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Review Article

Viral & Bacterial Meningitis: Usefulness of Molecular Assays for the Determination of the Etiological Agents

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Abstract: The review article includes the clinical significance of Human herpesviruses, herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) and other bacteria responsible for central nervous system (CNS) infections in humans. Clinical usage of the Molecular Diagnosis assays has been of immense significance in such diseases.
Keywords: Real Time PCR, Encephalitis, Polymerase Chain Reaction; Human herpesviruses; Aseptic meningitis; Spinal tap.

INTRODUCTION

Meningitis is an acute inflammation of the delicate membranes covering the brain and spinal cord known collectively as the meanings [1]. It can be due to viruses, bacteria, or other microorganisms, and less commonly by certain drugs [2]. Meningitis can be life-threatening because of the inflammation to the brain and spinal cord; therefore, the condition is classified as a medical emergency [2, 3]. The infection occurs most often in children, teens, and young adults. The most common symptoms of meningitis are headache and neck stiffness associated with fever, confusion or altered consciousness, vomiting, and an inability to tolerate light (photophobia) or loud noises (phonophobia). It is contagious germs that can be passed from one person to another through coughing and sneezing and through close contact.

Viral meningitis

Aseptic meningitis, common in occurrence, is rarely life threatening. It is generally not a reportable disease (depending on whether a reportable etiologic agent-such as mumps-is identified). For this reason, the actual incidence of viral meningitis is not known. However, seasonal increases occur in late summer and early autumn and are mainly attributed to arbovirus and enterovirus activity. Identification of the viral agent is challenging-under ideal circumstances, serologic and virology isolation methodology may yield specific etiologic agent identification for about half of the cases diagnosed. Symptoms are similar to those for bacterial meningitis and include fever, headache, photophobia, pain upon moving the eyes, meningismus and sometimes a vesicular/petechial rash (rubella-like if echoviruses and coxsackieviruses are causative). Other symptoms are malaise, myalgia, anorexia, nausea, abdominal pain, diarrhea-may accompany fever. Occurrence of stupor, marked confusion or coma is rare, and these symptoms generally are not indicative of meningitis with a viral cause. Gastrointestinal and respiratory symptoms may occur when infection is caused by enteroviruses.

Table 1: Bacteria & viruses responsible for meningitis

Bacteria	Virus
Escheriachia coli, Neisseria meningitis	Adenovirus, Coxsackievirus
Group B streptococci ,Pseudomonas species	Herpes simplex, Mumps, Enteroviruses (picornaviruses)
Haemophilius Influenza ,Streptococcus pneumonia	Arbovirus, Varicella-zoster virus (VZV)
Listeria monocytogenes	Measles, Echovirus, Lymphocytic Choriomeningitis Virus (LCMV)

Table 2: Less common and rare viruses responsible for meningitis

Common	Less common	Rare
Enteroviruses	Herpes Simplex Virus -1	Influenza
Herpes Simplex Virus-2	CMV	Parainfluenza
HIV	Epstein–Barr virus (EBV)	Rotavirus
LCMV	VZV	Vaccinia
Arbovirus	Adenovirus	Encephalomyocarditis virus
Mumps	Measles, Rubella	

Herpes Simplex Virus (HSV)

