Original Article

Diagnostic value of latex agglutination test in diagnosis of acute bacterial meningitis

Syeda Fasiha Mohammadi, Asha B. Patil, Shobha D. Nadagir, Namrata Nandihal, S. A. Lakshminarayana[1]

Departments of Microbiology, Karnataka Institute of Medical Sciences, Hubli, Karnataka, [1]Microbiology, Sree Gokulam Medical College, Kerala, India

Abstract

Objectives: To know the incidence of bacterial meningitis in children below five years of age. To compare conventional culture and antigen detection methods (Latex agglutination test). **Materials and Methods:** 100 CSF samples of clinically suspected meningitis cases in children below 5 years of age were included. The samples were subjected to cell count, Gram stain, culture and LAT. The organisms isolated in the study were characterized according to standard procedures. **Results:** Of the 100 cases studied, 31 cases were diagnosed as ABM by Gram stain, culture and latex agglutination test as per WHO criteria. The hospital frequency of ABM was 1.7%. 15 (48.38) cases were culture positive. Gram stain was positive in 22(70.96) cases and LAT in 17(54.83) cases. Haemophilus influenzae was the most common causative agent of acute bacterial meningitis followed by S.pneumoniae. Case fatality rate was 45.16%.The sensitivity and specificity of LAT was 66.66% and 87.91% respectively. **Conclusion**: Bacterial meningitis is a medical emergency and early diagnosis and treatment is life saving and reduces chronic morbidity. LAT was more sensitive compared to conventional Gram stain and Culture technique in identifying the fastidious organisms like H.influenzae, S.pneumoniae and Group B Streptococcus. However, the combination of Gram stain, Culture and LAT proved to be more productive than any of the single tests alone.

Key Words

Acute bacterial meningitis, culture, latex agglutination

For correspondence:
Dr. Asha B. Patil, Department of Microbiology, Karnataka Institute of Medical Sciences, Vidyanagar, Hubli - 580 021, Karnataka, India. E-mail: drashapatil@rediffmail.com

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Introduction

Bacterial meningitis is an important cause of childhood death and neurological sequelae in countries with limited resources.[1] The incidence of this disease varies widely in different parts of the world ranging approximately 3.0/100,000 population in the United States to as much as 45.8/100,000 population in Northeastern Brazil.[2] The community incidence of acute bacterial meningitis (ABM) in India varies from 0.5% to 2.6%.[3,4]

Clinical diagnosis of meningitis is difficult as its early clinical manifestations are often not specific, especially in babies and small children. On the other hand, even if it is recognized early and prompt treatment is given, the mortality rate is still high ~30% in children and 20-30% in neonates.[5]

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The production of clinically useful bacteriological report depends on the time it takes for the organism to grow under test and may result in a delay of 18 hours or longer in case of culture. Even after such a delay, cultures may fail to yield growth because of the previous antimicrobial treatment.[6] Hence, tests with shorter turnaround time and good sensitivity and specificity are important for early and accurate diagnosis. In recent years, there is considerable interest in rapid antigen detection tests that provide rapid species identification.

This study is carried out to establish the diagnostic value of latex agglutination (LAT) test for the rapid specific etiological diagnosis of bacterial meningitis.

Materials and Methods

This prospective study was carried out during the period of December 2008 to November 2009. A total of 100 clinically suspected cases of ABM in children below 5 years of age constituted the study group.

Inclusion criteria

Children below 5 years of age who were admitted with the clinical suspicion of ABM.

Exclusion criteria
Children developing meningitis following head trauma or neurosurgical procedure.

Sample collection
Cerebrospinal fluid (CSF) samples were collected prior to administration of antibiotics whenever possible. About 1-2 ml of CSF was collected in a sterile container, by lumbar puncture carried out with all aseptic precautions. Samples were processed within half an hour to 1 hour.

