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Detection of Meningitis pathogens prevalent in culture negative paediatric CSF samples using Real-time Multiplex PCR

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Abstract

Background

Meningitis remains a public health priority with approximately 100 thousand deaths per year in India. Currently available diagnostic methods are less sensitive and hindered by the use of antibiotics. Consequently, culture negative CSF are of diagnostic dilemma for physicians. Rapid progression of clinical manifestations and need of 48 hrs for culture identification often encourages indiscriminate use of antibiotics. In the study we present the use of Fast-track diagnostics multiplex PCR for pathogen detection.

Methods

120 bacterial culture negative CSF samples were subjected to testing. These samples were collected from children clinically suspected of meningitis and subjected to multiplex real time PCR using with FTD bacterial meningitis and Neuro 9 kit.

Results

A bacterial or viral agent was identified in 35(29%) of the 120CSF samples. *S.pneumoniae* was detected in 19 samples. *H. Influenzae* and *Neissieria meningitidis* was present in one sample each. Mixed infection of *S.pneumoniae* and *Neissieria meningitidis* was present in one sample. ParvovirusB19 was most common, followed by Varicella Zoster Virus and Epstein Barr Virus, Human Herpes Virus-6,7 and Adenovirus

PCRSeqTyping identified Serotypes 6B,19F,1,14 and 20 in 19samples positive for *S.pneumoniae*.

Conclusion

The results of our preliminary study point out the usefulness of qmPCR based assay to establish the etiology of meningitis in settings where substantial number of specimens are culture negative. The findings suggest that pneumococcal meningitis is more prevalent in India than was previously suspected. The diagnostic molecular tool provides simultaneous detection of viral pathogens which is a neglected parameter.

Abbreviations: LA: Latex agglutination; CSF: cerebrospinal fluid; FTD: Fast-track diagnostics; PCR: Polymerase chain reaction; GBD: Global Burden of Disease; LAT: latex agglutination testing; B19V: Human parvovirus B19 (B19V); Hib: *H. influenzae type b*; IAP: Indian Academy of Pediatrics; VZV: Varicella zoster virus; ssDNA: single stranded DNA; UIP: Universal Immunization programme

Introduction

Meningitis, a debilitating disease with many deaths and serious long-term effects for patients, remains a major global public health problem [1]. It is a major cause of morbidity and mortality among infants and children below 5 years of age [2]. Despite advances in vaccine production and the availability of potent newer antibiotics, the death rate due to meningitis remains significantly high in India [3,4]. The 2016 Global Burden of Disease (GBD) report estimated that the number of cases of global meningitis rose from 2.50 million (95% UI 2.19–2.91) in 1990 to 2.82 million (2.46–3.31) in 2016 [3]. Although global meningitis deaths decreased by 21.0% from 1990 to 2016, the overall burden of meningitis remains high [1].

It is necessary to know the aetiology, along with their susceptibility profile, so that patients with least potential mortality can be optimally treated. Accurate etiological diagnosis is often not possible due to weak culture facilities, prior antibiotic therapy, delayed crop plating, unavailability of uniform quality media, and low bacterial load. However, detection, characterization and serogrouping of strains is important for the investigation of outbreaks, events, contact management and implementation of vaccination strategies. There is a need for a periodic examination of cases of meningitis, as the pathogens responsible for the infection differ in time, geography and patient age.

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Microbiology laboratories play a critical role not only in the early detection of the causative bacterium and its pattern of antibiotic susceptibility, but also in the providing valuable information regarding the common incriminating pathogens and also on the start of empiric therapy [5]. The definite laboratory diagnosis of bacterial meningitis requires the isolation of bacteria by the culture of CSF, which takes at least 12 to 48 hours [6]. In addition, around 50% of suspected cases of bacterial meningitis are not confirmed by culture, mainly because of pre-analytical problems and low clinical sample quality due to delayed sample processing and/or the use of antibiotics before the lumbar puncture. Rapid methods such as Gram staining or latex agglutination testing (LAT) are available for the detection of certain agents but are not sufficiently sensitive [6]. The standardization of a molecular biology technique to identify major etiological agents of bacterial meningitis will contribute to improvements in the diagnosis, especially in cases where there is a low concentration of the microorganism, the patient is already being treated with antimicrobials, or when culture results are not satisfactory. Detection of nucleic acid in CSF of suspected cases by nucleic acid amplification test is considered an important diagnostic tool which has a high sensitivity, specificity, simplicity in execution, rapidity, and interpretation [7]. Multiplex molecular assays are an attractive option for detection of several microbial targets simultaneously and are now routinely used for bloodstream, respiratory, and gastrointestinal infections [8].

