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RESEARCH ARTICLE

# Efficiency of Real-Time PCR in the Diagnosis of Community-Acquired Bacterial Meningitis in Children

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## ABSTRACT

**Objectives:** Objectives: Community-acquired bacterial meningitis (CABM) is a life-threatening condition and remains a public health concern despite various efforts to prevent it. This study aimed to detect the bacteria causing CABM in children by Real-Time PCR.

**Methods:** In total, 178 Cerebrospinal fluid (CSF) samples from suspected meningitis cases were collected and subjected to cell count, biochemical, microbiological, and molecular analysis. Bacteria grown on blood and chocolate agar were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). DNA from CSF was extracted and used to detect bacteria by Real-Time PCR using TaqMan Probe.

**Results:** Fifty (28.1%) patients were diagnosed with confirmed meningitis. Of them, 46 (25.8%) were Real-Time PCR, and four (2.3%) were culture and Real-Time PCR positive. Out of 50 bacteria detected, S. pneumoniae (n=35, 19.7%) was the leading causative bacteria and was followed by H. influenzae (seven, 3.9%), E. coli (five, 2.8%), S. agalactiae (two, 1.1%), and N. meningitidis (one, 0.6%). Most of the S. pneumoniae (18 isolates, 51.4%) were isolated from 3-24 months of children, and in neonates, E. coli was the predominant bacteria. When CSF culture was the gold standard for diagnosis, the sensitivity and specificity of Real-Time PCR for S. pneumoniae were 100% (95%CI: 15.8-100%) and 81.3% (95%CI: 74.7-86.7%), respectively.

**Conclusion:** Streptococcus pneumoniae remains the leading organism of CABM in children despite vaccination and advancement in diagnosis. Real-time PCR has emerged as a vibrant diagnostic molecular appliance. Hence, Regular surveillance is crucial to curb the burdens and trends of CABM in children. *J Microbiol Infect Dis 2022; 12(2):47-53.*

***Keywords:*** *Streptococcus pneumoniae, Community-acquired bacterial meningitis, CSF, Real-Time PCR*

## INTRODUCTION

Meningitis is an infectious disease caused due to inflammation of the meninges in the brain, spinal cord, and subarachnoid space. Meningeal inflammation is responsible for the meningitis symptoms, i.e., headache, fever, neck rigidity, and pleocytosis in CSF [1]. Despite advances in treatment and vaccination, community-acquired bacterial meningitis (CABM) remains one of the most important infectious diseases worldwide [2,3]. Similarly, CABM is, adding an immense burden to the health system. It continues to be one of the crucial factors responsible for significant morbidity and mortality in neonates and children [3]. Due to CABM, 180,000 deaths occur per year in the pediatric population [4-7]. Hence, prompt diagnosis and

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treatment are crucial to overcoming the severe consequences.

Organisms implicated in CNS infections are geographically diverse, extensive, and variable. In neonates, *Escherichia coli, Streptococcus agalactiae*, and *Listeria monocytogenes* are the most common bacteria, while in children and adults, *Streptococcus pneumoniae* and Neisseria meningitidis are the predominant organisms [8,9]. *Haemophilus influenzae* causes meningitis in all age groups and is dominant in children below five years of age [10,11]. Geographically, the well-defined meningitis belt of the world is in Sub-Saharan Africa that stretching from Senegal in the East to Ethiopia in the West, which experiences a high rate of endemics of meningitis. *Neisseria meningitidis* was the primary organism in that region. However, the occurrence of the organism is changing due to pulse vaccination [12]. Hence, spectra of causative agents of meningitis should be regularly followed and updated throughout the world.

Diagnosis of CABM is confirmed by various laboratory tests. The culture of CSF is considered the 'Gold Standard'. However, due to the intake of antibiotics before CSF collection, organisms are sparsely detected by it. Nowadays, molecular diagnostic test, especially Real-Time PCR, is getting due attention and gaining momentum for diagnosis because of their potential to detect genetic material even from nonviable organism [13]. Therefore, this study was designed and conducted to find the incidence of the organism responsible for community-acquired bacterial meningitis in children by using Real-Time PCR.

