Original Article

Study on isolates of acute meningitis in a tertiary care centre in Assam

Abstract

Background: Meningitis is an inflammatory affection of the membranes surrounding the brain and spinal cord, which occurs as either a primary disease or secondarily to disease in some other part of the body. The epidemiological trend of acute meningitis varies with time and geography. **Objective:** To isolate the various agents of acute meningitis in all age group patients and to know the antimicrobial susceptibility pattern of bacterial isolates. **Materials and Mathods:** In this prospective study, a total of 316 cerebrospinal fluid (CSF) specimens were collected from patients showing signs and symptoms of acute meningitis and processed by standard microbiological methods in a tertiary care hospital in Guwahati, Assam over a period of one year, from August 2009 to July 2010. **Results:** Out of 316 CSF samples, bacterial and fungal culture positivity rate was found to be 16.13%. The most common bacterial isolate was *Staphylococcus aureus*, 29.41%. Isolation rate of *Cryptococcus neoformans* was 8%. All the Gram positive isolates were 100% sensitive to linezolid and vancomycin, whereas Gram negative isolates were 92% sensitive to polymyxin B. **Conclusion:** This study gives an idea about the changing trend of acute meningitis along with the changing *in vitro* antimicrobial susceptibility pattern of the bacterial isolates, which can help the clinicians to formulate the initial empiric therapy for patients of acute meningitis.

Key words: Acute meningitis, MRSA, sensitivity pattern

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INTRODUCTION

Meningitis is an inflammatory affection of the membranes surrounding the brain and spinal cord, which occurs as either a primary disease or secondarily to disease in some other part of the body. It is associated with significant morbidity and mortality in all parts of the world, particularly, in pediatric age group.[1] Causative agents of acute meningitis can be viral, bacterial or fungal pathogens. Among these, bacterial meningitis can be quite severe and may result in brain damage, hearing loss or learning disability and death.[2]

Among the bacterial agents causing meningitis, the most common agents responsible for community-acquired bacterial meningitis are *Streptococcus pneumoniae* (~50%), *Neisseria meningitidis* (~25%), Group B *Streptococcus* (15%) and *Listeria monocytogens* (~10%). *Hemophilus influenzae* now accounts for less than 10% of cases of bacterial meningitis. The epidemiology of bacterial meningitis has changed significantly in recent years, reflecting a dramatic decline in the incidence of meningitis caused by *Hemophilus influenzae* and *Neisseria meningitidis,* following the introduction and increasingly widespread use of vaccines for both these organisms. Enteric Gram negative bacilli are increasingly common cause of meningitis in individual with chronic and debilitating diseases like diabetes, cirrhosis or alcoholism and in those with chronic urinary tract infection. *Staphylococcus aureus* and coagulase-negative staphylococcus are important causes of meningitis that follow invasion of neurosurgical procedures.[3]

In most studies, besides conventional pathogens other bacteria like *Klebsiella* species, *Escherichia coli*, *Pseudomonas* species, *Acinetobacter* species, *Citrobacter* species, *Enterococcus* species etc. have been isolated from admitted patients.

In partially treated meningitis, CSF may become clear with predominant lymphocytes; culture may also be sterile.[4]

Cryptococcal meningitis caused by environmental fungus *Cryptococcus neoformans*, is a common opportunistic infection in human immunodeficiency (HIV)-infected individuals, particularly in Southeast Asia, Southern and East Africa.[5,6] It usually infects the HIV-infected patients with CD4 count <100 cells/μl. These patients present with features of acute, sub-acute or chronic meningitis or meningoencephalitis.[6]

MATERIALS AND METHODS

A total of 316 CSF samples were tested in the Department of Microbiology in a tertiary care hospital in Guwahati, Assam over a period a period of one year, from August 2009 to July 2010.

CSF samples were collected as a part of the routine clinical management from patients with signs and symptoms of acute meningitis and were admitted in different wards of the hospital that include: Medicine wards, pediatric wards, intensive care units (ICU), neonatal intensive care units (NICU) etc.

The CSF samples were collected in sterile containers by attending physicians and delivered to the Microbiology laboratory as soon as possible. CSF of 2-3 ml was transferred to a sterile centrifuge tube and was centrifuged at 3000 rpm for 5 minutes. The supernatant part was transferred to a clean container for chemical examination.[7]

The CSF deposits were seeded on to blood agar, chocolate agar, and chocolate agar medium with vitamino growth supplements, modified (twin pack) (HiMedia Laboratories, FD215), Mac Conkey agar and glucose broth. The Mac Conkey agar and glucose broth were incubated aerobically while blood agar, chocolate agar, chocolate agar medium with supplements were incubated in the presence of 5-10% carbon dioxide in candle jar at 37°C for 24 hours. The plates and broth were examined after overnight incubation and if there was no growth the plates were re-incubated for further 24 hours and examined. The broth was sub-cultured on the above solid media and examined.

