



Study of Acute Bacterial Meningitis Cases With Phenotypic Characterization and AntibioGram of CSF Pathogens in A Tertiary Care Hospital, India

*** Dr. Rajani Sharma**

M.D. Microbiology, Senior Resident, Dept. of Microbiology, PGIMER & Dr. RML Hospital, New Delhi, , Senior Resident, Department of Microbiology, PGIMER & Dr. R.M.L. Hospital, New Delhi.110001

Anuradha

M.D. Microbiology, Assistant Professor, Dept. of Microbiology, PGIMER & Dr. RML Hospital, New Delhi.

Duggal Nandini

M.D. Microbiology, HOD & Professor, Dept. of Microbiology PGIMER & Dr. RML Hospital, New Delhi.

ABSTRACT

Context: Bacterial meningitis remains a major cause of mortality and long term neurological sequelae worldwide. It is important to know the regional bacterial etiology in developing countries like India along with their sensitivity profile to allow optimum management of such patients with least possible mortality.

Aims: Our aim was to study the bacterial profiles and antimicrobial susceptibility patterns of the CSF isolates which were obtained from patients of acute bacterial meningitis in our area.

Material and Methods: Present study was undertaken from Jan 2015 to December 2015 and included 770 CSF samples of clinically suspected acute meningitis cases in all age groups. The samples were processed and organisms isolated in the study were characterized by standard procedure and antibiotic susceptibility tests according to CLSI guidelines.

Results: Total 68 bacteria were isolated from 770 cases with isolation rate of 8.83%. The most commonly isolated bacteria were Acinetobacter spp. 21 (30.8%) and K. pneumonia 9 (13.2%), followed by Pseudomonas aeruginosa 5 (7.3%), E. coli 3 (4.4%) and Citrobacter spp. 1 (1.4%). Among gram positive organisms, S. aureus in 18 (26.4%), CoNS in 6 (8.8%) and Enterococci spp. 5 (7.3%) isolated. All gram negative isolated were 100 % sensitive to Imipenem and Polymyxin B. Amongst Gram positive isolates Enterococcus spp. and Staphylococcus aureus were 100% sensitive to almost all the antibiotics tested.

Conclusion: To conclude, an entirely different trend in etiology in bacterial meningitis was observed in this study. Gram negative bacilli were found to be more frequently isolated in our series of patients with Acinetobacter being the most common isolate. Established organisms of meningitis such as Haemophilus influenzae and Neisseria meningitidis were not isolated at all, despite all our efforts. The high prevalence of drug resistant pathogens is a cause for worry and should be dealt with by rational use of antimicrobials.

KEYWORDS

Bacterial meningitis, Antimicrobial sensitivity, CSF

Introduction:

Bacterial meningitis remains a common disease worldwide, which is usually caused by viral, bacterial or fungal pathogens. It remains a major cause of mortality and long term neurological sequelae worldwide^{1, 2}, if not treated early. The clinical signs and symptoms can't be always relied upon, laboratory support is imperative to achieve early diagnosis. Laboratory surveillance of isolates is crucial to identify targets for formation of rational empirical treatment for potentially fatal bacterial meningitis. Published studies on meningitis are very few in the Southeast Asian region^{3,4,5}. Thus, we hope this study will add information to the existing data for the purpose of reducing mortality rates. We reviewed the microbiological records of patients with bacterial meningitis to determine frequency of pathogens causing bacterial meningitis, to find out etiological agents and their susceptibility pattern.

Materials and Methods:

A retrospective analysis of clinically suspected cases of meningitis, admitted during a span of one year from Jan 2015 to December 2015 was undertaken. All the clinically suspected cases of meningitis were included in this study. CSF samples of varying quantity of 3 to 5 ml were collected aseptically in sterile container and were transported at ambient temperature to the laboratory without delay.

