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Research Article

Bacteriological profile of acute bacterial meningitis at a tertiary care hospital of North India

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ABSTRACT

Background: Acute bacterial meningitis (ABM) is one of the most severe and potentially life-threatening infectious diseases. It is defined as an inflammation of the meanings, globally distributed as either sporadic or epidemic forms. ABM remains a major cause of mortality and long-term neurological sequel worldwide. Objective of the present study was undertaken to evaluate the bacteriological analysis in term of pathogens frequency and their sensitivity pattern in the cerebrospinal fluid of acute meningitis patients at a tertiary care hospital in eastern Uttar Pradesh, India.
Methods: The study was carried out at a tertiary care hospital from June 2014 to November 2015 irrespective of age group. A total of 3803 samples of cerebrospinal fluid (CSF) from clinically suspected cases of meningitis were subjected for bacteriological analysis.
Results: During the study period, a total of 3803 CSF samples were studied. Out of these, 343 were confirmed as bacterial meningitis based on Gram staining and or culture showing 9.01% incidence. ABM was more common in paediatric patients than adults. The most common organisms were Gram positive (66.18%) bacteria.
Conclusions: Acute bacterial meningitis is a medical emergency and making an early diagnosis and providing early and accurate treatment, are lifesaving and to reduce morbidity. This study may play an important role in the diagnosis and more accurate treatment for the ABM patients.

Keywords: ABM, CSF, GPC, Sporadic or epidemic, Morbidity and mortality

INTRODUCTION

Meningitis is a very serious infection of the meninges that surround the brain and the spinal cord usually caused by viral, bacterial and fungal pathogens. Knowing whether meningitis is caused by a virus or bacterium is important because the severity of illness and the treatment differ for them. Viral meningitis is generally less severe and resolves without specific treatment, while acute bacterial meningitis (ABM) can be quite severe and may result in mental illness, hearing loss, learning disability and death if not treated early. Delay in diagnosis and initiation of treatment can result in poor outcome of the disease.[1,2]

ABM remains a major cause of mortality and long-term neurological sequel worldwide.[2] According to World Health Report by World health organization, morbidity and mortality rates with this disease remain high.

Apart from epidemics, at least 1.2 million cases of bacterial meningitis are estimated every year among which 135,000 of them are fatal. Approximately 500,000 of these cases and 50,000 of the deaths are due to meningococci.[3,4] Despite the availability of potent newer antibiotics, the mortality rate due to ABM remains significantly high in India and other developing countries, ranging from 16-32%.[1,4] In India, meningococcal disease is endemic rather than other etiological agents are newly emerging trends of ABM in recent years.[5] Although the rate of disease associated with meningitis is lower than other major causes of childhood mortality, the high case fatality rates and neurologic sequelae in survivors result in considerable emotional and financial burden on the family and presents a major challenge to the health care system in financial and human resources.[6] There is a need for a periodic review of ABM worldwide, since the pathogens responsible for the infection vary with time, geography and patient's age. Increased awareness, availability and usage of vaccines may also reflect as a change in the epidemiological pattern of these pathogens.[2,7]

The etiological agents of community-acquired meningitis are differing from that causing nosocomial meningitis. Nosocomial infection is defined as a positive bacterial infection not present at the time of hospital admission, clinical evidence of an infection no sooner than 48 hours after admission or clinical evidence of meningitis within one month after discharge from hospital where the patient had received an invasive procedure, especially a neurosurgical procedure.

Otherwise, the patient was considered to have community-acquired infection. Microbiology laboratories play an important 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 also which drugs to start empiric treatment.[7] This prospective observational study was conducted in BRD Medical College and associated Nehru hospital, Gorakhpur, India to evaluate the bacteriological analysis in the cerebrospinal fluid of clinically suspected patients with bacterial meningitis also determine frequency of common pathogens and their sensitivity pattern causing bacterial meningitis at a tertiary care hospital of Eastern Uttar Pradesh.

METHODS

A prospective observational analysis of 3803 CSF samples clinically suspected for meningitis was carried out at a tertiary care hospital of Eastern Uttar Pradesh, India during a period of one and half year from June 2014 to November 2015. All the CSF samples were collected aseptically in a sterile container and transported to Department of Microbiology, Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh, India.