Organisms were identified by standard microbiological methods, which included colony morphology, as well as staining, and biochemical tests.[1,8,9]

The criteria for diagnosis of *Cryptococcus neoformans* were based on positive India ink preparation of CSF, culture in Sabouraud dextrose agar and Bird seed agar, assimilation test, biochemical test and latex agglutination test.[10]

The criteria for diagnosis of *Candida albicans* were based on KOH mount of CSF, culture in Sabouraud dextrose agar and CHROM agar, assimilation reaction and germ tube test.[11]

Antibiotic sensitivity test was conducted on pure culture isolates employing the disc diffusion method for the commonly used antibiotics per disc: Penicillin G (P, 10 μg), gentamicin (G, 10 μg), amikacin (AK, 30μg) erythromycin (E, 5μg)), vancomycin (V, 30 μg), linezolid (LZ, 30 μg), ciprofloxacin (CF, 5μg), amoxicillin-clavulanic acid (AC, 10 μg), piperacillin- tazobactum (PZ, 100/10 μg), cefotaxime (CE, 30 μg), ceftriaxone (CI, 30 μg), cefoperazone (CS, 75 μg), ceftazidime (CZ, 30 μg), cefoxitin (CN, 30 μg) and polymyxin B (Pb, 300 U), (HiMedia). The diameters of growth inhibition around the discs were measured and interpreted as sensitive, intermediate or resistant as per the Clinical and Laboratory Standards Institute (CLSI) guideline 2010.[12] Reference strains used as controls were *E. coli* ATCC 25922 and *S. aureus* ATCC 25923.

RESULTS

A total of 316 CSF samples were investigated during the study period from August 2009 to July 2010.

In this study, out of total 316 CSF samples, 163 (51.58%) samples were collected from patients of the age group 0-10 years, followed by 41 (12.97%) in the age group of 11-20 years. Only one sample (0.32%) was collected above the age of 70 years.

Out of the total 316 cases, 212 (67.09%) were males and 104 (32.91%) were females; the male to female ratio is 2:1.

Fever (92.72%), altered mental status (74.36%), headache (61.71%) and convulsion (57.6%) were the most common clinical manifestations of meningitis among the patients in the present study. Frequencies of clinical manifestations of patients are shown in Table 1.

Out of 316 CSF samples, bacterial as well as fungal pathogens were isolated from 51 samples showing an isolation rate of 16.14%. The patients showing CSF culture positivity also had some predisposing conditions, with altered CSF biochemical levels as well as pathological counts.

Out of total 51(16.14%) culture positive samples, 44 (86.27%) samples were positive for bacteria whereas 7 (13.72%) samples were positive for fungus. No sample was found to be positive for Mycobacteria.

Table 1: Clinical manifestation of acute meningitis in 316 cases

Clinical features	No. of cases (%)
Fever	293 (92.72)
Altered mental status	235 (74.36)
Headache	195 (61.71)
Convulsion	182 (57.59)
Vomiting	178 (56.33)
Irritability	167 (52.85)
Neck rigidity	138 (43.67)
Kernig's sign	110 (34.81)
Bulging fontanel	51 (16.14)
Refusal to feed	49 (15.51)
Skin rash	5 (1.58)

DISCUSSION

There is a need for periodic review of acute meningitis worldwide, since the pathogens responsible for infection vary with time, geography and patient's age. Increase in awareness, availability of vaccines may also reflect a change in the epidemiological pattern of these pathogens. The etiological agents of community-acquired meningitis may differ from hospital- acquired meningitis. Delay in diagnosis and initiation of treatment can result in poor outcome of the disease.[13] Thus, the data presented in this study can provide information of immediate public health importance to clinicians on the selection of antimicrobial agents for the treatment of patients suffering from acute meningitis.

Out of total suspected cases of meningitis, 163 (51.58%) CSF samples were collected from patients of the age group 0-10 years, followed by 41 (12.97%) in the age group of 11-20 years. Only one sample (0.32%) was collected above the age of 70 years. This was similar to the study of Emele[14] where 68% cases were in the age group 1-9 years and this, however contrasted with Mani *et al.*[15] where 86.8% were adults and 13.2% were below the age of 12 years.

Out of the total 316 cases, 212 (67.09%) were males and 104 (32.91%) were females; the male to female ratio is 2:1. Similar observations were made by Mani *et al.*[15] [male 293 (76.1%) and female 92 (23.9%)].

