

Diagnostic Evaluation of Latex Agglutination Test and Study of Spectrum of Bacterial Pathogens in Neonatal Meningitis

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Abstract: Acute bacterial meningitis continues to be a major public health problem. Pretreatment with antibacterial agents in patients of purulent meningitis may modify the clinical picture and CSF findings. Culture of CSF takes 24-48 hours for isolating the causative organism. Latex Particle Agglutination Test (LAT) since antigen may persist in the CSF even after lysis of bacteria. These immunological test could prove useful in partially treated patients. The present study was therefore designed to evaluate the utility of Latex Agglutination Test (LAT).

Aims and Objectives

- Identification and antibiotic sensitivity pattern of Etiological Organisms in neonatal meningitis
- To evaluate the diagnostic utility of LAT

Material and Methods

- **Study design** : Descriptive study/Evaluation of diagnostic test
- **Study Period** : June – 2011 – May 2012
- **Study Place** : Meenakshi medical college &R.I

Study Population: All neonates admitted in newborn ward in the institute with clinical suspicion of sepsis or meningitis.

Results And Analysis; Most frequent organism isolated was *E.coli* in 11(40.7%) followed by *Klebsiella* in 7 (25.9%) neonates. The overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the LAT were 100%, 65.7%, 29.4%, 100% respectively.

Keywords; etiology, LAT, Newborn, meningitis, sepsis,

I. Introduction

Acute bacterial meningitis continues to be a major public health problem. Mortality in the acute stage is high, survivors are often left with crippling neuro developmental sequelae. Prognosis is largely determined by early diagnosis and prompt institution of specific antimicrobial therapy. Pretreatment with antibacterial agents in patients of purulent meningitis may modify the clinical picture and CSF findings. So distinctive etiological diagnosis becomes difficult. Gram stain of CSF can provide a rapid preliminary identification of infective organisms, but is liable to misinterpretation particularly in inexperienced hands. culture of CSF takes 24-48 hours for isolating the causative organism. Furthermore culture may not be always positive especially in children who have received antibiotics prior to hospitalization.

Bacterial polysaccharide antigen of capsulated micro-organism can be detected by newer immunological test like Counter Immunoelectrophoresis (CIE), Latex Particle Agglutination Test (LAT) since antigen may persist in the CSF even after lysis of bacteria. These immunological test could prove useful in partially treated patients. The present study was therefore designed to evaluate the utility of Latex Agglutination Test (LAT), to study the spectrum of bacterial pathogens causing acute bacterial meningitis in neonatal period.

1.1 Latex particle Agglutination Test (LAT)

Latex agglutination test is simple to perform and very useful for rapid diagnosis of bacterial antigen in CSF. The test uses the technique of absorbing antiserum for specific antigen onto latex particles of uniform diameter, generally 0.8 microns. Latex particles are then washed to remove unabsorbed antiserum and are resuspended in an appropriate dilution. When the "sensitized" latex particles are placed in solution such as CSF, supernatant of blood containing specific antigen, visible agglutination occurs.

Latex agglutination test require that known positive and negative control specimens be incubated with the sensitized Latex particles, in addition, control latex particle which have not been sensitized are incubated with patient specimens and with negative and positive control in order to detect non-specific agglutination. False positive results are observed in sera containing rheumatoid factor.

LAT detect 0.5ng/ml of Polyribose phosphate¹³ in CSF as well as in serum. It detects antigenemia reliably with as little as 10² colony forming unit of H. influenza type b per ml of serum. LAT detects antigen in CSF earlier in the course of meningitis.

LAT is more sensitive than CIE in identifying group and type specific antigen of GBS¹³.

LAT appears to be a sensitive and specific technique for the detection of bacterial and fungal polysaccharide antigens.

Other immunological test for rapid diagnosis of bacterial meningitis

- ❖ Counter immuno electrophoresis (CIE)
 - ❖ Staphylococcal coagglutination test
 - ❖ Fluorescent antibody staining
 - ❖ Capsular swelling test – S.pneumoniae
 - ❖ Limulus amoebocyte lysate – endotoxin assay for gram negative bacteria.
- ESR, CRP, haptoglobin, fibrinogen, nitroblue tetrazolium dye, IL6, procalcitonin and leukocyte alkaline phosphatase are not specific for meningitis, but evidence for inflammatory response.

