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Diagnostic Utility of Latex Agglutination Test and Antibiogram Of Isolates Causing Acute Bacterial Meningitis in Pediatric Age Group.

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ABSTRACT

To evaluate the gram stain, culture, latex agglutination test (LAT) and C-reactive protein (CRP) for the diagnosis of acute bacterial meningitis (ABM) and to determine the pattern of antibiogram of isolates. Cerebrospinal fluid **(**CSF) samples from 60 probable meningitis cases were processed by gram stain, culture, antigen detection by LAT, antibiogram and CRP. 21 cases were diagnosed as ABM by using 3 methods (gram stain, culture, LAT), among them 14(66.66%) were diagnosed by LAT, 13(61.90%) by gram stain and 09(42.85%) by culture respectively. CRP was positive in 42.85% of the cases. 66.66% cases had history of antibiotics prior to admission. Both sensitivity and specificity for LAT was 83.33%. *S.pneumoneae* 07(33.33%) was the predominant causative agent followed by Group B Streptococcus 05 (19.04%). Antibiogram showed that all the isolates were 100% sensitive to third and fourth generation cephalosporins (cefoperazone and cefepime) and 66.66% resistant to cotrimoxazole. Case fatality rate was 19.04%. ABM is a life threatening illness; rapid diagnosis and immediate treatment results in higher cure rate and lowers the incidence of potentially fatal complications. LAT was more sensitive than gram stain and culture in identifying the fastidious organisms like *H.influenzae, N.meningitidis* with abnormal CSF parameters and in pre treated cases. Cephalosporins (cefoperazone and cefepime) were most sensitive drugs for treating bacterial meningitis. Periodic review of cases to assess the local changing trend of the infecting organisms and their antibiogram is absolutely essential to determine the type of organism and their resistance pattern, to reduce the morbidity.

Keywords-Acute bacterial meningitis, Latex agglutination test, *S.pneumoniae*, CRP**.**

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INTRODUCTION

Bacterial meningitis is an important cause of mortality and morbidity in pediatric population.[1] Children are particularly vulnerable to ABM because of their relatively immature immune systems, particularly their impaired immunity to the polysaccharide capsule of bacteria commonly associated with ABM.[2] Almost all microbes that are pathogenic to human beings have the potential to cause meningitis, but a relatively small number of pathogens (*Group B Streptococcus, Escherichia coli, Listeria monocytogenes, Haemophilus influenzae type b, Streptococcus pneumoniae, and Neisseria meningitidis*) account for most cases of ABM in neonates and children.[3] The clinical features of bacterial meningitis in infants and children can be subtle, variable, non-specific, or even absent, so rapid examination of CSF is considered as an essential step in early diagnosis.[3] Also as a result of emergence of antimicrobial resistance being reported, recommendations for therapy are changing, so laboratory surveillance of isolates is crucial to identify targets for immunization, chart preventive stratergies & to formulate the rational empirical treatment for potentially fatal bacterial meningitis.[4] Among currently available diagnostics tests; CSF culture though a definitive diagnostic test, requires 24-48 hours and can gives false negative results due to improper specimen collection, transportation and known for poor positive reports under Indian conditions[5,6], the gram stain is rapid and less expensive, but the detection of microorganisms depends on of organisms present, staining technique and the observers skill. Though available literatures have shown that, the detection of soluble antigen in CSF has clinical advantage of high sensitivity and specificity and easy to perform but there are limited and inconsistent data regarding diagnostic accuracy, false positivity and effect of prior antibiotics. Hence, there is a need for more systematic studies to generate valid data for improved quality of care and therefore the present study was taken up.

MATERIALS AND METHODS

This prospective study was done to evaluate the gram stain, culture, latex agglutination test (LAT) and C-reactive protein (CRP) for the diagnosis of acute bacterial meningitis and to determine the pattern of antibiogram of isolates. After approval and clearance from the Institutional Ethics Committee, 60 CSF samples from clinically suspected cases of meningitis in pediatric age (Newborn to 18 year) were collected by pediatricians from subjects attending pediatric ICU and emergency wards at KIMS Hospital and Research Centre, Bangalore from January 2012 to June 2013. Clinical details were recorded and written informed consent was obtained from all the parents of study subjects after fully explaining the study procedure to their satisfaction, in both English and vernacular language. As per WHO criteria[7] of a probabale case of bacterial meningitis, subjects fulfilling the following inclusion criteria were included into the study;

- Clinically suspected cases of meningitis with CSF examination showing at least one of the following : (a). Leukocytosis (> 100 cells/mm3) (b) Leukocytosis (10-100 cells/ mm3) and either an elevated protein (> 100 mg/dl) or decreased glucose (< 40 mg/dl) .

