

Evaluation of Direct Antigen Detection (LAT), CRP In Bacterial Meningitis

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Abstract: Infection within the subarachnoid space or throughout the leptomeninges is called meningitis. Based on host's response to the invading microorganism it is divided into two major categories: purulent and aseptic meningitis. Bacterial organisms are usually the cause of purulent infections. An important host defense mechanism within the CNS is the blood-brain barrier.¹ There is an urgent need for the laboratory diagnosis of suspected bacterial meningitis, because bacterial meningitis is life threatening and requires appropriate antibiotic therapy at the earliest possible moment.² patient suspected of having meningitis should have a specimen of cerebrospinal fluid (CSF) examined in the laboratory by Direct antigen detection systems (LAT) for detection of the polysaccharide capsular antigens having specificity and sensitivity in the 90-97% range and when used in conjunction with the direct staining techniques often provide rapid presumptive diagnosis on which specific antibiotic therapy can be based. CSF white blood cell count and differential count were the best predictors of meningitis. Latex agglutination test is more sensitive than culture in the diagnosis of bacterial meningitis in patients who had received previous antibiotic therapy presumably because bacterial growth is impaired.⁴ C-reactive protein (CRP), an acute phase reactant has been used to diagnose and follow the course of infection. So Quick diagnosis and effective antibacterial treatment is the key to success in bacterial meningitis. This study therefore undertaken to aid in rapid diagnosis of acute bacterial meningitis cases by LAT, CRP and Gram's stain along with culture. A total of 100 clinically suspected cases of meningitis aged from 1 day to 75 years admitted in Government General Hospital, Guntur. They are tested for both CRP and Latex agglutination test. In the present study of 100 suspected cases of meningitis, 38 cases (38%) were positive by Gram stain, CRP test and Latex agglutination test (5 antigen test). Out of 38 positive cases, Direct Gram stain positive in 24 cases (63.15%), Culture positive in 24 cases (63.15%), LAT positive in 26 cases (68.42%). The sensitivity and specificity LAT was 100% and 100%. The sensitivity and specificity for CSF CRP was 52% and 100% LAT and CRP when added together detects almost all cases of meningitis early and suggests prompt treatment of bacterial meningitis at early stages to prevent deaths and complications, disability (morbidity and mortality).

Key words: Bacterial meningitis, CSF CRP, Direct antigen detection systems (LAT)

I. Introduction

Infection within the subarachnoid space or throughout the leptomeninges is called meningitis. Based on host's response to the invading microorganism, it is divided into two major categories: purulent and aseptic meningitis. Bacterial organisms are usually the cause of purulent infections. There is an urgent need for the laboratory diagnosis of suspected meningitis, because bacterial meningitis is life threatening and requires appropriate antibiotic therapy at the earliest possible moment.²

WHO criteria: WHO criteria define a proven case of bacterial meningitis as:

A case that is laboratory confirmed by growing (i.e., culturing) or identifying (i.e., by Gram stain or antigen detection methods) a bacterial pathogen in the CSF with a clinical syndrome consistent with bacterial meningitis.³ Every patient suspected of having meningitis should have a specimen of cerebrospinal fluid (CSF) examined in the laboratory.² Direct antigen detection systems (LAT) available for direct detection of the polysaccharide capsular antigens have specificity and sensitivity in the 90-97% range and when used in conjunction with the direct staining techniques often provide rapid presumptive diagnosis on which specific antibiotic therapy can be based. CSF white blood cell count and differential count were the best predictors of meningitis. Latex agglutination test is more sensitive than culture in the diagnosis of bacterial meningitis in patients who had received previous antibiotic therapy presumably because bacterial growth is impaired.⁴

C-reactive protein (CRP), an acute phase reactant has been used to diagnose and follow the course of infection. So Quick diagnosis and effective antibacterial treatment is the key to success in bacterial meningitis. Thus early detection by clinical diagnosis supported with laboratory diagnosis (CSF analysis, Gram's stain, Culture, serological tests (LAT)) helps in timely intervention by the clinician to institute appropriate antibiotic therapy as early therapy is imperative in reducing morbidity and mortality.⁵ This study therefore undertaken to aid in rapid diagnosis of acute bacterial meningitis cases by Gram's stain and CRP, LAT and to carry out the evaluation of CRP and Antigen detection (LAT) in CSF in cases of acute bacterial meningitis

Aims And Objectives Of The Study

1. For early detection and treatment of Bacterial meningitis cases to prevent complications.
2. To Evaluate CRP (C-reactive protein) and LAT (Latex agglutination test) in bacterial meningitis cases.

II. Material and Methods:

This prospective study was done in the Department of Microbiology, Guntur Medical College / Government General Hospital, Guntur during the period of 2013-14.

A total of 100 clinically suspected cases of meningitis aged from 1 day to 75 years admitted in Government General Hospital, Guntur constituted the study group.

Cerebrospinal fluid (CSF) samples collected from pediatric, medical and neuromedical and emergency wards of Government General Hospital, Guntur

They are tested for both CRP and Latex agglutination test along with conventional microscopy

Inclusion Criteria: Children and adults admitted to Government General Hospital with history of fever with chills, rigors and neck rigidity and vomiting, drowsiness and altered sensorium were included in the study. All cases with or without antibiotic usage included.

Exclusion Criteria:

All cases of meningitis secondary to trauma and iatrogenic excluded.

