It accounts for 5% of all cases of extra pulmonary tuberculosis and their peak incidence is in children below the age of 4 years (Farer et al. 1979). However the number of adult patients presenting with TBM has increased substantially due to the HIV epidemic. TBM has a mortality rate ranging from 20% to 67% even with appropriate antituberculous therapy; however it can go up to 60% in patients with HIV (Marais 2010). TBM has varying degrees of clinical course and classically often presents with a sub acute clinical course which is no different from other forms of meningitis (Farinha et al. 2000; Thwaites and Tran 2005 and Verdon et al. 1996). In countries with a high incidence of tuberculosis, TBM can cause up to one-third to one half of all cases of meningitis and constitutes about 1% of all extra pulmonary tuberculosis. In developing countries, TBM is more common in children and infants (Muzumdar et al. 2018). Once neurological symptoms develop in the advanced stages of TBM, the diagnosis is very clear, but the prognosis is poor. Many patients are correctly diagnosed only late because initial symptoms are non-specific and diagnostic methods have low sensitivity or poor turnaround time (Nhu et al. 2014). Early diagnosis and treatment are therefore the most important factors in determining the outcome of the disease (Philip et al. 2015). Moreover, because of the overlap of clinical symptoms, an accurate differential diagnosis of TBM from other forms of meningitis/meningoencephalitis is necessary for instituting anti tuberculous treatment. This is especially true in countries like India where tuberculosis is highly endemic (Muzumdar et al. 2018).
Laboratory diagnosis of TBM is a complex and complicated issue. Paucibacillary nature of the disease makes the diagnosis more difficult resulting in high mortality of the disease (Cresswell et al. 2018). CSF is the specimen of choice. Developed more than 100 years ago, acid-fast staining by Ziehl-Neelsen (ZN) method is routinely used all over the world for the diagnosis of TBM (Torok 2015); despite this, the ability of this technique to detect M.tbc is highly variable with a sensitivity of 10 to 20% (Garg 1999 and Cresswell et al. 2018). Reportedly, approximately 10,000 organisms are necessary for smear positivity (Torok 2015). In one retrospective chart review, Dural et al. (1997) reported that ZN positivity was 42% in TBM among patients with HIV (n = 26) while it was 0% in HIV-non infected patients (n = 10). It has been reported that the sensitivity can be increased up to a maximum of 60% by increasing the volume of CSF up to 7 mL and increasing the time of screening each slide. In another study, Nhu et al. (2014) reported a very high smear positivity which they attributed to examination of each slide up to 30 minutes per slide; this will not be feasible in busy routine diagnostic or public health laboratories. Fluorescent microscopy has higher sensitivity and throughput, but both the equipment and ultra violet bulb are more expensive (Steingart et al. 2006). A combination of ZN staining with fluorescent microscopy without fluorescent staining has been reported to enhance the sensitivity of the microscopical positivity (Zou et al. 2016). Development of LED microscopy has solved some of these issues and is now recommended by World Health Organization for the routine examination of acid-fast stained smears (WHO 2011). Since smear sensitivity is directly associated with the number of bacilli present, the amount of CSF collected is critical in making a diagnosis by smear examination. However the amount of sample collected is often so small, especially in children, that no bacilli can be detected even if it is sedimented or treated with Triton as has been shown in some studies (Chen et al. 2012). Therefore a negative ZN does not exclude a diagnosis of TBM.
Even in the best of conditions, conventional TB culture has a sensitivity of 50% to 60% (Boulware 2013). Growth of M.tbc on Lowenstein – Jensen medium takes three to four weeks to appear which is totally useless as far as the diagnosis is concerned. Microscopically observed drug-susceptibility assay (MODS) which uses a liquid culture assay using Middlebrook 7H9 broth and an inverted microscope has been reported to be more sensitive than smear examination, but takes a median time to positivity of 6 days (Caws et al. 2007). This method has been found to be useful for the diagnosis of tuberculosis and drug susceptibility testing. It has been evaluated for TBM and has been found to be more sensitive than CSF smear and conventional culture. However a positive result can be obtained only in 6 days and therefore it is not of much value in making a quick and differential identification of TBM.

In context of poor sensitivity of diagnostic methods of TBM, attempts have been made to investigate biomarkers to diagnose TBM (Torok 2015). Characteristic CSF findings such as cell count, protein and glucose levels are seen in varying proportions of patients depending upon the region where the studies were undertaken (Jha et al. 2015 and Aher et al. 2018); however they were characteristically seen only in under two-thirds of TBM cases in HIV infected patients (Croda et al. 2010). CSF pleocytosis with lymphocyte proportion of >30% count have been found to help in the early diagnosis of TBM (Youseff et al. 2006). CSF lactate levels have been shown to be a diagnostic marker for CNS infections. The levels of lactate, a metabolic product of anaerobic bacteria are increased in CSF in patients with bacterial meningitis and TBM (Thwaites et al. 2003). Reports from Vietnam suggest that CSF lactate levels of 5–10 mmol/L support a diagnosis of TBM, and that high levels are associated with death. However this marker is yet to be validated as a diagnostic test for TBM. Adenosine deaminase assay (ADA) has
been evaluated as an aid in the diagnosis, but is not specific enough to differentiate TBM from other forms meningitis (Tuon et al. 2010).

