



Aetiology of Acute Bacterial Meningitis At A Tertiary Care Hospital

KEYWORDS

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Introduction-

Bacterial meningitis is a life threatening illness that is prevalent worldwide. Prior to the introduction of antibiotics in the 1940s, case fatality rates for epidemic and endemic bacterial meningitis exceeded 70%. Since then, antibiotic use has reduced case fatality rates of bacterial meningitis to 25% or less⁽¹⁾.

Despite advances in vaccine development and chemoprophylaxis, bacterial meningitis remains a major cause of death and long term neurological disabilities. Microbiology laboratories play a critical role not only in the early identification of the causative bacterium and its antibiotic susceptibility pattern but also in providing valuable information regarding the common incriminating pathogens in that area and the empiric treatment. Regional information regarding trends in terms of aetiology and antimicrobial susceptibility are essential for correct and timely management of meningitis ⁽¹⁾. The most common route is from haematogenous dissemination of microorganism from the distant site of infection, often from the respiratory tract followed by infections of paranasal sinuses and otitis media. More than 2/3rd of cases of meningitis occur in the first two years of life owing to decrease in immunity and high vascularity of brain ⁽²⁾. Three organisms predominate in causing community-acquired bacterial meningitis, Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae ⁽³⁾.

This study was carried out to assess the burden of bacterial meningitis in our area, by estimating the incidence of various organisms responsible for meningitis through prospective hospital-based case detection, to aid in rapid diagnosis of meningitis cases by Gram stain and latex agglutination test and to carry out the comparative evaluation of Gram stain, culture and antigen detection in CSF in cases of Haemophilus influenzae meningitis. This study also intends to determine antibiotic susceptibility pattern of the organism causing meningitis to help in formulating an antibiotic policy for meningitis.

Objectives-

- 1) To determine the pathogens responsible for acute bacterial meningitis and study their Antibiotic susceptibility pattern.
- 2) To detect capsular polysaccharide antigen of the commonly prevailing pathogens in CSF by latex agglutina-

tion tests.

- 3) Comparison of latex agglutination test with Gram's stain and culture.

Study Design

Descriptive study

Inclusion Criteria

1. Presence of clinical picture compatible with a diagnosis of acute pyogenic meningitis
2. All CSF samples with Neutrophils > 10/hpf & or Protein > 40mg/dl & or sugar < 40mg/dl

Exclusion Criteria:

1. Cases of post traumatic CSF
2. Meningitis developed after cranial surgery
3. Chronic meningitis
4. CSF with no pus cells.

Age group-

0 to 12 Years

Material and Methods-

200 clinically suspected cases of acute bacterial meningitis admitted in the Department of Paediatrics, Government Medical College Aurangabad, Maharashtra from the year November 2011 to October 2013. The CSF sample collected under aseptic precautions in a sterile container was subjected to centrifugation at 1500 rpm for 10 minutes. After centrifugation the supernatant was transferred to another test tube and used for serological tests like latex agglutination for bacterial antigens. The sediment was used for gram stain and culture. For culture CSF sample was inoculated on sheep blood agar, chocolate agar and MacConkey agar (in case the smear shows gram negative bacilli). Also subcultures from BHI broth were done after 8 hrs and 24 hrs, on above mentioned media. The inoculated plates were incubated at 37°C with 5% CO₂ using candle jar. The culture plates were observed for any growth next day. The isolated organisms were identified by standard biochemical reactions. Antibiotic sensitivity test was done by the Kirby-Bauer's disc diffusion method^(4,5,6).

The criteria to declare laboratory confirmed cases as acute bacterial meningitis along with presence of pus cells in gram stain include either one of the following⁽⁵⁾ :

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

Latex agglutination test:

Detection of polysaccharide surface antigen by latex agglutination test was also performed. The detection kit used in our study is manufactured by Bio-Rad Pastorex, France. It detects the presence of meningococcus, Streptococcus pneumoniae, H. influenzae type B, Group B streptococcus and E. coli in CSF. The procedure was performed as per the manufacturer's guidelines.

Result -

A total of 200 children clinically suspected of acute bacterial meningitis were investigated for laboratory diagnosis of acute bacterial meningitis during the study period. In total screened 200 samples 120 were males and 80 females. Male: female ratio was 1.5:1. The maximum numbers of the samples were from the age group of > 1 year- 6 years (68) followed by > 1 month to 1 year (60). Out of 200 isolates 122 samples had no finding on Gram stain, Culture and Latex agglutination test and rest 78 samples were laboratory confirmed cases of acute pyogenic meningitis.