II. Aims and Objectives

- 2.1 Identification and antibiotic sensitivity pattern of Etiological Organisms in neonatal meningitis
- 2.2 To evaluate the diagnostic utility of latex agglutination test.

III. .Material And Methods

3.1 Study design : *Discriptive study/Evaluation of diagnostic test*

3.2 Study Period : *June – 2011 – May 2012*

3.3 Study Place : *Meenakshi medical college and R.I*

3.4 Study Population: All babies of both sexes belonging to Neonatal period, admitted in newborn ward in the institute with strong clinical suspicion of sepsis or meningitis.

3.5 Inclusion criteria

Clinical features suggestive of sepsis/meningitis in the form of irritability, lethargy, poor feeding, convulsions, with or without fever and with or without bulging anterior fontanelle and atleast two of five following positive CSF laboratory test.

- 1) Spinal fluid pleocytosis > 5 polymorphs / mm³
- 2) Decreased spinal fluid sugar < 40 mg% (or) < 50% of a simultaneously obtained blood sugar
- 3) Spinal fluid protein >120 mg%
- 4) Definite bacteria on stained smear
- 5) Positive spinal fluid culture

Irrespective of pretreatment with antibiotics.

3.6 Exclusion criteria

- Congenital malformation of spinal cord/ central nervous system.
- Birth Asphyxia.
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3.7 Sample size

40 neonates fulfilling the above inclusion criteria were recruited over a period of one year.

IV. Maneuver

All neonates of both sexes presenting with clinical features of meningitis / sepsis admitted in the newborn unit during the study period were subjected to detailed physical examination and CSF analysis. 40 neonates fulfilled the criteria for bacterial meningitis and were recruited. These neonates were followed up for clinical outcome.

Sepsis screening done for the study population included hematological investigation – complete blood count, peripheral smear, biochemical investigations – blood sugar, calcium, electrolytes and microbiological investigation including non enteric culture.

The CSF sample for analysis was obtained by Lumbar puncture under strict aseptic precaution in a sterile container. The physical character of the sample, whether clear, turbid or purulent were noted. The sample was stained with methelene blue and presence and number of Polymorphonuclear cells / hpf were counted. The CSF sample was also subjected to culture. CSF was also tested for bacterial antigen by latex agglutination test by using wellcogen bacterial antigen kit (Murex – UK).. This kit provide a series of rapid latex test for qualitative detection of antigen from group B streptococci, H.influenzae type b, Streptococci pneumonia, N.meningitides A, C, Y and W 135, E- coli K1, present in CSF as a consequence of infection.

4.1 Principle of the Procedure

Wellcogen reagent consist of polystyrene latex particles which have been coated with antibodies to the bacterial antigen. The streptococcus and H.influenzae reagents are group B and type b specific respectively. S.pneumoniae reagent sensitized with antibodies purified from omnivalent serum, N.meningitides reagent are polyvalent. Meningococci group B and E-coli K1 are structurally and immunologically same.

4.2 Description of reagent and recommended storage conditions

All components should be stored at 2-8°C.

4.2.1 Test Latexes

0.5% suspension of polystyrene latex particles in buffer containing 0.1% sodium azide as a preservative. The latex particle coated with the appropriate rabbit antibody, except for the N.meningitides group B / E.coli K1 coated with murine monoclonal antibody.

4.2.2 Control Latex

Latex particle coated with an appropriate preparation of rabbit globulin (or) in the case of N.meningitides group b / E.coli K1 latex particle coated murine monoclonal antibody raised against bordetella bronchiseptica.

4.2.3 Polyvalent Positive Control

Freeze dried bacterial extracts containing antigen from representative strains of each bacterial species for which latex is provided. Reconstituted antigen stored at 2-8°C for upto 6 months.

4.3 Specimen Collection and Storage

Body fluid sample (CSF, Serum, Urine) should be tested as soon after collection as possible, if the fluid cannot be tested immediately it may be stored overnight at 2-8°C (or) for longer period frozen at 15 to 25°C.

4.4 Blood culture

Tested after 18-24 hours incubation at 37°C and (or) as soon as bacteria growth is observed.

4.5 Plate culture

Isolated colonies may be tested after overnight incubation at 37°C.

4.6 Interpretation of Results

Clear agglutination of a single test latex accompanied by negative reaction with all other test latex reagents and control latex, indicates presence and identity of a bacterial antigen in the test sample.

