ORIGINAL ARTICLE

Utility of latex agglutination test (LAT) in detecting acute bacterial meningitis against culture as gold standard

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ABSTRACT

Background: Bacterial meningitis is a medical emergency. It is a major cause of death and disability worldwide. Immediate steps must be taken to establish the cause and initiate effective therapy. Though Gram staining is essential in diagnosis of meningitis, some cases may be missed. Culture and latex agglutination tests (LATs) help to overcome this disadvantage. **Objective:** This study was conducted to find out the sensitivity of the LAT in acute bacterial meningitis against culture as the gold standard. **Materials and Methods:** Cerebrospinal fluid (CSF) samples were collected aseptically in dry, sterile bottles from all cases of bacterial meningitis by lumbar puncture from August 2011 to December 2013. They were subjected to cell count, Gram staining, culture and LAT. **Results:** Out of 538 samples, aetiological agents were identified by Gram stain and culture or LAT in 52 cases. *Streptococcus pneumoniae* was isolated in 22 (42%), *Haemophilus influenzae* type b in 14 (26.9%), *Neisseria meningitidis* in three (5.6%), Group B streptococci in three (5.6%), *Klebsiella pneumoniae* in six (11.5%), *Acinetobacter baumannii* in two (3.84%) and *Escherichia coli* in two (3.84%) cases. In the present study, the LAT for *H. influenzae* type b; *Streptococcus pneumoniae; Neisseria meningitidis* A, B, C, Y and W135; Group B streptococci; and *E. coli* K1 antigen had a sensitivity of 74.1%, specificity of 95.8%, positive predictive value 52.1% and negative predictive value 98.3%. **Conclusion:** LAT cannot replace the utility of culture. Despite its drawbacks, LAT is a simple, rapid procedure, suitable to be used as an adjunct laboratory test for establishing the aetiological diagnosis of bacterial meningitis, particularly in partially treated cases.

Key words: Acute bacterial meningitis, culture, latex agglutination

INTRODUCTION

Acute bacterial meningitis remains a major cause of mortality and morbidity worldwide. The mortality rate due to acute bacterial meningitis remains significantly high in India and other developing countries, in the range of 16-32%.[1] Even though a number of pathogens can cause bacterial meningitis, the three most common meningeal pathogens are *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*.[2] Delay in diagnosis and initiation of antimicrobial therapy may result in a poor outcome. As clinical signs of meningitis cannot always be relied upon, laboratory support is imperative to achieve an early diagnosis. The reporting time for culture and sensitivity depends on the time it takes for the organism to grow and may result in a delay of 18 h or longer. Even after such a delay, cultures may fail to yield growth because of previous antimicrobial treatment. Therefore, tests with shorter turnaround time, such as the latex agglutination test (LAT), with good sensitivity and specificity are important for early diagnosis.

The LAT can detect the antigen of *Haemophilus influenzae* type b, *Streptococcus pneumoniae, Neisseria meningitidis, Escherichia coli* K1 and Group B streptococci within 30 min. This study aims to assess the accuracy of LAT as a diagnostic test in cases of bacterial meningitis.

Access this article online

Quick Response Code:

Website: www.jacmjournal.org

DOI: 10.4103/0972-1282.171888

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How to cite this article: Jyothi R, Ramani Bai JT. Utility of latex agglutination test (LAT) in detecting acute bacterial meningitis against culture as gold standard. J Acad Clin Microbiol 2015;17:84-8.

MATERIALS AND METHODS

Aim

The aim of this study was to determine the diagnostic test parameters of the LAT in acute bacterial meningitis against culture as the gold standard.

Period

This prospective study was carried out during the period from August 2011 to December 2013.

Setting: Medical College, Thiruvananthapuram, Kerala, India, a tertiary care teaching hospital.

Inclusion criteria

All patients admitted with signs and symptoms of acute bacterial meningitis, such as headache, vomiting and fever during this period.

Exclusion criteria

Meningitis occurring postoperatively or as a post-traumatic complication, and cerebral abscess were exclusion criteria for the study.

Sample collection

Cerebrospinal fluid (CSF) samples were taken from patients with typical signs and symptoms of meningitis prior to administration of antibiotics whenever possible. The samples were collected aseptically by lumbar puncture in dry, sterile bottles and processed immediately. CSF was aliquoted into two sterile test tubes. One of the tubes was centrifuged for 20 min at 2000 rpm.[3] The sediment was used for culture and Gram staining. The supernatant was used for bacterial antigen detection using LAT, for *H. influenzae* type b; *Streptococcus pneumonia; Neisseria meningitidis* A, B, C, Y and W135; Group B streptococci; and *E. coli* K1 antigen (BD Directigen meningitis comboTest).