In the light of these concerns and limitations, the study aimed to determine the etiological agents that cause meningitis using molecular diagnostic methods to improve the laboratory diagnostic assessment of the patients. The assay detects 14 pathogens known to cause meningitis, including three bacteria and twelve viruses.

Methods

2.1 Study design and population:

The study was conducted in two tertiary care teaching hospitals in Chennai and Kanpur. 120 CSF samples were collected from paediatric patients aged 6 months to 5 yrs presenting to the paediatric outpatient department with children clinically suspected of meningitis. Informed consent was obtained from each parent/guardian prior to performing study-specific procedure. The protocol was reviewed and approved by local Ethics Committees, and the study was conducted according to Good Clinical Practice and the Declaration of Helsinki.

2.2 Study procedures:

Children with clinically suspected meningitis presenting to study physicians were anonymously recorded in screening logbooks and assessed for eligibility. Demographic characteristics, medical history, antimicrobial therapy and general symptoms of eligible children were collected.

2.3 Sample collection, handling and transport

CSF samples were collected by lumbar puncture by specialized professionals and samples collected were transported to microbiology laboratory within 1 hr in an ice box.

2.4 Bacterial culture:

CSF samples were directly inoculated onto 5% Sheep Blood agar, Chocolate agar and MacConkey agar (HiMedia, India). The MacConkey agar plates were incubated aerobically while chocolate and blood agar plates were incubated for 24–48 h at 37°C in 5% carbon dioxide. The plates were observed for the colony characters of bacterium, Gram-staining and biochemical reaction using the standard methods [6].

2.5 Molecular analysis:

2.5.1 Extraction of Total nucleic acids:

Total nucleic acids were extracted from the CSF samples using QIAamp viral RNA Mini Kit with automated DNA extractor, QIAcube (Qiagen, Germany), as per manufacturer's protocol. Quantity and quality of the extracted nucleic acid was measured spectrophotometrically at 260 nm absorbance with Nanodrop 2000 (Thermo Fisher Scientific, USA).

2.5.2 Meningitis multiplex PCR panel:

Total nucleic acids extracted were subjected to multiplex PCR using the Fast-track Diagnostics Neuro 9 panel (for Virus) and Bacterial meningitis panel. FTD Bacterial meningitis is an in vitro test for the quantitative detection of bacterial nucleic acid in cerebrospinal fluid (CSF) and blood samples for detecting *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae type b*. FTD Neuro9 is an in vitro test for the qualitative detection of viral nucleic acid as an aid to the evaluation of infections with adenovirus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1 & 2, varicella- zoster virus, enterovirus, parechovirus, human herpes virus 6 and 7 and parvovirus B19.

2.5.3 Serotyping of *S. pneumoniae* by PCRSeqTyping:

S.pneumoniae positive samples were subjected for serotyping by PCR amplification and sequencing of cpsB region as detailed elsewhere. Briefly, PCR amplification and sequencing was performed using cpsB primers [9,10]. The sequence data was used to interrogate the GenBank database (http://www.ncbi.nlm.nih.gov/blast/) and assigned to serotype using the criteria as per protocol [9]. Serotype of the cpsB nucleotide sequence from GenBank with the highest BLAST bit score was assigned, provided that sequence identity was >99% with the query amplicon nucleotide sequence.

Results

3.1 Study Population:

The study population was divided into 4 categories of age group. 25% (n=30) of the population belonged to 0-2yr of age group, 32% (n=38) were from 2-5yr age group, 31% (n=37) were from 5-10yr and 13% (n=15) belonged to 10-15yrs of age. 53% of the study population were male and 47% were female. No cells were found in 72 % (n=86) of the samples. Out of 34 samples which had cell count, average cell count was 134. None of the samples were positive for bacterial culture.

Geographically, 67% of the samples (n=80) were collected from South region (Chennai) and 33% of the samples (n=40) were collected from north region (Kanpur).

3.2 Bacterial culture:

No evidence of bacterial growth was observed in CSF samples. This could be due to prior antibiotic treatment received before hospital admission.

