ORIGINAL RESEARCH

CSF analysis for Bacterial culture and identification among under-five children suspected for meningitis

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Abstract
Bacterial meningitis, marked by inflammation of the meninges, poses a critical medical emergency with rapid progression and life-threatening consequences. This study aimed to assess bacterial isolates and their antimicrobial resistance patterns in cerebrospinal fluid (CSF) among under-five children in a tertiary care hospital in Madhya Pradesh. Over one year, 77 CSF samples were collected and analyzed. Most cases presented with fever, seizure, altered sensorium, and refusal to feed. The identified bacterial isolates included *S. pneumoniae, Pseudomonas, K. pneumoniae, Enterococcus, and E. coli*. Mortality was notably high in lab-confirmed S. pneumoniae infections, emphasizing the urgency of effective management. Antimicrobial susceptibility revealed sensitivity to various antibiotics, although penicillin resistance was a concern. The study underscores the importance of swift diagnosis, immediate antibiotic therapy, and public awareness to mitigate the global burden of bacterial meningitis, especially among vulnerable populations like children.
Keywords: CSF analysis, bacterial culture, under-five children, meningitis, antimicrobial resistance, antibiotic sensitivity, public awareness and vaccination

Introduction

Bacterial meningitis is a severe infection characterized by inflammation of the meninges, the protective membranes surrounding the brain and spinal cord. This medical emergency demands prompt attention due to its potential for rapid progression and life-threatening consequences. Primarily caused by various bacteria, including *Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae*, bacterial meningitis often presents with symptoms such as fever, severe headache, neck stiffness, and altered mental status.

Globally, bacterial meningitis is a significant public health concern, causing an estimated 288,649 deaths annually. Among these, 94,883 deaths occur in children under the age of five. The disease's severity is compounded by its potential to leave survivors with long-term complications; up to 24% may suffer from sequelae such as epilepsy, mental retardation, or sensorineural deafness, particularly when the infection occurs during early childhood.

AIM: The aim of this study was to assess bacterial isolates of CSF samples and their antimicrobial resistance patterns among under-five children in a tertiary care Hospital.

Material and Methods

Study area and Study period: This was a Hospital based cross-sectional study design conducted in the Department of Microbiology of a tertiary care hospital over a period of 1 year from August 2021 to July 2022

Study design
This was a Hospital based cross-sectional studyunder-five children suspected ofmeningitis.

Study population
Under-five children who was suspected for meningitis.

Ethical consideration: Study was approved by Institutional Ethics committee. A written consent from

parents of all eligible patients was collected before enrolling the patient in the study.

Procedure

Cerebrospinal Fluid sample collection and Identification of bacteria: CSF samples from suspected cases was collected after taking written consent, by lumber puncture in a sterile container under aseptic precaution. Samples were dispensed inthree screw capped vial for Bacterial culture and sensitivity, Routine Biochemistry and Haematology (Cell count) and sent to respective lab within 30 min of collection.

Microbiological analysis of CSF sample: After reaching to Microbiology lab sample was centrifuged and Sediment of was inoculated to Blood Agar plate, Chocolate Agar plate and MacConkey Agar and was incubated aerobically at 37 °C for 24-48 hrs Bacterial isolation and identification. Chemical analysis and cytological analysis was conducted based on standard operating procedures. Microscopy was done using Gram's stain simultaneously using freshly prepared Gram's stain reagents.

Isolation and identification of bacteria: After overnight incubation of the inoculum all culture media plates were examined for growth of colonies. All positive CSF cultures was identified and characterized on the basis of morphology, cultural characteristics, and standard biochemical testing. Gram positive and gram negative organisms were identified by series of biochemical tests like catalase test, coagulase test, Triple Sugar Iron slant agar (TSI), indole test and urease test (Oxoid) and Simmons Citrate test.

Antibiotic sensitivity test: The antibiotic resistance patterns of bacterial isolates were conducted on Mueller Hinton agar (MHA) using Kirby baur disc diffusion method as per the CLSI guideline and incubated at 37°C; for 24 hrs. For the susceptibility testing the following eight antimicrobial drugs and concentration was used: penicillin G (10µg), chloramphenicol (30 µg), cefotaxime (5µg), Cotrimoxazole, Erythromycin, Vancomycin (15µg) and Oxacillin(1µg).

Multidrug resistance was considered when resistance to 2 or more drugs belonging to different classes of antibiotics tested.

Table 1: Distribution of cases according to Clinical Features

Clinical Features	Number	Percentage (n=77)
Fever		90.70%
Seizure		47.90%
Altered Sensorum		40.00%
Refusal to feed		37.90%
Vomiting		28.60%
Meningeal signs		15.00%
Tachypnea		45.00%
Lethargy		60.00%

Table 2: Distribution of Isolates Grown in CSF (n-77)

Isolates grown	Number	Percentage%
E.coli	1	1.30%
Enterococcus species	1	1.30%
K. pneumoniae	1	1.30%
Streptococcus pneumoniae	2	2.60%
Pseudomonas species	1	1.30%
Total	6	7.79%

Table3: Distribution of Isolates grown in CSF on basis of antibiotic history

Isolates	History of Antibiotic administration						Total
	Given		Not Given		Not Known		
	Number	Percentage	Number	Percentage	Number	Percentage	
E.coli	0	0	0	0	1	33	1
Enterococcus	0	0	0	0	1	33	1
K.pneumoniae	1	50	0	0	0	0	1
Streptococcus pneumoniae	0	0	1	100	1	33	2
Pseudomonas spp.	1	50	0	0	0	0	1
Total	2	100	1	100	3	100	6

Results and Discussion

A total of 77 CSF samples werereceived during study period. Neonates are specially prone to Meningitis and sepsis due to their immature humoral and cellular response. Immaturity ofneonatal immune system impaired phagocytic ability of neutrophil and

monocytes, and diminishing maternal antibody all contribute to increased risk of infections. In this study 57.9% were males and 42.1% were females. Shreshtha*et al.* reported 55.2% males and 44.8% females from their study.