All CSF samples were inoculated on 5% sheep blood agar with staphylococcal touch colony, chocolate agar with added IsoVitaleX, MacConkey agar and brain-heart infusion broth. When organisms were seen in the Gram smear, a direct sensitivity test was done on appropriate media.

Blood agar with staphylococcal touch colony and chocolate agar were kept in candle jar with 10% CO_2, and all media were incubated at 37°C.

The culture was inspected after overnight incubation. Growth was identified and antibiotic sensitivity done. All the plates were incubated for a minimum of 48 h when there was no growth.

Statistical analysis

Diagnostic test evaluation of LAT was done using culture as the gold standard.

RESULTS

During the 2 years and 4 months study period, a total of 538 samples were collected from patients clinically suspected to have meningitis. Of these, 208 were males, 330 were females and there were 336 children. Out of 538 samples, 56 were positive by any one test, i.e., 56 were positive by Gram smear, 31 cases by bacterial culture, and 44 cases by LAT.

Out of 538 samples, the exact aetiological agent was identified in 52 cases, of which *Streptococcus pneumoniae* was seen in 22 (42%), *Haemophilus influenzae* type b in 14 (26.9%), *Neisseria meningitidis* in three (5.6%), Group B streptococci in three (5.6%), *Klebsiella pneumoniae* in six (11.5%), *Acinetobacter baumannii* in two (3.84%) and *Escherichia coli* in two (3.84%) [Table 1]. Among the aetiological agents identified in this study, *Streptococcus pneumoniae* was the predominant organism. LAT was positive for *Streptococcus pneumoniae* in 22 (4.1%), for *Haemophilus influenzae* type b in 14 (2.6%), for Group B streptococci in three (0.56%), *Neisseria meningitidis* in three (0.56%) and *E. coli* K1 in two (0.37%). Out of the 44 LAT positive samples, culture yielded *Streptococcus pneumoniae* in 16 out of 22 LAT positives, *Haemophilus influenzae* type b in four out of 14, *Neisseria meningitidis* in one out of three and *Escherichia coli* in two out of two LAT positive cases [See Table 2].

Table 1: Bacterial aetiological agents identified by culture and LAT

Organism	Number (%)
Streptococcus pneumoniae	22 (42)
Haemophilus influenza type b	14 (26.9)
Klebsiella pneumoniae	6 (11.5)
Neisseria meningitidis	3 (5.6)
Group B streptococci	3 (5.6)
Escherichia coli	2 (3.84)
Acinetobacter baumannii	2 (3.84)
Total	52 (100)

Table 2: Bacterial aetiological agents by LAT and culture

Organism	LAT-positive	Culture-positive
Streptococcus pneumoniae	22	16
Haemophilus influenzae type b	14	4
Neisseria meningitidis	3	1
Group B streptococci	3	—
Escherichia coli	2	2
Total	44	23

Out of the 52 cases with bacterial aetiology, 30 (57.8%) were males and 22 (42.2%) were females, 34 (65.38%) were children and 18 (34.62%) were adults.

In two cases, where the smear showed Gram-positive diplococci with pus cells, antigen detection and culture were negative. In two cases where the smear showed Gram-negative bacilli, both antigen detection and culture were negative.

DISCUSSION

Most often, therapy for bacterial meningitis has to be initiated before the aetiology is known. The choice of initial antimicrobial therapy is based on the most common pathogen prevalent in a particular geographical area, age group, and its antibiotic sensitivity pattern. In the present study, samples from 538 patients with diagnosis of acute bacterial meningitis were collected and processed. Analysis of meningitis cases with bacterial aetiology showed that the bacterial pathogen could be demonstrated by Gram stain of CSF samples in 56 (10.4%) and culture in 31 (5.7%). Latex agglutination test for antigen detection of *Streptococcus pneumoniae, Haemophilus influenzae* type b, *Neisseria meningitidis*, Group B streptococci and *E. coli* K1 could find out 44 (8.1%) cases, out of which culture was positive in 23 (4.2%). Out of 538 samples cultured, 16 yielded *Streptococcus pneumoniae* (2.9%), which was the predominant organism. Higher occurrence of the three primary pathogens has been reported by Sumangala Bai and Raja in 1997 in Kerala.[4]