A number of studies in the past have looked at the usefulness of detecting antibodies or their antigens within CSF (Torok 2015). One such study looked at detecting a panel of M.tbc antigens using real-time PCR and ELISA in Indian children (Haldar et al. 2012). Sensitivity and specificity for definite and possible TB were reported to be 96% to 100% respectively. Another looked at the use of polyvalent M.tbc specific rabbit IgG to stain antigen-specific CSF leucocytes (Shao et al. 2011). A diagnostic evaluation of 393 CSF samples revealed a sensitivity of 73.5% and a specificity of 90.7%. Detection of lipoarabinomannan (LAM) antigen using ELISA and lateral flow assay for the diagnosis of TBM has been reported (Cox et al. 2015 and Bahr et al. 2016). Detection of LAM-IgG in 51% of 73 patients with TBM as against 0% in 36 controls was reported by Xue et al. (1999). However none of these tests did find sufficient laboratory application for helping to make a differential clinical diagnosis of TBM.

In the past few years molecular diagnostic techniques have been implemented for the rapid detection of uncultivable and slow growing microorganisms as well as those which require special techniques (Ruskova and Raclavsky 2011). Because of the slow growth characteristics of *Mycobacterium tuberculosis* (*M.tbc*), PCR based nucleic acid amplification tests have found a special place in diagnostic microbiology. Nucleic acid based amplification tests (NAATs) can detect < 10 organisms and therefore are ideal for the identification of *M.tbc* in CSF samples (Torok 2015). Currently available modifications include quantitative / real-time PCR (qPCR), isothermal, strain displacement or transcription-mediated amplifications and ligase chain reaction
(Dinnes et al. 2007). The extensive review of literature on NAATs has shown that their specificity is high but sensitivity depends upon number of bacilli in the smear (Torok 2015 and Dinnes et al. 2007). Sensitivity is highest in smear-positive respiratory samples and lower in smear-negative samples and in non-respiratory disease. Thus, a negative result does not rule out the diagnosis of TB in these situations.

Multiplex PCR for the diagnosis of TBM has received a lot of attention in the past (Sun et al. 2017). Although most studies report high sensitivity and specificity in smear and / or culture positive TBM cases, their sensitivity in paucibacillary cases are still in doubt (Kulkarni et al. 2005; Rafi et al. 2007; Kusum et al. 2011 and Patel et al. 2013). WHO has officially endorsed the use of line probe assays and Xpert MTB/RIF for routine diagnostic use and are in routine use in many countries globally (Torok 2015). Some low income countries where TB is endemic have also adopted these techniques (Vassal et al. 2011). Globally Xpert MTB/RIF has found wider acceptance because of its ease of use and rapid turnaround time. Moreover, it is a very convenient and sensitive method to detect MDR-TB in a few hours though there are concerns of lower positive predictive value. In a recently reported study from Vietnam (Heemskerk et al. 2018), the Gene Xpert was positive in 72.3% of culture positive cases while conventional ZN stain and modified ZN with Cytospin were positive in 66.4% and 67.5% respectively. It has been reported that some Indian strains of M.tbc lack the gene corresponding to the primer rpoB gene used in Xpert MTB/RIF. Thus in a large number of cases the organism will go undetected which is one disadvantage of Xpert MTB/RIF. In recent times, HRMA has been evaluated as an effective molecular diagnostic test. Relatively less cost, rapidity and absence of a probe makes HRMA a preferred PCR-based test.
2.10. VIRAL MENINGITIS

With the introduction of vaccines for *S.pneumoniae*, *H.influenzae* and *N.meningitidis*, the incidence of acute pyogenic meningitis due to these bacteria is on the decline in many parts of the world (Sadarangani 2015). This is accompanied by a proportionate increase in the incidence of meningitis due to a variety of viruses; however, in many instances their epidemiology is largely unknown. In regions where vaccination to these pathogens is not common, bacterial pathogens predominate and consequently those of viral etiology are comparatively less (Dash et al. 2009).

