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Original Work

Prevalence of Gram-negative Pathogens and their antimicrobial susceptibility in bacterial meningitis in pediatric cases

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ABSTRACT: The present study was conducted to find out the prevalence and spectrum of Gram negative pathogens causing bacterial meningitis and their antimicrobial susceptibility pattern in a tertiary care hospital. The cerebrospinal fluid (CSF) (3-5 ml) was collected from 638 admitted children clinically suspected of septic meningitis. Bacterial isolates were identified and antimicrobial susceptibility was assessed by the Kirby-Bauer disk diffusion method. Of the 638 samples tested 102 (15.99%) were culture positive. Male to female (M:F) ratio was 1.62:1. The maximum incidence of 45(44.12%) cases was found in children (1-12 yrs); in institutional deliveries the incidence was 58 (56.86%) cases. Further, the incidence of 51 cases was found from May to August. *Escherichia coli (E. coli)* were commonest, seen in 9 (25%) cases followed by *Acinetobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. with 6 (16.67%) cases each. *Enterobacter* spp., *Neisseria* spp. and *Pseudomonas aeruginosa* were isolated in 3 (8.33%) cases each. *E. coli*, *Acinetobacter* spp, *Citrobacter* spp and *Klebsiella* spp isolates were 100% susceptible to meropenem, piperacillin-tazobactam and cefoperazone-sulbactam and 100% resistant to cotrimoxazole and tetracycline. All strains of *Neisseria* spp, *Enterobacter* spp and *Pseudomonas* spp. were 100% susceptible to meropenem followed by gatifloxacin. These were 100% resistant to tetracycline and cotrimoxazole. *Neisseria* spp. were also 100% susceptible to pristinamycin. In septic meningitis Gram negative organisms are less common (35.29%). Of the isolates, more common Gram negative isolates included *E. coli*, *Acinetobacter* Spp., *Citrobacter* Spp., and *Klebsiella* spp. and these isolates were 100% susceptible to meropenem, piperacillin-tazobacatam and cefoperazone-sulbactam. Hence, empirical therapy should be formulated according to antimicrobial susceptibility patterns.

KEY WORDS: ***Gram negative organisms; Antimicrobial susceptibility; Cerebrospinal fluid***

INTRODUCTION

Acute bacterial meningitis is not only a very serious infection occurring in infants and children but also an important medical emergency. Despite effective antimicrobial therapy death and long-term disabilities are common outcomes.[1-4] Moreover; low specificity of the clinical features associated with bacterial meningitis is a diagnostic challenge. This is exacerbated in areas with malarial endemicity by overlap of signs and symptoms of cerebral malaria.[5] Further a large variety of organisms have been described for septic meningitis cases with wide fluctuation in their prevalence rates and antimicrobial susceptibility pattern and most often the organisms are multidrug resistant. Therapy should be initiated immediately after the result of a lumbar puncture procedure are obtained, or even immediately after lumbar

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puncture itself if the clinical suspicion is very high.[6]

The initial treatment of bacterial meningitis should be empirical and involves choosing antimicrobial agent(s) which have bactericidal effect in CSF and should be based on epidemiological knowledge of the most common organisms for each age group and the local antimicrobial susceptibility pattern[7-9]. CSF culture provides confirmatory evidence of acute bacterial meningitis (ABM) and is essential for selecting the appropriate antibiotic for the etiological organism.[10] Admittedly, the identification of pathogens in bacterial meningitis and their susceptibility to antibiotics may help transition of empirical therapies into pathogen-specific therapies.[11]

The objectives of the present study were to find out the spectrum of pathogens causing bacterial meningitis, the prevalence of Gram negative pathogens, their antimicrobial susceptibility pattern, and to formulate empirical therapy in bacterial meningitis.

METHODOLOGY

This prospective tertiary care hospital based study was conducted at Children Hospital, Allahabad, UP, India. Our study group included 638 admitted children of both sexes and aged up to 12 years, clinically suspected to be suffering of septic meningitis. The study was carried out from September 2007 to October 2008. Ethical clearance was obtained for the study.