CSF was collected by lumbar puncture with all aseptic precautions. The CSF was taken into a sterile test tube and centrifuged at 3000 rpm for 20 minutes. The supernatant was used for Bacterial antigen detection test (LAT) and CRP test. The sediment was used for preparing Gram stained smears

CRP test:

Latex agglutination slide test was used for qualitative detection of CRP in CSF using commercial test kit of Ensure Biotec private limited

Procedure: Using the disposable plastic dropper, one drop of CSF was placed within the circled area on the reaction plate provided within the kit. One drop of latex CRP reagent was added to the CSF and mixed thoroughly with to and fro rotation of the slide manually for 2 minutes and examine for macroscopic agglutination under direct light source. Positive and negative controls were put up simultaneously. Interpretation of results: Positive-Agglutination within 2 minutes.

Negative- No agglutination.

Latex Agglutination Test (LAT):

CSF samples were tested for bacterial antigen detection using Biorad's pastorex bacterial antigen kit, a latex test to detect antigens of the following 5 organisms.

- E coli K1 antigen
- N meningitidis A,B,C,Y,W 135 antigen
- Streptococcus pneumonia antigen
- Group B streptococcus antigen
- H influenza type b antigen.

Meningococcus group B antigen being structurally and immunologically related to E coli K1 antigen is provided as a single test latex reagent and depending on the age of the patient. Positive reaction in neonatal specimen would suggest E coli infection and in older children and adults meningococcus B group is more likely infection and correlating with direct smear examination of CSF.

Procedure: CSF was preheated to 100°C in a water bath for 5 minutes, cooled to room temperature and centrifuged to remove the proteinaceous material that would react with the antigen to minimize non-specific reactions. The supernatant was then used for LAT test.

Disposable reaction cards containing 9 separate circles with the colour code of different latex reagents (colored monoclonal antibodies) provided with the kit.

One drop (40µl) of CSF was placed on the separate circle of the reaction card and one drop of five latex reagents were added to the separate circles, mixed thoroughly and manually rotated for 5 minutes and observed for agglutination. Positive and negative control latex tests were put up simultaneously. Positive test was identified by agglutination reaction on the test card and seen visually under direct light source.

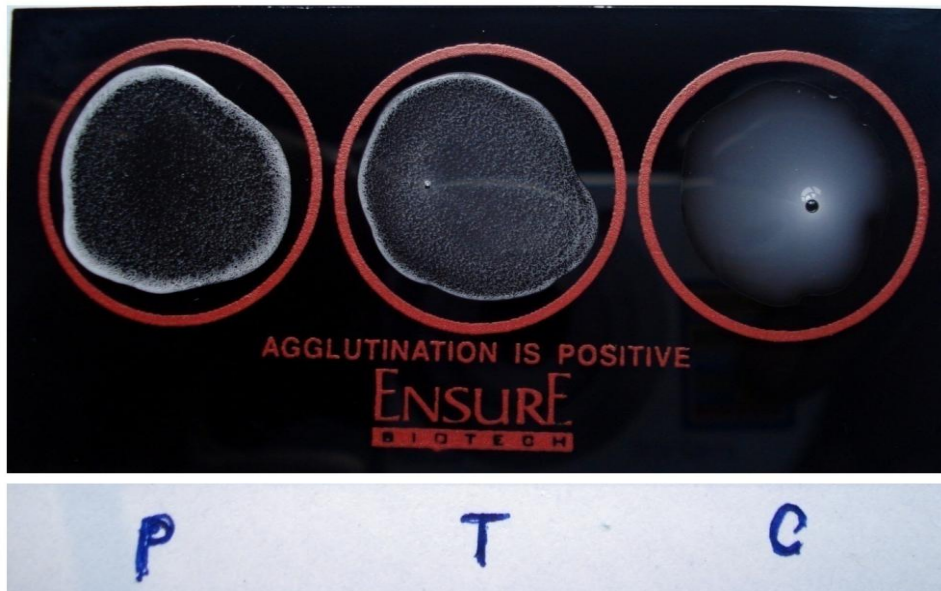
Interpretation of Results:

Positive - Fine agglutination (Clumps) visible to the naked eye.

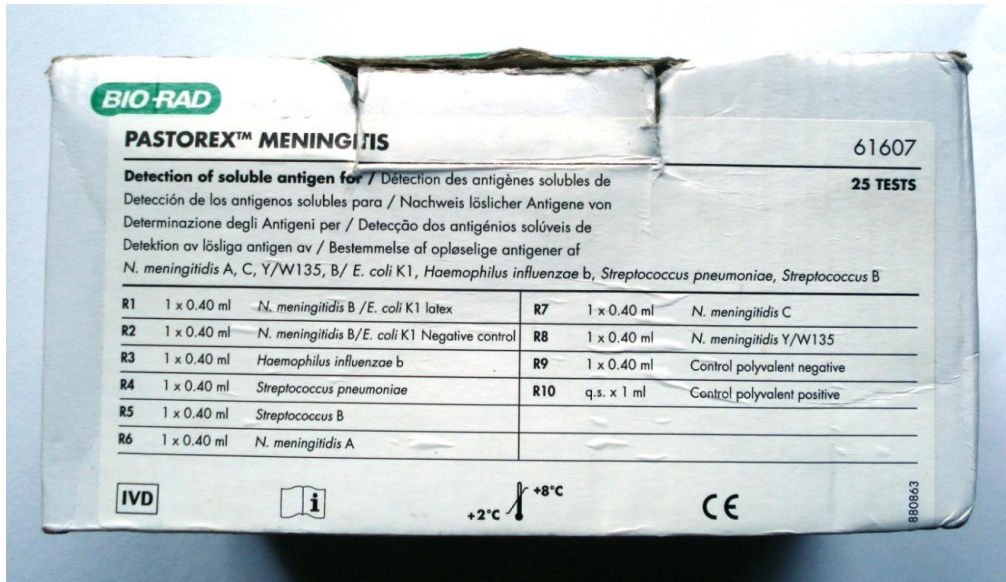
Negative - Homogenous suspension without clumps.