Fever (92.72%), altered mental status (74.36%), headache (61.71%) and convulsion (57.6%) were the most common clinical manifestations of meningitis among the patients in the present study [Table 1]. Similarly, fever was observed by Chinchankar *et al.*[16] in 52 cases (96%), on the contrary, *Abdul Khaliq*[17] reported fever only in 7 (33.33%) cases.

Out of total 316 cases, predisposing conditions were recorded in 143 (45.25%) patients. The most common predisposing condition was diabetes 45 (14.24%), followed by pre-term delivery 36 (11.39%) among neonates. HIV/AIDS were recorded in 17 (5.38%) patients [Figure 1]. Similarly, Tang *et al.*[18] mentioned various pre-disposing conditions, e.g. diabetes mellitus, head injury and/or neurosurgical procedure, and malignancy in 71.8% of patients.

The isolation rate of 16.14% found in the present study is in agreement with a various studies by different authors.[19,20] Even though all febrile patients with signs and symptoms of acute meningitis have underwent lumbar puncture to rule out acute meningitis, the bacterial isolation rate was found to be low. The predominant organism isolated from CSF culture was found to be *Staphylococcus aureus* 15 (29.41%) [Table 2]. Higher prevalence of meningitis due to *Staphylococcus aureus* may be due to predisposing factors as well as co-morbidities associated with the patient. Similar finding was observed by Wen-Neng Chang (23.8%)[21], Rasoul *et al.* (13.1%).[22] However, the study was dissimilar to the findings observed by Mani *et al* (1.8%).[15]

Among the Gram negative isolates, *Klebsiella sp.* was found to be the most common isolate, 8 (15.7%). Other Gram negative bacilli were *Escherichia coli* 6 (11.76%), *Acinetobacter sp.* 4 (7.84%), *Pseudomonas sp.* 3 (5.88%) and *Citrobacter sp.* 2 (4%). The change in the epidemiological trend of isolated organisms in meningitis is due to hospital-acquired infection associated with increasing immunocompromised status of

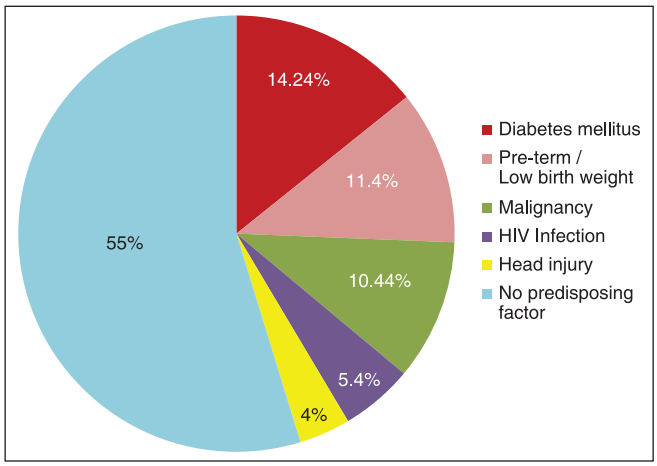

Figure 1: Predisposing conditions associated with 143 (45.25%) cases of acute meningitis

Table 2: Distribution of CSF isolates according to age and sex

Isolated organisms	No. (%)	Male	Female	newborn to 3 months	>3 months to 10 years	11-45 years	>45 years
Staphylococcus aureus	15 (29.41)	12	3	3	6	3	3
Klebsiella sp.	8 (15.7)	7	1	1	4	1	2
Escherichia coli	6 (11.76)	3	3	3	2	1	0
Acinetobacter sp.	4 (7.84)	4	0	0	0	2	2
Cryptococcus neoformans	4 (7.84)	3	1	0	0	2	2
Pseudomonas sp.	3 (5.88)	1	2	0	0	3	0
Candida albicans	3 (5.88)	3	0	2	0	0	1
Listeria sp.	2 (4)	2	0	1	1	0	0
Niesseria meningitidis	2 (4)	1	1	0	1	0	1
Streptococcus pneumoniae	2 (4)	1	1	0	0	1	1
Citrobacter sp.	2 (4)	2	0	2	0	0	0
Total	51 (100)	39	12	12	14	13	12

the patients like diabetes, HIV/AIDS, pre-term delivery, malignancy etc. Prolonged hospital stay, improper hand hygiene practice by the care givers, inappropriate patient-health care workers ratio were some other causes of hospital-acquired meningitis by these organisms.