Clinical information of cases was recorded such as date of admission, date of discharge, date of birth, associated illnesses, presence or absence of malnutrition, history of intake of antimicrobial agents prior to hospital admission and other relevant details were noted from patients bedside folder.

Patients diagnosed repeatedly with events of bacterial meningitis due to structural defects of the central nervous system and cases of meningitis caused by *Mycobacterium tuberculosis* were excluded.[12]

Lumbar puncture was carried out routinely in all children who were admitted with clinical suspicion of sepsis or neurologic impairment and who showed any of the following clinical criteria: neck stiffness or meningeal signs, bulging fontanel, impaired consciousness, history of convulsions without known epilepsy, focal neurologic signs, irritability or drowsiness, or presence of pyrexia with suspected clinical sepsis. In addition, CSF samples were collected from all newborns who were admitted to hospital with fever or suspected neonatal sepsis.

The CSF (3-5ml) was collected by lumbar puncture with complete aseptic precautions; the rate of collection was 3-5 drops per minute.[13] The CSF specimen was transported to the microbiology laboratory as soon as possible, preferably within 30 minutes with all prescribed precautions. Delay in transportation of samples may result in the death of delicate pathogens such as meningococci and the disintegration of leucocytes. Samples were not kept in a refrigerator as this may kill *H. influenzae*. If delay for a few hours was unavoidable, the specimen was kept in an incubator at 37°C[13]. For better and accurate results of culture and sensitivity examinations, CSF should be collected preferably prior to administration of antimicrobial agents.

A portion of the samples was inoculated on blood agar, chocolate agar and Mc Conkey agar by a standard loop method. The culture plate was incubated under 5-10% CO_2 at 37°c for 18-24 hours. The remaining CSF sample was incubated in 5ml brain heart infusion (BHI) broth and then incubated over night. Next day, the culture was observed for turbidity, if turbidity appeared and there was no growth on any of the solid media then subculture was performed from BHI broth.

The bacterial growth was interpreted by semi quantitative method in terms of heavy, moderate or scanty growth on primary plating. No quantification was done if growth was observed after enrichment. Specimens that showed no bacterial growth even after 48 hours of incubation in any of the culture media were labeled 'sterile'. Culture showing growth of *Candida* spp. was not further evaluated. Identification of bacterial isolates was carried out by Gram staining, motility, colony characteristics and biochemical tests. Antibiotic susceptibility test and interpretations were carried out for bacterial isolates by the Kirby – Bauer's disk diffusion method following NCCLS guidelines.[14,15]

The test was carried out by applying commercially available (Hi Media laboratories Pvt. Limited Mumbai, India) filter paper disc impregnated with specific amount of an antibiotic on to Mueller-Hinton agar/ blood agar surface, over which a saline suspension of microorganism had been inoculated.

The strains under test were reported as susceptible, intermediate or resistant comparing the diameter of zone of inhibition to the standard antimicrobial sensitivity chart.

RESULT

Clinical presentation(s) of the patient suspected of septic meningitis at the time of admission showed not only marked variability in clinical signs and symptoms but also in their specificity. Among neonates abnormal body temperature, either fever or hypothermia, was the most frequent presentation in 25 cases, followed by convulsions in 22 and refusal to feed in 19 cases. Among infants convulsions was the most frequent symptom in 19,

followed by fever 18, shrill cry 17 and irritability in 15 cases. In older children the most frequent symptom was convulsions in 42 cases followed by fever in 40, altered sensorium in 39, headache in 27, vomiting in 24, neck rigidity in 18 and photophobia in 11 cases. Thus there is no specificity in symptomatology.

CSF samples from 638 clinically suspected cases of septic meningitis were collected. Of these, 102 (15.99%) samples showed single bacterial growth and were characterized as culture positive. In none of the 102 culture positive cases there was growth of two different bacterial isolates. Totally 505 (79.15%) samples were sterile (showed no bacterial growth) and 31 (4.86%) samples were grossly contaminated (exhibiting three or more bacterial growths). Thus, altogether 536 (84.01%) samples were unwanted for the purpose of the study. It was noted that the rate of bacterial isolation was affected by antibiotic(s) use prior to lumbar puncture, and that the rate was increased if direct plating of CSF was carried out at the bedside.