C-reactive Protein Test Kit



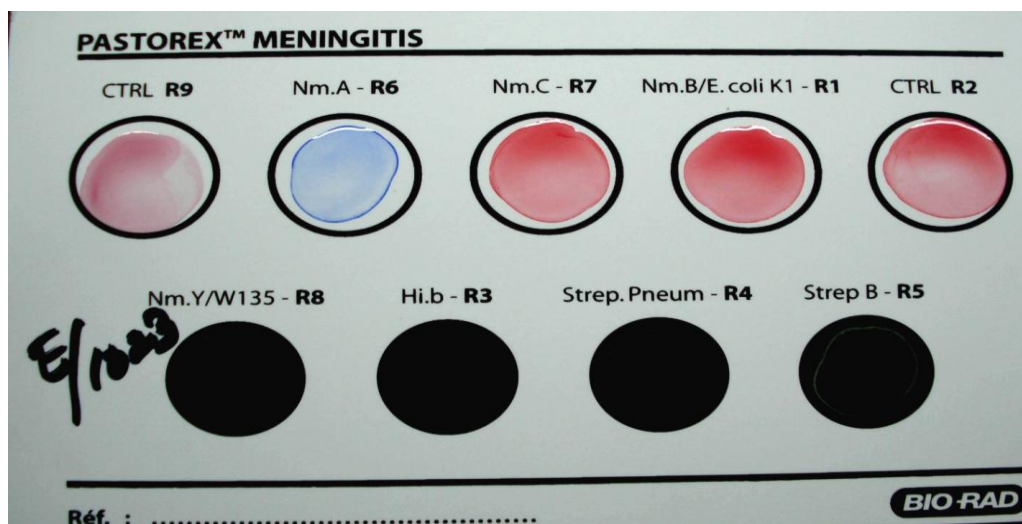
C-reactive Protein Test Result



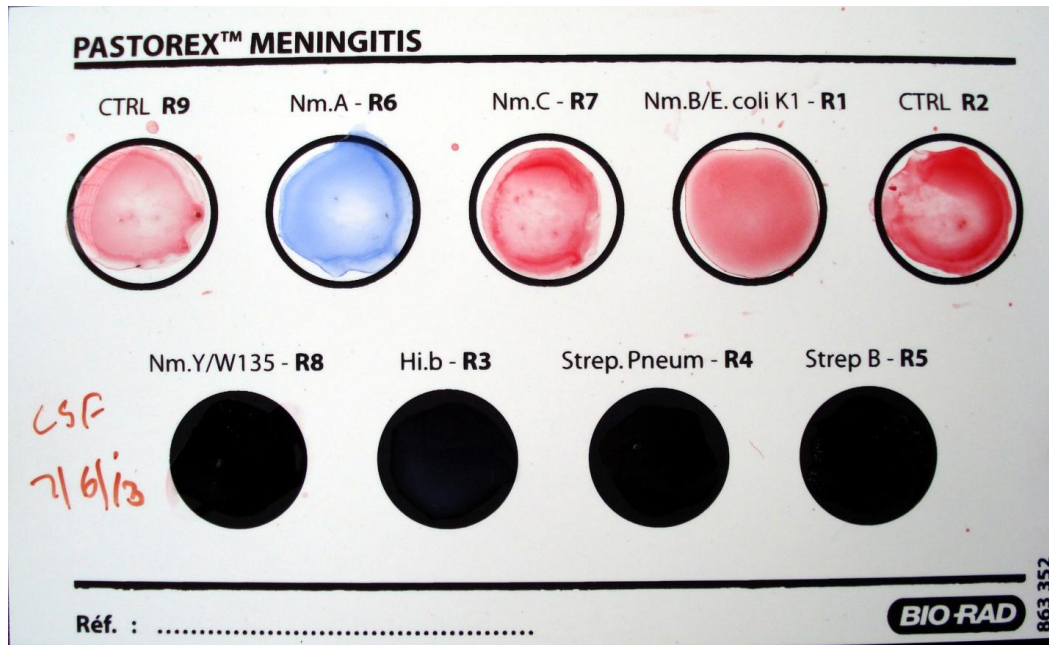
Biorad's Pastorex meningitis



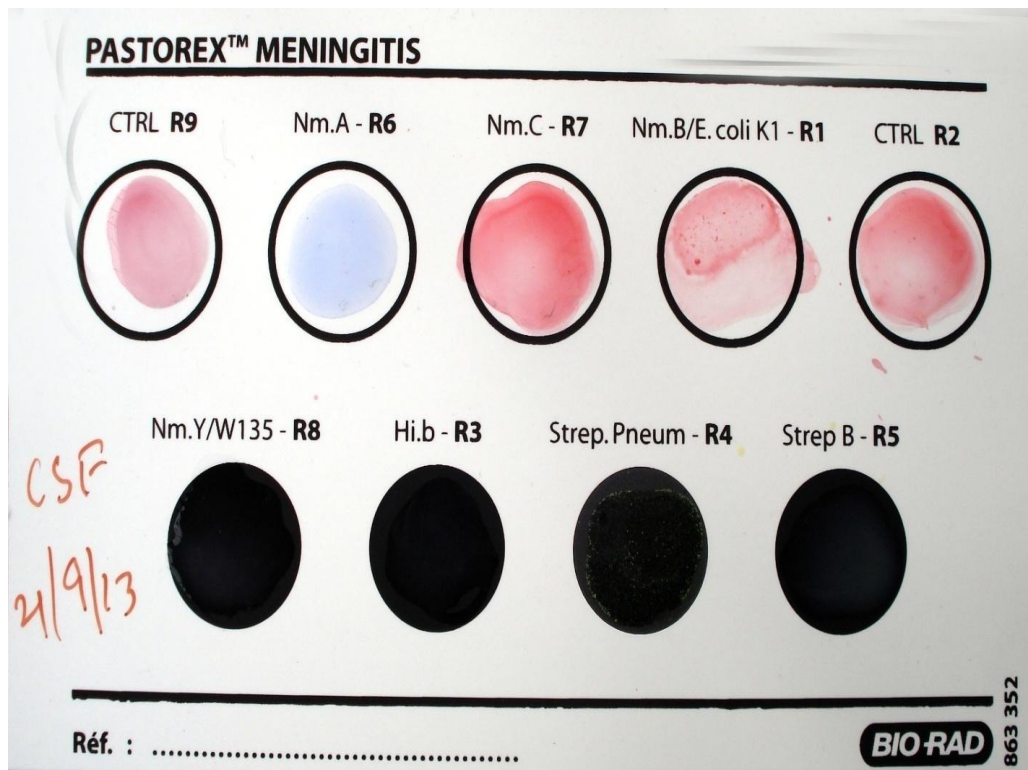
Pastorex meningitis Kit reagents



Neisseria meningitides A – Positive
Group B Streptococci – positive



E coli K1 – positive



Streptococcus pneumoniae – positive

III. Results

The present study was carried out in the Department of Microbiology, Guntur Medical college, Guntur for a period of one year ,100 cases of suspected meningitis aged from 1 day to 75 years who were admitted into NICU, PICU of Paediatric department, Medical and Neuromedicine wards of Government General Hospital, Guntur were investigated for laboratory diagnosis of meningitis by collecting CSF samples

Gram stain,C-reactive protein test (CRP), and Antigen test (Latex agglutination test for 5 antigens) (LAT) carried out for the above 100 samples of CSF In the present study of 100 suspected cases of meningitis, 38 cases (38%) were positive by Gram stain, CRP test and Latex agglutination test (5 antigen test) (Table-

I).In the present study of the 100 suspected cases, 60 (60%) were males and 40(40%) were females, making a ratio of 1.5:1.

Results show that out of 100 cases, 38 cases(38%) positive for bacterial meningitis. The male and female ratio for positive cases was 1.375:1 (males 22, 57.89%) (females16, 42.1%)

In the neonatal age group, out of 8 cases 4 (10.5%) were positive.

In infant age group, out of 12 cases, 6(15.78%) cases were positive.

In children age group (2-15yr), out of 26 cases, 12 (31.57%) were positive.

In the adult age group (16-55yr), out of 52 cases, 16 cases (42.1%) were positive.

