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# Comparative Analysis of Gram Stain, Culture and Bacterial Antigen Detection in Cerebrospinal Fluid Samples for Laboratory Diagnosis of Acute Bacterial Meningitis in Pediatric Population in A Tertiary Care Hospital

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## Abstract

**Acute bacterial meningitis (ABM) is a life threatening infection in children, associated with long term complications and high mortality rate.[1,2] Gram staining and culture are routinely used for diagnosis of ABM. Antigen detection by latex agglutination can provide prompt results thereby facilitating early initiation of empirical antibiotic treatment. To estimate the proportion of Laboratory confirmed cases among children admitted with clinical suspicion of acute bacterial meningitis in a tertiary care hospital. To compare and analyse the diagnostic efficacy of Culture, Gram stain and antigen detection by Latex agglutination in Cerebrospinal fluid (CSF) samples for laboratory detection of Acute bacterial meningitis. CSF samples from pediatric patients with clinical suspicion of ABM were analysed by Gram stain, culture and Antigen detection by Latex agglutination method. Results were recorded and analysed. Of the 50 clinically suspected cases, 13(26%) were confirmed as Acute bacterial meningitis by laboratory investigations. Among the organisms identified, *Streptococcus pneumoniae* was the most common isolate in 5(38.46%) cases followed by *Neisseria meningitidis, Klebsiella pneumoniae* and *Acinetobacter baumannii* in 2(15.38%) cases each and *Escherichia coli* and Group B *Streptococcus* in 1(7.69%) case each. Among the confirmed cases, 7(53%) samples showed culture positivity while Gram stain identified 8(61.53%)cases. Latex agglutination test showed positivity in 9(69.23%) cases. In life threatening infections like acute bacterial meningitis, where early diagnosis and prompt treatment is of utmost importance, Latex agglutination test can provide results within minutes facilitating early initiation of empirical therapy, making it an effective adjunct to gram stain and culture.**

**Keywords:** Laboratory Diagnosis, Acute Bacterial Meningitis, Gram Stain, Culture, Rapid Diagnosis, Latex Agglutination Method

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## INTRODUCTION

Acute bacterial meningitis(ABM) is a life threatening infection especially in children, associated with long term complications and high mortality rate.[1,2] Though ABM contributes to only 1.5% of all hospital admissions, mortality rate associated with ABM is significant ranging from 16% to as high as 45% necessitating early diagnosis with prompt initiation of appropriate antibiotics.[3-6] *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* type b, Group B *Streptococcus* and *Escherichia coli* are the pathogens frequently associated with ABM in children.[1,5-7] Rapid laboratory diagnosis with early initiation of appropriate antibiotics is crucial in reducing the mortality and long term sequelae associated with ABM.[1,3,4] Gram staining and culture are routinely used for diagnosis of bacterial meningitis. Factors like delay in transport of samples, delay in processing, low bacterial load and non-viable bacteria due to prior antibiotic therapy contribute towards reducing the sensitivity of culture and microscopic detection methods.[4,8,9] Molecular methods of diagnosis are currently being employed as the gold standard in developed countries due to its advantages like rapid turnaround time, accurate diagnosis and the ability to detect multiple pathogens (bacterial, viral and fungal pathogens).[10] However, factors like high cost, need for special infrastructure and trained personnel limit the use of such molecular diagnostic methods in resource poor settings. Rapid, cost effective and reliable diagnostic tests for ABM are the need of the hour for early initiation of appropriate antibacterial therapy.[3,4,7,11] Detection of antigen by latex agglutination test (LAT) can serve as a useful adjunct to routine microscopic examination for rapid screening of these cases. LAT can also detect antigen even in non-viable bacteria, which is beneficial in patients pre-treated with antibiotics, where culture is mostly negative.[4]

The main purpose of this study is to determine whether Latex agglutination test (LAT) is an effective adjunct to gram stain and culture which would help in making an early diagnosis of acute bacterial meningitis with early initiation of appropriate antibiotics, thereby reducing morbidity and mortality associated with bacterial meningitis.