- Willingness of the patients/parents or legal representatives to give the written informed consent. Patients with the following conditions were excluded from the study; No alteration of CSF parameters in clinically suspected meningitis cases.

Using all aseptic precautions, about 1-2 ml of CSF was collected in a sterile container, (preferably prior to the administration of antibiotics wherever possible) by lumbar puncture and sent to the clinical microbiology laboratory for confirmation of bacterial meningitis. Immediately after the arrival the macroscopic appearance of the CSF was recorded for turbidity. CSF was aliquoted into three test tubes. Sample from first test tube was used to analyze total WBC count, cell type, protein, glucose and CRP.

Sample from second test tube was centrifuged at 1500-3000 x g for 20 minutes and to prevent the possible contamination, the sediment was used first to inoculate the culture media and then direct smear was made by gram stain by using following techniques.

For culture and identification, the sediment of the centrifuged CSF was inoculated into the following media:

- Chocolate agar and sheep blood agar plate, and incubated at 370 C in 5-10% CO2.
- MacConkey agar plate and Brain heart infusion(BHI) broth and incubated aerobically at 370C

Inoculated primary plates were incubated for 48 to 72 hours. The plates were examined daily for 72 hours before discarding as negative. BHI broth was incubated for 7 days and examined daily for presence of growth or turbidity. The tube showing turbidity was sub-cultured on Chocolate agar plate and MacConkey agar plate. Tubes that remained non turbid were sub-cultured on the 7th day before discarding. Any growth on the above mentioned media were identified on the basis of their colony morphology, cultural characteristics and biochemical reactions according to standard techniques. The smears are made by placing 1 or 2 drops of sediments of CSF on an alcohol rinsed slide, allowing the drop to form a large heap and air dried. Air dried smears were heat fixed and stained by Gram's stain and observed for the presence of pus cells and organisms.

Antibiotic susceptibility testing was done by standard Kirby Bauer disk diffusion method as per the Clinical and Laboratory Standards Institute(CLSI) guidelines using the commercially available antibiotic discs from HiMedia(Mumbai,India). The antibiotics used for gram positive cocci were cloxacillin(1 µg), gentamycin (10µg), amoxicillin/clavulinic acid(30µg), erythromycin (15µg), ciprofloxacine(5µg), cefoperazone(75µg), cefepime(30µg), cotrimoxazole(23.75µg), clindamycin(2µg),tetracycline(30µg), vancomycin(30µg), linezolid(30µg). The antibiotics used for gram negative bacilli were ampicillin(10 µg), gentamycin(10µg), amikacin (30µg),amoxicillin/clavulinic acid(30µg), levofloxacin(5µg), ciprofloxacine(5µg), cefoperazone(75µg), cefepime(30µg), cefuroxime (30µg), cotrimoxazole(23.75µg), imipenem(10µg), piperacillin/ tazobactum(100/10µg).screening of possible ESBL production was done by using ceftriaxone(30µg), cefoperazone(75µg).Those isolates with zone diameters <25mm for ceftriaxone and <22mm for cefoperazone were considered for ESBL production.

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The third test tube was used for Bacterial antigen detection test (latex agglutination test); CSF samples were tested for bacterial antigen detection using WELLCOGEN bacterial antigen kit manufactured by Remel Europe Limited, UK to detect antigens of 5 organisms: *S.pneumoniae*, *Group B streptococcus, N. meningiditis ABCYW 135* , *H.influenzae type b* and *E coli K1* antigen. Test was performed and reports were interpreted according to the manufacturer's instructions.