3.2 CSF FTD Panel Results:

A bacterial or viral agent was identified in 35 (29%) of the 120 CSF samples. 18/40 (45%) of the samples from North region and 17/80 (21%) of the samples from South region were positive for FTD panel.

Of the 35 positive sample, the most commonly detected organisms were *S.pneumoniae* (19 cases, 54% of FTD positives) and Parvovirus B19 (B19) (13 cases, 37% of FTD positives). *H.Influenzae and Neissieria*

meningitidis was present in one sample each. Mixed infectious pathogens (virus and bacteria) were observed in 7 cases. Apart from B19, Varicella Zoster Virus (3 cases), Human Herpes Virus-6 (1 case), Human Herpes Virus-7 (1 case), Epstein Barr Virus (2 cases) and Adenovirus (2 cases) were identified. In one patient from north region, FTD panel detected mixed infection with *Neissieria meningitidis, S.pneumoniae* and Varicella Zoster Barr Virus.

S.pneumoniae was observed in 44% of the FTD positive samples (8/18) from north region while it was seen in 65% (11/17) of the FTD positive samples from south region. Prevalence of B19 was 61% (11/18) and 12% (2/17) of the FTD positive samples from North and South region.

PCRSeqTyping analysis of *S.pneumoniae* positive samples identified the following serotypes – 6B (n=7), 19F (n=5), 1 (n=3), 14 (n=3) and 20 (n=1)

Discussion

Recently, the introduction of newer Hib and Pneumococcal vaccines has improved the epidemiology of Bacterial Meningitis compared to the pre-vaccine period, when both morbidity and mortality were comparatively high [11]. Extremely successful antimicrobial treatment, when started early, results in a positive outcome. However, the emerging antibiotic resistance is of increasing concern [12]. The phenomenon of indiscriminate antibiotic use and repeated pre-treatment with antibiotics makes the isolation of the causative organism difficult and restricts the option of antibiotics. Hence, there is a growing dependency on newer techniques such as latex agglutination antigen detection or DNA-based polymerase chain reaction (PCR) [13].

Annually about 700,000 to 1 million deaths attributable to pneumococcal disease occur globally with majority of them occurring in the developing countries. Currently licensed PCV10 and PCV13 formulations are likely to offer coverage for serotypes prevalent in India and have been recommended by Indian Academy of Pediatrics (IAP) since 2012 [14]. The Government of India introduced PCV13 in the UIP during 2017 in high incidence state of India, which likely to reduce the incidence of pneumococcal disease in India.

The objective of this study was to determine the clinical applicability of Multiplex real-time PCR tests to detect viral and bacterial meningitis pathogens from CSF samples. In our study, *S.pneumoniae* emerged as the single most prevalent bacterial pathogen that caused meningitis in children followed by Parovirus B19 using multiplex real time PCR panels. These findings are corroborated by data from other Indian studies where *S. pneumoniae* was the most common aetiology in clinically suspected meningitis [15,16].

Serotyping performed on the *S. pneumoniae* positive CSF samples identified five different serotypes. The predominant pneumococcal serotypes identified in this study included 6B, 19F, 1 and 14 and 95% of these serotypes are covered by PCV-13. This finding is in agreement with the profile of pneumococcal serotypes recently published from India [16]. One *H.influenzae type b* (0.8%) and One Meningococci (0.8%) was also seen among CSF samples albeit at a low level when compared to the previous reports [17].

The decrease in the incidence of *H. influenzae type b* with concomitant increase in pneumococcal infections can possibly reflect the impact of the addition of Hib vaccine as part of the Pentavalent vaccine in the UIP from 2012 in India. Immunization of young children might not only have conferred protection against invasive H. influenzae type b disease but could also have indirectly provided protection to unvaccinated individuals through interruption of disease transmission thereby reducing risk of acquiring Hib infection [18,19]. There is likelihood for a similar shift in levels of pneumococcal disease in the post-PCV introduction scenario.

Among all patients, parvovirus B19 mono-infection was detected in 10 (8.3%) cases and mixed infection with *S.pneumoniae* was detected in 3 cases, suggesting that B19V could be involved in the disease etiology. In addition, in the literature, B19V mono-infection in a case of neurological disorders has been considered as a causative agent [20,21]. In India, Parovirus B19 is reported in non-traumatic arthropathy group, ESRD patients, febrile illness, children with aplastic anaemia/ leukaemia and chronic haematological disorders [22]. There are no reports from India reporting B19V in neurological disorders.