Out of 200 cases studied 78 proved to have pyogenic etiology. S.pneumoniae was the commonest organism that caused meningitis i.e., (35 cases) followed by Group B streptococci (Streptococcus agalactiae) eight cases and then followed by E.coli and, Acinetobacter baumannii six

cases each and five cases of H.influenzae

Table 1-Comparison of Gram stain, LAT and Culture showing Comparative Analysis of Gram stain, LAT and Culture positivity.

Organisms Identified	Total	LAT positive	Gram staining positive	Culture positive.
H.influenzae	5	5	5	4
Streptococcus pneumoniae	35	28	32	8
Group B Streptococci	8	7	8	2
E.coli	6	1	6	6
Acinetobacter baumannii	6	NA	6	6
N.meningitidis W/Y 135	4	4	1	0
N.meningitidis A	3	3	1	1
Enterococcus Faecalis	2	NA	2	2
Enterococcus faecium	2	NA	2	2
Staphylococcus aureus	2	NA	2	2
Aeromonas sobria	1	NA	1	1
Klebsiella pneumoniae	1	NA	1	1
Sphingomonas paucimobilis	1	NA	1	1
Pantoea spp.	1	NA	1	1
Pseudomonas aeruginosa	1	NA	1	1

NA = Not Applicable as latex agglutination test is not available for these isolates

In our study for isolates, Gram stain positivity was 88% (i.e., positive in 69 cases out of 78 cases). Culture positivity was 49% (i.e., positive in 38 cases out of 78 cases) and latex agglutination test positivity was 79% (i.e., positive in 48 cases out of 61 cases). LAT was not considered for the remaining 17 isolates (1 isolates of K. pneumoniae, 6 isolates of Acinetobacter baumannii, 2 isolates of Staphylococcus aureus and one isolate of pseudomonas aeruginosa, Aeromonas sobria, Pantoea spp. Sphingomonas paucimobilis & 4 Enterococci spp. as latex coated antibodies are not available)

Antibiogram of various isolates- Susceptibility pattern is as follows -

All the isolates were tested for drug resistance by Kirby Bauer disc diffusion and Group A and Group B drugs prescribed by CLSI 2012-13 were used.

- ESBL strains were confirmed phenotypically by combined disc test only i.e. by using criteria of ≥ 5 mm zone accentuation with beta lactamase inhibitor (clavulanic acid) compared with third generation cephalosporin (Ceftazidime and Cefotaxime)
- MBL strains were tested by Meropenem and Meropenem + EDTA discs a zone accentuation of ≥ 7 mm was

considered as positive.

Regarding the *Staphylococcus aureus* isolates, the drug Penicillin was used for disc diffusion in this case and not ampicillin. As penicillin is group A drug for *Staphylococcus*.

- The cefoxitin disc was used for the screening of MRSA (Methicillin Resistant *Staph aureus*) none of the two isolate were found to be MRSA
- *Streptococcus pneumoniae* was found to be susceptible to penicillin in 75% of the cases, 100% sensitive to linezolid, vancomycin cefotaxime and 50% cases showed sensitivity to cotrimoxazole.
- In case of Gr. B streptococci the two strains positive on culture were sensitive to Penicillin, linezolid & cefotaxime.
- Enterococcal spp. was found to be resistant to penicillin as they are known to be intrinsically resistant to it. In all the four cases vancomycin was found to be 100% sensitive.
- The *Staphylococcus aureus* was found resistant to penicillin, and cotrimoxazole and 100% sensitive to linezolid.
- In case of gram negative bacilli isolated most common was *E.coli* was found to be 66% sensitive to 3rd generation cephalosporins, 50% for ampicillin & gentamycin & 100% sensitive to cefepime, Ceftazidime- clavulanic acid combination and meropenem. Two isolates were Extended Spectrum Beta Lactamase (ESBL) producer.
- *Acinetobacter baumannii* was found resistant to all the drugs and was isolated from NICU patients. It was found 40% sensitive to gentamycin, cefotaxime, cefepime, ceftazidime- clavulanic acid combination and 60% sensitive to meropenem.
- One isolate of *N.meningitis A* was found sensitive to cefotaxime and meropenem.
- *Pseudomonas aeruginosa* was also found to be sensitive to all the drugs namely gentamycin, ceftazidime, meropenem and ceftazidime- clavulanic acid combination.
- *Sphingomonas paucimobilis* isolate was identified on API was found to be sensitive to all the drugs tested- gentamycin, ceftazidime, cefepime, meropenem and ceftazidime- clavulanic acid combination.
- *Klebsiella pneumoniae* was resistant to gentamycin, cefotaxime, ampicillin and sensitive to cefepime, meropenem and ceftazidime- clavulanic acid combination. (ESBL producer by Double disc synergy test).
- *Aeromonas sobria* isolate was identified on mini API was found to be sensitive to all the drugs tested- gentamycin, ceftazidime, cefepime, meropenem and ceftazidime- clavulanic acid combination
- Penicillin resistance was about 25% in *Streptococcus pneumoniae*, 50% of isolates showed resistance to cotrimoxazole.
- The strains of Group B streptococci were found to be sensitive to all the drugs including Penicillin.
- *Staphylococcus aureus* was found sensitive to Linezolid and cefoxitin (MSSA)
- Enterococcal spp were found to be resistant to penicillin but sensitive to vancomycin and Linezolid.
- In case of Gram negative bacilli two strains of *E.coli* were ESBL (Extended Spectrum Beta Lactamase) producer i.e. 33% (2 out of 6)
- One *K.pneumoniae* was isolated from the patient who had frank pus and hydrocephalus the strain was also ESBL producer and the baby didn't survive.
- In case of *Acinetobacter baumannii* one strain was MBL (Metallo Beta Lactamase) producer (confirmed pheno-

