Original Article

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Microbiology Section

Study of Bacterial Meningitis in Children Below 5 Years with Comparative Evaluation of Gram Staining, Culture and Bacterial Antigen Detection

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ABSTRACT

Context: Bacterial meningitis is one of the most serious infections seen in infants and children, which is associated with acute complications and chronic morbidity. Infections of Central Nervous System (CNS) still dominate the scene of childhood neurological disorders in most of the developing tropical countries.

Aims: To isolate, identify and determine the antibiotic susceptibility patterns of pathogens associated with bacterial meningitis. We also aimed to comparatively evaluate of Gram staining, culture and bacterial antigen detection in cerebrospinal fluid samples.

Materials and Methods: Present comparative study included 100 CSF samples of children below the age of 5 years, who were clinically suspected meningitis cases. The samples were subjected to Gram staining, culture and Latex agglutination test (LAT). The organisms isolated in the study were characterized and antibiotic susceptibility test was done according to standard guidelines.

Statistical Analysis: It was done by using Gaussian test.

Results: Of the 100 cases, 24 were diagnosed as Acute bacterial meningitis (ABM) cases by.

Gram staining, culture and latex agglutination test. 21 (87.5%) cases were culture positive, with 2 cases being positive for polymicrobial isolates. Gram staining was positive in 17 (70.53%) cases and LAT was positive in 18 (33.33%) cases. *Streptococcus pneumoniae* was the predominant organism which was isolated and it was sensitive to antibiotics.

Conclusion: In the present study, male to female ratio was 1.27:1, which showed a male preponderance. With the combination of Gram staining, culture, and LAT, 100% sensitivity and specificity can be achieved ($p < 0.001$). Gram staining and LAT can detect 85% of cases of ABM. Bacterial meningitis is a medical emergency and making an early diagnosis and providing treatment early are life saving and they reduce chronic morbidity.

Keywords: Bacterial meningitis, Neurological disorders, Latex agglutination test, *S. Pneumoniae*

INTRODUCTION

Bacterial meningitis results from the haematogenous dissemination of microorganisms from a distant site of infection. More than 2/3rd of cases of meningitis occur in the first 2 years of life, owing to decreased immunity and high vascularity of the brain [1]. The clinical presentation is vague, due to the immaturity of CNS in infants and children. Hence, no pathognomonic signs or symptoms can help in accurately diagnosing the cause of meningitis, and making the aetiological diagnosis mainly depends on CSF analysis and culture. Antigen detection by latex particle agglutination is a useful adjunct to routine Gram staining and it can detect non viable bacteria in CSF, thus permitting the detection of bacterial antigen in patients who are pre-treated with antibiotics, in whom culture is negative [2]. Untreated bacterial meningitis is highly fatal, leaving serious neurological sequelae in survivors and crippling children for their lifetime. Thus, an early detection aids clinician in providing a timely intervention, to institute, appropriate antibiotic therapy, as providing therapy early is imperative in reducing the morbidity and mortality. This study was therefore undertaken to aid in making a rapid diagnosis of acute bacterial meningitis cases, to isolate and identify the pathogen and also to do a comparative evaluation of Gram staining, culture and LAT in clinically suspected cases of ABM.

SUBJECTS AND METHODS

This comparative study was conducted for a duration of 3 years, in the Department of Microbiology of a tertiary care hospital which was attached to Bangalore Medical College, Bangalore, India. A total of 100 clinically suspected cases of bacterial meningitis, children who were below 5 years of age, who were admitted to the Children's Hospital, constituted the study group. Institutional ethical clearance and written informed consents of the parents of the children were obtained according to the guidelines set for the study.

Inclusion Criteria

Children who were below 5 years of age, who had signs and symptoms of meningitis. Children who had received treatment before the first lumbar puncture and had not shown any improvement or had not responded to therapy, but whose diagnoses were still indicative of bacterial meningitis were included in the study.