### Aims and objectives

1. To estimate the proportion of Laboratory confirmed cases among children admitted with clinical suspicion of acute bacterial meningitis in a tertiary care hospital.
2. To compare and analyse the diagnostic efficacy of Culture, Gram stain and antigen detection by Latex agglutination in Cerebrospinal fluid (CSF) samples for laboratory detection of Acute bacterial meningitis.

## MATERIALS AND METHODS

This study was done in the Department of Microbiology, Stanley Medical College, Chennai, for a period of 3 consecutive months from April 2022 – June 2022. Ethics committee clearance was obtained from the institution. CSF samples were collected from 50 children ≤ 12 years admitted with clinical suspicion of Acute bacterial meningitis. In our study, cases were categorised as suspected, probable or laboratory confirmed in accordance with WHO criteria.[12] Any patient admitted with clinical features of acute bacterial meningitis (acute onset fever, headache, neck stiffness, altered mental state or meningeal signs) was included as a suspected case of acute bacterial meningitis.[12] Suspected cases with atleast one of the following findings in CSF sample – turbidity on macroscopic examination, increased leucocyte count, raised protein levels (> 100 mg/dl) or decreased glucose (< 40 mg/dl) were considered as probable cases of bacterial meningitis.[12] A suspected case that is confirmed in the laboratory by CSF culture or Gram stain or detection of antigen in CSF or detection of pathogen in blood was defined as a confirmed case of bacterial meningitis.[12]

### Sample size

50.

### Type of study

Prospective study.

**Inclusion criteria**

Children ≤ 12 years admitted with clinical features suggestive of Acute bacterial meningitis.

**Exclusion criteria**

Children with clinical suspicion of Tuberculous meningitis or non-bacterial cause of meningitis.

**Procedure**

1-2 ml of CSF was collected by lumbar puncture following strict aseptic precautions from children ≤ 12years, admitted with clinical suspicion of Acute bacterial meningitis. Samples were processed immediately on reception of the sample.

Macroscopic appearance of the CSF sample was noted. Biochemical parameters like CSF protein and Glucose level along with the Leucocyte count was noted. CSF was aliquoted into 2 sterile test tubes. After centrifuging, sediment from the 1st tube was subjected to culture and microscopic examination by Gram stain. The sediment was inoculated onto blood agar and chocolate agar which were incubated at 37°C with 5 -10% $CO_2$ and onto Nutrient agar and Mac Conkey agar plates which were incubated at 37°C. It was also inoculated into BHI broth and incubated at 37°C. Tubes with turbidity were sub-cultured immediately onto Chocolate agar and blood agar, incubated at 37°C with 5 -10% $CO_2$ and Nutrient agar and MacConkey agar plates which were incubated at 37°C. Tubes without turbidity were sub-cultured on day 7 before discarding. The isolates were identified using standard microbiological techniques.[4,13] Blood culture was also done for all the clinically suspected cases. Antibacterial susceptibility testing was done for all isolates according to CLSI guidelines.[14]

**Antigen detection by latex agglutination test**

The second CSF sample was used for bacterial antigen detection. Bacterial Antigen detection was done using Pastorex Meningitis kit, which is an agglutination test used for qualitative detection of soluble antigens of *Streptococcus pneumoniae, Haemophilus influenzae* type b, Group B *Streptococcus, Neisseria meningitidis* groups A, C, Y, W135 and *Neisseria meningitidis* group B / *Escherichia coli* K1. The samples were processed according to the instructions given in the kit insert.

The CSF sample was heated at 100°C for 3 minutes. Once it reached room temperature it was centrifuged at 3000 g for 5 minutes. 50 µl of the supernatant was dispensed into each circle of the agglutination card. 1 drop of the corresponding latex reagents were added and mixed. The card was rotated gently using a rotator for 10 minutes to look for visible agglutination. The results were recorded and analysed statistically.

**RESULTS**

50 CSF samples collected from children with clinical suspicion of Acute bacterial meningitis were included in the study.

Age and gender distribution is tabulated in Table 1.

Among the clinically suspected cases of ABM, a male: female ratio of 1.1:1 was noted with a slight male preponderance. Male: female ratio in Laboratory confirmed cases was 1:1.2 with a slight preponderance of females.

