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Research article

ANTIBIOTIC SUSCEPTIBILITY PATTERN FROM PATHOGENS CAUSING ACUTE PYOGENIC MENINGITIS IN A TEACHING HOSPITAL, SOUTH INDIA.

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ABSTRACT

Background and Objectives: Acute pyogenic meningitis is one of the most serious infections in infants and children. It is associated with serious complications and risk of morbidity and mortality. The purpose of present study was to identify the pathogen in acute pyogenic meningitis and to determine its antibiotic susceptibility pattern.

Methods: Present study was undertaken for a period of one year from July 2009 to June 2010 included 100 CSF samples of clinically suspected acute pyogenic meningitis cases in children below 12 years. The samples were subjected to Gram's stain, culture and antibiotic sensitivity test. The cases positive in either of Gram stain or culture were diagnosed as acute bacterial meningitis cases. Results were tabulated and antibiotic sensitivity pattern was compared.

Results: Of the 100 cases studied, 26 cases were diagnosed as acute bacterial meningitis. Gram's stain positivity was 73% (19/26 cases), culture positivity was 100%. The most common organism isolated in the study was *Streptococcus pneumoniae*, followed by *Haemophilus influenzae*, *Escherichia coli* and *Klebsiella pneumoniae*. Aminoglycosides, cefotaxime and cotrimoxazole showed high sensitivity.

Interpretation and conclusion: Though Gram stain is very essential in diagnosis of meningitis, it may miss some cases. Culture and latex agglutination tests overcome this disadvantage. Streptococcus pyogenes still remains predominant pathogen. Antibiogram of the bacteria causing meningitis is also slowly undergoing a change. This calls for change in the empirical therapy for bacterial meningitis cases.

Key words: Acute pyogenic meningitis; Grams stain; Culture; antibiotic susceptibility pattern.

INTRODUCTION

Acute pyogenic meningitis is one of the most serious infections in infants and children. It is associated with serious complications and risk of morbidity. It occurs both in epidemic and sporadic pattern.(Kliegman and Nelson 2007) Before the discovery and use of antibiotics, bacterial meningitis generally was fatal. Although antibiotic therapy has improved dramatically the prognosis in patients afflicted with bacterial meningitis, it still continues to be a significant cause of morbidity and mortality in children(Cherry et al. 2014). The age distribution of patients with bacterial meningitis has not changed much during the past 40 years (Cherry et al. 2014). But emergence of antibiotic resistance among pathogens has led to narrowing down of the therapeutic options available for the treatment of meningitis cases. Hence study was conducted to identify the pathogen in acute pyogenic meningitis and to determine its antibiotic susceptibility pattern and suggest suitable options for empirical therapy of acute bacterial meningitis cases.

MATERIAL AND METHODS

The study was carried out in the Department of Microbiology, J.J.M. Medical College, Davangere. A total of 100 clinically suspected meningitis patients between age group 1 day to 12 years admitted to Department of Paedatrics in Chigateri General Hospital and Bapuji Child Health Institute were selected for this study.

After taking history and clinical examination, cerebrospinal fluid (CSF) sample was collected under aseptic precautions from patients clinically suggestive of acute pyogenic meningitis. Upto 3-5ml of CSF was collected in a sterile container for various microbiological, cytological and biochemical investigations.(Mandell, Bennett, and Dolin 2010) Few drops of CSF were directly collected into Brain heart infusion broth with isovitalex (Ananthanarayan 2009). Upon receipt of the CSF samples in the laboratory, the fluid was inspected grossly for turbidity, colour and xanthochromia. Wet mount was done immediately and observed for any possible presence of bacteria, yeasts or parasites etc.(Cheesbrough 2005) CSF samples were subjected to centrifugation at 1500 rpm for 10 minutes and the sediment was used for Grams stain and culture. Grams stain was observed for the presence of possible organism and pus cells. The specimen was heavily inoculated into brain heart infusion broth, sheep blood agar, chocolate agar with isovitalex (X and V) factor and Mac Conkey's agar media. Also subcultures from the BHI broth were done after 6-8hrs and 24 hrs on above mentioned media. The inoculated plates were incubated at 37^0C with 5% CO_2 using a candle jar in incubator. Bacteria grown were identified using biochemical reactions.

Depending on the presumptive identification of the organisms, antibiotic sensitivity test was done using Kirby bauer methods (Forbes 2007). Results were tabulated and antibiotic sensitivity pattern was compared. Gram stain and culture were compared and Mc nemars test was done to assess significance.

RESULTS

A total of 100 cases of acute bacterial meningitis were studied. Out of 100 children, 26 were proven by lab investigations as pyogenic meningitis.

Table 1. Age and sex distribution of positive cases:

Age in Years	Male (%)	Female (%)	Total (%)
0-1	6 (60)	4 (40)	10 (100)
2-6	6 (75)	2 (25)	8 (100)
7-12	3 (37.5)	5 (62.5)	8 (100)
Total	15 (58)	11 (42)	26 (100)

Out of the 26 cases of pyogenic meningitis males were affected more than females. 15 children (58%) were males and 11 (42%) were female. Most of the cases were between the age group 1 month to 1 year.

Table 2. Organisms grown from the cases

Organism isolated	No (%)
S. pneumoniae	7(26.92%)
H. influenzae	5(19.23%)
E. coli	3(11.5%)
K. pneumoniae	3(11.5%)
Acinetobacter	2(7.6%)
Group B Streptococci	2(7.6%)
Staphylococcus aureus	2(7.6%)
Coagulase negative Staphylococcus	1 (3.8%)
Pseudomonas species	1(3.8%)
Total `	26

Out of 100 cases studied 26 proved to have pyogenic etiology. *S.pneumoniae* was the commonest organism that caused meningitis i.e., 7 cases (26.9%) followed by *H. influenzae* 5 (19.2%) cases and then followed by *E. coli* and *K. pneumoniae* 3 (11.5%) cases each, Group B streptococci and *Acinetobacter* 2 (7.6%) cases each and a case of coagulase negative staphylococcus and *Pseudomonas* was isolated.