Processing of sample
Macroscopic appearance of CSF was observed. CSF was aliquoted into two sterile test tubes. One of the tubes was centrifuged at 1500-3000 ×*g* for 20 minutes and the sediment was used to inoculate culture media first and then direct smear was made for examination by gramgram-stain to prevent contamination. The supernatant was used for bacterial antigen detection. The other tube was used for cell count.[7]

Culture and identification
The sediment of the centrifuged CSF was inoculated on to chocolate agar plate incubated at 37°C in 5-10% CO_2 and Mac Conkey agar and BHI broth.[7]

Inoculated primary plates were incubated for 48-72 hours. BHI broth was incubated for 7 days and examined daily for the presence of growth or turbidity and was considered negative at the end of 7 days of incubation. Tubes showing turbidity were subcultured on chocolate agar and Mac Conkey agar. Tubes that remained non-turbid were subcultured on the 7th day before discarding. The isolates were identified as per standard techniques.[7]

LAT test
CSF samples were tested for bacterial antigen detection by using the bacterial antigen kit (BD Directigen Meningitis Combo Test). It is a LAT test to detect antigens of 5 organisms: *Escherichia coli* K1 antigen, *Neisseria meningitidis* ABCY or W 135 antigens, *Streptococcus pneumoniae* antigen, Group B *streptococcus* (GBS) antigen and *Haemophillus influenzae* type B (Hib) antigen

Meningococcus group B antigen being structurally and immunologically related to *E. coli* K1[6] antigen is provided as a single test latex reagent and depending on the age of the child, a positive reaction in a neonatal specimen would suggest *E. coli* K1 infection and in older children *meningococcus* group B is a more likely infection.

Statistical analysis
The results were analyzed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and were calculated as per standard statistical methods using the SPSS software 17th version.

Results

As per World Health Organization (WHO) criteria,[8] 31(31%) cases were laboratory confirmed as cases of ABM [Table 1]. CSF cell count was most commonly in the range of 51-500 cells/cumm, i.e., in 15 (48.38%) cases, 2 (6.45%) cases had a cell count of >1000 cells/cumm. Neutrophils were seen predominantly in 20 (64.5%) of cases.

Table 1: Laboratory confirmed cases of ABM as per WHO criteria (*n*=31)

Tests	No. of cases positive (%)
Gram stain+LAT+culture	3 (9.67)
Gram stain+LAT	9 (29.03)
Gram stain+culture	5 (16.12)
Culture+LAT	3 (9.67)
Only Gram-stain	5 (16.12)
Only culture	4 (12.90)
Only LAT	2 (6.45)
Total	31

ABM=Acute bacterial meningitis, LAT=Latex agglutination test, WHO=World health organization

Table 2: Etiological agents identified in CSF by various methods (*n*=31)

Organisms identified	Total (%)	Gram-stain positive (%)	Culture positive (%)	LAT positive (%)
H. influenza	9 (29.03)	7 (77.77)	3 (33.33)	8 (88.88)
S. pneumonia	7 (22.58)	5 (71.42)	4 (57.14)	7 (100)
GBS	2 (6.4)	1 (50)	0	2 (100)
Other GNB	8 (25.80)	4 (50)	8 (100)	0
Species unidentified	5 (16.1)	5 (100)	0	0
Total	31 (100)	22 (70.96)	15 (48.38)	17 (54.83)

CSF=Cerebrospinal fluid, LAT=Latex agglutination test, GBS=Group B *streptococcus*, GNB=Gram-negative bacilli, *H. influenzae=Haemophilus influenzae*, *S. pneumonia=Streptococcus pneumonia*

Gram-stain was positive in 22(70%) cases of ABM, 8(25.80%) were gram-positive *cocci* in pairs, 1(3.22%) was gram-positive *cocci* in chain and 13(41.93%) were gram-negative bacilli (GNB).

CSF culture results in the present study showed that 15(48.38%) cases of ABM were culture positive. *S. pneumoniae* was the most common organism isolated, 4(12.90%) cases, followed by *Haemophillus influenzae* and *Klebsiella pneumoniae*, i.e., 3(9.67%) cases each. *Citrobacter koseri, Acinetobacter baumannii* and *Pseudomonas aeruginosa* were isolated in 1(3.22%) case each. CSF LAT was positive in 17(54.83%) cases of ABM. 8(25.80%) were Hib, 7(22.58%) were *S. pneumoniae* and 2(6.45%) were GBS [Table 2].

The comparative analysis of gram-stain, culture and LAT in the present study showed that culture was positive in 48.38%, gram-stain in 70.96% and LAT in 54.83% of the 31 ABM cases [Table 2].

Overall considering all tests together (Grams-stain, culture and LAT), *H. influenzae* was the most common isolate in the present study positive in 9(29.03%) cases followed by *S. pneumoniae* in 7(22.58%) cases. A total of 2 cases of GBS were identified by LAT only [Table 2].