The study was approved by the Institutional Ethical Committee of Baba Raghav Das Medical College, Gorakhpur Uttar Pradesh, India (Approval No: XXVIII - B/P2/2014). A written informed consent was obtained from all subjects before enrolment into the study. After observing CSF samples of varying quantity (1 to 3 ml) physically for blood and colour of CSF samples aliquoted in three sterile vials first for cyto-chemical analysis second for staining and third for culture. All the cases clinically suspected were processed immediately (within 30 minutes) for cell counts, biochemical analysis, Gram staining and inoculated on Sheep Blood agar, Chocolate agar and MacConkey agar, aerobically incubated at 37^{o}C in incubator for bacteriological culture. The culture plates were observed routinely on the daily basis, if there is growth of micro-organism present then it was further confirmed by Gram stain, colony characteristics and biochemical reaction. The pathogens were identified on the basis of standard methods and protocol as per guideline.[8,9]

Antibiotic sensitivity to relevant antibiotics was determined by the Kirby-Bauer disk diffusion method as per the standard guidelines Clinical and Laboratory Standards Institute (CLSI, 2012) using the commercially available antibiotic disks from Hi-Media (Mumbai, India). Extended spectrum beta-lactamases (ESBL) production and methicillin resistant Staphylococcus aureus (MRSA) were tested as per standard CLSI protocol. ATCC reference strains, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were included as control strains.[10] Clinical presentation of disease and patients outcomes were also analyzed in this study.

RESULTS

During the study period, a total of 3803 CSF samples were included for this study. Out of these 343 were found confirmed as acute bacterial meningitis based on Gram staining and culture result showed 9.01% positivity rate. The male and female ratio among all culture positive cases was 1.67:1 as shown in Table 1.

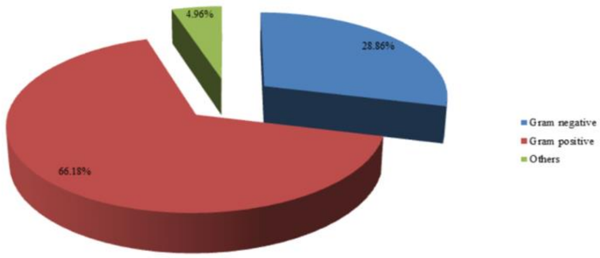

Figure 1: Distribution of different groups of microorganism among positive cases of ABM.

Among 343 culture positive cases, 215 (62.68%) were male and the most common age group was 0-2 years and remaining 128 (37.32%) were female and the age group 5-10 years were more prone to acute bacterial meningitis. Out of total 343 positive cases 111 (32.37%) belongs to 0 to 2 years of age group follows as 2 to 5 years; 87 (25.37%), 5 to 10 years; 97 (28.28%), 10–20 years; 38 (11.07%), 20 to 50 years; 8 (2.33%) and >50 years; 2 (0.58%). The age and sex demographical distribution pattern was shown in Table 1. Most common clinical presentation was the fever 96.5%, vomiting and nausea 42.8%, following by headache 39.8%, altered consciousness 43.2%, nick stiffness 13.7%, seizures 9.4%, and other neurological sign including Brudzniski's sign, kerning's sign 2.1% were observed. Out of 343 isolates, Gram positive bacteria 227 (66.18%) were the most common cause of ABM as compared to Gram negative 99 (28.86%) and others 17 (4.96%) as shown in Figure 1.

Table 1: Age and sex wise demographical distribution of suspected ABM cases.

Demographic distribution of clinical isolates from ABM									
Age group (years)	Male (2129)	Positive (215)	% of positivity	Female (1674)	Positive (128)	% of positivity	Total (3803)	Positive (343)	% of positivity
0 – 2	635	74	34.41	392	37	28.90	1027	111	32.37
2 – 5	564	57	26.51	454	30	23.43	1018	87	25.37
5 – 10	544	55	25.58	450	42	32.81	994	97	28.28
10 – 20	333	24	11.16	327	14	10.93	660	38	11.07
20 – 50	42	5	2.32	42	3	2.34	84	8	2.33
> 50	11	0	0	9	2	1.56	20	2	0.58

Table 2: Antibiotic sensitive pattern in gram positive isolates.