## METHODS

A prospective study was conducted at the Department of Microbiology, All India Institute of Medical Sciences, New Delhi. Four hundred CSF samples were collected from suspected meningitis cases from March 2015 to January 2019. Of the total CSF samples, 178 were from children under five years. Patient details were collected from ward medical records. The patients were excluded from the study when they were suggestive of tubercular/viral/fungal meningitis based on the history of disease and clinical, radiological, or microbiological examinations. CSF samples were processed for cell count, biochemical tests, and microbiological and Molecular (Real-Time PCR) analysis. Hospital-acquired infection was defined as a positive bacterial infection that was not present at hospital admission or clinical evidence of infection no sooner than 48 hours after entry. Apart from that, the patient was considered to have a community-acquired infection.

### Gram staining and culture

CSF samples were centrifuged at 10,000 rpm for 10 minutes; sediments were used for Gram staining and culture. Blood, chocolate, and Mac Conkey Agar were inoculated with sediment of CSF as well as a part of CSF was added with BHI-broth for the enrichment and incubated at 37 $^{0}$C for 24-48 hours with 5% $CO_2$. Subculture was carried out from BHI-broth in case no growth was observed on the plates inoculated with sediment of CSF. Culture plates were monitored daily to look for the growth of bacteria. The bacteria grown on plates were identified by MALDI-TOF (Matrix-assisted laser desorption/ Ionization Time of flight (Biomerieux, France). Isolates were further subjected to an antibiotic susceptibility test.

### Identification of the isolates by MALDI-TOF

MALDI-TOF made identification of bacterial isolates; for this, bacteria were taken with a wooden stick from the isolated colony grown on a culture plate, and the smear was made on a MALDI-TOF slide; in addition, 0.50 µl matrix (α-cyano- 4 hydroxycinnamic acid) was put on the smear then slide was kept at room temperature for 5 minutes to dry. Finally, the slide was placed in the MALDI-TOF machine (VITEK MS Biomerieux, France) for acquisition and identification.

### Extraction of DNA from CSF

DNA was extracted from CSF with QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following manufacturer protocol. DNA was eluted in 100 µl Qiagen elution buffer and stored at -20 $^{0}$C for further use. Similarly, DNA was also extracted from ATCC strains of *S. pneumoniae* (49619), *H. influenzae*e (33391), *N. meningitidis* (13090), and *Group B Streptococcus* (12403) & *E. coli* (25922). DNA extracted from ATCC strains was quantified by mass spectrophotometer (Nanodrop, TECAN, Switzerland) and was used for determining the lower limit of detection and as the positive control.

### Determination of lower limit of detection (LLD)

The lower detection limit was determined using DNA extracted from ATCC strains of *S. pneumoniae*, *H. influenzae*e, *N. meningitidis*, *S. agalactiae*, and *E. coli*. Similarly, the concentration of DNA has adjusted to 100 ng/ml, from which ten-fold serial dilution of genomic DNA was prepared in autoclaved milliq water and amplified by Step One Plus Real-Time PCR. The lower limit of detection was calculated using formulae: Genome copies/µl = concentration (ng/µl) × 6.02 × $10^{23}$× $10^{-9}$/660× whole-genome nucleotide number [14]. The lower limit of detection of each bacteria is given in table 3.

### Testing of CSF sample by Real-Time PCR

The ply gene of *S. pneumoniae*, ctrA gene of *N. meningitidis*, bexA gene of *H. influenzae*e [15], sip gene of *S. agalactiae* [16], and 16SrRNA of *E. coli* [14] were used as species-specific targets, positive result was indicated by an exponential increase in fluorescence in separate reaction along with amplification. The primers and probes used are listed in table 1. The assay was performed in 25 µl reaction volume with 0.45 µl (50 µM) of each primer, 0.625 µl (10 µM) probe, and two µl DNA extracted from CSF and TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA) as per manufacturer instructions. DNA amplification was done in Step One Plus Real-Time PCR system (Applied Biosystems, USA) following temperature program: 50 $^0$C for 2 minutes, 95 $^0$C for 10 minutes, followed by 40 cycles of 95 $^0$C for 15 seconds, and 60 $^0$C for 1 minute. The instrument software analyzed amplification data. Specimen were considered positive, with a cycle threshold (CT) value of <35, and negative with CT values >35 or no amplification. Nuclease-free water was included as a negative control. Similarly, DNA extracted from the ATCC strain was used for positive control.

