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BACTERIOLOGICAL PROFILE OF ACUTE BACTERIAL MENINGITIS AMONG NEONATES IN A TERTIARY CARE CENTRE IN ASSAM

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ABSTRACT **Introduction:** Meningites is an inflammation of the membranes surrounding the brain and spinal cord. It is a life threatening clinical syndrome. Pyogenic meningitis occurs all ages, but is commonest among neonates **Objective:** To study the trends in etiology and the antimicrobial susceptibility pattern of the pathogens causing acute bacterial meningites (ABM) prevalent in Lower Assam over a period of 1 year from September 2021-2022 **Materials and Methods:** The study was carried out in the Department of Microbiology, FAAMCH A total of 66 suspected cases of ABM were included in the study CSF samples were collected from the patients and inoculated on chocolate agar, blood agar and MacConkey agar Any growth was identified using colony characteristics and standard biochemical tests. CSF Latex agglutination test was also performed. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method as CLSI standards **Results:** Of the 66 cases studied, 28 cases were diagnosed as ABM by Gram stain and Culture Male to Female ratio was 1.5:1 showing male preponderance, S pneumoniae (31 81%) was the most common causative agent of acute bacterial meningitis followed by Group B Streptococcus (18.18%). In the present study, the antimicrobial sensitivity pattern of the isolates showed that all Gram positive and Gram-negative organisms were 100% sensitive to cefoperazone & cefepime, Cotrimoxazole was the least effective and Amoxyclav was effective in >50% of Isolates. **Conclusion:** ABM prevalence in neonates was 28 cases amongst 66 suspected cases. S pneumoniae and Group B Streptococcus were the most isolated. organism and Cefoperazone & cefepime were most sensitive antibiotics whereas Cotrimoxazole was highly resistant. With the use of the information obtained during the study, a strategy for treating patients with bacterial meningitis can be developed Morbidity or death could be avoided with an early and precise diagnosis accompanied by appropriate management

KEYWORDS : acute bacterial meningitis, CSF, Latex agglutination, antimicrobial susceptibility

INTRODUCTION

An infection of the meninges that cover the brain and spinal cord is known as bacterial meningitis; acute complications and morbidity risk are connected to it. Both epidemic and sporadic patterns of occurrence exists[1]. Before the development and widespread use of medicines, bacterial meningitis typically resulted in death. Although antibiotic treatment has significantly improved the prognosis for individuals with bacterial meningitis, the disease still ranks among the leading causes of morbidity and death in children[2].

One of four ways—direct hematogenous spread, transit through the choroid plexus, rupture of superficial cortical abscesses, or contiguous dissemination of an adjacent infection—allows bacterial organisms to enter the meningial area[3].

The most frequent method is the haematogenous spread of microorganisms from the distant site of infection, frequently from the respiratory system, followed by infections of the paranasal sinuses and otitis media. Due to lowered immunity and the high vascularity of the brain, more than two thirds of cases of meningitis occur in the first two years of life[4].

The clinical presentation is hazy since newborns and young children's CNS are still in developing stage. Therefore, clinically, no pathognomic sign or symptom will reliably identify the origin of meningitis; instead, CSF analysis and culture are the primary diagnostic tools for determining the aetiology.

MATERIALS & METHODS

Inclusion criteria

Sample collected from patients attending FAAMCH with clinical signs and symptoms suggestive of acute bacterial meningitis (fever,headache, neck stiffness, vomiting , kernig sign, bulging frontanel , refusal to feed) within the age group of zero days to 18 years.

Exclusion criteria

Patients with clinical features suggestive of meningitis with risk factors like recent head injury or neurosurgery were to be excluded from the study.

Specimen Collection

CSF samples were collected by the doctors in NICU on immediate arrival of the case with clinical suspicion of meningitis, prior to administration of antibiotics whenever possible. About 1-2 ml CSF was collected by lumbar puncture, done with all aseptic precautions. Informed consent was obtained from the parents for performing Lumbar puncture.