Table 3: Comparison of results of Gram's stain versus culture

Gram stain	Culture		Total	Mc Nemar's test
	Positive	Negative		
Positive	19	0	19	0.015
Negative	7	0	7	
Total	26	0	26	

Antibiogram

Antibiotic susceptibility testing was done on all the culture positive isolates of which 12 were Gram positive bacteria and 14 were Gram negative bacteria.

Table 4: Antibiotic susceptibility pattern of Gram positive bacteria

Antibiotic	Sensitive	Percentage sensitive
Ampicillin	3	25
Cotrimoxazole	7	58
Ciprofloxacin	4	33
Cephalexin	6	50
Cefotaxime	5	42
Amikacin	11	92
Gentamicin	8	67
Erythromycin	6	50

Table 5: Antibiotic susceptibility pattern of Gram negative bacteria

Antibiotic	Sensitive	Percentage sensitive
Ampicillin	5	36
Cotrimoxazole	9	64
Ciprofloxacin	7	50
Cephalexin	7	50
Cefotaxime	11	79
Amikacin	11	79
Gentamicin	6	43

DISCUSSION

Acute Bacterial meningitis is definitely a life threatening disease and its rapid diagnosis in the laboratory forms the basis for the quick initiation of effective treatment. Especially in the developing world, an in-ordinate delay from the time of development of symptoms to the initiation of antimicrobial therapy may contribute to the higher mortality rate observed in these countries.(Kliegman and Nelson 2007; Cherry et al. 2014)

The present study was undertaken to study the bacteriological profile and antibiogram of the isolates of bacterial meningitis and also to do a comparative evaluation of Gram stain and culture in clinically suspected cases in children below 12 years.

In our study, of 100 clinically suspected cases, laboratory evidence could be seen in 26 cases only. Remaining 74 cases did not yield any growth. Mc Nemar test for significance showed 0.015 indicating that it is significant.

This probably reflects that either patients were infected with fastidious bacteria like *Neisseria meningitidis* that cannot be grown with conventional methods or had received antibiotics before collecting specimen. Though we attempted to collect specimen before antibiotic administration, many patients had already received few doses before they landed up at our hospital as ours is a tertiary care hospital.

After antibiotic dose is given, the bacteria experience a post antibiotic effect where their growth is inhibited or they are killed and hence they fail to grow in culture. (Forbes 2007). This disadvantage can be overcome with use of latex agglutination tests. Latex agglutination tests have 2 advantages: 1. They can detect antigen of dead bacteria which do not grow on culture 2. Some fastidious pathogens like meningococci which are difficult to grow in the laboratory can be diagnosed with help of these latex agglutination kits. (Patil et al. 2013; Mishra, Mahaseth, and Rayamajhi 2013)
In our study, gram stain detected 73% (19/26) of cases of culture confirmed bacterial meningitis cases. The finding can be explained as we know that culture is superior to microscopy. Microorganisms are seen in the smear examination only if their concentration is more than 10^5 per ml of specimen, while culture can detect organisms even at lower concentrations like 10^3 organisms per ml. Our study correlates with studies of the other Indian authors, who report 67% and 74% gram stain positivity respectively.(Chinchankar et al. 2002; Mirdha, Gupta, and Bhujwala 1991)

In our study, of all 26 culture positive cases, predominant isolate was S. *pneumoniae* causing 7 cases (26.92%) followed by *H.influenzae* (19.2%). T.A. Ogunlesi et al, in their study also report *S. pneumoniae* as predominat pathogen, (33.9%), followed by *H. influenzae* (33.9%). (Ogunlesi, Okeniyi, and Oyelami 2005) Another study reports H. influenzae (33.8%), S.pneumoniae (26%). (N M Shembesh 1998) Viswanath G. et al in their study report *H. influenzae* (22.5%) was the main isolate followed by S. *pneumoniae*. (Viswanath et al. 2007).
In the antibiogram of Gram positive bacteria, aminoglycosides (amikacin 92% & gentamicin 67%) had highest sensitivity percentage followed by Cotrimoxazole (58%). This trend is probably because, aminoglycosides being injectable antibiotics, are used only when prognosis is poor, hence less exposure of bacteria to the antibiotic stress. However, cephalosporins and macrolides available as oral drugs also, are used more frequently leading to selection pressure among organisms leading mutation and higher prevalence of resistance. This is probably also the reason for cotrimoxazole emerging as 3rd alternative option. (Forbes 2007; Ananthanarayan 2009). As resistance to cotrimoxazole was high in the past, the drug was slowly discontinued from usage. When the usage decreases, selection pressure on the bacteria is withdrawn and mutation is reversed and sensitive pattern is re-established. Among gram negative bacteria, amikacin (79%), cefotaxime (79%) and cotrimoxazole (64%) tested highest sensitivity percentage. This result highlights the changing pattern of antibiogram among pathogens of meningitis and calls for change in empirical treatment of meningitis cases.

CONCLUSION

Bacterial meningitis being a medical emergency needs urgent medical attention. Institution of antibiotic treatment promptly decreases the mortality and morbidity. Though Gram stain is very essential in diagnosis of meningitis, it may miss some cases. Culture and latex agglutination tests overcome this disadvantage. *Streptococcus pyogenes* still remains predominant pathogen in our setup. Since, antibiogram of the bacteria causing meningitis is also slowly undergoing a change which calls for change in the empirical therapy for bacterial meningitis cases.

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