### Statistical Analysis

Statistical analysis of these comparisons performed was done by Wilcoxon Singed–rank test. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using Medcalc diagnostic test.

## RESULTS

A total of 400 cases of suspected bacterial meningitis were included in the study of which 178 (44.5%) were children (Figure 1). Of the total 178, 127 (71.4%) were male and 51(28.7%) were female. The cardinal clinical features were observed as fever in 173 cases (97.2%) followed by altered sensorium in 131 (73.6%) and headache in 97 (54.5%) (Table 2). The mean value of CSF glucose and protein was 47.8 ± 22.5 and 123.6 ± 81.2, respectively. An increased number of Polymorph nuclear leukocytes, maximum 2-3 PMN/100 high power field was observed in 3 (21.9%) CSF.

The mean age of the patients was 1.53 ± 2.52 years (ranging from 1 day and 5 years). Out of a total of 178 CSF, 50 (28.1%) were positive by Real-Time PCR of which 46 samples (25.9%) were only positive by Real-Time PCR and four samples (2.3%) were positive by culture as well as RT-PCR. Of the total positive samples, 35 (70.0%) were *S. pneumoniae* followed by *H. influenzae*e (seven, 14.0%), and (five, 10.0%) *E. coli*. Similarly, the majority of *S. pneumoniae* (18, 51.4%) was observed in children from three to 24 months. In neonates, *E. coli* (three, 6%), and *S. agalactiae* (two, 4.0%) were observed, details are given in Figure 2.

The lower limit of detection (LLD) of different bacteria causing CABM was determined by Real-Time PCR. LLD of *E. coli* was 20 fg and had the least genome copies/µl i.e. 3 followed by *N. meningitidis* i.e. 8 copies/µl. LLD and genome copies/µl is depicted in Table 3. The number of whole-genome nucleotides: *E. coli*-5498450-bp, *S. pneumoniae*-2046115-bp, *S. agalactiae*-2160267-bp, *H. influenzae*e-1830138-bp, and *N. meningitidis*-2194961-bp.

The sensitivity, specificity, positive predictive value, and negative predictive value of Real-Time PCR were calculated by Medcalc diagnostic test-taking culture as 'Gold Standard' for the diagnosis. However, only *E. coli* and *S. pneumoniae* were detected by culture. Sensitivity and specificity of *E. coli* was 100% (95% CI: 15.81-100%) and 98.30% (95%CI: 95.10-99.65%) respectively. Similarly, for *S. pneumoniae*, it was found 100% (95%CI: 15.81-100%) and 81.25% (95%CI: 74.69-86.73%) (Table 4).