Infection in adults is usually benign (e.g. oral cold sores). When it occurs in critical anatomical sites, for example ocular or central nervous system, or acquired by the neonate during parturition, the sequelae may lead to serious complications. One central feature of HSV infection is reactivation from the sensory nervous system of latently infected humans, although the triggers for this are not well defined. Infection with HSV is thus life long with unpredictable reactivations in which lesions may not always be manifested. An echo (Enteric Cytopathic Human Orphan) virus is a type of RNA virus that belongs to the species Enterovirus B, genus *Enterovirus* of the *Picornaviridae*. Echoviruses are found in the gastrointestinal tract and exposure to the virus causes other opportunistic infections and diseases. Echovirus is highly infectious, and its primary target is children. It is a leading cause of acute febrile illness in infants and young children, and is the most common cause of aseptic meningitis. Infection of an infant with this virus following birth may cause severe systemic diseases, and is associated with high infant mortality rates. It is also known as orphan virus means that is not associated with any known disease [4]. Coxsackievirus is a virus, belongs to a family of non enveloped, linear, positive-sense RNA viruses which includes poliovirus and echovirus. It shares many characteristics with poliovirus. They are divided into group A and group B viruses. Group A consists of 23 serotypes [1–22, 24] and group B consists of six serotypes [1–6] are recognized. It is the leading causes of aseptic meningitis. It is best known as the cause of infectious mononucleosis (glandular fever). It is also associated with particular forms of cancer, such as Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and conditions associated with human immuno deficiency virus (HIV) such as hairy leukoplakia and central nervous system lymphomas [5, 6]. There is evidence that infection with the virus is associated with a higher risk of certain autoimmune diseases especially dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and multiple sclerosis [7]. Poliovirus: the causative agent of poliomyelitis, is a human enterovirus. It is composed of an RNA genome and a protein capsid. The genome is a single-stranded positive-sense RNA genome that is about 7500 nucleotides long [8]. The viral particle is about 30nm in diameter icosahedral symmetry. Poliovirus is one of the well-characterized viruses, and has become a useful model system for understanding the biology of RNA viruses [9].

Bacterial Meningitis

Bacterial meningitis is the most common form of meningitis. It needs to be treated right away to prevent brain damage and death. Infection causes swelling around the brain. Commonly found in the environment, nose and respiratory system [10]. Children up to 2yrs are most susceptible to bacterial meningitis .Adults with risk factors are weakened immune system, head injury, ear infections, brain or spinal surgery, widespread blood infection. Symptoms include high fever, headaches, chest, confusion, irritability, increasing drowsiness. Seizures and stroke may occur. A general intravenous antibiotic is used with a corticosteroid to bring down the inflammation. Neisseria meningitis is a parasitic, aerobic, Gram-negative, non-endospore forming nonmotile, coccal bacterium that is responsible for causing meningitis, inflammation of the meninges layer covering the brain. Upon Gram staining, it appears as a Gram-negative diplococcus and cultures of the bacteria test positive for the enzyme cytochrome c oxidase. *N. meningitidis* is an obligate commensal residing in the human nasopharynx [11-13]. The highest incidence of nasopharyngeal carriage of *N. meningitidis* is in adolescents especially those residing in overcrowded spaces. Particularly prone are school-going children and college students, household contacts of meningococcal patients and also military recruits . Other factors that may predispose to meningococcal carriage include lower socio-economic status and concurrent viral or bacterial respiratory tract infection. In such individuals the carriage rates can be as high as 34%. Recent estimates show that the global incidence of meningococcal disease is 500,000 per annum with a worldwide mortality rate of 10%. *N. meningitidis* can exist with or without a polysaccharide capsule. However, nearly all of the meningococcal meningitis infections are caused by the capsulated form. Based on the polysaccharide characteristics *N. meningitidis* can be divided into at least 12 different serogroups. Serogroups A, B, C, W135, X and Y are isolated in almost 90% of the infections. The serogroup distribution is often related to the age of the patient and more importantly to the geographical location. *S. pneumoniae* is one of the most common causes of bacterial meningitis worldwide. It is a capsulated bacterium which has 93 serotypes based on the different polysaccharide 6 characteristics of the capsule. Most of

the serotypes are capable of causing disease but majority of the infections in the developing countries are caused predominantly by serotypes 1. It usually affects small children under the age of 2 years. The other age group with high susceptibility to pneumococcal infection is the old age. The high rates of pneumococcal infections may partly be due to the high carriage rates among the general population. Children under 6 years of age have the highest rates of nasopharyngeal carriage. Due to this reason pneumococcal disease has become the leading cause of vaccine preventable deaths in that age group. Pneumococcal meningitis incidence may exhibit mild seasonal variations. Although some strains of *S. pneumoniae* have been implicated in large outbreaks, causing widespread epidemics is not considered typical of pneumococcal disease [14-16].