Table 1 depicts age, sex, seasonal distribution, culture positivity besides place and mode of delivery of cases, and vaccination status of the cases. Out of 102 culture positive samples, 63 (61.76%) were males and 39 (38.24%) females with M:F ratio 1.62:1. Maximum numbers of culture positive CSF samples 45 (44.12%) were from children of age group 1-12 years with M: F ratio 0.67:1, followed by neonatal age group (0-28 days) with 36 (35.29%) culture positive samples and M:F ratio 11:1. The lowest number of culture positive cases 21 (20.59%) were from infant age group (1 month-1 year) with M:F ratio of 1.33:1. Thus, a variability in M:F ratio existed amongst different age groups.

Maximum cases of septic meningitis were noted during the four months of May to August (51) and minimum cases were observed from September to December (15) suggesting higher prevalence of septic meningitis during summer- rainy season. Among institutionally delivered cases 58 (56.86%) cases were found culture positive compared to 44 (43.14%) home delivered cases. Incidence was higher in vaginally delivered cases: 79 (77.45%) compared to caesarean cases: 23 (22.55%). Place of delivery in vaginal mode was either home or institution. Of all cases, 74(72.55%) were vaccinated whereas 28 (27.45%) were not immunized (**Table 1**).

Table 1: Age, sex, seasonal distribution, culture positivity, place and mode of delivery of culture +ve cases

Age	**0-28 days**	**1 month – 1 year**	**1-2 years**	**Total**	
No. (%)	36 (35.29)	21 (20.59)	45 (44.12)	102 (100)	
Sex (M/F)	33/3	12/9	18/27	63/39	
Season	**Sep-Dec 2007**	**Jan-Apr 2008**	**May-Aug 2008**	**Sep-Oct 2008**	**Total**
No. (%)	**15**	**24**	**51**	**12**	102 (100)
Organism	**Gram -ve**		**Gram +ve**		**Total**
No. (%)	36 (35.29)		66 (64.71)		102 (100)
Place of delivery	**Home**		**Institutional**		**Total**
No. (%)	44 (43.14)		58 (56.86)		102 (100)
Organism	17 (16.66) Gram -ve		19 (18.63) Gram -ve		36 (35.29)
Mode of delivery	**Vaginal**		**Caesarean**		**Total**
No. (%)	79 (77.45)		23 (22.55)		102 (100)
Organism	29 (28.43) Gram -ve		7 (6.86) Gram -ve		36 (35.29)
Vaccination status	**Vaccinated**		**Non-vaccinated**		**Total**
No. (%)	74 (72.55)		28 (27.43)		102 (100)

Of all cases, 66 (64.71%) were Gram positive and 36 (35.29%) cases Gram negative implying a predominance of Gram positive cases. Regarding distribution of Gram negative isolates among culture positive cases, *E. coli* was noted in 9 (25%) cases followed by *Acinetobacter* spp, *Citrobacter* spp and *Klebsiella* spp. with 6 (16.67%) cases each. *Enterobacter* spp, *Neisseria* spp, and *Pseudomonas aeruginosa* each were isolated in 3 (8.33%) cases.

It may be stated that intermediate susceptible organism upon judicious exposure to higher concentration of an antimicrobial agent may become susceptible hence intermediate susceptible organisms were considered susceptible to that antimicrobial agent for the purpose of the present study .[16,17]

Overall, the susceptibility pattern of Gram negative CSF isolates to various antimicrobial agents exhibited that all the isolates were 100% susceptible to meropenem and to pristinamycin (recommended only for *Neisseria* among Gram negative bacteria), followed by piperacillin-tazobactam (94.44%) . These isolates were 100% resistant to tetracycline and cotrimoxazole, followed by amoxicillin (86.11%), cefuroxime (80.56%) and chloramphenicol (69.44%)

Table 2 shows that *E. coli* were 100% susceptible to meropenem, piperacillin-tazobactam and cefoperazone- sulbactam. All *E. coli* isolates were 100% resistant to ceftazidime, tetracycline and cotrimoxazole and were 66.67% resistant each to cefotaxime, ceftriaxone, gatifloxacin and levofloxacin.