Drug	CONs (101)	%	*Staphylococcus aureus* (44)	%	*Streptococcus spp.* (82)	%
AMX	32	31.68	10	22.73	23	29.11
AZ	27	26.73	17	38.64	40	50.63
C	56	55.45	29	65.91	42	53.16
CL	28	27.72	10	22.73	25	31.65
OF	39	38.61	11	25	26	32.91
P	13	12.87	6	13.64	16	20.25
SXT	18	17.82	7	15.91	12	15.19
GF	47	46.53	8	18.18	34	43.04
LI	101	100	40	90.91	54	68.35
VM	101	100	38	86.36	57	72.15
AS	34	33.66	11	25	8	10.13
CX	56	55.45	31	70.45	36	45.57

Gram positive disks: AMX= Amoxicillin; AZ= Azithromycin; C=Choloramphenicol; CL= Ciprofloxacin; OF= Ofloxacin; P= Penicillin-G; SXT= Co-trimoxazole; GF= Gatifloxacin; LI= Linezolid; VM= Vancomycin; AS = Ampicillin-Sulbactum; CX= Cefoxitin

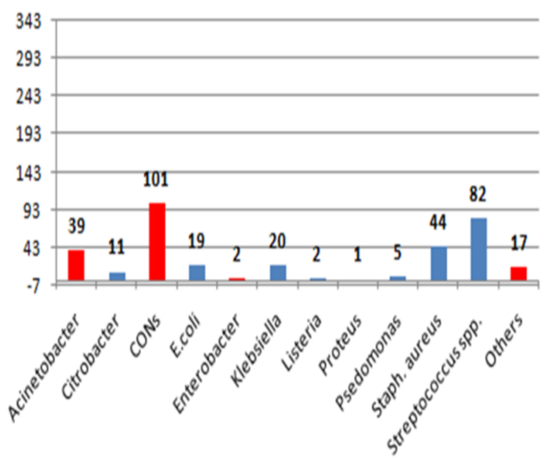

Figure 2: Distribution of microorganism among all positive cases of ABM.

The most common pathogen among 227 Gram positive bacteria were *Coagulase negative Staphylococcus spp.* (CONs) 101 (44.50%) predominant in all age groups followed by *Streptococcus spp.* 82 (36.12%) and *Staphylococcus aureus* 44 (19.38%). Among 99 Gram negative microorganism, *Acinetobacter spp.* 39 (39.40%) was predominant followed by *Klebsiella spp.*

20 (20.20%), *E.coli* 19 (19.20%), *Citrobacter spp.* 11 (11.11%), *Pseudomonas spp.* 5 (5.05%), *Listeria spp.* 2 (2.02%), *Enterobacter spp.* 2 (2.02%) and *Proteus spp.* 01 (1.01%). Out of 343 cases, 17 (4.96%) were positive for mixed infection, in which *Neisseria spp.* was found in 03 cases with Gram negative isolates. The distributions of different micro-organisms causing ABM were shown in Figure 2. The cell counts of the CSF samples ranged from no cells to sheets of cells, which could not be counted on the haemocytometer. A predominance of

neutrophils was the common feature which was seen in all cases with high cell counts. The mean sugar levels in the CSF samples were tested with the range of 32.2±3.4 mg/dl, while a high mean level of protein (90.2±11.5 mg/dl) was detected in clinical samples.

Table 3: Antibiotic sensitive pattern in gram negative isolates.