Human parvovirus B19 (B19V) is a member of Parvoviridae, a family of small (~25 nm) non-enveloped viruses containing a linear single stranded DNA (ssDNA) genome of 5 to 6 kb [23]. These parvoviruses are considered as human pathogens and are associated with a wide range of pathologies. B19V causes fifth disease, persistent anaemia, transient aplastic crisis, hydrops fetalis, and arthropathy [24]. B19V infection has been associated with various neurological complications such as encephalitis, meningitis, stroke, neuropathy, status epilepticus, and encephalopathy. While B19V infection is more frequently found among immunocompromised hosts, it can occur in the immunocompetent, apparently healthy children and adults as well [25,26].

Infections by herpes viruses (Varicella zoster virus [VZV]) are frequent in humans, and such viruses tend to persist within cranial nerves, dorsal roots, and autonomic ganglia causing latent infections by virtue of reactivation [27]. VZV infection of the central nervous system (CNS) such as encephalitis, meningitis, myelitis, or vasculitis occurs rarely but is feared because of the numerous unfavourable outcomes of such presentations. Meningitis is a rare complication of VZV infection and very few cases have been described in India so far [28, 29]. Our study demonstrated the presence of VZV in 2.5% of the cases. Mono-infection of VZV was detected in 2 cases and interestingly was detected as coinfection with *N.meningitidis* and *S.pneumoniae* in one case. All cases are from Northern part of India. This finding is comparable to results other Indian studies where they have prevalence of 0.1% [30] 3% [31]; 3.3% [32] 4.4% [33,34].

We report an unusual case of PCR positive case of pneumococcal and meningococcal mixed meningitis in a 5-month-old boy. The patient was recruited in Paediatrics department of medical college in Kanpur with no underlying condition before the onset of the symptoms suggesting meningitis. Varizella-Zoster Virus was also detected in the CSF sample along with other two bacteria. Similar observation was made in studies from Marchandin et al [35], Nejad et al [36] and Downs et al [37]. A review of the literature suggests that 1% of all cases of meningitis are caused by more than one bacterial species. Before 1950 such cases occurred predominantly in children and were caused by combinations of bacteria commonly associated with meningitis. Since 1950 a largely adult population has been affected by mixed bacterial meningitis, with a higher incidence of gram-negative bacillary organisms cultured from the cerebrospinal fluid. Mortality was 26% for cases occurring before 1950 and 63% for those occurring after 1950. Failure to recognize one of the organisms present in the cerebrospinal fluid may result in the initiation of inadequate therapy in as many as 67% of cases [37].

Given the associated risks of excessive antimicrobial exposure, existing diagnostic problems, prolonged hospital stays, costs, and severe morbidity and mortality of undiagnosed and untreated CNS infections, we are in desperate need of rapid and reliable diagnosis of meningitis [38]. Significant recent developments have been made in novel diagnostic platforms for CNS infections, including multiplex PCR assays such as ME panel, FTD panel, 16s ribosomal DNA sequencing, and metagenomic deep sequencing [39]. The appeal of these methods is the ability to assess several pathogens at one time. Data from case reports and case series demonstrates the possible value of these methods for the detection of treatable pathogens [40-42]. These exciting aspects are countered by the high costs of new diagnostic platforms, the local resources needed for research, including technological expertise and support, and the difficulty of interpreting results. It is important that we assess the performance of such tests against gold standard testing and also understand the processes by which these diagnostic methods are used to improve patient care, antimicrobial stewardship, hospital related costs and patient results.

Conclusion

Early diagnosis and treatment of meningitis are very critical for reducing its mortality and morbidity. Early identification of responsible agents with molecular methods, such as PCR, may be possible in patients with suspected meningitis. Significant economic benefits can be achieved by avoiding excessive use of antibiotics and hospitalisation through early identification of microbial agents. Multiplex PCR of CSF samples has proven to be a valuable tool for improving the speed and precision of the diagnosis of bacterial meningitis, even in cases with CSF cytochemical characteristics of acute bacterial meningitis but with negative culture findings.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