Among the 50 clinically suspected cases, 13 (26%) cases were Laboratory confirmed cases as per WHO criteria for bacterial meningitis.

Distribution of Laboratory confirmed cases of Acute bacterial meningitis is given in Table 2.

Among the laboratory confirmed cases (n = 13), turbid appearance was seen in 8 (61.5%) samples. Elevated leucocyte count was seen in 7 cases (53.9%), predominantly Neutrophils. Raised protein levels were noted in 5 samples (38.5%) and decreased glucose levels were noted in only 4 samples (30.8%).

3 isolates (23%) were detected by all 3 methods, 4 isolates (31%) were detected by both Gram stain and culture, 1 isolate (7%) was identified by LAT and Gram stain and not by culture while 5 cases (38%) were detected only by Antigen detection using LAT.

Organisms identified along with the age distribution is given in Table 3.

Distribution of the organisms identified by the method of detection is given in Table 4.

**Table 1.** Age & gender distribution of ABM Cases (n=50)

| Age | Clinically Suspected Cases | | | Laboratory Confirmed Cases | | |
|---|---|---|---|---|---|---|
| | Male | Female | Total | Male | Female | Total |
| ≤28 days | 0 | 3 | 3 (6%) | 0 | 0 | 0 |
| >28 days –≤ 1 year | 14 | 6 | 20 (40%) | 0 | 0 | 0 |
| >1 year – ≤ 5 years | 4 | 6 | 10 (20%) | 1 | 2 | 3 (23.07%) |
| >5years – ≤ 12 years | 8 | 9 | 17 (34%) | 5 | 5 | 10 (76.92%) |
| Total | 26 | 24 | 50 | 6 | 7 | 13 |

## DISCUSSION

Acute bacterial meningitis is a life-threatening condition in children associated with long term complications and high mortality rate, necessitating early diagnosis with prompt treatment.

In our study, a male:female ratio of 1.1:1 was noted among the clinically suspected cases with a slight preponderance of males, similar to other studies.[2,3] Most of the clinically suspected cases were in the age group >28 days – ≤ 1 year with 20 (40%) cases followed by >5years – ≤ 12 years with 17 cases (34%).

Among the 50 clinically suspected cases included in the study, 13 (26%) cases were Laboratory confirmed as Acute bacterial meningitis. Similar findings were noted in studies by Kala Yadav *et al.* (24%) and Viswanath *et al.* (26%).[2,15] Study by Mohammadi *et al.* showed a higher proportion of laboratory confirmed cases (41%).[4] Laboratory confirmed cases showed a male: female ratio of 1:1.2 with a slight preponderance of females. Of the 13 laboratory confirmed cases, 10 (76.9%) were in the age group>5years – ≤ 12 years and 3 cases (23.1%) were in the age group >1 year-≤5 years.

Death was observed in 1 among the 13 confirmed cases. Our study showed a lower case fatality rate of 7% comparable to findings by Bingen *et al.* (9.2%).[16] Most studies show a higher mortality rate ranging from 30%-45%.[3,4,6]

Among the organisms identified by all methods, *Streptococcus pneumoniae* was the most common isolate in 5 (38.5%) cases followed by *Neisseria meningitidis, Klebsiella pneumoniae* and *Acinetobacter baumannii* in 2 cases (15.4%) each and *Escherichia coli* and Group B *Streptococcus* in 1(7.7%) case each similar to other studies.[1,4] However study by Bingen *et al.* noted *Neisseria meningitidis* (55.3%) as the most common isolate followed by *Streptococcus pneumoniae* (33.4%).[16] There were no isolates of *Haemophilus influenzae* detected in our study. This could possibly be attributed to effective vaccination against *Haemophilus influenzae.* Of the 50 suspected cases, 48 were vaccinated. Of the confirmed cases, all were vaccinated against *Haemophilus influenzae*. None of the cases had received vaccination for *Streptococcus pneumoniae* or *Neisseria meningitidis*.