Drug	*Acinetobacter spp. (39)*	%	*Citrobacter spp. (11)*	%	*E. coli spp. (19)*	%	*Klebsiella spp. (20)*	%	*Pseudomonas aeruginosa (5)*	%
OF	23	59	9	81.8	4	21.05	9	45	3	60
CL	21	53.8	8	72.7	2	10.53	8	40	5	100
AK	26	66.7	7	63.6	12	63.16	7	35	3	60
CAZ/CZ	8	20.5	3	27.3	1	5.263	7	35	1	20
CRO/FR	9	23.1	4	36.4	5	26.32	3	15	1	20
GM	15	38.5	4	36.4	6	31.58	1	5	3	60
MF	24	61.5	11	100	10	52.63	11	55	2	40
GF	22	56.4	6	54.5	1	5.263	5	25	1	20
CS	19	48.7	10	90.9	10	52.63	11	55	2	40
LV	18	46.2	11	100	3	15.79	6	30	2	40
PT	12	30.8	3	27.3	13	68.42	3	15	2	40
IM	36	92.3	10	90.9	19	100	16	80	5	100

Gram negative disks: OF=Ofloxacin; Cl= Ciprofloxacin; AK=Amikacin; CAZ= Ceftazidime, GM=Gentamycin; MF=Moxifloxacin, GF=Gatifloxacin, CS=Cefoperazone-sulbactum; LV=Levofloxacin; PT= Peperacillin-tazobactum; IM=Imipenem-cilastatin.

Antibiotic sensitivity test

All the Gram positive microorganisms were highly sensitive to linezolid and vancomycin while amoxicillin, penicillin G, and co-trimoxazole were less sensitive drugs in Gram positive cocci (GPC). Out of 44 *S. aureus*, 13 (29.55%) isolates were MRSA positive by standard cefoxitin disc diffusion screening method. Other *Streptococcal* isolates showed variable sensitivity against different antibiotics which were tested, as has been depicted in Table 2. In present study, among Gram negative bacteria the most common isolate was *Acinetobacter spp.*, against this pathogen, imipenem (92.3%), amikacin (66.7%), moxifloxacin (61.5%) were highly sensitive while the less sensitive drugs were ceftazidime (20.5%), ceftriaxone (23.1%). The *E. coli* showed lowest sensitivity towards ceftazidine and gatifloxacin (5.26%), ciprofloxacin (10.53%), levofloxacin (15.79%), ofloxacin (21.05) whereas their sensitivity to amikacin (63.16%), piperacillin-tazobactum (68.42%) was relatively much higher.

In other Gram negative microorganisms like *Citrobacter spp.* in which ceftazidime and peperacillin-tazobactam were similarly less sensitive (27.3) and highly sensitive against moxifloxacin (100%), levofloxacin (100%), cefoperazone–sulbactam and imipenem (90.9%). gentamicin (5%), ceftriaxone and peperacillin-tazobactam (15%) showed very low sensitivity against *Klebsiella spp.* but imipenem (80%) was found to be highly sensitive against same pathogen. In case of *Pseudomonas spp.*, it was highly sensitive to ciprofloxacin and imipenem (100%) as compared with ceftazidime and ceftriaxone (20%). Those isolates with zone diameters less than 25 mm for ceftriaxone and less than 22 mm for cefoperazone were subsequently confirmed for extended spectrum beta-lactamases (ESBL) production by double disc diffusion synergy test (DDST).[11] The all 99 Gram negative isolates were tested for production of ESBL by DDST and found 19.2% overall prevalence. The antimicrobial sensitivity pattern of the Gram negative isolates have been summarized in Table 3. The mortality rates in our study were 11% that was most commonly seen in early paediatric age group (0-2 years).

DISCUSSION

Worldwide, acute bacterial meningitis is occurring sporadically and in small outbreaks, most common in developing countries. According to World Health Report, WHO, morbidity and mortality rates due to the bacterial meningitis remain high. Acute bacterial meningitis is a medical emergency, leads to serious neurological sequelae. Making an early diagnosis and providing treatment early are lifesaving and reduce morbidity. Apart from epidemics, at least 1.2 million cases of bacterial meningitis are estimated to occur every year; 135,000 of them are fatal.[12]

The World Health Report by WHO states that, meningitis death statistics by worldwide region: about 20,000 deaths from meningitis in Africa followed by 18,000 in America, 73,000 in South East Asia, 15,000 in Europe, 25,000 in Eastern Mediterranean and 20,000 in Western Pacific. Meningitis caused 0.9% of deaths of infants in USA.[3,12] In India, Meningitis disease is endemic; cases of meningococcal meningitis are reported sporadically or in small clusters. During 2005, total 8367 cases of bacterial meningitis were reported in India in which 485 death cases were marked. Majority of the cases were reported from Andhra Pradesh (3734 cases, 36 deaths), Madhya Pradesh (1039 cases, 6 deaths), Uttar Pradesh (659 cases, 124 deaths), West Bengal (702 cases, 64 deaths), Delhi (292 cases, 50 deaths), Maharashtra (394 cases, 100 deaths) and Karnataka (464 cases, 19 deaths).[5]

Table 4: Comparative table showing different clinical presentations among ABM patients in different studies.