Transport

In a sterile screw capped containers CSF Sample was collected and sent for analysis for CSF cell count and type, protein and glucose Sample which were received in Microbiology laboratory was immediately processed without delay on arrival. Samples collected during night hours were kept in incubator and processed without delay the following day. Macroscopic appearance of CSF observed for turbidity/ purulent (pus) and whether blood stained. CSF was transferred to three sterile test tubes. One of the test tube was subjected to centrifugation at 2000rpm for 20minutes and gram stain smear was prepared from the deposit. Second test tube was also similarly treated and inoculated on Blood, MacConkey and Chocolate agar. Remaining third test tube was stored for further analysis if required.

Gram Stain:

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 stained by Gram's stain and observed for the presence of pus cells and organisms[4].

Culture [4,5]

The sediment of the centrifuged CSF was inoculated onto the following media namely Sheep Blood agar Chocolate agar and MacConkey agar plate.

Inoculated primary plates were incubated for 48 to 72 hours. The plates were examined daily for 72 hours before discarding as negative. 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. Antibiotic susceptibility testing was done by standard Kirby bauer disk diffusion method and controls were put up according to CLSI guidelines[6].

Tests used for the identification of isolates [4,5]

1)Streptococcus pneumoniae: Gram positive cocci in pairs, lanceolate shape, encapsulated, Optochin sensitive, Bile soluble and Catalase test negative

2)Group B streptococcus: Gram positive cocci, in pairs or chains.

Chain formation is most pronounced in broth media, Bacitracin sensitive, Camp test positive and Catalase test negative

3) Enterococcus fecalis: Gram positive cocci in pairs, Ferment esculin, mannitol, sucrose, sorbitol. Grows on tellurite blood agar producing black colonies and relatively heat resistant, surviving 60° C for 30minutes.

4) N.meningitidis: Gram negative cocci in pairs, with the adjacent sides flattened , Catalase and oxidase positive. Glucose and maltose are utilised, but not sucrose or lactose, producing acid but no gas. Indole not produced and Nitrate not reduced.

5) H.influenzae :Gram negative pleomorphic bacilli , Oxidase positive and Sattelitism positive.

6) Enterobacteriaceae

a) E coli: Indole produced, Methyl red test positive, Citrate not utilized, Urea not hydrolysed, Triple sugar iron agar-acid slant by acid butt with gas and without H_2S.

b) Klebsiella pneumoniae: Indole not produced, Methyl red test negative, Citrate

utilized as a sole source of carbon, Urea hydrolysed, Triple sugar iron agar-acid slant by acid butt with gas and without H_2S

c) Citrobacter koseri: Indole produced, Methyl red test positive, Citrate utilized as a sole source of carbon Urea not hydrolysed , Triple sugar iron agar-alkaline slant by acid butt with gas and without H_2S.

ANTIMICROBIAL SUSCEPTIBILITY TEST

The antimicrobial susceptibility testing of the bacterial isolates were performed using Kirby- Bauer disc diffusion method on Mueller - Hinton agar and zone diameters were interpreted according to the CLSI guidelines 2021[6]. Inoculum: 0.5 McFarland turbidity. Medium used is MHA (Mueller- Hinton agar) plate. Method used is Lawn culture. Incubation at 37°C ambient air and incubated for 16-18 hours.

Latex agglutination test:

CSF samples were tested for bacterial antigen detection using PASTOREXTM MENINGITIS by Bio-Rad Laboratories[7].
To detect antigens of 5 organisms: S.pneumoniae antigen, Group B streptococcus antigen , N. meningiditis

ABCYW 135 antigen, H. influenzae type b antigen, E coli K1 antigen.

RESULTS

This prospective study was taken up in the Department of Microbiology, Fakhruddin Ali Ahmed Medical College & Hospital, Barpeta, for a period of one year from September 2021 to August 2022.A total of 66 clinically suspected of acute bacterial meningitis were investigated for laboratory diagnosis of acute bacterial meningitis during the study period.Out of 66 cases, 28 were proven by lab investigations as pyogenic.

Male to Female ratio was 1.5:1 showing male preponderance (male 17, female 11)

Clinical presentation of cases

Symptoms	Total no of cases	Percentage%
Fever	28	100
Seizure	26	92.8
Refusal to feeds	08	28.5
Vomitting	09	32.1

In the current study, out of 28 instances, fever was most frequently present (100%) in 28 cases, followed by seizures in 26 cases (92.8%), refusal to feeds in 8 (28.5%), and vomiting in 9 cases (32.1%).