Table 3: Comparison of sensitivity, specificity, PPV and NPV of CSF Gram-stain and CSF LAT with culture as gold standard

Test	Sensitivity %	Specificity %	PPV %	NPV %
Gram-stain	53.33	83.52	36.36	91.02
LAT	66.66	87.91	35.29	96.38

PPV=Positive predictive value, NPV=Negative predictive value, CSF=Cerebrospinal fluid, LAT=Latex agglutination test

CSF gram-stain and LAT were compared against CSF Culture. Gram-stain showed a sensitivity of 53.33%, specificity of 83.52%, PPV of 36.36% and NPV of 91.02%. LAT had a sensitivity of 66.66%, specificity of 87.91%, PPV of 35.29% and NPV of 96.38% [Table 3].

Discussion

ABM is a medical emergency, which warrants early diagnosis and aggressive therapy. Though the common pathogens associated with ABM are *S. pneumoniae*, *H. influenzae* and *N. meningitidis*, the etiological agents and their relative frequency may vary in different geographical areas. Some changing trends in the epidemiology of ABM have also been reported worldwide over the past few decades.[9] For instance, *S. pneumoniae* is a major cause of childhood bacterial meningitis in countries where Hib disease has been eliminated by vaccination.[10]

Identification of the isolate is crucial for initiating appropriate therapy. Although bacterial culture is considered to be the standard method, the negative effect of prior antimicrobial drug use on its sensitivity necessitates non-culture techniques for diagnosis.[10]

From the middle 70s, simple, fast and cheap methods to identify etiologic agents in purulent meningitis were made available with the development of immunochemical techniques such as latex particle agglutination test (LPAT).[11]

The LPAT is highly sensitive and specific, simple to perform no special equipment required, technically easy, results are available in 10 minutes.[12]

In our study, ABM constituted 1.7% of all pediatric ward admissions in children below 5 years of age. The reported frequency of occurrence of ABM in hospital admissions among children below 5 years of age is 0.5-2.5%.[3,4] It is noteworthy to mention that a majority i.e. 61(61%) children were below 1 year of age, of which 24(24%) were neonates. The male and female ratio in our study correlated with other studies who have reported 1.75:1.[12]

As per the WHO criteria of a proven case of bacterial meningitis,[8] 31/100 (31%) cases were laboratory confirmed as bacterial meningitis in the present study [Table 1].

Excluding 5 cases, which were positive only on gram-stain and could not be speciated and considering all tests together *H. influenzae* and *S. pneumoniae* were the predominant etiological agent. Similar results were observed by other authors.[12-14] GBS was the etiological agent in 2/31 (6.45%) cases of ABM in the present study. Similar findings have been reported by other workers.[12,13] Two children beyond 1 month of age, presented with *E. coli* and *C. koseri* meningitis and both gave a positive history of Chronic suppurative otitis media. *Acinetobacter baumannii* and *P. aeruginosa* were the causative agents in one case each of neonatal meningitis (3.22%). Although these isolates are often associated with hospital acquired infections, their presence in the community is no longer uncommon.

In the present study, culture detected 48.38% of the confirmed cases of ABM [Table 2]. Our study correlates with studies of the other Indian authors, who reported 50% and 42.8% culture positivity.[3,15] In our study, the isolation rates were higher than that reported by another study.[12] Majority of our isolates were gram-negative organisms 73.33%. Rao *et al*,[16] in their study have reported predominance of gram-negative bacteria (63.3%) while gram-positive bacteria were found only in 36.7% of cases.

Reasons as reviewed in other studies for low CSF culture yield are low bacterial load,[14] use of antimicrobial agents prior to CSF collection,[4] poor culture media,[4] poor culture facility such as non-availability of special media, stored in unsatisfactory conditions, samples refrigerated before plating, delayed and faulty inoculation, lack of transport media and inadequacy in processing of CSF specimens[12,14] autolytic enzymes,[17] lack of 24 hours facility for processing CSF samples.[9] These factors highlight the need for prompt inoculation of CSF on culture media plates at the bedside by residents rather than transporting the CSF specimen to laboratory, if transported then using transport media is recommended.[4,5] In spite of the above limitations, the utility of culture in terms of species identification and the ability to perform antimicrobial susceptibility testing makes it superior to gram-stain and LAT.