H. influenzae is a common respiratory pathogen which can occur either as capsulated or un-capsulated form. The difference in structure of the polysaccharide capsule is the basis for the division of *H. influenzae* into 6 serotypes; a, b, c, d, e and f. Out of these 6 serotypes, serotype b is associated with most of the meningitis infections.. The infection occurs usually in children less than 5 years of age and is rare in adults. The incidence varies in different parts of the world but it is generally estimated to be higher in Africa where the incidence is around 46 per 100, population . In unimmunized patients, the mortality rate is estimated to be almost 43%. Group B Streptococcus, also known as 'Streptococcus agalactiae' and more colloquially as Strep B and group B Strep, can cause serious illness and sometimes death, especially in newborn infants. Streptococcus agalactiae is a gram-positive streptococcus characterized by the presence of Group B Lancefield antigen. The CAMP test is an important test for identification of GBS. It is characterized by the presence of group B Lancefield antigen and by its ability to hydrolyze sodium hippurate. It is also sensitive to bile, and will lyse in its presence. It is found normally in the intestine, vagina, and rectal area in 15%-45% of all healthy women. Group B strep infections can affect neonates and adults. The infection is spread to infants before or during delivery. Signs and symptoms may include fever, breathing problems, lethargy, and poor feeding. Diagnosis of GBS infection is made by isolating the organism from body fluids..The treatment for GBS infection is antibiotics. Complications of GBS infection include sepsis, pneumonia, meningitis, or occasionally death [17, 18].

Differential Diagnosis of Bacterial Meningitis

Bacterial meningitis is a significant cause of mortality and morbidity worldwide. Neurological outcome and survival depend largely on damage to CNS prior to effective antibacterial treatment.

Examination of CSF

A needle is inserted into the spinal cord to extract a sample of cerebrospinal fluid (CSF) that envelops the brain and spinal cord. The CSF should arrive still warm and either be examined immediately or placed in an incubator for examination within an hour, an examination of CSF involves the following) Macroscopic examination, Cytological examination, Examination of Gram stained smear, Culture and antimicrobial susceptibility testing, Latex agglutination test for antigen detection [19]. Other laboratory tests include; Blood cultures can be useful in a situation where CSF cannot be obtained before the administration of antimicrobials. Blood cultures can enhance the identification of the causative organism which follow haematogenous route to reach the meninges (WHO). Blood cultures are often positive and valuable to detect the causative organism and establish susceptibility patterns if CSF cultures are negative [20]. Flow cytometry: Recently, flow cytometry with a dedicated bacterial channel has (Sysmex UF-1000i) possible application in automated cell counting and this novel approach in the differential diagnosis of meningitis has been explore [21].

Molecular Diagnosis

PCR assays are highly sensitive and specific and have been evaluated for their effectiveness in detecting the presence of bacterial DNA in CSF from patients with suspected and proven bacterial meningitis [22]. PCR-based assays are useful adjuncts to conventional bacterial culture and antigen detection methods in establishing the bacterial etiology in meningitis in settings where substantial numbers of specimens are culture negative. PCR for H. influenzae, S. pneumoniae, and N. meningitidis had an overall specificity of 100 percent. The sensitivity for H. influenzae, S. pneumoniae and N.meningitides, was 92%, 100%, and 88%; respectively. By multiplex assay, it is found that in bacterial meningitis patients defined by positive CSF culture, positive CSF Gram stain, and based on clinical suspicion with negative cultures, PCR has high sensitivities for H. influenzae, S. pneumoniae, and N. meningitides with a specificity of 100% for all three microorganisms [23].