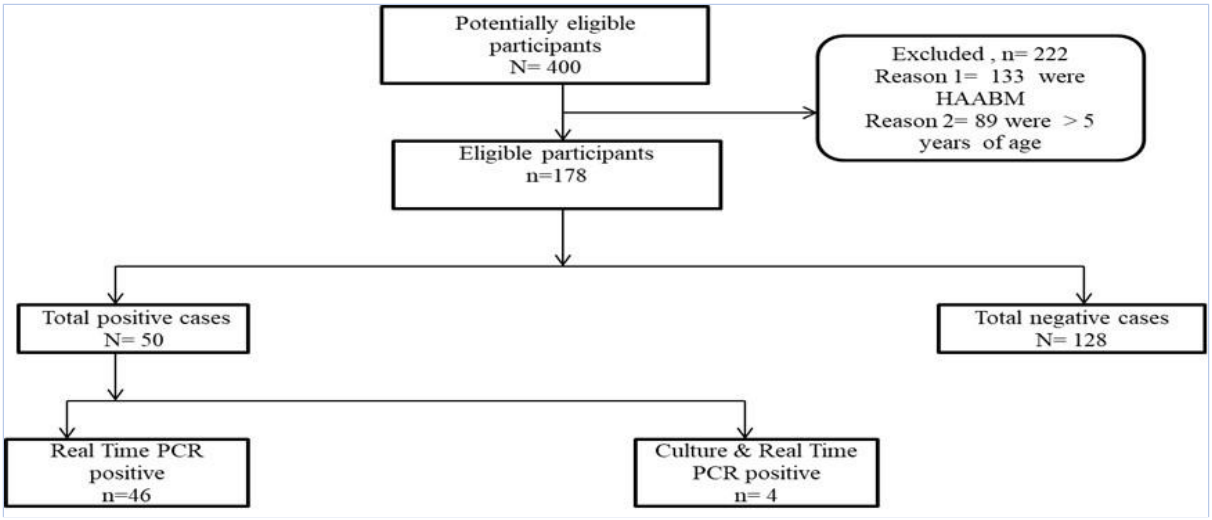


Figure1 Flow diagram of enrolled cases of suspected meningitis and positive cases.

Table1 Primers and Probes used for Real-time PCR

| Organisms | Target gene | Primers and Probes Sequences | References |
|---|---|---|---|
| *S. pneumoniae* | ply forward | 5'-TGCAGAGCGTCCTTTGGTCTAT-3' | 15 |
| | ply reverse | 5'-CTCTTACTCGTGGTTTCCAACTTGA-3' | |
| | ply probe | 5'-VIC-TGGCGCCCATAAGCAACACTCGAA-3'-TAMRA | |
| *N. meningitidis* | ctrA forward | 5'-GCTGCGGTAGGTGGTTCAA-3' | |
| | ctrA reverse | 5'-TTGTCGCGGATTTGCAACTA-3' | |
| | ctrA probe | 5'-FAM-CATTGCCACGTGTCAGCTGCACAT-3'-TAMRA | |
| *H. influenzaee* | bexA forward | 5'-GGCGAAATGGTGCTGGTAA-3' | |
| | bexA reverse | 5'-GGCCAAGAGATACTCATAGAACGTT-3' | |
| | bexA probe | 5'TET-CACCACTCATCAAACGAATGAGCGTGG-3' TAMRA | |
| *S. agalactiae* | sip forward | 5'-ATCCTGAGACAACACTGACA-3' | 16 |
| | sip reverse | 5'-TTGCTGGTGTTTCTATTTTCA-3' | |
| | sip probe | 5'-FAM-ATCAGAAGAGTCATACTGCCACTTC-3'-TAMRA | |
| *E. coli* | 16SrRNA forward | 5'-GGGAGTAAAGTTAATACCTTTGC-3' | 14 |
| | 16SrRNA reverse | 5'-CTCAAGCTTGCCAGTATCAG-3' | |
| | 16SrRNA probe | 5'-FAM- CGCGATCACTCCGTGCCAGCAGCCGCGGATCGCG-3'-QSY7 | |

## DISCUSSION

Globally, Community-acquired bacterial meningitis remains a significant cause of morbidity and mortality among children under five despite the advances in diagnosis, treatment, and vaccination. In this study, a total of 178 CSF samples were collected from the children suspected of meningitis. The main presenting complaints observed in children were fever (97.19%) and altered sensorium (73.59%). Cellular analyses of CSF showed an increased cell count in 21.91% of the cases. An Increased protein and decreased glucose levels were observed in 82.02% of the CSF samples.

In the study, causative agents of community-acquired bacterial meningitis were detected using Real-Time PCR and culture methods.