RESULTS

Out of 21 (35%) laboratory confirmed cases of ABM (Figure- 1), majority of the subjects 13(61.90%) were under one year of age, of which 5(23.80%) were neonate as shown in Table 1, with a male to female ratio of 1.6:1. History of antibiotic administration prior to admission was observed in 66.66% cases. CSF analyses shown that, maximum cases of ABM of total WBC count was in the range of 101-500 cells /mm3 in 52.38%, CSF protein in the range of 151-200mg/dl in 47.61% and CSF glucose was in the range of 21-30mg/dl in 42.85% cases. Out of 60 cases tested CSF CRP was positive in 28.33% and of 21confirmed cases of ABM, 42.85% cases were positive for CSF CRP. In subjects with positive gram stain for ABM, pus cells and gram positive cocci in pairs was seen in 47.61%, and pus cells and gram negative bacilli in 14.28% cases. 38.02% cases of ABM were negative for gram stain. *S.pneumoniae* was the most predominant organism isolated by culture 14.28% cases followed by *Group B Streptococcus* (9.52%). *E.coli, K.pneumoniae, C.koseri and E.fecalis* isolated in 4.76% of each case. 51.14% cases of ABM were culture negative. Of the 14 LAT positive cases of ABM, *S.pneumoniae* was the most predominant organism identified in 28.57%, followed by *S.agalactiae* (19.04%), *H.influenzae b* (9.52%), *E.coli and N.meningitidis* in 4.76%. LAT was negative in 33.33% cases.

Table-1: Distribution of cases according to age and causative agent among the laboratory confirmed cases of ABM

Etiological agent	Total (%)	0-1month (neonate)	1m-3m	3m-1yr	1yr-5yr	5yr-10yr	10yr-18yr
S.pneumoniae	7(33.33%)	-	1(14.28%)	3(42.85%)	1(14.28%)	1(14.28%)	1(14.28%)
S .agalactiae	4(19.04%)	3(75.00%)	1(25.00%)	-	-	-	-
E.coli	1(4.76%)	-	1(100%)	-	-	-	-
H.influenzae	2(9.52%)	-	-	1(50%)	1(50%)	-	-
N.meningitidis	1(4.76%)	-	-	-	1(100%)	-	-
E.fecalis	1(4.76%)	1(100%)	-	-	-	-	-
K.pneumoniae	1(4.76%)	1(100%)	-	-	-	-	-
C.koseri	1(4.76%)	-	-	-	-	-	1(100%)
Unidentified species*	3(14.28%)	-	-	1(33.33%)	-	1(33.33%)	1(33.33%)
Total	21(100%)	5(23.80%)	3(14.28%)	5(23.80%)	3(14.28%)	2(9.52%)	3(14.28%)

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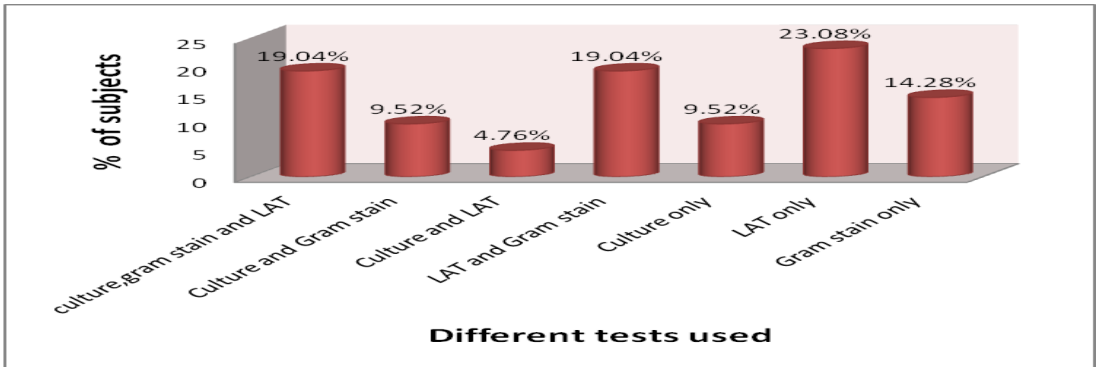
Figure-1: Laboratory confirmed cases of ABM as per WHO criteria



CSF LAT could identify the maximum number of ABM as shown in Table 2. Gram stain showed a sensitivity of 77.77% and specificity of 88.23% with positive predictive value of 53.84% and negative predictive value of 95.74%. Both sensitivity and specificity of LAT was 83.33% with positive predictive value of 35.71% and negative predictive value of 97.82%.(Table 3). Antibiogram showed that all the isolates were sensitive to third and fourth generation cephalosporins (cefoperazone and cefepime) while 66.66% were resistant to cotrimoxazole. (Table 4). Case fatality rate was 19.04%.