Differential Diagnosis of Viral Meningitis: The list of diagnostic tests mentioned in various sources as used in the diagnosis of viral meningitis includes; i) Lumbar puncture (spinal tap) - Viral meningitis is usually diagnosed by laboratory tests of spinal fluid obtained with a spinal tap. It can also be diagnosed by tests that identify the virus in specimens collected from the patient ii) Blood culture - Blood tests for virus to rule out viral meningitis iii) Meningococcal rash pressure test - test a skin rash for the "failure to whiten under pressure" property of a rash from meningococcal. iv) Other tests may include: a)A neurological exam to test nerve, motor, and sensory function; hearing, speech, and vision; balance; mental status b) Throat culture c) Computed tomography (CT), magnetic resonance

imaging (MRI), electroencephalography (EEG) to spot problems in the brain [24].

Species-specific Real-Time PCR assays

PCR detection of *N. meningitidis, H. influenzae*, and *S. pneumoniae* can be achieved by amplification of several potential gene targets. The following assays have been developed and validated to be used on DNA extracted from clinical specimens (typically, blood and CSF) and bacterial isolates. *N. meningitides;* Two genes can be targeted in *N. meningitidis* species-specific assays, *ctr A* and *sod C*. The capsule transport to cell surface gene, *ctr A*, is highly conserved among isolates responsible for invasive meningococcal infections and has been used in both real-time and conventional PCR to detect *N. meningitidis.* It is a gene within the capsule locus. However, since at least 16% of carried meningococci lack *ctr A,* a real-time PCR assay to detect all meningococci. This assay targets the Cu, Zn superoxide dismutase gene, *sod C*, which is not genetically linked to the capsule locus. The *sodC* assay detects encapsulated meningococci, but it is also useful for detecting non groupable meningococci that do not contain an intact *ctrA*. For this reason, it is recommended that *sod C* be used for detection of *N. meningitides. H. influenzae:* The protein D encoding gene, *hpd*, encodes protein D, a highly conserved, surface-exposed lipoprotein that is present in all encapsulated and non-encapsulated *H. influenza*.The conserved nature of this gene and its presence in all strains of *H. influenzae* characterized to make it a highly attractive gene target for the development of a *H. influenzae* species-specific real-time PCR assay. The recently developed and validated *hpd* real-time PCR assay is capable of detecting all six serotypes (a-f) and nontypeable (HiNT) *H. influenzae* with high sensitivity and specificity. Real-time PCR assays targeting *bex A* were developed and distributed because *bexA* is present in all six serotypes of *H. influenzae*. However, though sensitive for detection of Hib, it is less sensitive for Hia, Hic, and Hid, and does not detect Hie, Hif, or HiNT and should no longer be used. *S. pneumoniae*: Both conventional and real-time PCR assays have been developed for the detection of *S. pneumonia* [8], and target genes have included the pneumolysin (*ply*), autolysin (*lyt A*), and pneumococcal surface adhesion (*psa A*) genes. However, false-positive results with *ply*-based PCR have been reported when applied to upper respiratory tract specimens. A suggested explanation for these false positives is the detection of non-pneumococcal alpha-hemolytic streptococci ,which are normally present in the respiratory flora (e.g., *Streptococcus mitis* group and *Streptococcus oralis*) which sometimes contain a *ply* gene [25, 26] .The PCR detection assay for *S. pneumoniae* using a specific segment of the autolysin gene (*lyt* A) is recommended because it is highly conserved within the species.The real-time PCR assay *lyt A* primers and probes that have been found to be extremely reliable for detection of *S. pneumoniae*. Due to recombination events that occur between pneumococci and closely related streptococci, there will probably be rare false-positives or false-negatives for virtually any real-time assay for pneumococcal identification.