Details		1	2	3	4	5	6	7
Author		Shrestha RG et al[22]	Modi S et al[14]	Khan F et al[11]	Farag HFM et al[23]	Yadhav KML et al[15]	Basri R et al[16]	Our study
Year		2015	2013	2011	2005	2014	2015	2015-16
Place		Nepal	Patna, India	Aligarh India	Alexandria, Egypt	Bangalore, India	Malaysia	Gorakhpur India
Journal		BMC Paediatrics	JCDR	Neurology Asia	IJMM	JCDR	Nagoya J. Med. Sci.	----
ABM Patients number/ Total patients enrolled		18/ 252	164/252	403/5859	202/310	24/100	125	343/3803
ABM prevalence		7.10%	65.07	6.87%	65. 16%	24%	N/A	9.01%
Clinical presentation (in %)	Fever	78	96.5	96.5	92.1	N/A	73.6	96.5
	Headache	N/A	99	33	55.7	N/A	24.8	39.8
	Vomiting & Nausea	N/A	90	N/A	75.2	N/A	48	42.8
	Neck rigidity	63	89.2	51	N/A	N/A	15.2	13.7
	Altered consciousness	N/A	16.3	62	N/A	N/A	N/A	43.2
	Meningeal sign	N/A	4	N/A	23.8	N/A	10.4	2.1
Outcome (mortality rate)		33.3	8.7	17.4	13.9	37.5	31	11

In the present study out of 3803 CSF samples only 343 (9.01%) patients were confirmed as bacterial meningitis based on gram staining and culture result in our study. Several studies from India report culture-negative cases of meningitis or a low CSF culture positivity, ranging from 6-50%.[11,13]

In the present study, males were found to suffer with ABM 1.67 times more frequently than females. This male preponderance which is seen with this infection has also been reported in several previous studies.[11,13,14] The present study also reflected that acute bacterial meningitis was more common in paediatric patients than adults which is similar to other studies based on bacterial meningitis in India.[6,15]

Clinical features including fever 96.5%, vomiting and nausea 42.8%, following by headache 39.8%, altered consciousness 43.2%, neck rigidity 13.7%, seizures 9.4% and other neurological sign including meningeal sign (Brudzniski's sign, kerning's sign and Meningeal irritation) in 2.1% which were also similar with other studies.[1,11,14,16] The clinical presentations of the meningitis were also shown in Table 4.

We found neutrophilic leucocytosis to be consistent finding in a majority of cases of ABM. The raised levels of CSF proteins and decreased CSF sugar levels were observed by us, which is similar to the findings in other studies.[3,6]

In our study the most common microorganism was Gram positive 227 (66.18%) as compared to Gram negative 99 (28.86%) and other 17 bacterial agents (4.96%), similar to other studies which were conducted in India.[7,11,16] Among all isolated microorganism, most common microorganism was *Staphylococcus spp.* as compared to other microorganisms responsible for ABM, which is in accordance with other recent studies.[16,17]

In this study, *Staphylococcus* spp. have emerged as the most common community as well as hospital acquired pathogen causing acute bacterial meningitis in all age groups. Other study also showed an increase in the incidence of staphylococcal infection causing acute bacterial meningitis.[17]

The other important pathogens causing acute bacterial meningitis (ABM) in this study were *Streptococcus spp. (α-Haemolytic Streptococci, Enterobacter spp.) Acinetobacter spp.* followed by *Klebsiella spp., Citrobacter spp., Listeria spp., E. coli*, whereas Pseudomonas *spp.* was the other most important pathogen for acute bacterial meningitis.