typically)

- These results signify the varying levels of drug resistance amongst the gram positive and the gram negative microbes, and the need to control the spread of these resistant strains before they reach the alarming levels in this region.
- **Biostatistics analysis**

We compared the sensitivity and specificity of LAT and Gram stain with Culture which is considered as the gold standard test⁽⁵⁾ Comparison of Culture and LAT showed Sensitivity = 76% and Specificity = 79% PPV = 33% and NPV = 96% (95% confidence limit).

Comparison of Culture and Gram stain showed Sensitivity = 100% and Specificity = 79% PPV = 54% and NPV = 100% (95% confidence limit).

Comparison is made between the LAT and Gram stain among which none is regarded as gold standard Kappa factor is calculated to study the degree of agreement between the two methods.

Number of observed agreement = 91.67%

Kappa = 0.794. The strength of agreement is considered to be good.

Discussion-

Comparative studies of gram Stain in acute bacterial meningitis shows a gram stain positivity of 88% in our study while the gram stain positivity was in 67% cases as shown by Mane et al⁽⁷⁾(2002), Vishwanath et al⁽⁸⁾ study showed positivity in 90% cases (2007), Chandramukhi et al⁽⁹⁾ 65% (2007) and in a recent study by Awari et al⁽¹⁰⁾ gram stain positivity was 98%(2012). LAT was positive in 79% of cases in our study which is in accordance with Mane et al⁽⁷⁾78% (2002), Vishwanath et al⁽⁸⁾ 90% (2007), Chandramukhi et al⁽⁹⁾ found sensitivity a bit less i.e in 54% cases (2007) while in study Awari et al (2012) it was 92%. Culture was positive in 46.5% cases in our study this can probably be due to prior antibiotic course given to patient, delay in transportation of the CSF sample, fastidious nature of the organisms causing meningitis. It was found to be low in studies by Mane et al⁽⁷⁾ (50%), Vishwanath et al⁽⁸⁾ (62%), Chandramukhi et al⁽⁹⁾ (41%) while a bit on higher side in study by Awari et al (9) (72%). We found *Streptococcus pneumoniae* to be the most common organism responsible for Meningitis in children in our region. Studies by R.Mani et al⁽⁷⁾ and Debnath et al⁽¹¹⁾ also had similar finding in their study.

We also found some rare causes of meningitis like *S.paucimobilis*, *Aeromonas sobria* and *Pantoea Spp*. In earlier studies Sonavane et al⁽¹²⁾ found a cases of meningitis due to *Sphingomonas paucimobilis*. *Aeromonas hydrophila* has been reported as a cause of meningitis in a 3 month old baby by Seetha et al⁽¹³⁾. This is probably first study from India showing *Pantoea spp*. to be responsible for meningitis *Sphingomonas paucimobilis* and *Pantoea spp* were isolated from CSF of NICU Patients. In majority of the cases gram stain correlation with culture and latex positivity was excellent. Proving that the age old practice of gram staining still holds firm roots in the diagnosis of various infectious conditions.

Conclusion-

Gram stain was found most sensitive indicator than culture and LAT Latex agglutination was also found to be sensitive and specific test for detection of antigen in CSF. We found *Streptococcus pneumoniae* as the most common cause of meningitis in our region. We also found some rare isolates

causing meningitis in our study namely. *Sphingomonas paucimobilis*, *Pantoea* spp. and *Aeromonas sobria*. Using the above information an approach to the patients with bacterial meningitis can be formulated. Rapid and accurate diagnosis and appropriate management could avoid the complications of meningitis like neurological defects, deafness or any morbidity or mortality.

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