Serogroup/serotype-specific Real-Time PCR assays:

The capsule gene loci of both *N. meningitidis* and *H. influenzae* have areas that are both unique and conserved within each serogroup (*N. meningitidis*) or serotype (*H. influenzae*) thus providing gene targets for the development or real-time PCR assays, designed to identify each specific serogroup or serotype. *N. meningitidis*: *N. meningitidis* is classified into 12 serogroups on the basis of the chemical composition and linkage type of saccharide subunits of the capsular polysaccharide that are expressed on the bacterial cell surface. Major disease-causing serogroups include A, B, C, Y, and W135, the latter four of which produce sialic acid containing capsular polysaccharides; whereas serogroup A produces a poly-α1-6-linked N-acetylmannosamine 6-phosphate capsule . Outbreaks caused by serogroup X meningococci, which express poly-α1-4-linked N-acetylglucosamine 1-phosphate capsule have also been reported [27]. Serogroup D is no longer recognized as a serogroup of *N. meningitidis*.

Haemophilus influenzae

The capsule locus of all six serotypes of *H. influenzae* (Hi a, b, c, d, e, and f) consists of three regions encoding functions for capsule polysaccharide synthesis, modification, and translocation. *Bex DCBA* in the ATP-driven export region (also known as Region I) code for protein components of an ATP-driven polysaccharide export apparatus. *hcsA* and *hcsB* in post polymerization modification region (also known as Region III) share high similarity with *lipA* and *lipB* (recently renamed *ctrE* and *ctrF*), respectively, which are involved in modification and export of meningococcal capsule polysaccharide [28]. The serotype-specific region (previously Region II) contains genes for capsule synthesis and is unique to each serotype. The serotype-specific genes are named *acs*, *bcs*, etc. for "a capsule synthesis", "b capsule synthesis", and so on. The *cap* locus encodes functions for *H. influenzae* capsule synthesis. The genetic organization of the *cap* locus has been well characterized in Hib and Hif, which belong to two phylogenetic divisions that are defined by multilocus enzyme electrophoresis typing. The ATP-driven export region includes most of Hia and Hib strains, and all of Hic, Hid, and Hie strains. Strains from this region have at least one completed *cap* locus flanked by insertion sequence (IS) element IS*1016*, except Hie.

***S. pneumoniae* serotyping PCR assays**

S. pneumoniae can be further classified into at least 93 serotypes based on the immunochemistry of their capsular polysaccharides. The high cost of antisera, subjectivity in interpretation, need for a

complete set of control strains, and technical expertise requirements associated with these serologic methods have resulted in the more recent development of PCR-based serotyping systems. PCR-serotyping has the potential to overcome some of the difficulties associated with serologic testing and the development of PCR-based assays for direct detection of serotypes from clinical specimens is a valuable aid in surveillance, particularly in situations where culture is insensitive. PCR assays (both conventional and real-time) for the detection of the more common serotypes are being developed. Multiplex conventional PCR assays for serotyping *S. pneumonia:* A multiplex PCR-based serotyping scheme that includes 40 serotype specificities has been developed. This PCR approach has the potential to greatly reduce reliance upon conventional serotyping and provides serotype-determining potential to laboratories that lack type-specific antisera and other reagents needed for conventional serotyping are being developed. The multiplex approach uses 9 reactions to identify 40 but also provides some flexibility that allows for altering combinations of serotypes included in each sequential reaction. These can be modified based on the most prevalent serotypes in any given geographic region but do require validation to ensure no cross reaction between serotype primer sets. Three such schemes based upon pneumococcal serotype prevalence in the USA, Africa, and Latin America has been designed. These schemes will continue to be refined as additional serotypes are added and primer sets updated to improve specificity and sensitivity. Real-time PCR assays for serotyping *S. pneumoniae: A* number of real-time PCR assays for serotyping *S. pneumoniae* are recommended for determining serotypes from clinical specimens when DNA may be present in low amounts and insufficient for conventional multiplex PCR serotyping. Multiplex real-time PCR for pathogen detection: Real-time PCR allows for development of multiplex assays for detection of several genes in the same reaction by using specific probes with different fluorescent dye labels. Multiplex real-time PCR assays are available for detection of *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* in a single reaction. In addition, assays for serogrouping *N. meningitidis* and serotyping *S. pneumoniae* using a multiplex approach have also been developed. However, they are not recommended for routine use. . Changes in DNA polymerase or PCR reagent concentrations may lead to loss of sensitivity.