In the present study, LAT could detect 17 lab confirmed cases of ABM (54.83%) [Table 2]. No case of *N. meningitidis* was detected in the present study. The detection of *N. meningitidis* Group B antigen by immunological techniques continues to pose a problem and this has been attributed to the poor immunogenicity of this particular antigen.[18]

Comparative studies of Singh *et al*,[15] Mirdha *et al*,[17] quote a higher detection rate of LAT in their studies because the majority of the organism in their study comprised of *meningococci*, *pneumococci* and Hib that were negative for culture as compared with our study where 40% of organisms were *Enterobacteriaceae* other than *E. coli* and non-fermenters that were culture isolated but the reagents to detect them were not included in the panel of the kit.

In developing countries like India where a majority of neonatal meningitis is caused by *Enterobacteriaceae*, culture appears to be superior to LAT.

Sensitivity and specificity of LAT has been reported to a range from 83.8% to 93.0% and 94.0% and 100% respectively.[11,19]

In contrast, the sensitivity in our study is quite moderate 66.66% [Table 3]. Using CSF culture as a gold standard for calculating sensitivity, specificity, PPV and NPV, has its own limitations, as CSF culture is less sensitive compared to serologic tests and LAT is not designed to detect all the organisms. Hence, a culture negative and LAT positive test result that is taken as false positive could actually be a true positive and vice-versa, which could influence the sensitivity, specificity and PPV adversely. For instance, though the sensitivity of LAT in our study appears to be moderate if we consider only the bacteria that can be detected by LAT and exclude the GNB other than *E. coli*, LAT could identify 17 of the 18 fastidious bacteria while culture identified 7. Among the 16 culture negative cases, LAT was positive in 11 cases [Table 3]. As is evident by the results, LAT was superior to culture in identifying fastidious bacteria such as *H. influenzae*, *S. pneumoniae* and GBS.

Among the 9 culture positive and LAT negative cases, 6 were GNB whose reagents were not included in the panel of the kit. Hence, LAT failed to detect these organisms. Among the remaining three culture positive cases (1 - *H. influenzae* and 2 - *E. coli*) LAT failed to detect the corresponding antigens in the CSF. It is also possible that the antiserum in diagnostic LAT kit does not detect all the capsular serotype prevalent in a geographical area or probably as yet unrecognized serotypes are the causative agents in such cases.[4] This probably explains the false negative LAT in our study. False positive LAT results may occur in case of recent immunization with Hib conjugate vaccine and infection with cross reacting organisms.[9] Despite the good specificity of LAT 87.91% in the present study, the low PPV 35.29% is of concern, especially in view of the high-cost of the test. Nevertheless, because of the high NPV of 96.38%, a negative LAT fairly rules out ABM in clinically suspected cases.

It is important to note that bacterial antigen testing was originally designed to be used in patients who demonstrated laboratory and clinical findings consistent with meningitis. Despite these initial intentions, this test has been used much too often as a screening tool in cases of suspected meningitis in patients whose CSF specimens have normal chemistries and cell counts. The indiscriminate use of LAT without consideration of the chemical and cytological profiles of the CSF is a misuse of valuable resources.[20] Several studies advocate the usefulness of LAT, especially in pretreated cases and to differentiate partially treated pyogenic meningitis from tuberculous meningitis, which is rampant in India.[9]

In the present study, the case fatality is 45.16% and is similar to the studies of other Indian authors.[3]

Conclusion

No test can replace the utility of culture especially so in neonates as LAT does not identify *Enterobacteriaceae* other than *E. coli*. Thus, CSF Culture is crucial to the diagnosis of neonatal meningitis regardless of the other laboratory results. Despite its drawbacks, we found LAT to be simple, rapid procedure suitable to be used as an adjunct laboratory test. It was also found to be valuable in detecting fastidious bacteria that are difficult to isolate on culture. However, it should not be used indiscriminately as a screening tool in the routine diagnostic laboratory and its use should be reserved for the detection of bacterial antigens in CSF specimens that fail to reveal the organism on gram-stain. Besides the high-cost of LAT is a prohibitive factor in resource poor countries. Considering the merits and demerits of the tests, selection of the tests based on the individual case histories appears to be prudent and will yield more productive results rather than any of the tests alone.

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