In elderly group, out of 2 cases, no case is positive. (Table-II).

Meningococci 8 cases(21.05%) were predominantly seen in the children age group.

Pneumococci were predominantly seen in the adult age group 10 cases (26.31%).

Group B Streptococci were seen in the infant age group 2 cases(5.26%). E coli 4cases(10.52%) were seen in the neonatal age group.

Staphylococcus aureus seen in the 4 cases(10.52%) infant age group. (Table-IV)

Out of 38 positive cases, Direct Gramstain positive in 24 cases (63.15%), .

LAT positive in 26 cases (68.42%).

CSF CRP positive in 20 cases (52.63%), Serum CRP positive in 16 cases (42.10%). (Table-V)

Out of 38 positive cases, 14 (36.84%) were S.pneumonia, 14 (36.84%) were N meningitidis. 4 (10.52%) were S aureus, 4 (10.52%) were Escherichia coli, 2 (5.26%) were Group B streptococci. (Table-VI).

In Direct smear examination of CSF, out of 24 (63.15%) Gram stain positive cases,14 (58.33%) were Streptococcus pneumonia , 6 were Neisseria meningitidis (25%). Other organisms like Staphylococcus aureus seen in 4 cases (16.66%), Escherichia coli and Group B streptococci reported nil.(Table-VII)Latex Agglutination Test detected 26 organisms (68.42%), of which 6 (23.07%) were S pneumonia, 14 (53.84%) were N meningitidis, 4(15.38%) were Escherichia coli, 2 (7.69%) were Group B streptococci. (Table-IX) Group B Streptococci (2 cases, 5.26LAT detected 14(36.84%) cases of meningococci but Gram stain and Culture detected only 6 (15.78%) cases of meningococci %) and E coli (4 cases,10.52%) were detected by LAT onlyThe sensitivity and specificity LAT was 100% and 100%. The sensitivity and specificity for CSF CRP was 52%and 100% and Serum CRP was 42% and 100%. (Table-XII).