Table 3: Countries with high endemic rates (>10 cases/100,000 population) and/or > =1 epidemic over the last 20 years [31]

Country	Year	Incidence per 100000 population	Predominant sero group
Angola	1994-2000	19-230	Data not available
Benin	1980-1999	6-57	
Burkina faso	2004-2009	26-187	
Burundi	2004-2009	158	
Cameroon	1980-1999	1-224	
Centrafique	2004-2009	3.3-19.4	
Chad	2004-2009	9.6-15.9	
Cote de ivoire		0-6	
Ethiopia		0-104	A
Gambia		4-165	
Ghana		0-108	
Guinea		0-17	
Guinea Bissau		0-133	
Kenya		267	
Mali		2.6-12.9	
Mauritania		0-14	
Namibia		4-165	
Niger		7.8-90.7	
nigeria	2004-2009	0.7-52.6	
RD congo		7.3-23.7	
Rwanda		0-28	
Senegal		0-53	
Tanzania		0-19	
Togo		6-13.2	
Uganda		0-18	
Sudan	2008	Not available	Data not available
Saudia Arabia	2000		A,W-135
Uruguay	2001	30(pre- vaccine) and 1.6 (post vaccine)	
New Zealand	1991-2000	17.4	B
Mongolia	1994-1995	80-90	A

Table 4: Countries with moderate endemic rates (2–10 cases/100,000 population per year)

Country	Year	Incidence/100000 population	Predominant sero group
South Africa	2000-2005	0.8-4	B in western cape
Belgium	1999-2010	2.9(pre-vaccine),0.85(post-vaccine)	B,C
Denmark	1999-2010	1.19-3.5	B
Greece		0.49-2	C
Ireland		14.3(pre-vaccine),2.19(post vaccine)	BC
Iceland		7.6(pre vaccine),0.6(post vaccine)	BC
Lithuania	2004-2010	1.4-2.6	*
Luxemburg	1999-2010	0.2-5.68	*
Malta	1994-2007	0.8-8.9	BC
Netherland	1999-2010	3.6(pre-vaccine),0.85(post vaccine)	BC
Norway	1992-2010	.8-4.6	B
Portugal	2000-2010	.74-3.0	BC
Spain	1999-2010	3.52(pre-vaccine),0.88(post-vaccine)	BC
Switzerland	1999-2004	1.16-2.36	C
Turkey	1997-2005	0.3-2.2	*
United kingdom	1999-2010	5.4(pre-vaccine),1.64(post-vaccine)	B,C
Brazile	1998-2006	1-4.5	BC
Cuba	1998-2003	3.4-8.5(pre-vaccine),<1(post-vaccine)	B
Australia	1995-2006		B

Table 5: Countries with moderate endemic rates (<2 cases/100,000 population per year)