The sensitivity of each latex in detecting bacterial antigen is 67% for Group b streptococci, 97% for H.Influenzae type b. 88% for Streptococci pneumonia. 71% for N.Meningitides A CY 135 and 65% for E-coli K1. The specificity of all 5 test latexes on CSF was greater than 98%.

4.7 Limitation of procedure

Positive result depend on the presence of detectable level of antigen in the body fluid. Positive results for group B streptococcus beyond 6 months of age should be interpreted with caution. Limited clinical data are available for the detection of antigen in urine (or) serum using N.Meningitides B/ E. Coli K1. Cross reaction is common with unrelated bacteria which posses common antigen and also with rheumatoid factor. Radiological investigation done included chest radiograph and cranial ultrasonogram. The presence of ventriculitis, ventriculomegaly and subdural effusion were noted.

The clinical progression of the disease and treatment during the stay in the hospital were monitored and immediate complications if any, were looked for.

Outcome measure noted were death due to the illness and recovery with without immediate complications.

Death due to illness was defined as the newborn dying at any time during hospital stay.

V. Results And Statistical Analysis

Sensitivity, specificity, positive predictive value and negative predictive value of latex agglutination test against the gold standard of culture were calculated.

Organisms identified in CSF samples by conventional method (gram stain and culture) as well as by Rapid diagnostic method were tabulated as follows

5.1 Identification of Etiological Organisms by Gram stain, Culture and Latex Agglutination test

Table – 1

Gram stain	N = 27
Gram Positive	2
Gram Negative	10
Total	12

Organism	Culture	Latex	Total
E-coli	4	11	11
Klebsiella	7	0	7
H.influenzae	0	4	4
Psudomonas	2	0	2
Pneumococci	1	2	2
Staph aureus	1	0	1
Total	15	17	27

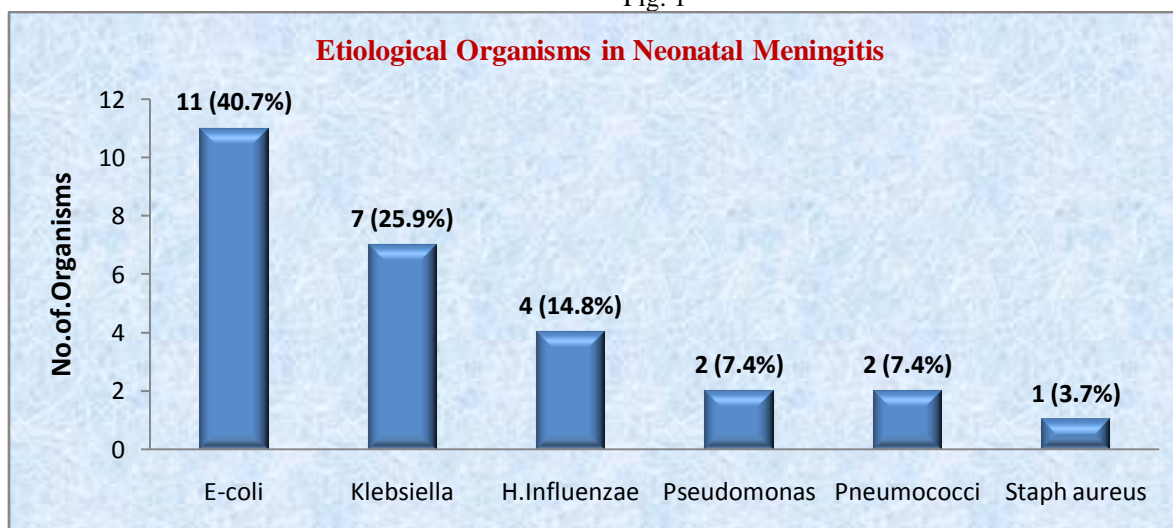
Of the neonates in the study, organisms were isolated from 27 (67.5%) neonates either by gram stain 12(30%) or culture 15 (37.5%) or LAT 17 (42.5%). Gram negative bacteria was found in 24(88.8%) neonates while gram positive bacteria was found 3(11.2%). 5(20%) neonates showed positively both for culture as well as LAT. 10(37%) neonates were positive only by culture and 12(44%) neonates were positive only by LAT.

Most frequent organism isolated was E.coli in 11(40.7%) followed by Klebsiella in 7 (25.9%) neonates. Other organisms isolated were H.Influenzae in 4 (14.8%), Pseudomonas in 2(7.4%), Pneumococcal in 2(7.4%) staphylococci in 1(3.7%). All the 11 neonates of E.coli meningitis were picked up by LAT and 4 were also identified by culture.