TABLE-I Prevalence of Bacterial meningitis cases:

Total no. of cases	Positives	Percentage(%)
100	38	38%

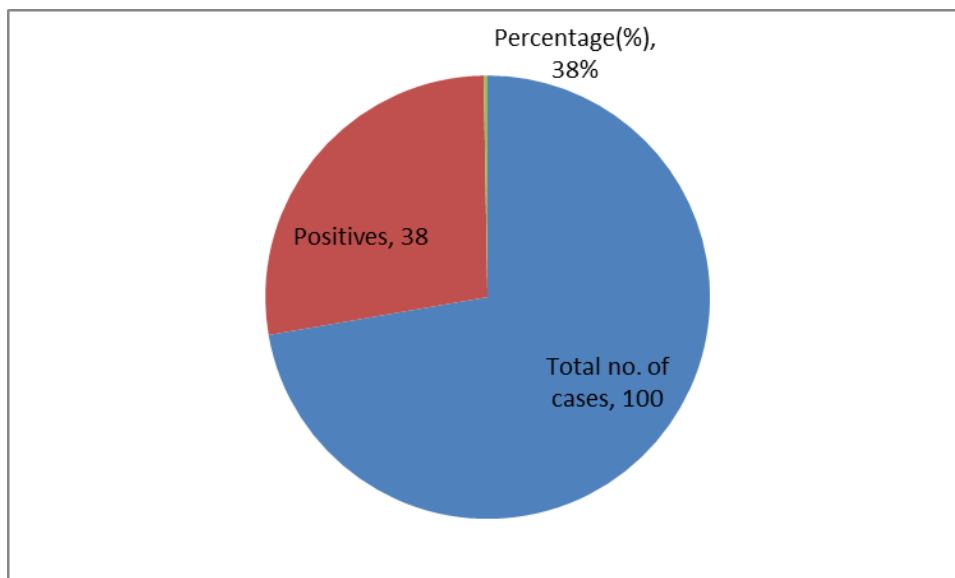


TABLE-II Age and Sex- wise distribution of Cases:

Age	Total no.of cases	Male	Positive cases (%)	Female	Positive cases (%)
0-1 month	8	4	2(5.26%)	4	2(5.26%)
2 month to 1 yr	12	4	2(5.26%)	8	4(10.5%)
2 yr – 15 yr	26	16	8(21.05%)	10	4(10.5%)
16 yr – 35 yr	36	20	8(21.05%)	16	4(10.5%)
36 – 55 yr	16	14	2(5.26%)	2	2(5.26%)
56 -75 yr	2	2	0	0	0
Total	100	60	22(57.89%)	40	16(42.1%)

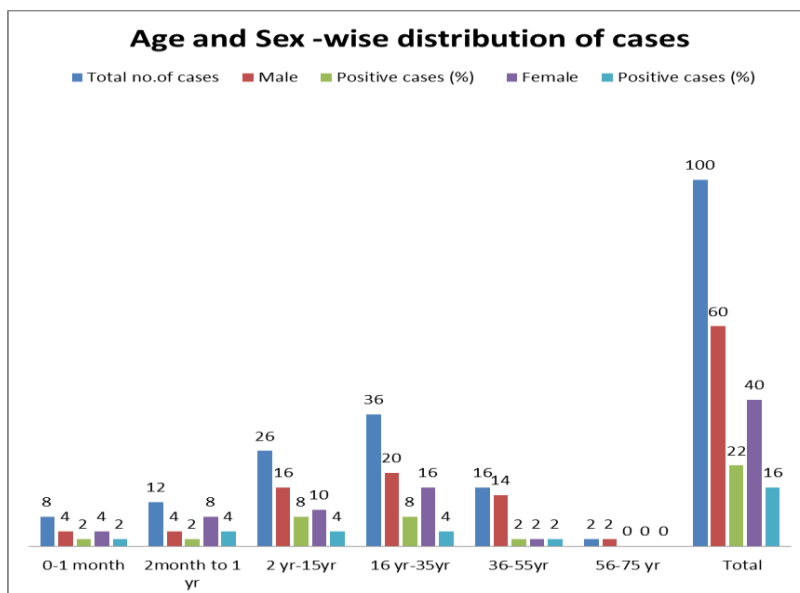


TABLE-V Laboratory confirmed cases of meningitis

Laboratory test	No. of cases positive	Percentage
Direct Gram stain	24	63.15%
Culture	24	63.15%
Latex agglutination test (LAT)	26	68.42%
CSF C-reactive protein (CRP)	20	52.63%
Serum C-reactive protein (CRP)	16	42.10%
Total Positive cases	38	100