Country	Year	Incidence/100000 population	Predominant sero group
Austria	1999-2010	1.20-1.2	BC
Bulgaria	2000-2010	0.11-1.1	*
Croatia	1997-2005	0.7-1.3	*
Cyprus	1997-2010	0.13-1.7	*
Czech republic	1999-2010	0.57-1	BC
Estonia	2001-2010	0.15-1.6	*
Finland		0.64-1.1	B
France	1999-2010	0.7-1.13	BC
Germany		0.47-0.73	BC
Hungary	2004-2010	0.3-0.4	*
Italy	1999-2010	0.25-0.55	BC
Latvia	2004-2008	0.25-1.03	*
Poland	1999-2010	0.17-0.84	B
Serbia	2000	0.9	*
Slovakia	2004-2010	0.59-0.9	*
Slovenia	1999-2010	0.3-1.2	*
Sweden	2004-2010	0.5-0.7	BC
Argentina	2009	0.6	B
Canada	1985-2006	1.4(pre-vaccine), 0.4(post vaccine)	C
Chile		0.8	B
Columbia	1998-2006	0.3	Y
Mexico		0.1	C
USA	2000-2009	0.8(pre-vaccine), 0.3(post vaccine)	Equal B,C Y
Venezuela		0.3	Y
Korea	2002-2008	<0.1	
Thailand	2007-2008	<0.1	
China	2000onward	<0.2	AC
Japan	1999-2004	<0.02	*
Philippines	2004-2008	<0.1	A
Singapore	2005-2009	0.1-0.2	
Taiwan	2000-2001	01.-0.2	A

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REFERENCES

1. Sáez-Llorens X, McCracken GH; Bacterial meningitis in children. Lancet, 2003; 361 (9375): 2139–2148.
2. Tunkel AR1, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM *et al*.; Practice guidelines for the management of bacterial meningitis. Clinical Infectious Diseases, 2004; 39 (9): 1267–1284.
3. Brouwer MC, Tunkel AR, van de Beek D, van de Beek D; Epidemiology, Diagnosis, and Antimicrobial Treatment of Acute Bacterial Meningitis. Clin Microbiol Rev., 2010; 23(3): 467-492.
4. Brouwer MC, Tunkel AR, van de Beek D; Epidemiology, Diagnosis, and Antimicrobial Treatment of Acute Bacterial Meningitis. Clin Microbiol Rev., 2010; 23(3): 467-492.
5. Ginsberg L; Difficult and recurrent meningitis. Journal of Neurology, Neurosurgery, and Psychiatry. 2004; 75 Suppl 1 (90001): i16–21.
6. Akepathi C, Bothamley GH, Brennan PJ, Ivanyi J; Levels of antibody to defined antigens of Mycobacterium tuberculosis in tuberculous meningitis. J Clin Microbiol., 1989; 27(5): 821-825.
7. Akepati C, Konstantin L, Haradara Bahubali V, Khanna N, Pier NB, Mandavalli G *et al*.; Evaluation of Sysmex UF-1000i for use in cerebrospinal fluid analysis *Clin Chim Acta*. 2008; 392(1-2): 30-33.
8. Carvalho MG, Tondella ML, McCaustland K, Weidlich L, McGee L, Mayer LW *et al*.; Evaluation and improvement of real-time PCR assays targeting *lytA*, *ply*, and *psaA* genes for detection of pneumococcal DNA. Journal of Clinical Microbiology, 2007; 45(8): 2460-2466.
9. Wang X, Mair R, Hatcher C, Theodore MJ, Edmond K, Wu HM *et al*.; Detection of bacterial pathogens in Mongolia meningitis surveillance with a new real-time PCR assay to detect *Haemophilus influenzae*. International Journal of Medical Microbiology, 2011; 301(4): 303-309.
10. Claus H, Maiden MC, Maag R, Frosch M, Vogel U; Many carried meningococci lack the genes required for capsule synthesis and transport. Microbiology, 2002; 148(Pt. 6): 1813-1819.
11. Sadler F, Fox A, Neal K, Dawson M, Cartwright K, Borrow R; Genetic analysis of capsular status of meningococcal carrier isolates. Epidemiology and Infection, 2003; 130(1): 59-70.
12. Mothershed EA, Sacchi CT, Whitney AM, Barnett GA, Ajello GW, Schmink S *et al*.; Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of *Neisseria meningitidis*. Journal of Clinical Microbiology, 2004; 42(1): 320-328.
13. Taha MK, Alonso JM, Cafferkey M, Caugant DA, Clarke SC, Diggle MA *et al*.; Interlaboratory comparison of PCR-based identification and genogrouping of *Neisseria meningitidis*. Journal of Clinical Microbiology, 2005; 43(1): 144-149.
14. Dolan-Livengood JM, Miller YK, Martin LE, Urwin R, Stephens DS; Genetic basis for nongroupable *Neisseria meningitidis*. Journal of Infectious Diseases, 2003; 187(10):1616-1628.
15. Janson H, Ruan M, Forsgren A; Limited diversity of the protein D gene (*hpd*) among encapsulated and nonencapsulated *Haemophilus influenzae* strains. Infection and Immunity, 1993; 61(11): 4546-4552.
16. Song XM, Forsgren A, Janson H; The gene encoding protein D (*hpd*) is highly conserved among *Haemophilus influenzae* type b and nontypeable strains. Infection and Immunity, 1995; 63(2): 696-699.
17. Murdoch DR, Anderson TP, Beynon KA, Chua A, Fleming AM, Laing RT *et al*.; Evaluation of a PCR assay for detection of *Streptococcus pneumoniae* in respiratory and nonrespiratory samples from adults with community-acquired pneumonia. Journal of Clinical Microbiology, 2003; 41(1): 63-66.
18. Whatmore AM, Efstratiou A, Pickerill AP, Broughton K, Woodard G, Sturgeon D *et al*.; Genetic relationships between clinical isolates of *Streptococcus pneumoniae*, *Streptococcus oralis*, and *Streptococcus mitis*: Characterization of "atypical" pneumococci and organisms allied to *S. mitis* harboring *S. pneumoniae* virulence factor-encoding genes. Infection and Immunity, 2000; 68(3): 1374–1382.
19. Gagneux SP, Hodgson A, Smith TA, Wirth T, Ehrhard I, Morelli G *et al*.; Prospective study of a serogroup X *Neisseria meningitidis* outbreak in northern Ghana. Journal of Infectious Diseases, 2002; 185(5): 618-626.
20. Satola SW, Schirmer PL, Farley MM; Complete sequence of the *cap* locus of *Haemophilus influenzae* serotype b and nonencapsulated b capsule-negative variants. Infection and Immunity, 2003; 71(6): 3639-3364.
21. Suzuki N, Yuyama M, Maeda S, Ogawa H, Mashiko K, Kiyoura Y; Genotypic identification of presumptive *Streptococcus pneumoniae* by PCR using four genes highly