All CSF specimens were processed according to the standard laboratory protocol adopted from the Clinical and Laboratory Standards Institute (CLSI)⁶. A portion of CSF samples were

centrifuged and deposits were subjected to Gram's stain, ZN stain, Negative staining with India Ink for Cryptococcus and culture. All the samples were inoculated on Chocolate agar, Blood agar and MacConkey agar. Remaining portions of CSF was put in Brain-Heart Infusion broth for enrichment from which subcultures were done on the above mentioned media. All the culture plates were incubated at 5% CO₂ and at 37°C in an incubator for 24-48 hours. The culture plates were observed daily for presence of growth. The isolates were identified by standard techniques and antibiotic susceptibility tests were done against locally available antibiotics by using Kirby bauer's disk diffusion method in accordance with CLSI 2014 criteria and interpreted accordingly.

Results:

Total 68 bacteria were isolated from 770 cases with isolation rate of 8.83%. Table 1 shows that out of the 68 confirmed meningitis cases, 40 (58%) were male and the remaining 28 (41.1%) were female. Male: Female ratio was 1.4:1. The age distribution patterns of the study subjects summarized in table-1. The age range was from 2 months to 75 years.

Table 1: Age & Sex wise Distribution of patients

Sex	
Male	40 (58%)
Female	28 (41.1%)
Age Group (In Year)	
Less than 1 Year	7

1-15 years	25
16-30 years	24
30-45 years	6
46-60	4
>60	2

The most commonly isolated bacteria were *Acinetobacter spp.* 21 (30.8%) and *K. pneumoniae* 9 (13.2%), followed by *Pseudomonas aeruginosa* 5 (7.3%), *E. coli* 3 (4.4%) and *Citrobacter spp.* 1 (1.4%). Among gram positive organisms, *S. aureus* in 18 (26.4%), CoNS in 6 (8.8%) and *Enterococci spp.* 5 (7.3%) isolated. [Table 2]

Meningitis caused by *Staphylococcus aureus* and coagulase negative staphylococci mainly seen in patients who had undergone any neurosurgical procedures or placement of CSF shunts.

Table 2: Bacterial Pathogens Isolated from CSF

Organisms Number	(n=68) (%)
<i>Acinetobacter spp.</i>	21 (30.8)
<i>Klebsiella spp.</i>	9 (13.2)
<i>Pseudomonas aeruginosa</i>	5 (7.3)
<i>E. coli</i>	3 (4.4)
<i>Citrobacter spp.</i>	01 (1.4)
<i>S. aureus</i>	18 (26.4)
CoNS	06 (8.8)
<i>Enterococcus spp.</i>	05 (7.3)

Antibiograms of these organisms have been shown in [Table 3]. All gram negative isolated were 100 % sensitive to Imipenem and Polymyxin B. Amongst Gram positive isolates *Enterococcus spp.* and *Staphylococci aureus* were 100% sensitive to almost all the antibiotics tested.

Table 3 .Antibiotic sensitivity patterns of isolates

Drugs	Acinetobacter	Klebsiella	Pseudomonas	E.coli	Citrobacter spp.	S. aureus	Enterococci	CoNS
Amp	-	-	-	-	-	66%	100	100
CX	-	78	-	75	-	66%	-	100
CAZ	40	55	64	65	0	-	-	-
Ci	40	55	-	65	0	-	-	-
AK	55	68	76	79	0	-	-	-
GEN ^a	58	40	52	41	0	-	100	-
CIP	43	71	76	61	0	50%	-	33
COT	64	31.9	-	37	0	50%	-	66
E	-	-	-	-	-	61	-	17
T	-	-	-	-	-	83	-	66
VA*	-	-	-	-	-	100	100	100
LZ	-	-	-	-	-	100	100	100
PC	-	-	48	-	-	-	-	-
PT	68	81	80	72	0	-	-	-
IMP	90	89	100	79	100	-	-	-
CPM	89	83	-	81	0	-	-	-
AT	-	71	63	69	0	-	-	-
NET	87	79	94	82	0	-	-	-
CL	100	100	100	100	100	-	-	-
PB	100	100	100	100	100	-	-	-