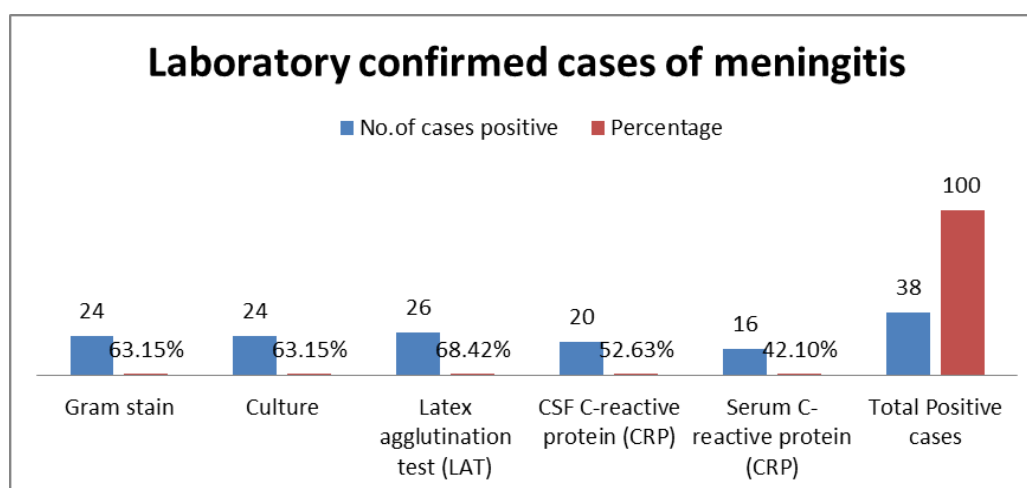


TABLE-VI Total number of organisms identified in the present study

Organisms	No. detected	Percentage (%)
S pneumonia	14	36.84%
N meningitidis	14	36.84%
S aureus	4	10.52%
Escherichia coli	4	10.52%
Group B streptococci	2	5.26%
	38	100%

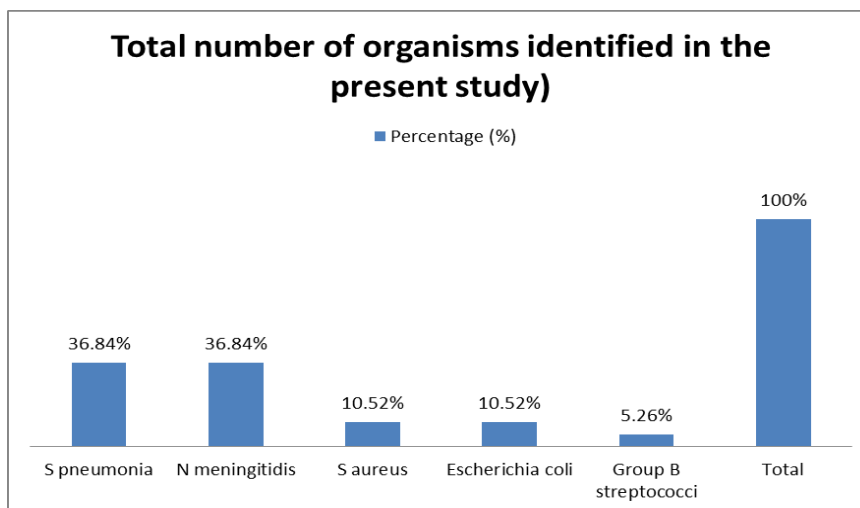


TABLE-VII Organisms detected by Direct Gram stain

Organisms seen in Direct Gram stain	Total no. of organisms seen	Percentage(%)
S pneumonia	14	58.33%
N meningitidis	6	25%
Staphylococcus aureus	4	16.66%
Escherichia coli	-	-
Group B streptococci	-	-
Total Gram stain Positive cases	24	100%

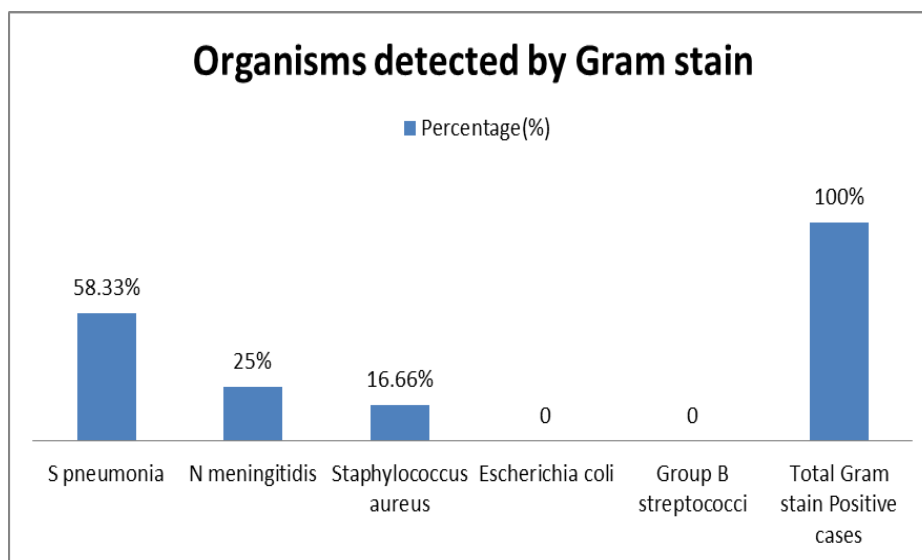


TABLE-IX Organisms detected by LAT in the study

Organisms detected by LAT	Total no. of organisms detected	Percentage (%)
Streptococcus pneumonia	6	23.07%
N. meningitidis	14	53.84%
Escherichia coli	4	15.38%
Group B Streptococci	2	7.69%
H influenzae B	-	-
Total positive cases	26	100%

