

Study of Bacterial Meningitis in Children under 5 Years with Comparison of Gram Staining and Bacterial Antigen Detection: A study in Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh

Ferdousi Begum^{1*}, Tanzila Farhana², Reaz Mobarak³, Md. Rafiqul Islam⁴, Md. Mizanur Rahman⁵, Md. Al-Mamun Hossain⁶

¹Registrar, High Dependency and Isolation Unit, Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh

²Medical Officer, High Dependency and Isolation Unit, Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh

³Prof. Head of Department, High Dependency and Isolation Unit, Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh

⁴Asst. Prof, High Dependency and Isolation Unit, Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh

⁵Medical Officer, Department of Cardiology, National Institute of Cardiovascular Diseases and Hospital (NICVD), Dhaka, Bangladesh

⁶Assistant Professor, (Hepatology), OSD, DGHS, Deputed Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

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*Corresponding author: Ferdousi Begum

Abstract

Original Research Article

Introduction: 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 countries. **Objective:** To isolate, identify and determine the antibiotic susceptibility patterns of pathogens associated with bacterial meningitis. To assess comparative evaluation of Gram staining, culture and bacterial antigen detection in cerebrospinal fluid samples. **Materials and Methods:** Present comparative study included 100 CSF samples of children under the age of 5 years, who were clinically suspected meningitis. 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 (70.83%) cases and LAT was positive in 8 (33.33%) cases. Streptococcus pneumoniae was the predominant organism which was isolated and it was sensitive to various antibiotics. **Conclusion:** In the present study, male to female ratio was 1.27:1, which showed a male and female 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 lifesaving and they will reduce chronic morbidity.

Keywords: Pyogenic Meningitis, Antigen detection, CSF.

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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, due 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]. Acute bacterial meningitis is seen more in children than in adults and it is caused by a variety of microorganisms; the most important among them are Haemophilus influenzae, Neisseria meningitides and Streptococcus pneumoniae [16]. Fever may be absent up to 30% of individuals and 20-30% does not have signs of meningism at presentation. Previous antibiotic therapy may also mask the

significance of the presenting illness [17]. 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 appropriate antibiotic therapy, as providing early therapy 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.

OBJECTIVE

To isolate, identify and determine the antibiotic susceptibility patterns of pathogens associated with bacterial meningitis. To assess comparative evaluation of Gram staining, culture and bacterial antigen detection in cerebrospinal fluid samples.

SUBJECTS AND METHODS

This comparative study was conducted for a duration of 2 years, in the High Dependency and Isolation Unit which was attached at Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh during July 2017 to June 2019. A total of 100 clinical suspected cases of bacterial meningitis, children who were under 5 years of age were admitted to the Dhaka Shishu (Children) 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 under 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 milliliters 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, MacConkey'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 37°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: (a) E coli K1 antigen (b) N. meningitidis ABCYW 135 antigen (c) Streptococcus pneumoniae antigen (d) Group B streptococcus antigen (e) 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 proteinaceous 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 (40µl) 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: (a) Positive for Gram staining, LAT and culture (b) LAT and Gram staining (c) Culture and LAT and (d) 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 and female preponderance. 62% male and 38% female cases of (100) gave a history of having been treated with antibiotics before the first lumbar puncture was done (Figure-1). Of the 100 clinically suspected cases of activity-based management, the laboratory confirmed cases were found to be only 24 (100%), 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-2). 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-2). 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-2). Of the 24 cases of ABM, 2(8.33%) cases showed polymicrobial organisms.

Table-1: Presenting symptoms with duration of the study patients (n=100)

Symptoms	Fever	Number	Duration of symptoms (days)	
			100	2
Convulsion		95	1	1.3
Vomiting		41	1	1.2
Lethargy		37	1	1.2
Excessive Crying		15	1	1.2
Unconsciousness		9	1	1.1

Table-2: Showing comparative analysis of culture, gram stain and latex agglutination test (LAT) (n=100)

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)

GNB isolated by culture only

*Pneumococci detected by GS and LAT +Hib detected by LAT comparative analysis of culture, gram stain (GS)

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 (Table-2).

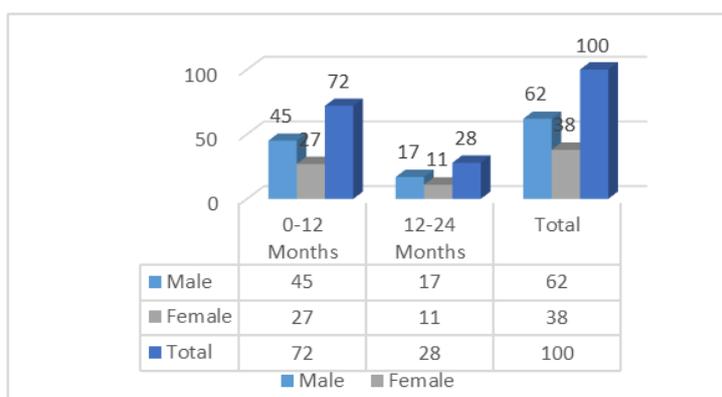


Fig-1: Distribution of the study patients by age & Sex (n=100)

Table-3: Showing number of organisms isolated by culture GS and LAT +Hib detected by LAT (n=100)

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

S. Pneumoniae was sensitive to Ampicillin, Gentamycin, Tetracyclin, Erythromycin, Chloramphenicol and Cotrimoxazole. One case showed a mixture of S. Pneumoniae and N meningitidis ACYW 135. 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 (Table-3).

Table-4: Showing percentage of sensitivity of the organisms (n=100)

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	-

A-Ampicillin, G-Gentamycin, T-Tetracyclin, E-Erythromycin, Ch-Chloramphenicol, Co-Cotrimoxazole, CP-Cephalexin, Cf-Ciprofloxacin/ceftriaxone, Ca-Ceftazidime, Va-Vancomycin, ceftriaxone. Table-4 Showing number of organisms isolated by culture Antibiogram was made by using antibiotic disks like Ampicillin(10µg), Gentamycin (10µg), Tetracycline (30µg), Erythromycin (15µg), Chlor- amphenicol (30µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Cef- ta-zi-dime- (30µg), Cephalexin (30µg) and Vancomycin (30µg). All GNB which were isolated in this study were sensitive to

Ciprofloxacin, except one isolate, 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. Acinetobacter 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-4).

Table-5: Sensitivity, Specificity, accuracy, positive and negative predictive values of the serology (LAT) and Gram stain (n=100)

Test of Validity	Serology(LAT)	Gram Stain
Sensitivity	100.0	75.4
Specificity	75.4	100.0
Accuracy	74.0	96.0
Positive predictive Value	34.9	100.0
Negative predictive Value	100.0	91.6

The validity of serology (LAT) and Gram Stain Correlated by calculating were sensitivity, specificity, Accuracy, Positive Negative predictive Values (Table-5).

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 had also 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. CSF culture is the “Gold standard” and these positive cases could be identified by other tests too, as being found here. Making an early diagnosis and providing appropriate antibiotic therapy are life saving and they also reduce chronic morbidity and mortality. The Study Showed that a high sensitivity (100%) and specificity (98-100%) of LAT compared to culture (40%) isolation and gram stain 25-30% positivity Maxson *et al.*, [15].

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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