Among 13 (3.79%) cases were found with mixed infection in which *Neisseria spp.* was found in 3 cases with Gram negative isolates. *Staphylococcus* is reported same as other studies held in India.[11,16] It would be fact that etiological pathogens do differ from region to region. In present study reiterates the finding of a low prevalence of meningococcal meningitis except during epidemics, in various Indian studies.[1,14,17,18]

Some authors have found high incidence of *H. influenzae* cause meningitis in the paediatric age group while others have experienced a low incidence in different geographical region. It appears that *H. influezae* and *N. meningitidis* are not the common pathogen responsible for acute bacterial meningitis in south East Asia when compared with the western countries. Another reason for low isolation of *Haemophilus spp.* might be to introduction of Hib vaccine in immunization programme.[1,18,19]

Various reasons cited in the literature for a low yield of bacteria on culture are improper technique of lumbar puncture, delay in transport of specimens to the laboratory, Non-availability of special media for specific pathogens in the emergency setting, Autolysis enzymes in CSF, fastidious nature of pathogen and antibiotic treatment prior to lumbar puncture.[1,15,20]

In this study, all the isolates of Gram positive cocci (GPC) were sensitive to linezolid and Vancomycin which is similar to other Indian study but amoxicillin, Penicillin G, and co-trimoxazole were less sensitive drugs for GPC.[11] Out of total 44 *Staphylocoocus aureus* 13 (29.55%) were methicillin resistant *Staphylococcus aureus* (MRSA) which is low as compared with a Indian study conducted in North India.[11] All the Gram negative isolates were highly sensitive to carbepenems and glycopeptides.

Among the Gram negative organisms, high resistance was seen with chloramphenicol and ceftriaxone and least with piperacillin-tazobactum and imipenem.

The resistance to different group of drugs can be correlated to the rampant indiscriminate use of antibiotics leading to a large-scale drug resistance. This can be attributed to the general tendency of the Indian population to prefer private practitioners or quacks that do not follow proper antibiotic prescription norms. It is particularly useful concern for the clinicians possess the susceptibility data on Gram positive and Gram negative bacteria rather than for particular organisms only. Along with a perceptible deterioration in the susceptibility to the various antibiotics, ESBL (19.2%), which is in accordance with a study; and MRSA (29.44%) production in ABM isolates were also noted in our study, which is low as compared with a similar Indian study conducted in North India.[11]

Emergence of resistant bacterial strains to conventional antibiotics such as chloramphenicol, penicillin and ampicillin has also been reported in other studies.[6,7,14] The overall sensitivity patterns of the different clinical isolates were observed by us, are similar to the findings of previous Indian studies.[1,9,21] The mortality rate was 11% and it was found most commonly in early paediatric age group (0 – 2 years) as compared with others studies as shown in Table 4.[16,22,23]

CONCLUSION

In the present study, bacterial pathogens were isolated in 3803 samples showing an isolation rate of 9.01%, male and female ratio was 1.67:1, which showed a male preponderance. Staphylococcus spp. was the most common emerging bacteriological etiological agent in all age groups in this study, while the spectrum of micro-organisms which causes acute bacterial meningitis varies with time, geographical region and patient’s ages.

Since clinical signs of meningitis are not always reliable, a laboratory support is imperative, to achieve an early diagnosis. So more studies (preferably community based studies or prospective studies in hospital settings) need to be conduct in this region to enrich our knowledge and understanding the emerging trends of acute bacterial meningitis (ABM) in eastern Uttar Pradesh, India especially in Gorakhpur region which is highly prevalent zone of encephalitis and we thus correlate with ABM to reduce the disease burden.

This study may play an important role in the diagnosis and more accurate treatment to the patients suffering with acute bacterial meningitis. Regular prevalence and antibiotic susceptibility studies will help to enhance antimicrobial stewardship thus minimizing the emergence and spread of antimicrobial resistance and it would also be helpful for clinicians choosing an appropriate empirical antimicrobial. Hence, continued surveillance with more detailed studies is warranted to know the actual magnitude of problem and the spectrum of diseases and antimicrobial resistance caused by these pathogens.

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