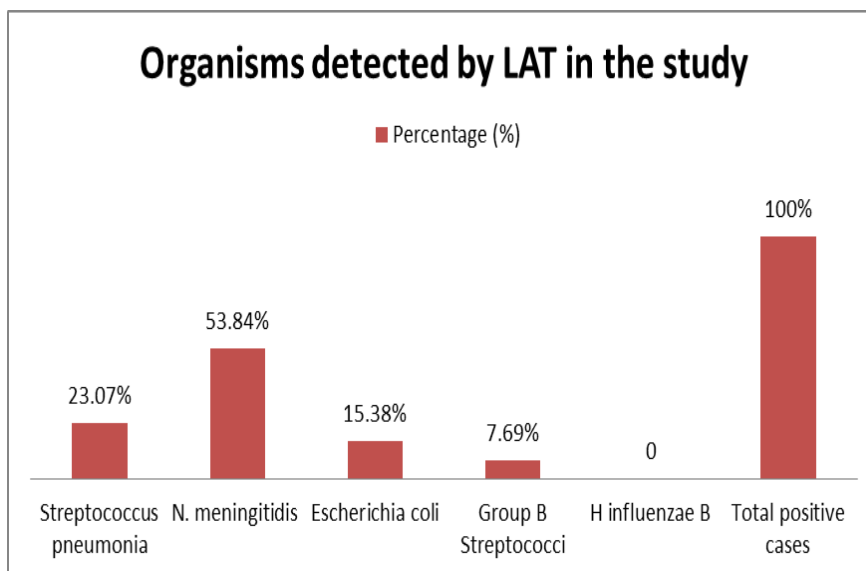


TABLE-X Comparative analysis of Direct Gram stain, culture and LAT

Organism identified	Total organisms isolated	Direct Gram stain positive	Culture positive	LAT positive
Pneumococci	14	14	14	6
Meningococci	14	6	6	14
Group B Streptococci	2	-	-	2
Escherichia Coli	4	-	-	4
Staphylococcus aureus	4	4	4	-
H influenza	0	0	0	0
	38	24	24	26

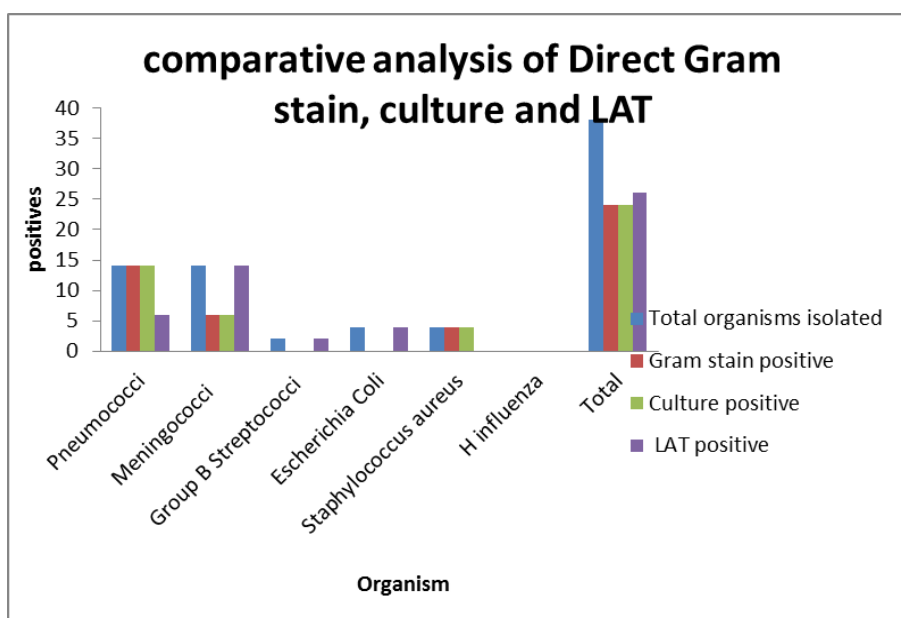
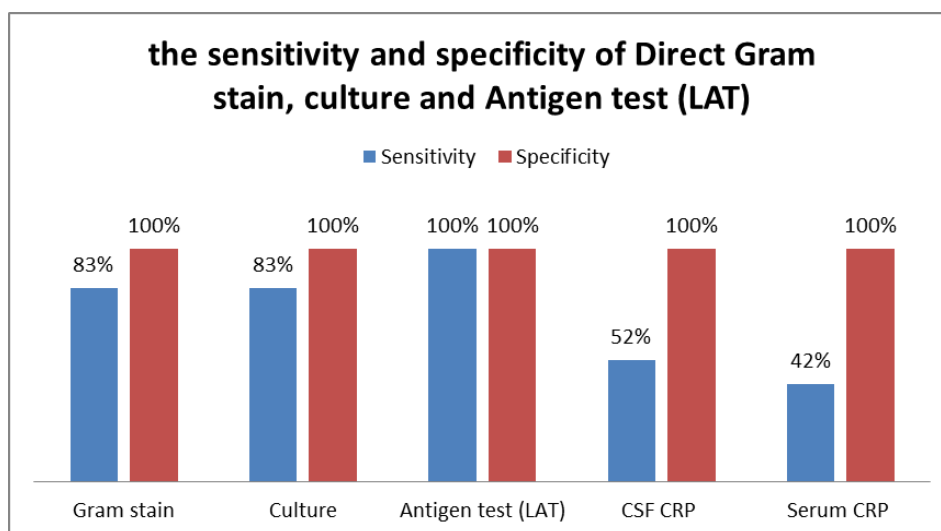


TABLE-XII The sensitivity and specificity of Gram stain, culture and Antigen test (LAT).