**Table 2.** Detection methods for confirmed cases of ABM (n=13)

| Laboratory Test | Positives | Percentage (%) |
|---|---|---|
| Gram stain + Culture + LAT | 3 | 23 |
| Gram stain + Culture | 4 | 31 |
| Gram stain + LAT | 1 | 7 |
| Only LAT | 5 | 38 |
| Total | 13 | |

As per Table 4, 8 cases (61.5%) were identified by Gram stain similar to observations by Kala *et al.* (70.8%), Chinchankar *et al.* (67%) and Mohammadi *et al.* (71%).[2-4] One isolate of *Streptococcus pneumoniae* not identified by culture was positive on microscopic examination by Gram stain. Similar study by Shrestha *et al.* has shown only 27.8% detection by Gram stain.[6]

Among the Laboratory confirmed cases, 7(53%) samples showed culture positivity, which correlates with similar findings by Mohammadi *et al.*, Chinchankar *et al.* and Singh H *et al.* who showed culture positivity of 50%,48.4%, and 42.8%, respectively.[3,4,17] Among the culture positives, *Klebsiella pneumoniae* and *Acinetobacter baumannii* were isolated in 2(15.4%) cases each and *Streptococcus pneumoniae, Escherichia coli* and Group B *Streptococcus* was isolated in 1 (7.7%) case each. With availability of effective vaccines

**Table 3.** Age wise distribution of organisms isolated (n=13)

| Organism | ≤ 28 days | >28 days – ≤ 1 year | >1 year– ≤ 5 years | >5years – ≤ 12 years | Total |
|---|---|---|---|---|---|
| Group B *Streptococcus* | - | - | 1 | - | 1 |
| *Streptococcus pneumoniae* | - | - | - | 5 | 5 |
| *Escherichia coli* | - | - | - | 1 | 1 |
| *Neisseria meningitidis* | - | - | 1 | 1 | 2 |
| *Haemophilus influenzae* type b | - | - | - | - | - |
| *Klebsiella pneumoniae* | - | - | 1 | 1 | 2 |
| *Acinetobacter baumannii* | - | - | - | 2 | 2 |
| Total | - | - | 3 (23.08%) | 10 (76.92%) | 13 |

**Table 4.** Organisms isolated by gram stain, culture and LAT(n=13)

| Organism | Gram Stain Positive | Culture Positive | LAT Positive | |
|---|---|---|---|---|
| Group B *Streptococcus* (n = 1) | 1 | 1 | 1 | |
| *Streptococcus pneumoniae* (n = 5) | 2 | 1 | 5 | |
| *Escherichia coli* (n = 1) | 1 | 1 | 1 | |
| *Neisseria meningitidis* (n = 2) | - | - | 2 | |
| *Haemophilus influenzaetype* b(n = 0) | - | - | - | |
| *Klebsiella pneumoniae* (n = 2) | 2 | 2 | * | |
| *Acinetobacter baumannii* (n = 2) | 2 | 2 | * | |
| Total (n=13) | 8 (61.53%) | 7 (53.84%) | 9 | (82%) |

*- Not included in the LAT panel

against *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*, Gram negative bacteria are emerging as major etiological agents of ABM in recent years. Though not commonly associated with ABM, similar studies by Kala *et al.*, Mohammadi *et al.* and Viswanath *et al.* have also identified *Klebsiella pneumoniae* and *Acinetobacter baumannii* as etiological agents of ABM.[2,3,15]

CSF antigen detection by Latex agglutination test was positive in 9 (69.23%) cases. Similar findings were also noted by Chinchankar *et al.* (78%), Mohammadi *et al.* (54.8%) and Finlay *et al.* (58%).[3,4,18] Study by Kala *et al.* has shown only 33.33% detection by Latex agglutination test2. *Streptococcus pneumoniae* was positive in 5 (38.46%) cases, followed by *Neisseria meningitidis* in 2 (15.38%) cases and *Escherichia coli* and Group B *Streptococcus* in 1 (7.69%) case each. 2 cases each of *Klebsiella pneumoniae* and *Acinetobacter baumannii* identified by culture and Gram stain could not be identified by LAT as it is not included in the Antigen test panel. All the other 3 culture positive cases were identified by LAT. Though bacterial detection by culture is used as the gold standard for laboratory diagnosis of bacterial meningitis, in our study, LAT identified 4 cases of *Streptococcus pneumoniae* and 2 cases of *Neisseria meningitidis* which were negative by culture. This could be attributed to factors like low bacterial load or non-viable bacteria as all these cases had received antibiotic treatment prior to lumbar puncture. Further studies are needed to rule out false positivity.