1. Defeating meningitis by 2030: baseline situation analysis, 2020
2. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, et al. (2017) Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 17: 873–881. [Crossref]
3. Zunt JR, Kassebaum NJ, Blake N, Glennie L, Wright C, et al. (2018) Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 17: 1061–1082. [Crossref]
4. Modi S, Anand AK (2013) Phenotypic Characterization and Antibiogram of CSF Isolates in Acute Bacterial Meningitis. *J Clin Diagn Res* 7: 2704-2708. [Crossref]
5. Giuliano C, Patel CR, Kale-Pradhan PB (2019) A Guide to Bacterial Culture Identification and Results Interpretation. *P T* 44: 192-200. [Crossref]
6. Gray LD, Fedorko DP (1992) Laboratory diagnosis of bacterial meningitis. *Clin Microbiol Rev* 5: 130-145. [Crossref]
7. Bestetti A, Pierotti C, Terreni M, Zappa A, Vago L, et al. (2001) Comparison of three nucleic acid amplification assays of cerebrospinal fluid for diagnosis of cytomegalovirus encephalitis. *J Clin Microbiol* 39: 1148-1151. [Crossref]
8. Chang SS, Hsieh WH, Liu TS, Lee SH, Wang CH, et al. (2013) Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis - a systemic review and meta-analysis. *PLoS ONE* 8: e62323. [Crossref]
9. Nagaraj G, Ganaie F, Govindan V, Ravikumar KL (2017) Development of PCRSeqTyping-a novel molecular assay for typing of Streptococcus pneumoniae. *Pneumonia (Nathan)* 9: 8. [Crossref]
10. Leung MH, Bryson K, Freystatter K, Pichon B, Edwards G, et al. (2012) Sequetyping: serotyping *Streptococcus pneumoniae* by a single PCR sequencing strategy. *J Clin Microbiol* 50: 2419–2427. [Crossref]
11. Raoot A, Dewan DK, Dubey AP, Seth S (2017) Introduction of new vaccines in State Immunization Schedule – Delhi's experience. *Indian Pediatr* 54: 271-274. [Crossref]
12. Bedi N, Gupta P (2016) Antimicrobial stewardship in pediatrics: An Indian perspective. Indian Pediatr 53: 293-298. [Crossref]
13. Dublish S, Singh P (2018) Bacterial Meningitis: Bugs' Story. *Indian Pediatr* 55: 903-904. [Crossref]
14. Indian Academy of Pediatrics Committee on Immunization (IAPCOI) (2012) Consensus recommendation on immunization and IAP immunization timetable 2012. *Indian Pediatr* 49: 549–564. [Crossref]
15. Jayaraman Y, Veeraraghavan B, Chethrapilly Purushothaman GK, Sukumar B, Kangusamy B, et al. (2018) Burden of bacterial meningitis in India: Preliminary data from a hospital based sentinel surveillance network. *PLoS One* 13: e0197198. [Crossref]
16. Manoharan A, Manchanda V, Balasubramanian S, Lalwani S, Modak M, et al. (2017) Invasive pneumococcal disease in children aged younger than 5 years in India: a surveillance study. *Lancet Infect Dis* 17: 305-312.
17. Chinchankar N, Mane M, Bhave S, Bapat S, Bavdekar A, et al. (2002) Diagnosis and outcome of acute bacterial meningitis in early childhood. *Indian Pediatr* 39: 914–921. [Crossref]
18. Madhi SA (2015) Pneumococcal conjugate vaccine and changing epidemiology of childhood bacterial meningitis. *J Pediatr (Rio J)* 91: 108–110. [Crossref]
19. Stephens DS (2011) Protecting the herd: the remarkable effectiveness of the bacterial meningitis polysaccharide-protein conjugate vaccines in altering transmission dynamics. *Trans Am Clin Climatol Assoc* 122: 115–123. [Crossref]
20. Watanabe T, Kawashima H (2015) Acute encephalitis and encephalopathy associated with human parvovirus B19 infection in children. *World J Clin Pediatrics* 4: 126–134. [Crossref]
21. Barah F, Vallely PJ, Chiswick ML, Cleator GM, Kerr JR (2001) Association of human parvovirus B19 infection with acute meningoencephalitis. *Lancet* 358: 729–730. [Crossref]
22. Vadivel K, Mageshbabu R, Sankar S, Jain A, Perumal V, et al. (2018) Detection of parvovirus B19 in selected high-risk patient groups & their phylogenetic & selection analysis. *Indian J Med Res* 147: 391-399. [Crossref]
23. Qiu J, Söderlund-Venermo M, Young NS (2017) Human parvoviruses. *Clin Microbiol Rev* 30: 43–113. [Crossref]
24. Heegaard ED, Brown KE (2002) Human Parvovirus B19. *Clin Microbiol Rev* 15: 485–505. [Crossref]
25. Douvoyiannis M, Litman N, Goldman DL (2009) Neurologic manifestations associated with parvovirus B19 infection. *Clin Infect Dis* 15: 1713–1723. [Crossref]
26. Barah F, Whiteside S, Batista S, Morris J (2014) Neurological aspects of human parvovirus B19 infection: A systematic review. *Rev Med Virol* 24: 154–168. [Crossref]
27. Gilden DH, Kleinschmidt-DeMasters BK, LaGuardia JJ, Mahalingam R, Cohrs RJ (2000) Neurologic complications of the reactivation of varicella-zoster virus. *N Engl J Med* 342: 635–645.
28. Gupta P, Ranjan R, Agrawal CS, Muralikrishnan K, Dave N, et al. (2016) Meningitis with polymerase chain reaction for varicella zoster positivity in cerebrospinal flid of a young immunocompetent adult. *J Neurosci Rural Pract* 7: 591-593. [Crossref]
29. Carey RAB, Chandiraseharan VK, Jasper A, Sebastian T, Gujjarlamudi C, et al. (2017) Varicella Zoster Virus Infection of the Central Nervous System - 10 Year Experience from a Tertiary Hospital in South India. *Ann Indian Acad Neurol* 20: 149-152. [Crossref]
30. Joshi R, Mishra PK, Joshi D, Santhosh S, Parida MM, et al. (2013) Clinical presentation, etiology, and survival in adult acute encephalitis syndrome in rural Central India. *Clin Neurol Neurosurg* 115: 1753-1761. [Crossref]
31. Beig FK, Malik A, Rizvi M, Acharya D, Khare S (2010) Etiology and clinic epidemiological profile of acute viral encephalitis in children of western Uttar Pradesh, India. *Int J Infect Dis* 14: e141-146. [Crossref]
32. Rathore SK, Dwibedi B, Kar SK, Dixit S, Sabat J, et al. (2014) Viral aetiology and clinico-epidemiological features of acute encephalitis syndrome in eastern India. *Epidemiol Infect* 142: 2514-2521. [Crossref]