specific for *S. pneumoniae*. Journal of Medical Microbiology, 2006; 55 (Pt 6): 709-771.
22. Pai R, Gertz RE, Beall B; Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. Journal of Clinical Microbiology, 44(1): 124-131.
23. Felissa LR, Durham JD; Emerging infectious diseases: trends and issues. Springer Publishing Company, 2nd edition, 2007.
24. Gomase VS, Chitlange NR; Immunoproteomics approach for development of synthetic peptide vaccine from Mycobacterium tuberculosis. International Journal of Immunology Research, 2009; 1: 7-12.
25. Maeda E1, Akahane M, Kiryu S, Kato N, Yoshikawa T, Hayashi N *et al.*; Spectrum of Epstein–Barr virus-related diseases: a pictorial review. Jpn J Radiol., 2009; 27(1): 4-19.
26. Cherry-Peppers G, Daniels CO, Meeks V, Sanders CF, Reznik D; Oral manifestations in the era of HAART. Journal of the National Medical Association, 2003; 95 (2 Suppl 2): 21S–32S.
27. Toussirot E, Roudier J; Epstein–Barr virus in autoimmune diseases. Best Practice & Research. Clinical Rheumatology, 2008; 22 (5): 883–896.
28. Scott, Kevin J; Molecular epidemiological characterisation of carried Neisseria meningitidis isolates in Scotland, 1974-2004 and a comparison with an invasive meningococcal disease strain collection. PhD diss., University of Glasgow, 2011.