Bacteriologic Methods in the diagnosis of Acute Bacterial Meningitis

PREETI SINGH, #SONAL SAXENA AND *ANJU SETH
From the Departments of Pediatrics and #Microbiology, Lady Hardinge Medical College, New Delhi, India.
**anjuseth.peds@gmail.com*

Acute bacterial meningitis continues to be a life-threatening neurological emergency that warrants rapid diagnosis and management to prevent mortality and serious neurological disability. The August 1966 issue of Indian Pediatrics had a Research article on 'Bacteriologic Methods in the diagnosis of Acute Bacterial Meningitis.' Through this communication, we present the changes in epidemiology and advances in the diagnosis of acute bacterial meningitis in last 50 years.

THE PAST

The reported article by Hughes, *et al.* [1] describes the CSF findings of three consecutive cases of acute bacterial meningitis, out of total 653 admissions to the Institute of Child Health during 13-month period from January 1965 through 1966. In this paper, the authors demonstrated the efficiency of improved bacteriologic cultures over routine cultures in isolating the causative agents of acute bacterial meningitis. The special bacteriological procedures were carried out in assistance with Baltimore Biological Laboratory Division (Baltimore, Maryland) and Difco laboratories (Detroit, Michigan). The three children described herein had CSF cell count >1000 cell/mm^3 with predominance of polymorphonuclear cells. Apart from CSF cell count, biochemistry, gram stain and routine cultures (use of sheep blood agar incubated in atmospheric air at 37 ^{0}C for 24h), specialized bacteriological cultures using sheep blood agar in trypticase soy agar base (BA) and chocolate agar (CA) plates were employed for isolating the etiological agents. 'Candle Jar' cultures were performed by placing culture plates or slants in biscuit tin having tight fitting lid which was sealed while the candle was left inside burning. All the cultures were maintained for at least 48 hours prior to being discarded as negative. Selected strains were further typed by agglutination with commercial antiserum.

The CSF from first two patients (age 6 mo and 11 mo) were non-contributory on Gram stain and failed to demonstrate growth on routine blood agar, except for positive satellitism. However, abundant growth was noted on CA after incubation in both atmospheric air and candle jar, and the colonies were characterized as *Haemophilus influenzae*. In Case 2, serological identification was established by agglutination with *H. influenzae* type b. The third case was a 20-day-old boy, and his CSF revealed Gram positive cocci on gram stain but failed to grow on ordinary BA incubated in air at 37^{0}C. The use of CA supported growth more than BA under similar incubation conditions but growth augmentation and appearance of β hemolysis was facilitated by incubation in candle jar. The colonies were moderately sensitive to bacitracin and designated as group A Streptococcus after precipitation in capillary tubes containing group A antiserum.

The reported article discussed the etiologic agents of acute bacterial meningitis from Children's Hospital, Boston (447 cases over a period 1956-60) which revealed *H. influenzae* as the most important bacteriological agent implicated. Indian studies in that era also reported it as the leading cause of non-tuberculous bacterial meningitis across all age groups [2]. It was realized that *H. influenzae* is fastidious organism and its isolation can be facilitated by the use of CA under candle jar conditions. This medium also supported the growth and hemolysis of Group A β-hemolytic Streptococci, *Neisseria meningitides* and some strains of Diplococci pneumoniae (*Streptococcus pneumoniae*). CA cultures incubated in candle jar were recommended as the most economical, convenient and efficient media over routine culture methods used for CSF analysis in cases of acute bacterial meningitis.

Historical Background and past knowledge: The earliest treatise on the association of meningitis with bacterial (meningococcal) infection was by an Austrian

bacteriologist Anton Weichselbaumin 1887, though the first reported outbreak of meningitis occurred much earlier in Geneva in 1805.The use of lumbar puncture for early CSF analysis was introduced by Heinrich Quincke in 1891 [3]. The etiological agents responsible for acute bacterial meningitis received recognition in the late 19th century [3]. In the pre-antibiotic era, bacterial meningitis had a uniformly fatal outcome, until the use of penicillin in 1944 [4].

THE PRESENT

Worldwide, the various epidemiological studies conducted after publication of the reported article have indicated *H. influenzae* type b (Hib), *Streptococcus pneumoniae* and *Neisseria meningitidis* to be the commonest etiological agents of acute bacterial meningitis in children. Till the end of the 20th century, the global burden of meningitis due to *H. influenzae* was huge, and it contributed a large proportion of under-five mortality and morbidity [5]. A multi-centre sentinel surveillance study from India found *H. influenza*e to be the predominant cause (70%) of bacteriologically confirmed meningitis in children under two years of age, while *S. pneumoniae* and group B Streptococcus were identified in 13% and 8% cases, respectively [6]. Introduction of Hib conjugate vaccine has led to virtual elimination of invasive disease due to *H. influenzae* from developed countries and a substantial decline in the incidence of meningitis in developing countries that have adopted it as part of routine immunization program. The widespread use of pneumococcal and meningococcal conjugate vaccines have further influenced the epidemiology of meningitis in developed countries.

Diagnosis of acute bacterial meningitis relies heavily on Gram stain, culture and antigen detection by latex agglutination testing. Culture of CSF continues to remain gold standard for diagnosis of acute bacterial meningitis. The age old candle jar continues to be in use, but has given way to special CO_2 incubators in modern settings. In India, the isolation rates of these pathogens is low as compared to West. Tropical environmental conditions in India probably do not allow the survival of relatively more fragile bacteria like *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, *S. agalactiae and Listeria monocytogenes* over hardy pathogens. The yield of CSF culture is further reduced in patients who have received antibiotic before lumbar puncture, delay in processing of samples or due to storage of these samples in refrigerator [7]. Recently MALDI-TOF MS (Matrix assisted laser desorption ionization- time of flight mass spectrometry) has emerged as a rapid automated, low cost and reliable tool based on mass spectroscopy for microbial identification and detection of antibiotic resistance [8].

In recent times, molecular diagnostics have drastically reduced the turnaround time for diagnosis. Nucleic acid detection techniques such as PCR can detect bacteria in culture negative patients. Broad range PCR and multiplex PCR have shown high sensitivity and specificity for detection of *H. influenzae, N. meningitides,* Group B Streptococci *and S. pneumoniae* [9]. Recently FDA has approved a fully automated DNA extraction and amplification system which has drastically reduced turnaround time for detection of DNA to just one hour. This system requires 200 µL of CSF to test for a multiplex panel of six bacteria (*H. influenzae, N. meningitides, Group B Streptococci, S. pneumoniae, Listeria monocytogenes* and *E coli* K1), eight viruses and two fungi [10]. However, their utilization in routine diagnostic practice is hindered by the high running cost. Overall, etiological diagnosis of acute bacterial meningitis still remains a challenge, especially in low- and middle-income countries.

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