Table-2: Comparison of CSF Culture, Gram stain and LAT

Test	Positive	Negative	Total
Culture	09(42.85%)	12(57.14%)	21(100%)
Gram stain	13(61.90%)	08(38.02%)	21(100%)
LAT	14(66.66%)	07(33.33%)	21(100%)

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

Tests	sensitivity	specificity	PPV	NPV
Gram stain	77.77%	88.23%	53.84%	95.74%
LAT	83.33%	83.33%	35.71%	97.82%

Table -4: Antibiogram of the isolates

Organisms	Most sensitive (100%)	Organisms were > 50% sensitive to following	Least sensitive (33.33%)
Gram positive bacteria *(S.pneumoniae, Group B Streptococcus, E.fecalis*)	vancomycin linezolid cefeoperazone	ciprofloxacin(55.55%) tetracycline(66.66%) clindamyci (50.00%)	erythromycin cloxacilline
Gram negative bacteria (Enterobacteriacea- *E.coli , K.pneumoniae, C.koseri*)	cefuroxime amikacin levofoxacin imipenem piperacilline/tazobactm	gentamicin (55.55%)	ampicillin
Both	cefepime	amoxyclav (66.66%)	cotrimoxazole

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DISCUSSION

Bacterial meningitis is one of the most common infectious disease emergencies involving the central nervous system. Despite the availability of potent antibiotics the mortality rate due to acute bacterial meningitis remains significantly high in India and other developing countries.[8] The rapid laboratory diagnosis of meningitis is of particular concern to the clinical microbiologist and clinician. Gram stain and culture remain the standard tests for the laboratory diagnosis of meningitis, however, they are often augmented with antigen detection (eg-latex agglutination) tests.

In patients with antibiotic therapy before lumbar puncture or where culture is negative, alternative methods of CSF study have been developed. LAT has been introduced for this purpose, as it can detect comparatively very small quantity of antigen present and is highly sensitive, specific, simple to perform and results are available rapidly in 10 minutes. [5

In the present study, as per WHO criteria of a proven case of bacterial meningitis,[7] 21/60 (35.00%) cases were laboratory confirmed as bacterial meningitis. More than half of the cases, 13(61.90%) were under 1year age. Of these 13cases of ABM , 5(23.80%) cases each were in the age group of below 1month (neonate) and 3 month to 1 year each and 3(14.28%) cases were in the age group of 1to 3month. 8(38.09%) cases diagnosed between 1-18 year age. It was in accordance with other studies. [4,5 Males 13(61.53%) were affected more than females 8(38.10%) with a male to female ratio of 1.6:1 and correlated with the other studies. [9,10]

Cases presented with wide array of symptoms, fever being the most common followed by seizures, altered sensorium, refusal of feeds and vomiting. Signs of meningeal irritation were present only in 19.04% of cases. Similar observations were made by other workers. [4,11]

Among the 21 laboratory confirmed cases of ABM, CSF appeared turbid in 12 (57.14%) cases and it was clear in 9 (42.85%) cases. None of the samples showed cobweb formation nor were blood tinged. Thus CSF turbidity was not reliable in ruling out acute bacterial meningitis.15(71.14%) cases of ABM showed polymorpho neutrophil predominance and is correlated with other studies.[4,12] In 4(19.04%) cases there was lymphocyte predominance and this could be due to partial treatment of the cases as this leads to change in the CSF picture.

Most common range of CSF WBC count, protein and glucose among the laboratory confirmed cases of bacterial meningitis was in the range of 101-500 cells /mm3 in 52.38% cases, 151-200mg/dl in 47.61% cases and of 21-30 mg/dl in 42.85% respectively, these findings was in accordance with other studies. [13,14 CRP is considered as a sensitive indicator of inflammation. In our study 42.85% of cases were CRP positive. This observation correlates with studies of Tankhiwala et al [15] and Chinchankar et al. [4]

Out of 9 (42.85%) culture positive isolates, *S.pneumoniae* was the most common isolate in 3(14.28%) cases followed by *Group B Streptoccus* in 2(9.52%) and *E.coli,*

K.pneumoniae.C.koseri, E.fecalis in 1(4.76%) case each. Culture was negative in 12 (57.14%). Similar isolation rates have been reported by other workers. [4,14]

Out of 13 (61.90%) gram stain positive cases, Gram positive cocci 10(47.61%) were identified more than gram negative bacilli 03(14.28%).Gram stain was negative in 9(38.09%) cases. It was in accordance with other studies. [4,14]

Out of 14 (66.66%) LAT positive cases, *S.pneumoniae* 6(28.57%) was the most common etiological agent detected by LAT, followed by Group B Streptococcus 4(19.04%), *H.ingfluenzae b* 2(9.52%) , *E.coli and N.meningitidis* in 1(4.76%) case each. In 7(33.33%) cases LAT was negative. Our observations correlate with studies of the other authors. [4,14,15] Overall considering all tests together (Gram stain, culture and LAT), our study identified maximum cases of *S.pneumoniae* 7(33.33%), followed by *Group B Streptococcus* 4(19.04%).