Laboratory confirmed cases of ABM as per WHO criteria

A diagnosis of bacterial meningitis in a patient with a clinical syndrome is considered laboratory-confirmed if a bacterial pathogen is grown (i.e., cultured) or found (i.e., by Gram stain or antigen detection methods) in the CSF or from the blood.

Test	Total no. of cases	Percentage
Culture and Gram stain and LAT	06	21.42%
Culture and Gram stain	04	14.28%
Culture and LAT	02	07.14%
LAT and Gram stain	08	28.68%
Culture only	03	10.71%
LAT only	08	28.68%
Total	28	100(%)

Distribution of cases according to causative agent among the laboratory confirmed cases of ABM

Etiologic agent	Total (%)
S.Pneumoniae	09(32.14%)
S. agalactiae	6(21.42%)
E.Coli	1(3.44%)
H.Influenzae	3(10.34%)
N.Meningitidis	1(3.44%)
E.Fecalis	3(10.34%)
K.Pneumoniae	3(10.34%)
C.koseri	2(6.89%)
Total	28(100%)

Etiologic agents identified in CSF by Culture and LAT

Etiological agents	TOTAL	Culture	LAT	Combined
S.pneumoniae	09(32.14%)	05(45.45%)	8(88.88%)	5
S. agalactiae	06(21.42%)	03(50.00%)	06(100%)	3
E. coli	1(3.44%)	01(100%)	01(100%)	0
H.influenzae	03(10.34%)	0	03(100%)	0
N.meningitidis	01(3.44%)	0	01(100%)	0
E.fecalis	03(10.34%)	03(100%)	0	0
K.pneumoniae	03(10.34%)	03(100%)	0	0
C.koseri	02(6.89%)	03(100%)	0	0
Total	28(100%)	18(64.28%)	19(75.86%)	8(28.13%)

S.pneumoniae was the most frequent isolate found in the current study by culture and LAT in 9/28 instances, or 32.14%,of these 5 out of 9 cases were both culture and LAT positive. It was followed by S.agalactiae in 6/28 cases, or 21.42% of total cases. In case of S.agalactiae 3 cases were positive for both LAT and culture.

Only LAT was alone able to diagnose 3 cases of H.influenzae and 1 case of N.meningitidis. In the overall scenario, LAT was more frequently used to identify fastidious bacteria than Culture, such as H.influenzae, N.meningitidis, S.pneumoniae, and S. agalactiae.

SUMMARY & CONCLUSION

In this study performed at FAAMCH Barpeta out of 66 cases with clinical suspicion of ABM, 28 cases were proven by laboratory investigations as ABM. The study period was of one year from September 2021 to August 2022. The male to female ratio was found to be 1.5:1. 100% of cases presented with fever and 92.8% with seizure.

The most frequent cause of ABM was Streptococcus pneumoniae followed S. agalactiae.

Antimicrobial Resistance pattern of the isolates

Antimicrobial agent	S.pneumoniae (5)	S.agalactiae (3)	E.fecalis (3)		K.pneumoniae (3)	C . koseri (3)
Penicillin G	5 (100%)	2 (66.66%)				
Ciproflox acin	5 (100%)	2 (66.66%)		0	3(100%)	
Amoxicillin	0					
Vancomycin	5 (100%)	3 (100%)	3 (100%)			
Ofloxacin	2 (40%)		3 (100%)			

Amikacin	2 (40%)	2 (66.66%	3 (100%	0	0	3(100 %)
Azithromycin	3 (60%)			-	-	-
Cefuroxime	4 (80%)			-	-	-
Chloramphenicol				1(100 %)	-	-
Linezolid			3	-	-	-
Peperacillin-Tazobactum			3(100 %)	-	3 (100%)	-
Gentamycin				-	-	0
Ceftriaxone	0	3 (100%)		0		
Imipenem	-	-	-	1(100 %)	3 (100%)	3(100 %)
Piperacilline /Tazobactam	-	-	-	1(100 %)		
Levofloxacin	-	-	-	1(100 %)	1(33.33%)	3(100 %)
Ampicillin	-	-	-	0		
Cefepime	5(100%)	3(100%)		1(100 %)	3(100%)	3(100 %)
Polymyxin B					3 (100%)	3(100 %)
Cefoperazone				1(100 %)	3(100%)	3(100 %)
Cotrimoxazole	0	0		0	0	0
Cefotaxime						1(50%)