One to 0.5 millilitres of CSF was collected in a sterile container and it was immediately processed. Macroscopic appearance of CSF was observed for volume/ turbidity/ purulence and it was also checked for blood staining. CSF was centrifuged at 1500 to 3000 x g for 20 minutes. The supernatant was used for bacterial antigen detection test and the sediment was first inoculated onto blood agar, chocolate agar, Mac Conkey's agar and BHI broth and then, a direct smear was made for examination by Gram staining, to prevent contamination. Inoculated primary plates were incubated at 370 C for 48 to 72 hours. BHI broth was incubated for 7 days, it was examined daily for presence of growth or turbidity and it was considered to be negative at the end of 7 days of incubation. All the organisms which were isolated were identified by standard procedures and antibiotic susceptibility testing (AST) was done according to CLSI guidelines [3].

Lat

CSF samples were tested for bacterial antigen by using WELLCO-GEN bacterial antigen kit (Remel Europe Ltd. UK), a latex test which

was used to detect antigens of 5 organisms:

– *E coli* K1 antigen

– *N. meningitidis* ABCYW 135 antigen

– *Streptococcus pneumoniae* antigen

– group B *streptococcus* antigen

– *H. Influenzae type b* antigen (Hib)

Meningococcal Group B antigen, being structurally and immunologically related to *E.Coli* K1 antigen, was provided as a single test latex reagent and depending on the age of the child, a positive reaction seen in a neonatal specimen would suggest *E.Coli* K1 infection and in older children, meningococcal Group B was a more likely infection and which correlated with direct smear examination of CSF.

Procedure: CSF was pre-heated at 100°C in a water bath for 5 minutes, cooled to room temperature and centrifuged to remove the proteinitious material that would cross react with the antigen (to minimize non specific reactions). The supernatant was then used for LAT. Disposable reaction cards containing six separate circles with the colour codes of different test latex reagents were provided with the kit. One drop each (40ml) of CSF were placed on the separate circles of the reaction card and one drop each of five different test latex reagents were added to the separate circles. These were mixed thoroughly and manually rotated for 3 minutes. They were then observed for agglutination. Positive and negative controls and control latex tests were put up.

The criteria used for declaring laboratory confirmed cases as acute bacterial meningitis, along with presence of pus cells in Gram staining included:

1. Positive for Gram staining, LAT and culture
2. LAT and Gram staining
3. Culture and LAT and
4. Culture only.

The results were compiled and statistically analyzed.

RESULTS

In the present study done on 100 cases, male to female ratio was 1.27:1, which showed a male preponderance. 44 (44%) cases gave a history of having been treated with antibiotics before the first lumbar puncture was done. Of the 100 clinically suspected cases of ABM, the laboratory confirmed cases were found to be only 24 (24%), on considering the above criteria. The comparative analysis done of Gram staining, culture and LAT showed that culture was positive in 87.5% cases, that Gram staining was positive in 70.83 % cases, and that LAT was positive in 33.33% cases among the 24 ABM cases, as has been shown in [Table/Fig-1]. Among 21 culture positive cases, 6(28.6%) showed gram positive isolates and 15 (71.4%) showed gram negative isolates. Gram staining was positive in 17 cases, among which 7 (41.18%) showed gram positive cocci, 9 (52.94%) showed gram negative bacilli and 1 (5.88%) case showed 2 organisms (polymicrobial) [Table/Fig-1].

Streptococcus pneumoniae was the predominant organism which was isolated in this study. It was isolated in 3 cases by culture, but Gram staining and LAT could detect *S. Pneumoniae* in 7 cases of ABM. *Enterococci* were isolated in 3 cases by using different media, but it was demonstrated only in 1 case by Gram staining. Gram negative bacilli (GNB) isolated in 14 cases were demonstrated in 9 cases by gram stain and LAT was positive in one case (Haemophilus influenzae b). The organisms which were isolated in the study have been shown in [Table/Fig-2].