Table 2: Prevalence and antimicrobial susceptibility of common Gram Negative Organisms in bacterial meningitis

Organisms	***E. coli***			***Acinetobacter* spp.**			***Citrobacter* spp.**			***Klebsiella* spp.**		
No.	**N=9**			**N=6**			**N=6**			**N=6**		
Susceptible	**No. (%)**			**No. (%)**			**No. (%)**			**No. (%)**		
Drugs	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amikacin	6 (66.67)	0 (0)	3 (33.33)	3 (50)	0 (0)	3 (50)	3 (50)	2 (33.33)	1 (16.67)	5 (83.33)	1 (16.67)	0 (0)
Gentamicin	5 (55.56)	0 (0)	4 (44.44)	0 (0)	0 (0)	6 (100)	1 (16.67)	0 (0)	5 (83.33)	3 (50)	2 (33.33)	1 (16.67)
Amoxicillin	1 (11.11)	0 (0)	8 (88.89)	0 (0)	0 (0)	6 (100)	1 (16.67)	0 (0)	5 (83.33)	1 (16.67)	0 (0)	5 (83.33)
Cefepime	6 (66.67)	0 (0)	3 (33.33)	6 (100)	0 (0)	0 (0)	4 (66.67)	0 (0)	2 (33.33)	4 (66.67)	0 (0)	2 (33.33)
Cefotaxime	1 (11.11)	2 (22.22)	6 (66.67)	0 (0)	6 (100)	0 (0)	2 (33.33)	1 (16.67)	3 (50)	1 (16.67)	0 (0)	5 (83.33)
Cefuroxime	3 (33.33)	3 (33.33)	3 (33.33)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)
Ceftazidime	0 (0)	0 (0)	9 (100)	3 (50)	0 (0)	3 (50)	1 (16.67)	1 (16.67)	4 (66.67)	3 (50)	1 (16.67)	2 (33.33)
Ceftriaxone	1 (11.11)	2 (22.22)	6 (66.67)	3 (50)	3 (50)	0 (0)	1 (16.67)	0 (0)	5 (83.33)	1 (16.67)	0 (0)	5 (83.33)
Meropenem	9 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)
Amoxicillin + Clavulanic Acid	4 (44.44)	0 (0)	5 (55.56)	0 (0)	3 (50)	3 (50)	3 (50)	0 (0)	3 (50)	3 (50)	0 (0)	3 (50)
Cefoperazone + Sulbactam	9 (100)	0 (0)	0 (0)	3 (50)	3 (50)	0 (0)	5 (83.33)	1 (16.67)	0 (0)	6 (100)	0 (0)	0 (0)
Piperacillin + Tazobactam	9 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)
Gatifloxacin	3 (33.33)	0 (0)	6 (66.67)	3 (50)	3 (50)	0 (0)	2 (33.33)	0 (0)	4 (66.67)	6 (100)	0 (0)	0 (0)
Levofloxacin	3 (33.33)	0 (0)	6 (66.67)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	6 (100)	0 (0)	0 (0)
Chloramphenicol	3 (33.33)	0 (0)	6 (66.67)	3 (50)	0 (0)	3 (50)	0 (0)	0 (0)	6 (100)	3 (50)	0 (0)	3 (50)
Co-trimoxazole	0 (0)	0 (0)	9 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)
Pristinamycin	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Tetracycline	0 (0)	0 (0)	9 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	6 (100)

S=Susceptible; I= Intermediate susceptible; R= Resistant; NR= Not recommended

All strains of *Acinetobacter* spp. were 100% susceptible to fourth generation cephalosporin-cefepime, meropenem and piperacillin-tazobactam and were 100% resistant to gentamicin, amoxicillin, cefuroxime, levofloxacin, cotrimoxazole and tetracycline.