In the present study, majority of cases presented with fever (90.7%),[Table 1] followed by seizure (47.9%), altered sensorium (40%) and refusal to feed (37.9%). About 28.6% reported vomiting and 14.3% had meningeal signs.In a study done by Wang *et al.*[1] most common presenting features were fever (100%), lethargy (100), impaired consciousness (88.50), neck stiffness (69.2%). seizures (53.8%), and headache (50%). Similarly, Jaroush*et al.*[1] reported fever (78%). stiff neck (47%), omitting (37%), poor feeding (19%), and Tiltability (16%) were the most frequently reported symptoms. In a study conducted in China [3]) fever, vomiting, and meningeal irritation were the most prevalent Symptoms in patients where all patients (100%) had fever.

In positive antibiotic history group (Table-2) samples, organisms detected were *K. pneumoniae* and *Pseudomonas*, while in those with no prior antibiotic usage, *S. pneumoniae* was detected in CSF sample. In those with unknown antibiotic usage, *S. pneumoniae, E.coli and Enterococcus* were detected[Table 3].

In this study, CSF samples showed growth of *S. pneumoniae* (2%), *Pseudomonas* (1.2%). *K.pneumoniae* (1.2%), *Enterococcus* (1.2%) and *E.coli* (1.2%). Shrestha *et al.* [4] reported *H. influenzae* (38.9%) followed by group B *Streptococcus*and *E. coli* each constituting 16.7% while *Pseudomonas* spp. in 1 (5.5%) case. Similarly, in the study done by Karode*et al*.most commonly isolated pathogens in CSF were *Klebsiella* (15.3%), *Enterococcus* (5.7%), *E. coli* (5.7%), *S. aureus* (3.8%), *Streptococcus pneumoniae* (1.9%), and *Acinetobacter* (1.9%) [5].

Owusu*et al.* in their study reported *S. pneumoniae* (77.70) as the most common bacteria, followed by *E. coli* (3.4%). *Salmonella* species (3.49%); *Neisseria meningitidis*(2.5%), *Pseudomonas species* (2.5%)[6].

In a study conducted by Jiang *et al.* in CSF samples of patients, the most prevalent pathogens were *E. coli* (28.5%), *Streptococcus pneumoniae* (17.8%), *Staphylococcus epidermidis* (10.0%), *Haemophilus influenzae* type b (9.59%), and group B *Streptococcus*(7.2%) [3].

In the present study. 50% mortality was reported in lab confirmed *S. pneumoniae*infection. Shrestha *et al.*[4] in their study reported case fatality rate of *S. pneumoniae* as 50%. In a study by Gouveia*et al.* S overall case-fatality among hospitalized patients with Pneumococcal Meningitis was 37% [7].

On CSF biochermistry, 50% of *S. pneumoniae*cases have leukocytes count 10-500 cells/ mm3; CSF protein 100-150 mgldL and CSF glucose 1-10 mg/dL while rest 50% *S. pneumoniae* cases have leukocyts in count of501-100 cells/mm^3,protein 150-300 mg/dL and Glucose 11-13 mg/dL. In a study done by Gouevia*et al.* risk factors for case-fatality beside extremes of age, coma on admıssion and penicillin resistance were CSF protein>300 mg/dl blood leukocyte count <15.000 cells/mm^3[7].

In the present study, all *S. pneumoniae* isolates were sensitive for Chloramphenicol, Vancomycin, Ceftriaxone, Cotrimoxazole, Erythromycin, Penicillin, Cefoxitin. Wang *et al.* so reported very high prevalence of penicilin resistant *S. pneumoniae* from several countries with resistance rates ranging from 53.4% to 73.4%.

Currentstudy failed to recover *H. influenzae*. In many studies, *H. influenzae* isolation ratevary from 0.9-12.6%, According to the results of extensive studies of the etiology of Meningitis that have been conducted in Nepal, French Guiana, the North American Arctic and Northern Togo, *S.pneumoniae* and *H.influenzae* type b were the pathogens most frequently [4,7, 8] isolated from the CSF of pediatric bacterial Meningitis cases [4,7, 8].

In the present study, no *N.meningitidis* were isolated on culture. Isolation of *N.meningitidis* in CSF is very low in India, 1% from Bengaluru [9] andvarying from 1-25% in western countries 7,9) Similar findings were obtained in various studies conducted over the years[10, 12].

Conclusion

Effective management of bacterial meningitis involves swift diagnosis and immediate initiation of antibiotic therapy. Vaccination against common causative agents has proven effective in preventing certain forms of bacterial meningitis. Recognizing the urgency of this condition is paramount, underscoring the need for public awareness, timely medical intervention, and preventive measures to mitigate the global burden of bacterial meningitis and safeguard vulnerable populations, especially children.

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