Polymicrobial isolates

Of the 24 cases of ABM, 2(8.33%) cases showed polymicrobial organisms. One case showed a mixture of *S. Pneumoniae* and N. meningitidis ACYW135. Both were culture negative and they were identified by Gram staining and LAT. Second case showed a mixture of *S. Pneumoniae* and *Enterobacter aerogenes*. *S. Pneumoniae* was culture negative and it was identified by Gram staining and LAT and *Enterobacter aerogenes* was isolated by culture as a pure isolate, both on primary solid media and in BHI broth. From the comparative analysis, the sensitivity and specificity of culture were found to be 87.5% and 100%, those of Gram staining were 70.8% and 100% and those of LAT were 100% and 100% respectively. Statistical analysis was done using Gaussian test, which related the difference between proportions of sensitivities for LAT and those for culture ($p < 0.001$) and between those for LAT and those for Gram staining ($p < 0.0001$), which was significant.

Organism identified	total	Culture +ve(%)	GS +ve(%)	LAT+ve (%)
Pneumococci	5	3 (60)	5(100)	5(100)
Enterococci	3	3(100)	1(33.33)	0
N. meningitis ACYW135+ *Pneumococci*	1	0	1 (100)	1 (100)
Pneumococci + GNB	1	1# (100)	1*(100)	1*(100)
GNB	14	14(100)	9(64.28)	1+ (7.14)
Total	24(100)	21(87.5)	17(70.83)	8(33.33)

[Table/Fig-1]: Showing comparative analysis of culture, gram stain (GS) and latex agglutination test (LAT)
GNB isolated by culture only
* *Pneumococci* detected by GS and LAT
+Hib detected by LAT

Sl.No.	Organism	No. Isolated	Percentage
1	*Klebsiella pneumoniae*	4	19.04
2	*S. Pneumoniae*	3	14.29
3	*Enterococcus faecalis*	3	14.29
4	*Enterobacter aerogenes*	3	14.29
5	*Acinetobacter sp.*	3	14.29
6	*Pseudomonas aeruginosa*	2	9.52
7	*H. Influenzae type b*	1	4.76
8	*Shigella Flexneri*	1	4.76
9	*Citrobacter freundii*	1	4.76
	Total	21	100

[Table/Fig-2]: Showing number of organisms isolated by culture

Antibiogram

Antibiogram was made by using antibiotic disks like Ampicillin(10µg), Gentamycin (10µg), Tetracycline (30µg), Erythromycin (15µg), Chloramphenical (30µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Ceftazidime (30µg), Cephalexin (30µg) and Vancomycin (30µg). *S. Pneumoniae* was sensitive to Ampicillin, Gentamycin, Tetracyclin, Erythromycin, Chloramphenicol and Cotrimoxazole. All GNB which were isolated in this study were sensitive to Ciprofloxacin, except one isolate, *Citrobacter freundii*, which was a drug resistant strain. Hib which was isolated in one case was sensitive to Ampicillin, Chloramphenicol and Cotrimoxazole. All isolates of Klebsiella species which were tested for ESBL production by double disc diffusion synergy test (DDST) were negative. Acenitobacter species were sensitive to Gentamicin, Ciprofloxacin and Ceftazidime. *Pseudomonas aeruginosa* was sensitive to Ciprofloxacin and Ceftazidime. Antibiograms of these organisms have been shown in [Table/Fig-3].

Case fatality in the present study

In our study, 9 of 24 (37.5%) children died within 24 hours of admission. 7 of 9 (77.78%) cases had a history of prior medication and were being treated with antibiotics. Out of 9 cases of ABM that died, 2 (22.22%) were mixed bacterial meningitis cases.