33. Jain P, Jain A, Kumar A, Prakash S, Khan DN, et al. (2014) Epidemiology and etiology of acute encephalitis syndrome in North India. *Jpn J Infect Dis* 67: 197-203. [Crossref]

34. Gunathilake KMD (2017) Prevalence of Varizella-Zoster Virus Infection among Encephalitis Study Groups World Wide. *Ann Clin Pathol* 5: 1118.

35. Marchandin H, Ventura V, Alonso JM, Van de Perre P (2005) Mixed bacterial meningitis due to Streptococcus pneumoniae and Neisseria meningitidis in an 18-month-old child. *J Clin Microbiol* 43: 1477-1479. [Crossref]

36. Mohammad Nejad AA, Shayestehpour M, Romani B, Namin BM, Vasmehjani AA, et al. (2018) A Rare Case of Pneumococcal and Meningococcal Mixed Meningitis in an Infant with Cardiac Dysfunction. *Arch Clin Infect Dis* 13: e65270.

37. Downs NJ, Hodges GR, Taylor SA (1987) Mixed Bacterial Meningitis. Reviews of Infectious Diseases, 9(4), 693-703. Retrieved November 16, 2020.

38. Radmard S, Reid S, Ciryam P, Boubour A, Ho N, et al. (2019) Clinical Utilization of the FilmArray Meningitis/Encephalitis (ME) Multiplex Polymerase Chain Reaction (PCR) Assay. *Front Neurol* 10: 281. [Crossref]

39. Tyler KL (2018) What's next (Generation) for the diagnosis of chronic meningitis? *JAMA Neurol* 75: 915–917. [Crossref]

40. Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, et al. (2014) Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med* 370: 2408–2417. [Crossref]

41. Naccache SN, Peggs KS, Mattes FM, Phadke R, Garson JA, et al. (2015) Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. *Clin Infect Dis* 60: 919–923. [Crossref]

42. He T, Kaplan S, Kamboj M, Tang YW (2016) Laboratory diagnosis of central nervous system infection. *Curr Infect Dis Rep* 18: 35. [Crossref]