Encephalitis refers to a serious form of neurological disease affecting the brain parenchyma while meningitis is the inflammation of the meninges (Ai et al. 2017). Of all etiologies of encephalitides, viral etiology is the most common among them (Barbadoro et al. 2012). Most commonly identified viral etiology in the United States, Italy and Australia is *Herpes simplex* virus, both in children and adults while in Southern Vietnam, *Japanese encephalitis* virus (JEV) was the leading cause of viral encephalitis in children (Ai et al. 2017). A huge epidemic of encephalitis in children due to *Japanese B virus* was reported from Vellore in mid 1960s which was confirmed in the laboratory by virus isolation and detection of hemagglutination inhibiting antibodies (Carey et al. 1969 and Carey et al. 1968). An outbreak of Japanese encephalitis was also reported in Assam during 1980 (Chakraborty et al. 1987) and from Haryana in 1990 and again in 2005 (Kar and Saxena 1998 and Rao et al. 2005). Japanese B encephalitis has been thus a growing problem in different parts of India and many parts of southeast Asia since mid 1960s (Bu’Lock 1986). In Uttar Pradesh, enterovirus was an important cause of encephalitis in children (Kumar et al. 2012) while *Herpes simplex* virus was the most commonly identified pathogen in children and adults in eastern India (Rathore...
et al. 2014). In West Bengal, a huge outbreak of 398 line listed cases of acute encephalitis syndrome (AES) was reported in July 2014. Of 271 cases tested, 49.4% were identified as Jap B genotype III (Gurav et al. 2016). In most of these cases, it was very difficult to differentiate between viral meningitis and meningoencephalitis for which a highly sensitive and specific test with minimal turn-around time is required for accurate diagnosis.

For several decades, identification of viruses as etiological agents of meningitis was hampered due to non availability of sensitive laboratory techniques. Even when available, conventional techniques such as cell culture/tissue culture and antibody techniques, most laboratories were not willing to perform these techniques for want of expertise and facilities. However, with the discovery of NAATs, the use of molecular techniques became easily available to more laboratories. With increasing use of PCR in laboratories across the world, cost of the equipment and reagents became less and thus more accessible to many more laboratories. With application of real-time PCR for the detection of many uncultivable microbes, detection of viruses in the laboratory became more easy and frequent. This led to more cases of meningitis due to viruses being identified (McGill et al. 2017). A study conducted in England and Wales during 2012 – 2014, 57% of 1126 patients enrolled had meningitis; of these, 231 (36%) were viral, 99 (16%) were bacterial and remaining were either due to unknown etiology or other causes. The viruses identified were Herpes simplex virus I / II, (HSV I / II) Varicella – Zoster virus (VZV), Epstein-Barr virus (EBV), and Cytomegalovirus (CMV). An early diagnosis of viral meningitis can help in initiating appropriate management protocols at the earliest possible time, minimize the unnecessary use of antibiotics and the length of hospitalization and institute antiviral therapy wherever possible. Quality of life in patients have been reported to decline after viral meningitis, with an excess of pain, anxiety, and depression, and reduced
activity levels up to 48 weeks after admission (Brouwer and van de Beek 2018). Earlier studies have also emphasized the negative impact of viral meningitis on cognitive functioning even in patients with an apparent favorable outcome. Thus viral meningitis is not a less serious or benign condition and new treatment protocols, better follow-up, and rehabilitation strategies should be developed for better results.

Conventional methods for the detection of viruses include immunoassays such as immunofluorescent assays, ELISA and the more cumbersome tissue culture methods are all limited by slow turnaround time, poor sensitivity / specificity and lack of standardization of reporting. These techniques are subject to inter-observer variations and therefore cause problems in standardization and interpretation. In recent times, these techniques have been replaced by PCR based amplification tests that are characterized by high sensitivity, specificity and fast turnaround time. PCR based techniques are particularly useful for detection of viruses because none of the conventional methods have the same advantages that PCR possesses; more importantly, most laboratories do not have facilities for diagnosis of viral infections. Since PCR is an open platform for identification of diverse microbes, additional protocols for viral identification into the system will not bear extra cost. With selection of specific primers and their proper evaluation, in-house PCR can easily be standardized as a valuable adjunct for the rapid diagnosis of viral meningitis.

The most widely used PCR-based method for the detection of viruses is the real-time or quantitative PCR (q-PCR). This is the based on the detection of amplicons at every cycle of amplification by a fluorescent dye. Kong et al. (2015) used q-PCR to identify enteroviruses as the most common pathogen in Cangzhou, China. Use of commercial PCR kits was found to
reduce the use of antimicrobials by identifying the viral etiologies wherever possible (Eichinger et al. 2018). Although the cost of the Q-PCR equipment has come down drastically due to their wide-spread use, the cost of the reagents, especially, the fluorescent dye can be prohibitive in low income settings.

In the field of virology, Lin et al. (2008) developed high resolution melting analysis for subtype differentiation of the influenza A virus and for detection of newly emerging variants. The Influenza B virus has also been studied using the melting curve analysis by Nakagawa et al. (2008), who demonstrated that the assay not only detected its antigenic variants but also allowed for more precise analysis of the antigenic shifts in the Influenza B virus. A HRMA assay detecting and genotyping noroviruses directly from stool samples was also developed and successfully validated by Tajiri-Utagawa et al. (2009).

Therefore, we expect the routine availability of the newly available elegant technique of high-resolution melting analysis (HRMA) in medical microbiology laboratories should provide a strong stimulus in this field. This is already envisioned by the growing number of specific HRMA applications published in medical microbiology.