The culture positivity rate of the total CSF processed samples was very low (2.24%). On the other hand, these samples were also found positive by Real-Time PCR. A similar finding was reported by Bonab ZH et al. [17], who had shown culture positivity of 2.5%. The culture positivity rate in CSF decreases by 60-70% in

cases of CABM when collected from the patients who have already been taken antibiotics. Low culture positivity was due to the fastidious nature of the organisms and to the intake of antibiotics by the patients before attending tertiary care centers [18]. However, in our study, the positivity rate was 28.1% by Real-Time PCR, having 70% *S. pneumoniae*, 14.0% *H. influenzae*, and 0.6% *N. meningitidis*. Our findings were compatible with Jayaraman's study from India [19]. They had shown positivity of *S. pneumoniae* in 82.9% and *H. influenzae* in 14.4%, respectively. However, *S. pneumoniae* positivity is low in this study, possibly due to the study's difference in sample size and geography.

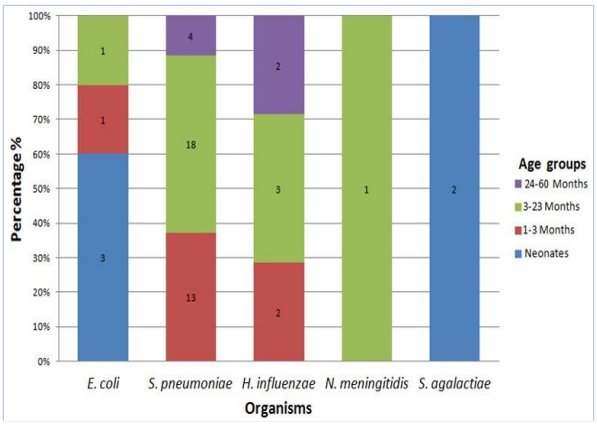


Figure 2 Showing distribution of organisms in different age groups

Table 2. Demographic details of meningitis patients.

| Clinical Features | Number of patients |
|---|---|
| Fever | 173 (97.19%) |
| Headache | 97 (54.49%) |
| Nuchal rigidity | 36 (20.22%) |
| Altered sensorium | 131 (73.59%) |
| Nausea / Vomiting | 41 (23.03%) |
| Kernig's and Brudzinski's sign | 32 (17.97%) |
| Bulging anterior fontanelle | 15 (8.42%) |

Table 3. The lower limit of detection of different bacteria.

| Organisms | Strain Id number | Target gene | Detection limit | Genome copies/µl |
|---|---|---|---|---|
| *E. coli* | ATCC 25922 | 16S rRNA | 20 fg | 03 |
| *S. pneumoniae* | ATCC 49619 | ply | 100 fg | 45 |
| *S. agalactiae* | ATCC 12403 | sip | 20 fg | 09 |
| *H. influenzae* | ATCC 33391 | bexA | 200 fg | 99 |
| *N. meningitidis* | ATCC 13090 | ctrA | 20 fg | 08 |

The number of whole-genome nucleotides: E. coli-5498450-bp, S. pneumoniae-2046115-bp, S. agalactiae-2160267-bp, H. influenzae-1830138-bp, and N. meningitidis-2194961-bp.

Table 4 Sensitivity and specificity of Real-Time PCR compared with culture.

| Statistics | *E. coli* (N=5,C=2 & RTPCR=3) | | *S. pneumoniae* (N=35, C=2 & RTPCR=33) | |
|---|---|---|---|---|
| | Value | 95% CI | Value | 95% CI |
| Sensitivity | 100% | 15.81-100% | 100% | 15.81-100% |
| Specificity | 98.30% | 95.10-99.65% | 81.25% | 74.69-86.73% |
| PPV | 40.00% | 17.84-67.18% | 5.71% | 4.27-7.62% |
| NPP | 100% | - | 100% | - |
| Accuracy | 98.37% | 95.15-99.65% | 81.46% | 74.96-86.66% |

N=Total number, C=culture & RTPCR= Real Time PCR

In the present study, out of the bacteria detected, 86.2% were *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*, which are the most common bacterial causes of CABM in children. This finding was in accordance with Wu's study that had shown 25.1% positivity of these pathogens [20]. Similarly, 51.2% of the total positive results were detected in 3-24 months children in our study. Compatible findings were reported from India and other countries [19, 21-23]. Detection of a sample number of bacteria in 3-24 month children

could be related to insufficient immunity status at these ages or not being competent immunity enough to fight with these pathogens that invades CNS. In addition, *S. pneumoniae* was found as the major bacteria in children below 5 years of age followed by *H .influenzae*. A similar finding had also been reported in India [19, 24]. The increased incidence of *S. pneumoniae* and decrease in the incidence of *H. influenzae*e type b might be the impact of the addition of the Hib vaccine as a part of the pentavalent vaccine in the national immunization program in India in 2012.