Among the organisms isolated, for S.pneumoniae 100% cases were sensitive to Penicillin G , Ciprofloxacin , Cefepime and Vancomycin. 80% sensitivity was seen in cefuroxime and 60 % resistance was seen to Ofloxacin and Amikacin. S.agalactiae has shown 100% sensitivity towards Ceftriaxone, cefepime and Vancomycin whereas Gentamycin , Penicillin G , Ciprofloxacin and Amikacin had shown 33.4% resistance.

E.Fecalis has shown 100% sensitivity was towards Vancomycin , Ofloxacin , Amikacin ,Cefepime , Chloramphenicol , Linezolid & Piperacillin-Tazobactum. C.koseri displayed high sensitivity towards Cefoperazone , Amikacin , Imipenem, Levofloxacin, Cefepime and Polymyxin B and resistance was reported towards Cefotaxime and Cotrimoxazole.

K.pneumoniae was 100% sensitive for Cefoperazone Piperacillin-Tazobactum , Imipenem, Cefepime and Polymyxin B but 50% resistance was found towards Levofloxacin , Ciprofloxacin and Amikacin. E.Coli highly sensitive towards for Cefoperazone ,Imipenem, Chloramphenicol , Piperacillin Tazobactum and Levofloxacin.

Resistance was reported Ceftriaxone and Ciprofloxacin.

Compared to Gram stain, LAT is better in identifying species. Additionally, LAT was more accurate in detecting the sensitive organisms including H. influenzae, Neisseria meningitidis, S. pneumoniae, and S. agalactiae when compared to traditional Gram stain and Culture techniques.

No test can fully replace the value of CSF culture, especially in newborns as LAT cannot detect Enterobacteriaceae other than E. coli. Therefore, regardless of the other laboratory results, CSF Culture is essential for the diagnosis of newborn meningitis. Additionally, we discovered that the CSF latex agglutination test is an effective method for determining the cause of acute bacterial meningitis quickly. A single test could not be used to diagnose acute bacterial meningitis since none of them had all the necessary characteristics. As a result, the combination of tests based on unique case history will produce more useful findings than any one of the tests by itself. With the use of the information obtained during the study, a strategy for treating patients with bacterial meningitis can be developed. Meningitis consequences such as neurological impairments, deafness, any other morbidity, or death could be avoided with an early and precise diagnosis accompanied by appropriate management.

REFERENCES

1. Nelsen. Text book of paediatrics. In : Part XVII – XXXIII. Kleiegman edt., 19thedn, vol. 2. Saunders 2011.
2. Feigin, Cherry, Kaplan D. Text book of pediatric infections diseases.8th edn : Chapter 38. Saunders Elesevier Science Section IV, 2018:pp.443-474.
3. Menkes JH, Sarnat HB. Child neurology. In : Infections of the nervous system. Chapter 6.11th edn., Lippincott Williams and Wilkins Philadelphia. 2005:pp.467- 499.
4. Collee JG, Marr W. Biochemical test. Mackie & McCartney Practical Medical Microbiology. 14th edition. New delhi (India). Elsevier. 2007. 131-150
5. BAILEY & SCOTT'S DIAGNOSTIC MICROBIOLOGY, 13th edition, 2014. Overview of Bacterial Identification Methods and Strategies, p 195-230
6. M100 Performance Standards for Antimicrobial Susceptibility Testing, 31st Edition.
7. Putta SD, Deshpande S, Bharadwaj R. Evaluation of Latex Agglutination Test Efficacy in Diagnosis of Acute Pyogenic Meningitis. Annals of Pathology and Laboratory Medicine. 2016 Oct;3(04).