All strains of *Citrobacter* spp. were 100% susceptible to meropenem, piperacillin-tazobactam and cefoperazone-sulbactam followed by amikacin (83.33%) they were 100% resistant to cefuroxime, levofloxacin, chloramphenicol, cotrimoxazole, and tetracycline. All strains of *Klebsiella* spp. were 100% susceptible to meropenem, cefoperazone-sulbactam, piperacillin-tazobactam, gatifloxin, levofloxacin and amikacin. These were 100% resistant to cefuroxime, cotrimoxazole and tetracycline.

Table 3 shows that all strains of *Enterobacter* spp. were 100% susceptible to meropenem and gatifloxacin, and 100% resistant to tetracycline, cotrimoxazole, chloramphenicol, cefuroxime, amoxicillin and gentamicin. All strains of *Pseudomonas aeruginosa* were 100% sensitive to meropenem followed by cefepime, piperacillin-tazobactam and gatifloxacin 66.67% each. These were 100% resistant to cefuroxime, ceftazidime, chloramphenicol and cotrimoxazole. Resistance each to gentamicin, amoxicillin, cefuroxime, ceftriaxone, amoxicillin-clavulanic acid, cefoperazone-sulbactam and levofloxacin was 66.67%.

All strains of *Neisseria* spp. were 100% susceptible to meropenem, piperacillin-tazobactam, cefoperazone-sulbactam, amikacin, gatifloxacin, cefepime, and pristinamycin. The resistance to each of amoxicillin and cefuroxime was 66.67%.

Table 3: Prevalence and antimicrobial susceptibility of common Gram Negative Organisms in bacterial meningitis

Organisms	***Enterobacter* spp.**			***Pseudomonas aeruginosa***			***Neisseria* spp.**		
No.	**N=3**			**N=3**			**N=3**		
Susceptible	**No. (%)**			**No. (%)**			**No. (%)**		
Drugs	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amikacin	1 (33.33)	1 (33.33)	1 (33.33)	0 (0)	2 (66.67)	1 (33.33)	3 (100)	0 (0)	0 (0)
Gentamicin	0 (0)	0 (0)	3 (100)	1 (33.33)	0 (0)	2 (66.67)	2 (66.67)	0 (0)	1 (33.33)
Amoxicillin	0 (0)	0 (0)	3 (100)	1 (33.33)	0 (0)	2 (66.67)	1 (33.33)	0 (0)	2 (66.67)
Cefepime	1 (33.33)	0 (0)	2 (66.67)	2 (66.67)	0 (0)	1 (33.33)	3 (100)	0 (0)	0 (0)
Cefotaxime	1 (33.33)	0 (0)	2 (66.67)	0 (0)	1 (33.33)	2 (66.67)	1 (33.33)	1 (33.33)	1 (33.33)
Cefuroxime	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	1 (33.33)	0 (0)	2 (66.67)
Ceftazidime	0 (0)	1 (33.33)	2 (66.67)	0 (0)	0 (0)	3 (100)	1 (33.33)	1 (33.33)	1 (33.33)
Ceftriaxone	0 (0)	1 (33.33)	2 (66.67)	0 (0)	1 (33.33)	2 (66.67)	2 (66.67)	0 (0)	1 (33.33)
Meropenem	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)
Amoxicillin + Clavulanic Acid	1 (33.33)	0 (0)	2 (66.67)	1 (33.33)	0 (0)	2 (66.67)	2 (66.67)	0 (0)	1 (33.33)
Cefoperazone + Sulbactam	1 (33.33)	0 (0)	2 (66.67)	1 (33.33)	0 (0)	2 (66.67)	3 (100)	0 (0)	0 (0)
Piperacillin + Tazobactam	2 (66.67)	0 (0)	1 (33.33)	2 (66.67)	0 (0)	1 (33.33)	3 (100)	0 (0)	0 (0)
Gatifloxacin	3 (100)	0 (0)	0 (0)	2 (66.67)	0 (0)	1 (33.33)	3 (100)	0 (0)	0 (0)
Levofloxacin	0 (0)	1 (33.33)	2 (66.67)	1 (33.33)	0 (0)	2 (66.67)	0 (0)	2 (66.67)	1 (33.33)
Chloramphenicol	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	2 (66.67)	0 (0)	1 (33.33)
Co-trimoxazole	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)
Pristinamycin	NR	NR	NR	NR	NR	NR	3 (100)	0 (0)	0 (0)
Tetracycline	0 (0)	0 (0)	3 (100)	0 (0)	0(0)	3 (100)	0 (0)	0 (0)	3 (100)