In the current study, only one (0.56%) *N. meningitidis* was detected; however, in India, six hospitals based study had reported 1.5% meningococcal meningitis [25]. Incidence of sporadic meningococcal disease in India is low compared to *S. pneumoniae*, and *H. influenzae*e type b as epidemics of *N. meningitidis* occur particularly in North India after 15-20 years of the interval [26]. Likewise, *E. coli* 3 (6.0%) was the leading organism in neonates, followed by *S. agalactiae*. A concurrent finding was reported by L. Ouchenir et al. [27]. Nowadays, the detection rate of *S. agalactiae* is reducing in neonates due to proper screening and prevention during the gestational period.

In this study, to detect the cut-off value of Real-Time PCR for different bacterial agents of CABM, the lower limit of detection (LLD) was determined using DNA extracted from ATCC strains, and bacterial genome copies/µl was calculated [14]. The minimum number of genome copies/µl was observed in *E. coli*, i.e., three copies/µl, followed by eight copies/µl in *N. meningitidis*. For a result to be positive by Real-Time PCR requires a low bacteria count. It has to have a high sensitivity of 100% and specificity of 81.25%-98.30% (Table 4) compared to the culture for the diagnosis of bacterial meningitis. In the current study, 23.6% more bacteria were detected by Real-Time PCR as compared to culture as well as most of the bacteria causing CABM in children were detected. However, only *S. pneumoniae* and *E. coli* were isolated by culture, 2.25%. Hence, more positivity by Real-Time PCR advocates its effectiveness in the CABM diagnosis in children.

## CONCLUSION

Globally, continuing efforts have been made to curb bacterial meningitis and its severe consequences in children. However, Streptococcus pneumoniae remains the leading organism of community-acquired bacterial meningitis. In addition, detecting *H. influenzae* and other bacteria and *S. pneumoniae* by Real-Time PCR decipher the incidence of strata of bacteria causing CABM and its profundity in children. Real-time PCR has emerged as an effective and crucial molecular appliance for CABM diagnosis as the bacterial detection rate has been enhanced by almost 23.6% by Real-Time PCR. Hence, Real-Time PCR should be implemented for regular surveillance and diagnosis that is empirical for accessing the burdens and trends of CABM in children.

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**Conflicts of interest:** The authors declare no potential conflict of interest relating to regarding article's research, authorship, and publication.

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## REFERENCES

1. Van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M. Clinical features and prognostic factors in adults with bacterial meningitis. N Engl J Med 2004; 351(28):1849-59.

2. Bijlsma MW, Brouwer MC, Kasanmoentalib ES. Community-acquired bacterial meningitis in adults in the Netherlands, 2006–14: a prospective cohort study. Lancet Infect Dis 2016; 16 (3): 339–347.

3. McIntyre PB, O'Brien KL, Greenwood B, Van de Beek D: Effect of vaccines on bacterial meningitis worldwide. Lancet 2012; 380(9854):1703-1711.

4. Klinger G, Chin CN, Beyene J, Perlman M: Predicting the outcome of neonatal bacterial meningitis. Pediatrics 2000;106(3): 477–482.

5. Stevens JP, Eames M, Kent A, Halkets S, Holt D, Harvey D. Long-term outcome of neonatal meningitis. Arch Dis Child Fetal Neonatal Ed 2003; 88(3): F179-184.

6. Chang CJ, Chang WN, Huang LT. Bacterial meningitis in infants: the epidemiology, clinical features, and prognostic factors. Brain Dev 2004; 26(3): 168-175.

7. De Louvois J, Halket S, Harvey D. Neonatal meningitis in England and Wales: sequelae at 5 years of age. Eur J Pediatr 2005;164(12): 730-734.