Isolation rate of *Streptococcus pneumoniae* and *Neisseria meningitidis* were less in the study. *Hemophilus influenzae* have not been isolated from CSF in this study. This may be because of fastidious nature of the organisms, vaccine implementation against these organisms or antibiotic treatment prior to lumber puncture. The CSF becomes sterilized within 4 hours of parenteral antibiotic treatment in case of pneumococcal meningitis.[23] The lowered incidence of meningococcal meningitis in the present study is probably due to the occurrence of meningitis during the inter-epidemic period.

Among the fungal isolates *Cryptococcus neoformans* 4 (7.84%) predominated over *Candida albicans* 3(5.88%). The single predisposing factor for *Cryptococcus neoformans* meningitis was found to be AIDS. Out of 4 patients, 3 (75%) responded to amphotericin B therapy whereas the other patient died of Cryptococcamea. *Candida albicans* were isolated from two pre-term neonates and one 49-year male patient with leukemia. All of them responded to fluconazole.

Among the 15 isolates of *Staphylococcus aureus*, 80% was found to be sensitive to amoxycillin-clavulanic acid followed by amikacin 60% each. Among the total 15 isolates of *Staphylococcus aureus*, 3 (20%) were methicillin-resistant *S. aureus* (MRSA). Inappropriate as well as excessive use of broadspectrum antibiotics and prolonged hospital stay are the other causes of emergence of MRSAs. Garcia-Arias *et al.*[24] isolated *S. aureus* in 3%, among which MRSAs was 47.6% which is almost similar to the observation of this study. MRSAs isolated in the previous study of the same institution were 14%.[25] Higher prevalence of meningitis due to *S. aureus,* in the present study, may be due to predisposing factors as well as co-morbidities associated with the patient. Inappropriate and injudicious use of broadspectrum antibiotics, prolonged hospital stay, overcrowding of the hospital environment, inadequate practice of hand hygiene, non-isolation of MRSA patients were the causes behind the emergence of higher prevalence of MRSAs.

However, all the Gram-positive isolates of CSF are found to be sensitive to newer antibiotics like linezolid and vancomycin [Table 3].

The *in vitro* susceptibility tests of the bacterial isolates revealed that polymyxin B 92% was the most effective antimicrobial agent against the entire spectrum of the Gram-negative bacilli. It was followed by imipenem 88% and piperacillin-tazobactum 64%. The sensitivity of each of ciprofloxacin and amikacin was 56%, followed by amoxycillin-clavulanic acid 52% [Table 4]. Sonavane *et al.*[13] found 2 isolates of multi-drug resistant (MDR) *Acinetobacter spp*., as they were resistant to higher antibiotic like imipenem.

CONCLUSION

Increasing resistance to empirically used antimicrobials in meningitis especially to cephalosporins (cefoperazone, ceftriaxone, cefotaxime, ceftazidime etc.) is an alarming condition. Resistance to newer drugs like imipenem is another alarming condition for the clinicians.

For public health concern, the etiological change in the trend of meningitis and the risk factors associated with meningitis should be reviewed from time-to-time. Along with these surveys, continued surveillance for resistance characteristics among the organisms is necessary. This study gives a clear idea about the prevalence of organisms related with meningitis in this part of the country along with the changing *in vitro* antimicrobial susceptibility pattern of the bacterial isolates. Producing updated information on local causative pathogens and their antibiotic sensitivity pattern can help the clinicians to formulate the initial empiric therapy. Prevention of the emergence of antimicrobial resistant bugs by promotion of judicious use of antibiotics can provide a long-term solution.

Table 3: Antibiotic sensitivity pattern of Gram-positive bacteria isolated from CSF

Antibiotics	P%	AC%	VA%	LZ%	G%	AK%	E%	CF%	CI%
Isolates									
S. aureus (15)	26.6	80	100	100	53.3	60	46.6	53.33	13.33
S. pneumoniae (2)	50	100	100	100	100	100	100	100	50
Listeria sp. (2)	nil	100	100	100	50	50	50	50	50

Table 4: Antibiotic sensitivity pattern of Gram-negative bacteria isolated from CSF

Antibiotics	P%	AC%	PT%	AK%	CF%	CI%	CE%	CS%	CZ%	I%
Isolates										
Klebsiella sp. (8)	nil	75	87.5	62.5	75	25	37.5	12.5	37.5	87.5
E. coli (6)	nil	66.6	83.3	83.3	66.6	16.6	33.3	16.6	50	100
Acinetobacter sp. (4)	nil	25	50	25	25	nil	25	nil	25	75
Pseudomonas sp. (3)	nil	nil	33.3	33.3	66.6	nil	33.3	nil	66.6	100
Neisseria meningitidis (2)	50	50	nil	50	50	50	50	nil	50	50
Citrobacter sp. (2)	nil	50	50	50	nil	nil	50	nil	50	100

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