**Table 5.** Comparative analysis of CSF gram stain and lat using culture as the gold standard

| Parameters | Gram Stain | Latex Agglutination Test |
|---|---|---|
| Sensitivity | 100% | 42.9% |
| Specificity | 97.6% | 86% |
| Positive Predictive Value (PPV) | 87.5% | 33.3% |
| Negative Predictive Value (NPV) | 100% | 90% |

Comparative analysis of LAT, Gram stain and culture was done using culture as the gold standard. As per Table 5, detection by LAT had a Sensitivity of 42.9% and Specificity of 86%. Positive predictive value (PPV) and Negative predictive value (NPV) were 33.3% and 90% respectively. Sensitivity of Gram stain was 100%, Specificity was 97.6%, Positive predictive value (PPV) was 87.5% and Negative predictive value (NPV) was 100% in comparison to culture. Sensitivity of LAT in our study was moderate. This could be due to the identification of 2 isolates each of *Klebsiella pneumoniae* and Acinetobacter which are not included in the LAT panel. If only organisms included in the LAT panel are considered, LAT shows a higher sensitivity, specificity, PPV and NPV in comparison to culture especially in detection of fastidious organisms like *Neisseria meningitidis* and *Streptococcus pneumoniae*.

Gram stain and culture of Cerebrospinal fluid (CSF) samples are routinely done in our Institution for diagnosis of bacterial meningitis. Our Institute being a tertiary care hospital mainly receives patients who have been treated outside with various antibiotics before referral, making identification of causative organisms by microscopy and culture difficult. Also, isolation and identification of organisms by culture takes 48-72 hours thereby delaying the initiation of specific antibiotic therapy. Despite these drawbacks, the importance of Gram stain and culture in laboratory diagnosis of acute bacterial meningitis cannot be undermined. Gram stain provides a cost-effective alternative for rapid and reliable detection of causative organisms in ABM. With availability of effective vaccines against *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*, Gram negative bacilli are emerging as the major etiological agents of ABM, which can only be detected by Gram stain and culture.

Latex agglutination test as a diagnostic modality in laboratory diagnosis of Acute bacterial meningitis offers various advantages. It can detect even low bacterial load or non-viable bacteria in children who had received antibiotic therapy prior to admission where culture is often negative. It also provides rapid results which is beneficial in treatment of cases with Acute bacterial meningitis. However, cost of each test by LAT is approximately Rs. 850 which is a major constraint in resource poor settings. So, a proper screening of clinically suspected cases of acute bacterial meningitis by biochemical parameters, cell count and Gram stain should be made mandatory before doing LAT to make it more cost effective.

## CONCLUSION

In life threatening infections like acute bacterial meningitis, where early diagnosis and prompt treatment is of utmost importance, Latex agglutination tests can provide results within minutes which can guide clinicians in initiating empirical therapy, thereby reducing the morbidity and mortality associated with these cases. However, factors like cost and inability to detect pathogens not included in the panel, would limit the use of LAT as a routine diagnostic test for ABM. Proper screening of clinically suspected cases of acute bacterial meningitis by biochemical parameters, cell count and Gram stain before Latex agglutination test would make antigen detection by Latex agglutination method an effective adjunct to gram stain and culture enabling earlier diagnosis with decreased cost and better outcome.

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None.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## AUTHORS' CONTRIBUTION

VD conceptualized and designed the work. JJ, KC and TM performed data collection. VD, JJ and KC performed data analysis and interpretation. VD, JJ and KC wrote the article. VD and JJ reviewed and revised the article. All authors read and approved the final manuscript for publication.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Government Stanley Medical College & Hospital, Chennai, India, with EC registration No. ECR/131/Inst/TN/2013/RR-19

## INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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