8. Brouwer MC, Tunkel AR, Van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clinical microbiology reviews 2010; 23 (3):467- 92.

9. Centres for Disease Control and Prevention (CDC) (2017) Bacterial Meningitis [updated January 25, 2017].

10. World Health Organization (WHO). Haemophilus influenzae type b (Hib) Vaccination Position Paper-July 2013. Releve epidemiologi quehebdomadaire.2013; 88(39): 413-426.

11. Hamborsky J, Kroger A, Wolfe C, editors. Centers for Disease Control and Prevention: Epidemiology and Prevention of Vaccine-Preventable Diseases. 13th ed Washington D.C.: Public Health Foundation; 2015.

12. Diallo AO, Soeters HM, Yameogo I, Sawadogo G, Ake F, Lingani C. Bacterial Meningitis epidemiology and return of Neisseria meningitidis serogroup A cases in Burkina Faso in the five years following MenAfriVac mass vaccination campaign. PLoS One 2017; 12(11): e0187466.

13. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis 2004; 4(6):337-348.

14. Wang Y, Guo G, Wang H, et al. Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis. BMC Pediatrics 2014;14: 224.

15. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol 2001; 39(4): 1553-1558.

16. Bergh K, Stoelhaug A, Loeseth K, Bevanger L. Detection of group B streptococci (GBS) in vaginal swabs using real-time PCR with TaqMan probe hybridization. Indian J Med Res 2004; 119 (4): 221-223.

17. Bonab ZH, Farajania S, Ghostasloy R, Nikkah E. Evaluation of nested PCR method for diagnosis of meningitis due to Neisseria meningitidis and Haemophilus influenzae. Turk J Biol 2012; 36:727-31.

18. Nigrovic LE, Malley R, Macias CG, et al. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. Pediatrics 2008; 122 (4):726–30.

19. Jayaraman Y, Veeraraghavan B, Chethrapilly Purushothaman GK, Sukumar B, Kangusamy B, Nair KA. Burden of bacterial meningitis in India: Preliminary data from a hospital based sentinel surveillance network. PLoS One 2018; 13(5): e0197198.

20. Wu HM, Cordeiro SM, Harcourt BM, Carvalho M, Azevedo J, Oliveira TQ. Accuracy of real-time PCR, Gram stain and culture for Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae meningitis diagnosis. BMC Infect Dis 2013; 13:26.

21. Khumalo J, Nicol M, Hardie D, Muloiwa R, Mteshana P, Bamford C. Diagnostic Accuracy of two multiplex real-time polymerase chain reaction assays for the diagnosis of meningitis in children in a resource-limited setting. PLoS One 2017;12(3): e0173948.

22. Oordt-Speets AM, Bolijn R, Van H RC, Bhavsar A, Kyaw MH. Global etiology of bacterial meningitis: A systematic review and meta-analysis. PLoS ONE 2018; 13(6): e0198772.

23. Debnath DJ, Wanjpe A, Kakrani V, Singru S. Epidemiological study of acute bacterial meningitis in admitted children below twelve years of age in a tertiary care teaching hospital in Pune, India. Med J DY Patil Univ 2012; 5: 28-30.

24. Manoharan A, Manchanda V, Balasubramanian S, Lalwani S, Modak M, Bai S: Invasive pneumococcal disease in children aged younger than 5 years in India: surveillance study. Lancet Infect Dis 2017; 17(3): 305-312.

25. Kabra SK, Praveen K, Verma IC, Mukherjee D, Chowdhary BH, Sengupta S. Bacterial meningitis in India: An IJP survey. Indian J Pediatr 1991; 58(4):505-11.

26. Sinclair D, Marie-Pierre P, Jacob JT, Brian G: The Epidemiology of meningococcal Disease in India. Trop Med Int Health 2010; 15(12):1421-35.

27. Lynda O, Christian R, Sarah K. The Epidemiology, Management, and Outcomes of Bacterial Meningitis in Infants. Pediatrics 2017; 140(1): e20170476.