Organism	No. Tested	A	G	T	E	Ch	Co	Cp	Cf	Ca	Va
		10µg	10µg	30µg	15µg	30µg	25µg	30µg	5µg	30µg	30µg
Streptococcus pneumoniae	3	3 (100%)	3 (100%)	1 (33.33%)	3 (100%)	3 (100%)	2 (66.67%)	3 (100%)	-	-	-
Enterococcus faecalis	3	3 (100%)	3 (100%)	-	-	-	-	-	-	-	3 (100%)
Klebsiella pneumoniae	4	0	2 (50%)	-	-	-	0	0	4 (100%)	0	-
Enterobacter aerogenes	3	0	0	-	-	-	3 (100%)	0	3 (100%)	1 (33.33%)	-
Acinetobacter Species	3	0	3 (100%)	-	-	-	1 (33.33%)	0	3 (100%)	2 (66.67%)	-
Pseudomonas aeruginosa	2	0	0	-	-	-	0	0	2 (100%)	2 (100%)	-
H. Influenzae type b	1	1 (100%)	-	-	-	1 (100%)	1 (100%)	-	-	-	-
Shigella Flexneri	1	0	0	-	-	-	0	0	1 (100%)	0	-
Citrobacter freundii	1	0	0	-	-	-	0	0	0	0	-

[Table/Fig-3]: Showing percentage of sensitivity of the organisms
A-Ampicillin, G-Gentamycin, T-Tetracyclin, E-Erythromycin, Ch-Chloramphenicol, Co-Cotrimoxazole, CP-Cephalexin, Cf-Ciprofloxacin, Ca-Ceftazidine, Va-Vancomycin

DISCUSSION

Meningitis is a serious emergency in which microbiological laboratory plays a critical role in early identification of the causative bacterium and its antibiogram. However, it is empirical to start antibiotic therapy before the complete laboratory results are available. Such a blind prescription requires knowledge on the most frequent aetiological agents of meningitis existent in the local population. Hence, the present study was undertaken, which showed a male preponderance of 1.27:1, which correlated with those seen in studies of Rao et al., [4] and Chinchankar et al., [5] who had also reported ratios of 1.2:1 and 1.07:1 in their studies.

Gram staining analysis done in our study (70.83%) correlated those done by other Indian authors like Mirdha et al., [6] (74%), Chinchankar et al., [5] (67%) and Mohammadi et al., (70.96%) [7]. Culture was positive in 87.5% cases in our study and similar isolations were reported by Sippel et al., [8] (72.83%) and Kalra et al., [9] (77.6%). Rao et al., [4] reported that 63.3% of GNBs and that 36.7% of gram positive organisms had been isolated in their study and Mohammadi et al., [7] reported that 73.33% GNBS and 26.67% gram positives had been isolated in their study, which were similar to our findings of 71.4% GNBs and 28.6% gram positive organisms.

S.Pneumoniae was the predominant organism which was isolated in our study. Relatively high incidences of Pneumococcal infections were noted by Grimwood et al., [10] and Bhat et al., [11].

Other studies done on LAT showed higher rates of isolations like those reported by Deivanayagam et al., [12] (84%) and Mirdha B R et al., [6] (100%), but in our study, LAT could detect only 33.33% isolations. This could be due to the predominant GNB isolated in our study, that were not included in the panel of the kit. But 100% sensitivity and specificity reported in our study correlated with those shown by Maxson et al., [13] and Devanayagam et al., who hadalso reported 100% sensitivity and specificity in their studies. Kumar et al., [14] showed a case fatality rate of 44%, with a history of prior medication in patients and Mohammadi et al., showed a rate of 45.16%. In our study, a 37.5% case fatality rate was seen in polymicrobial meningitis cases and in cases with a history of prior medication. This showed that identification of aetiological agent was a must, before starting the patients on any medication. Making an early diagnosis and providing appropriate antibiotic therapy are life saving and they also reduce chronic morbidity and mortality.

CONCLUSION

Bacterial meningitis is a medical emergency. Antibiotics have a profound effect on the clinical course and prognosis of meningitis. They are also found to alter the CSF findings and reduce the sensitivity of culture. Hence, antigen detection is a better adjuvant to conventional methods and culture, though a gold standard requires 48 hours, trained medical personnel and an equipped laboratory. Gram staining and LAT can detect up to 85% of cases, as was seen in our study. This would help in making an early diagnosis and initiation of therapy, as providing a timely intervention was life saving and as it could help in reducing morbidity.

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