Streptococcus pneumoniae remains the most common aetiological agent in all age groups, accounting for 22 cases (42%) in the present study, reflecting a similar trend reported in an earlier study. R. Mani *et al.* has reported that 61.8% of meningitis cases in a 10-year retrospective study for 1996-2005, was due to *Streptococcus pneumoniae*.[1] Muralidharan in 2007 analysed the isolates from CSF samples from various centres in South India and found 17 isolates of *Streptococcus pneumonie* out of 307 isolates.[5] Schlech *et al.* had claimed that 13.3% of meningitis cases were due to *Streptococcus pneumoniae* in their study for 1971-1981 in USA.[6] Schuchat *et al.* reported a pneumococcal meningitis rate of 46% during a period of 1 year in 1995, in USA.[7]

Meningitis caused by *H. influenzae* type b has almost been eliminated from the Western world following routine vaccination with Hib conjugate vaccine. Carolyn V Gould *et al.* have stated that the conjugate vaccine has been extremely effective in reducing the incidence of *H. influenzae* type b meningitis worldwide, often by more than 90%.[8] There are 14 (2.6%) cases of *H. influenzae* meningitis in the present study, all being children aged between 2 months and 4 years. Mani *et al.* has reported *H. influenzae* type b as 1.8% of all cases of meningitis and claimed that the incidence has remained low for the past several decades. Muralidharan S. reported seven *H. influenzae* type b out of 307 isolates[5] However, the commonly held view of *H. influenzae* disease being rare in Asia has been challenged by a research study by Peltola in 1999, who reported that 11% of meningitis cases were due to *H .influenzae* type b and recommends large-scale vaccination for *H. influenzae* type b in Asian countries.[9]

In the present study, three cases of meningococcal meningitis (0.56%) was detected. Schlech *et al.* had reported 19.6% cases of *Neisseria meningitidis* among all the meningitis cases during their study of bacterial meningitis in USA during 1977-1981.[6] Schuchat *et al.* had reported 25% of meningitis cases being meningococcal meningitis during 1995 in the USA.[7] Only 1% of meningitis cases were meningococcal meningitis in a study conducted by Mani *et al.* and, according to them, meningococcal meningitis has a low prevalence except during epidemics[1] Muralidharan S reported one *Neisseria meningitidis* out of 307 isolates.[5]

Escherichia coli (*E. coli*) was one of the other aetiological agents detected in neonates in this study, i.e., two cases (0.37%). In one case, meningitis developed after the neonatal period and there was no history of immunosuppression, injury, invasive procedure or complicated delivery. One similar case has been reported by Borgina from the University of Toronto, where a 2-month old baby developed *Escherichia coli* meningitis. This was traced to be due to *E. coli* bacteriuria in the mother before delivery, and the child was on treatment for sepsis from the time of birth. Here no such source could be identified. Anna *et al.* had reported that *E. coli* causes about 20% of all cases of neonatal meningitis but less than 2% of cases of meningitis at all other ages.[10]

Group B streptococci was identified from three cases (0.57 %) of neonatal meningitis. Domingo *et al.* have reported the prevalence of Group B streptococcal meningitis of 4.3% among all meningitis cases in adult patients. In recent years, Group B streptococci have been known to cause meningitis in neonates, is being increasingly recognised as a cause of meningitis in adults and is associated with a high case fatality.[11] Schuchat et al. have reported the incidence of neonatal sepsis and meningitis due to Group B streptococci in 0.5-3 cases per 1000 live births, although there are substantial geographical and racial differences.[7]

A simple Gram smear can offer immediate clues to aid in the diagnosis of pyogenic meningitis. The interpretation

of Gram smear is easier, less time-consuming and can give a good idea of the infecting agent. In the present study, 56 cases were Gram smear-positive, of which 31 yielded growth on culture. Some studies have reported a CSF Gram stain sensitivity of 60-90% and a high specificity of >97%, stressing its importance in the rapid and accurate diagnosis of causative organism in predicting the outcome, and advocating Gram staining of CSF as a routine procedure for prompt identification of the pathogen. Mani *et al.* reported that Gram staining provided evidence of the causative bacteria in 65.7%.[1] The yield of bacteria on a Gram smear depends on several factors such as the number of organisms present, prior use of antibiotics, technique used for smear preparation (centrifuged or direct smear), staining techniques, and the observer's skill and experience. A study by Dunbar *et al.* (1998) has also concluded that microscopic examination of a Gram-stained concentrated CSF is highly sensitive and specific in the early diagnosis of meningitis. In that study, examination of Gram smear of a specimen revealed the causative organism in 88% of cases. If the patients who had received effective antimicrobial therapy prior to lumbar puncture are excluded, CSF Gram smear positivity is 92%.[12]