2 cases of *H.influenzae b* and one case of *N.meningitidis* were identified by LAT only. In 3 (14.28%) cases of ABM, organism could not be speciated as they failed to grow in the subsequent culture nor they were identified by LAT. They were identified only on Gram stain, 2 out of these 3 cases showed pus cells and gram positive cocci in pairs and one case showed pus cells and gram negative bacilli. Hence gram stain ay could also provide useful information even if culture is not available.

The sensitivity, specificity, positive predictive value and negative predictive value of Latex agglutination test were 83.33%, 83.33%, 35.71%, 97.82% respectively. Comparable efficacy was also observed in various studies. [1,5,6] Culture is superior to LAT in neonatal meningitis as LAT is not designed to detect Enterobacteriaceae other than *E.coli,* but culture can detect these pathogens, which are emerging as common causes of neonatal meningitis, besides, the cost of LAT is the major limiting factor.

We found 66.66% of cases presented with history of antibiotics prior to lumbar puncture. CSF LAT detected a marginally higher number of cases 10/14 (71.42%) of ABM when compared to Gram stain 8/14(57.14%) and Culture 3/14 (21.42%) in arriving at a diagnosis once antibiotic treatment has started. Medical colleges being a referral hospital, majority of cases received are partially treated before hospitalization. Similar observations were made by other authors

Overall the antimicrobial sensitivity pattern of all isolates was 100% sensitive to and third and fourth generation cephalosporins (cefoperazone & cefepime) and least sensitive (33.33%) to cotrimoxazole. Amoxiclav was sensitive in 66.66% of all isolates. It is usefull for the clinician to possess the susceptibility data on gram positive and gram negative bacteria rather than for particular organism alone. In addition to above antibiotic sensitivity pattern, all Gram positive bacteria were also 100% sensitive to vancomycin and linezolid. , all Gram negative bacteria were also 100% sensitive to amikacin, levofloxacin, cefuroxime, imipenem and piperacillin/tazobactam. Ciprofloxacin, tetracycline, clindamycin sensitivity was 55.5%, 66.66%, 50.00% respectively for gram positive bacteria. In addition to cotrimoxazole being a least sensitive (33.33%) drug for *S.pneumoniae* other drugs penicillin, erythromycin and clindamycin also showed 33.33% sensitivity. In addition to cotrimoxazole, ampicillin was

sensitive only in 33.33% of Enterobacteriaceae. A high percentage of ampicillin resistant Enterobacteriaceae were isolated in the present study. Similar findings have been observed by other workers.[11,17] No ESBL producers were noted. Resistance of isolates might be a reflection of the inappropriate use of antibiotics, or unavailability of a guidance regarding the selection of drugs. Therefore producing updated information on local pathogens and their antibiogram is essential in overcoming such a global problem.

Overall mortality rate was 19.04% and correlated with the other studies.[4,5,11,16] Fatality rate was highest in case of *H.influenzae b* meningitides 1/2(50%) and lowest in cases *S.pneumoniae* 1/7(14.28%) . 33.33% fatality rate was found with unidentified species which was positive only on Gram stain which detected gram negative bacilli. The case fatality for Group B Streptococcus was 25%.

CONCLUSION

LAT was found to be useful in identifying fastidious bacteria that are difficult to grow on culture. As it can detect only specific pathogens and negative LAT does not rule out other causative agents of bacterial meningitis, culture is essential to detect changing microbial spectrum. Despite its drawbacks LAT can be used as an adjunctive laboratory test with abnormal CSF parameters (WBC, glucose, protein, others like CRP) indicate bacterial meningitis particularly in pretreated cases. Cephalosporins (cefoperazone and cefepime) were most sensitive drugs for treating bacterial meningitis but other commonly used antibiotic may still have role in treating bacterial meningitis. Continuous monitoring of microbial spectrum of bacterial meningitis and updated information of their antibiotic susceptibility pattern is required for effective management.

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