	Direct Gram stain	Culture	Antigen test (LAT)	CSF CRP	CRP
Sensitivity	83%	83%	100%	52%	42%
Specificity	100%	100%	100%	100%	100%



IV. Discussion

The present study was taken up in the Department of Microbiology, Guntur Medical College, Guntur for a period of one year

100 cases of suspected meningitis aged from 1 day to 75 years who were admitted into NICU, PICU of Paediatric department, Medical and Neuromedicine wards of Government General Hospital, Guntur were investigated for laboratory diagnosis of meningitis by collecting CSF samples, Gram stain, C-reactive protein test (CRP), Antigen test (Latex agglutination test). In the present study, as per WHO criteria of a proven case of bacterial meningitis, 38 cases (38%) out of 100 suspected cases of bacterial meningitis were laboratory confirmed as bacterial meningitis for 5 antigens carried out for the above 100 samples of CSF. The Gram stain is rapid and less expensive, but the detection of microorganisms depends on number of organisms present, prior use of antibiotics, staining technique and observer's skill.

In the present study, 52.63% of cases were CSF CRP positive, CRP positivity decreases in patients with partially treated bacterial meningitis.⁵ so the decrease in positivity can be explained by the pretreatment of bacterial meningitis. As GGH, Guntur is a tertiary care hospital, most of the cases referred from peripheral hospitals and treated elsewhere, CSF CRP positive cases should be considered as pyogenic meningitis unless proved otherwise. Routine use of this simple, reliable and inexpensive test is recommended for rapid diagnosis and differential diagnosis of meningitis. CSF CRP test shows more sensitivity and specificity (100%), Assays of CRP may be used as differential tests for bacterial meningitis versus viral meningitis, when assay is done before the antibiotic treatment, being sufficient sensitive and easy to perform.

The value of CRP lies in the management of Direct Gram stain negative bacterial meningitis cases in whom delay in the specific treatment till the culture report were available will be devastating.

The present study showed LAT positivity of 68.42%, Antigen may persist in the CSF even after lysis of bacteria with pretreatment of bacterial meningitis cases. Positive result depends on the presence of detectable level of antigen in the body fluid

The increase in percentage of LAT in relative to the Gram stain can be explained by the pretreatment of bacterial meningitis cases. Since identification of organism is very important to institute appropriate antibiotics, rapid diagnostics methods like LAT have an important role in the management of bacterial meningitis cases.

With the LAT a rapid bacteriological diagnosis is possible within 15 minutes and the kit is able to identify bacterial antigens even with negative culture and after initiation of antibiotic treatment.

In patients with antibiotic therapy before lumbar puncture or where culture is negative, LAT can detect comparatively very small quantity of antigen present and is highly sensitive and specific, simple to perform and results are available rapidly in 10 minutes. reported by non-specialized laboratory technicians and no alteration of results by prior antibiotic therapy.

Negative test never rules out bacterial meningitis.

LAT is a useful diagnostic test for bacterial meningitis especially in developing countries where laboratory facilities are limited.

The non-specific nature of clinical presentation, especially in children and lack of laboratory facilities can delay or obscure diagnosis.

LAT is more useful in neonatal and children with bacterial meningitis who frequently present with non-specific symptoms.

LAT is a cost effective and rapid test compared to culture and can be useful in early institution of therapy and ensure better prognosis.

LAT is rapid, and simple to perform, yielding serotype data (Pneumococci) directly by testing of cerebrospinal fluid.

LAT was more sensitive than Gram stain and culture in identifying the fastidious organisms like H influenza, N meningitidis with abnormal CSF parameters and in pretreated cases.

V. Conclusions

Bacterial meningitis is an important serious illness world-wide and is a medical emergency. It still remains major cause of death and long term neurological disabilities. Aim of the study was to do a comparative evaluation of CRP, LAT with Gram stain of Bacterial meningitis. Prompt diagnosis and aggressive management are the goals for which we need laboratory diagnosis as early signs and symptoms are often non-specific. Culture is a gold standard technique but it takes 24-48 hours for reporting. Gram's stain detects most of the cases immediately. LAT detects more cases, more sensitive and detects cases even not reported by culture due to pretreatment of cases and reports immediately, hence most useful in detecting organisms in bacterial meningitis quickly, and is a useful adjunct to Gram's stain and Culture. CSF CRP are non-specific and can be raised in inflammations also but is useful in combination with LAT in detecting bacterial meningitis cases from other causes.

So Gram's stain, LAT and CRP when added together detects almost all cases of meningitis early and suggests prompt treatment of bacterial meningitis at early stages to prevent deaths and complications, disability (morbidity and mortality).

Meningococci and pneumococci are still the important causes of bacterial meningitis in both children and adults.

The study of bacterial meningitis is important as it reflects the diagnostic abilities of various tests available to detect early and also to prevent deaths and complications. Vaccine preventable organisms causing bacterial meningitis can be tried in immunization schedules in India particularly in children because it is cost-effective.

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