Mani et al. have reported 40.8% culture-positive cases in their study.[1] Kabra *et al.* claimed that the incidence of culture-negative cases of meningitis was 6-50%.[13] The various reasons cited for a low yield of bacteria on culture are prior antibiotic treatment, delay in transport of specimens to the laboratory, non-availability of special media for specific pathogens and presence of autolytic enzymes in CSF. More recently, it has been suggested that CSF sterilisation may occur more rapidly after initiation of parenteral antimicrobial therapy. Complete sterilisation of CSF containing meningococcus occurs within 2 h, and of pneumococcus occurs within 4 h.[14]

Kabra *et al.* have observed that the false negative LAT could be possibly because of low antigen titres in the CSF.[13] Perkin *et al.* have questioned the clinical usefulness of antigen detection kits.[15] Though capsular serotyping of our isolates was not performed, it is possible that the antiserum in diagnostic LAT kits does not detect all the capsular serotypes prevalent in our geographical area or probably, as yet unrecognised serotypes are the causative agents in such cases. A negative result on the LAT does not rule out infection, and a false positive result may be due to recent immunisation with *Haemophilus influenzae* type b conjugate vaccine and infection with cross-reacting organisms.[1]

In the present study, LAT for *H. influenzae* type b; *Streptococcus pneumoniae*; *Neisseria meningitidis* A, B, C, Y and W135; Group B streptococci; and *E. coli* K1 antigen had a sensitivity of 74.1%, specificity of 95.8%, positive predictive value of 52.1% and negative predictive value of 98.3% [Table 3], taking culture as the gold standard. Syeda *et al.* have reported that the LAT had a sensitivity of 66.66%, specificity of 87.9%, positive predictive value of 35.29% and negative predictive value of 96.38%.[16] Despite its drawbacks, the LAT is a simple, rapid procedure suitable for use as an adjunct laboratory test, but it needs to be interpreted cautiously, taking the patient's clinical condition into account.[1]

Table 3: Correlation of antigen detection by latex agglutination test with culture

Antigen detection by latex agglutination	Culture		Total
	Positive	Negative	
Positive	23 (a)	21 (b)	44
Negative	8 (c)	486 (d)	494
Total	31	507	538

Sensitivity (a/a + c × 100) = 74.2; Specificity (d/b + d × 100) = 95.8; Positive predictive value (a/a + b × 100) = 52.1; Negative predictive value (d/c + d × 100) = 98.3

Gram smear-negative CSF samples with neutrophilic pleocytosis in patients with a clinical suspicion of bacterial meningitis warrant antigen testing with the LAT. Surinder *et al.* have concluded that along with greater ease of performance, the LAT for specific bacterial antigens compared favourably with the conventional tests and is rapid enough to guide the clinician for institution of proper antibiotics, hence it can be considered as an adjunct laboratory test for establishing the aetiological diagnosis of bacterial meningitis, particularly in pretreated cases.[17]

CONCLUSION

The findings of the study showed that primary pathogens still rank first among the aetiological agents of acute bacterial meningitis (75%). *Streptococcus pneumoniae* remains the major aetiological agent of meningitis in both adults and children (42%).

No test can replace the utility of culture, especially in neonates, as LAT does not identify Enterobacteriaceae other than *E. coli*. Thus, CSF culture is crucial to the diagnosis of neonatal meningitis. Despite its drawbacks, LAT is a simple, rapid procedure, suitable for use as an adjunct laboratory test for establishing the aetiological diagnosis of bacterial meningitis, particularly in the following:

1. Acute bacterial meningitis.
2. Gram smear-negative CSF samples with polymorphonuclear leucocytosis and clinical suspicion of acute bacterial meningitis, prior antibiotic treatment.
3. Partially treated meningitis.

However, it should not be used indiscriminately as a screening test in the routine diagnostic laboratory, and its use should be reserved by considering Gram smear, cell count and biochemical parameters. Culture is time-consuming and can give false negative results if the specimen has been transported and stored under unsatisfactory conditions or if antibiotic therapy had been initiated before the specimen was taken.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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