In the study, 4 cases of H.Influenzae were picked up only by LAT, 7 cases of Klebsiella, 2 cases of Pseudomonas, 1 case of staphylococci identified only by culture. The rapid antigen test for the above organisms could not be done due to the lack of availability of these antigens in the panel of the test kit.

In study population 14 cases had received pretreatment with antibiotics for 48 – 72 hours prior to admission. Of these culture was positive in 1 (7%) case and LAT identified the organism in 4 (28.6%) cases.

Fig: 1



The diagnostic efficacy of LAT was compared to the gold standard of culture.

5.2 Sensitivity and specificity analysis of LAT by culture

Table-2

		Culture		Total
		Positive	Negative	
Latex Agglutination Test	Positive	5 (a)	12(b)	17
	Negative	0 (c)	23 (d)	23
	Total	5	35	40

Sensitivity - 100%

Specificity - 65.7%

Positive Predictive Value - 29.4%

Negative Predictive Value -100%

Total culture positive cases were 15, of which 3 organisms namely klebsiella(7), Pseudomonas (2), staph aureus (1) constitute 10 cases. The antigen of these organisms were not present in the panel of rapid diagnostic kit, those were excluded to evaluate the diagnostic efficacy of LAT. The overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the LAT were 100%, 65.7%, 29.4%, 100% respectively.

5.3 Sensitivity Pattern of organisms to various antibiotics depicted as tabular form as follows

Table – 3

Antibiotics	N=15(%)
Cefotaxime	10 (66.6%)
Ciprofloxacin	5 (33.3%)
Amikacin	8 (53.3%)
Ceftazidime	5 (33.3%)
Cotrimaxazole	4 (26.6%)
Ampicillin	2 (13.3%)
Garamycin	2 (13.3%)
Cefotaxime (or) amikacin	14 (93.3%)
Cefotaxime (or) Ampicillin	10 (66.6%)
Cefotaxime (or) amikacin (or) Ampicillin	14 (93.3%)

Out of 15 culture positive CSF samples, 10 (66.6%) samples were sensitive to cefotaxime, 8(53.3%) were sensitive to ciprofloxacin, 5(33.3%) samples were sensitive to Amikacin and 5(33.3%) sensitive to Ceftazidime. Sensitivity to Cotrimaxazole was obtained in 4(26.6%) samples. 93.3% of organisms were covered by combination of Cefotaxime and amikacin as well as combination of Cefotaxime, amikacin and ampicillin. While Cefotaxime and ampicillin combination covered only 66.6% of the organisms.

VI. Discussion

The neonate is extremely vulnerable to infections in the first 28 days of life. Death and morbidity during this period are very high. Sepsis accounts for 25-40% of all neonatal death, meningitis can occur alone or as a part of sepsis in neonatal period. Meningitis in newborn has varied clinical presentation and a rapid and often fatal course. Hence early diagnosis of meningitis is important to influence the outcome.

Since identification of organism is very important to institute appropriate antibiotics rapid diagnostic methods like LAT have an important role in the management of neonatal meningitis. The evaluation of LAT on its ability to pick up the etiological organism has been done by various authors and published namely, H.C Whittle et al.,³, Joel I et al.,⁴, Bijay et al.,⁶, Harcharan singh et al.,⁵, N.Deivanayagam T.P et al.,⁷, B.K. Das et al.,⁸

This study was done to evaluate the diagnostic utility of one such kit namely the Wellcogen antigen kit to identify five organisms namely group B streptococcus, haemophilus influenza type b, streptococcus pneumonia, neisseria meningitides (Group A, C, Y W 135) E.coli K1.

In our study gram negative bacteria were the most common causative organism. Which is comparable to the results of J.S Yu et al.,⁽¹⁾ James overall et al.,⁽²⁾. Several factors account for the susceptibility of the neonate to infection with these agents. The fetus is frequently exposed to enteric bacteria during the course of maternal peripartum infection. Postnatally the infants are exposed to gram negative organism through humidification apparatus; resuscitation equipments or article used in daily care. The newborn has been shown to have a lack of serum bactericidins against gram negative bacilli.