^ain case of *Enterococcus* high dose genamycin (120 ug) used
*vancomycin sensitivity by MIC

Discussion:

Bacterial meningitis is a potentially life threatening disease that consists of inflammation of the meninges and the underlying subarachnoid CSF. The successful management of bacterial meningitis depends upon the isolation of organisms and the selection of an appropriate antibiotic regimen. Thus, this study could be helpful to clinicians in North India on the selection of antimicrobial agents for the treatment of meningitis.

CSF samples from only 68 (8.83%) patients were positive on culture in our study. Several studies from India report culture-negative cases of meningitis or a low CSF culture posi-

tivity, ranging from 6-50%^{1,7,8,9}. The common gram negative pathogens isolated were *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter spp.* and *Citrobacter spp.* These findings were different from those in other studies, probably because this study included all age groups unlike the other studies, which included only children^{8,9,10}.

As compared to western studies, the relative incidence of meningitis caused by *Haemophilus influenzae*, *Neisseria meningitidis* and *Listeria spp.* is less in South East Asia.^{11,12} On the contrary Gram negative bacilli such as *K. pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter spp.* are increasingly being recognized as important pathogens. These gram negative isolates are also reported as pathogens of bacterial meningitis in a recent study conducted at NIMHANS Bangalore in 2006¹. Among Gram positive bacteria isolated in the present study commonest was *Staphylococcus aureus* and *coagulase negative staphylococci*. The difference in etiology from the temperate West may be due to the fact that India is a semitropical country, where hardy bacteria like *S. aureus* and gram negative bacilli flourish, and the relatively more fragile bacteria like *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, *S. agalactiae* and *Listeria monocytogenes* in comparison do not have a survival advantage. Secondly unfortunately most of the patients turn to the tertiary care centre after taking treatment from local practitioners which may lead not only to culture negative results but also to lower isolation of *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, *S. agalactiae* and *Listeria monocytogenes* which have not developed significant resistance to the usual antimicrobials. These results highlight the very different etiological profile in India in comparison to that of the West, pointing to the fact that Asian epidemiology is distinctly different from the West.

This also explains why most of the latex agglutination gave negative results. Latex agglutination tests are mainly used to determine specific community acquired meningitis but not to determine hospital acquired meningitis. The cases in our study were a mixture of hospital and community acquired meningitis which was expected as our hospital is the referral centre.

The high incidence of sterile CSF (91.17%) may be due to empirical antibiotic use as access to drugs, prescribed or otherwise is unrestricted. This problem can be readily overcome if newer methods of diagnoses which do not require the growth of live pathogens such as polymerase chain reaction are incorporated into medical laboratory practice in the region.

The problem of antibiotic misuse may lead to the increasing resistance to the common anti-meningitis drugs i.e., ampicillin, penicillin and chloramphenicol.¹³ In our study most of the enterobacteraceae members and pseudomonas were susceptible to piperacillin with tazobactam, carbapenems. The only isolate of *Citrobacter* spp. was found to be multidrug resistant. In case of gram positive bacteria, the highly effective antibiotics were tetracycline, vancomycin, and linezolid.

A limitation in the study includes loss of some fastidious bacteria leading to high culture negative CSF because of frequent inappropriate use of antibiotics by patients before presentation, delay in processing of CSF specimens and paucity of newer method of diagnoses which do not require the growth of live pathogens such as polymerase chain reaction in the medical laboratory practice in our set-up.

The second limitation is that our study was limited to one tertiary centre hospital. The patients in this study do not represent the population of meningitis cases within India. Further research is needed to obtain more understanding of hospital bacterial meningitis, especially in comparing between types of hospitals, patients' risk factors, and outcomes among survivors.

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