The sensitivity, specificity, positive predictive value and negative predictive value of Latex agglutination test were 100%, 65.7%, 29.4%, 100% respectively. Comparable efficacy was also observed in various studies including. H.C. Whitte et al.,⁽³⁾ Joel I et al.,⁽⁴⁾, Bijay et al.,⁽⁶⁾ Harcharan singh et al.,⁽⁵⁾, N.Deivanayagam T.P et al.,⁽⁷⁾, B.K. das et al.,⁽⁸⁾.

Latex agglutination test with few antigens in the panel (E.coli, H.influenzae, S.pneumoniae, N.Meningitides, group B Streptococcus) alone may not be sufficient to identify the organisms in neonatal meningitis. However, the diagnostic utility of LAT improve with culture and gramstain. With the sensitivity of 100% and negative predictive value of 100%, it may be useful to identify neonate who present with symptoms suggestive of sepsis, it also helps to differentiate meningitis from other condition like inborn errors of metabolism which also has similar clinical symptoms.

The sensitivity pattern of organisms to antibiotics was analysed. The combination of cefotaxime and amikacin covered 14(93.3%) culture positive CSF samples out of 15. In view of rapidly emerging resistance to ampicillin in gram negative bacilli and opinion of expert from various centres in india, S.K.Kabra et al., recommended one of the third generation cephalosporin with an aminoglycoside for empirical therapy of bacterial meningitis during the first 3 months of life. Empirical therapy for neonatal meningitis administered in our newborn unit was consistent with these recommendation.

The overall mortality rate was 35% which is comparable to McCarthy. et al.,⁽⁹⁾ Victor A.E et al.,⁽⁹⁾ the overall mortality rate decreased from 60% as documented by James overall et al.,⁽²⁾ J.S. Yu et al.,⁽¹⁾ to 35%, as reported by recent studies^(9, 10) over a period of past 4 decades with the advent of newer antibiotics and early diagnosis by rapid methods.

VII. Summary and Conclusion

7.1 Gram negative organisms account for majority of the cases. Majority of them were sensitive to combination of one of the cephalosporins or aminoglycosides. The combination of antibiotics decreases the overall mortality rate.

7.2 Though the sensitivity of LAT was high (100%), Latex Agglutination Test with the current panel may not be adequate especially for neonatal meningitis. It need inclusion of other gram negative organisms like klebsiella, pseudomonas for better yielding. It can be used as an adjunctive test to conventional methods in the diagnosis of neonatal meningitis.

References

- [1]. J.S. Yu and A.Gravaog. Purulent meningitis in the neonatal period. Arch. Dis. Childhealth. 1963, 38:391.
- [2]. James C.Overall Jr.M.D. Neonatal bacterial meningitis. Journal of Paediatrics. April 1970: 76 : 499 – 511.
- [3]. H.C Whittle P.Tugwell, L.J Egler, B.M Greenwood. Rapid bacteriological diagnosis of pyogenic meningitis by latex agglutination test. The lancet Sep.14: 1974: 619-621.
- [4]. Joel I, Ward, M.D., George, R.Siber, M.D. Rapid diagnosis of Haemophilus influenza type b infections by latex particle agglutination and counter immune electrophoresis. The Journal of Pediatrics 1978: 93: 37-42.
- [5]. Harcharan Singh R.Sarkar. Immunological test in acute bacterial meningitis. Indian pediatrics Vol.25: 188.
- [6]. Bijay R. Mirdha, U.Gupta and R.A. Bhujwala Latex agglutination test. An adjunct to the laboratory diagnosis of pyogenic bacterial meningitis. Indian Journal of Paediatrics. 199 : 58 : 521 – 524.
- [7]. N.Deivanayagam, T.P Ashok, K.Nedunchelian, S.Shaffi Ahamad. N.Mala. Bacterial meningitis diagnosis by latex agglutination test Indian Pediatrics. Vol.30 : 1993: 495-499.
- [8]. B.K das Rajesh Lal Gurubhacharya, I.M Mohapatra and O.P Mishra Bacterial antigen detection test in meningitis. Indian Journal of Pediatrics. 2003: 70 (10) : 799-801.
- [9]. McCarthy A.E Victor G. Ramotar K. Toye B 1994 Risk factor for Acquiring ampicillin resistant enterococci and clinical outcomes at candian tertiary care hospital. Journal of clinical microbiology 32: 2671 – 2676.
- [10]. Nulder C.J.J Van Alphen L. Zanon H.C 1984 neonatal meningitis caused by E.coli in Netherlands. Journal of infections diseases, 935 – 940.