S=Susceptible; I= Intermediate susceptible; R= Resistant; NR= Not recommended

DISCUSSION

Septic meningitis is an emergency and is one of the most important causes of morbidity and mortality among children including neonates. A wide spectrum of microorganisms has been described for cases of septic meningitis and this spectrum is subject to geographic variation. Moreover, the organisms isolated are very often resistant to multiple antimicrobials which makes the treatment more difficult leading to consequences such as increased hospital stay as well as mortality. Area specific bacteriological monitoring studies aimed to provide knowledge about the type of pathogens responsible for septic meningitis and their susceptibility patterns may help clinicians choose correct empirical treatment. Moreover, any delay in the initiation of empirical therapy or wrong choice of antimicrobial agent(s) may lead to adverse consequences including fatality. Once the specific pathogen is known by culture and sensitivity then a specific definitive therapy may be instituted.

CSF was collected from a total of 638 children including neonates, clinically suspected of septic meningitis. Of these 102 (15.99%) were culture positive. Various comparable studies reported different culture positivity rates owing to multiple factors. Our findings are in conformity with those of Kalghatgi et al[18] (15%), Agnihotri et al[19] (19.2%) and approximate with those of Sonavane et al[20] ranging between 6-50%, Surinder et al[21] (23.1%), salam[22] (27.27%), Singhi et al[23] (30%) and Keshari et al[24] (58.9%). In contrast Roca et al[25] reported 7% incidence of bacterial meningitis. Al khorasani et al[26] and Mani et al[27] reported a high incidence of 95.6% and 73.8% respectively. This disparity is primarily because of previous antibiotic intake before the patient reports to a tertiary care hospital. Also, there are many other clinical conditions like aseptic meningitis, tubercular meningitis etc which clinically simulate septic meningitis and need to be differentiated. From a laboratory perspective, the limitations of the bacterial culture of CSF samples included lack of sensitivity and requirement of specific reagents, facilities and expertise. Sensitivity of CSF culture may further be decreased (by <30%) if patients had started antibiotic treatment before lumbar puncture.[1,7] To address these methodological limitations, non-culture approaches, such as latex agglutination may be used to verify the etiologic agents.[1] Moreover certain CSF characteristics may be considered as indicators of probable bacterial meningitis, but these characteristics do not provide etiological information[1,7].

Neonatal risk factors commonly observed included hospitalization for more than one week, low birth weight, prematurity, exposure to ventilator, invasive procedures, perinatal asphyxia. Our findings are in agreement with those of Guerina[28].

The maternal risk factors observed were premature labour, premature rupture of membranes and maternal intra-partum fever. Our findings are in conformity with those of Dutta.[29] No relationship between primary immunization and neonatal septicemia was found though primary immunization definitely reduced the incidence of neonatal septicemia. It may be mentioned that *Haemophilus influenzae* type b (Hib), pneumococcal and meningococcal vaccines were not routinely administered to infants immunization schedule at our hospital though BCG vaccination was carried out for all children during their first days of life.

In the present study, 505 (79.15%) samples were culture negative (sterile) and 31 (4.86%) were grossly contaminated. This emphasizes the relevance of proper aseptic precautions by clinicians. An increased number of sterile cultures were because some organisms cannot survive more than an hour delay in transportation. This again emphasizes need for better training in proper sample handling and timely transportation.

In the present study M:F ratio among 102 culture positive cases was 1.62:1 signifying incidence of bacterial meningitis was more in males than females. Our findings are in agreement with those of Sonavane et al[20] (M:F ratio of 1.35:1) and Keshari et al[24] (M:F ratio of 1.7:1) , but at variance with Singhi et al[23] (M:F ratio 3.21:1). Maximum 44.12% culture positivity was noted in children (1-12 yr) with M:F ratio 0.67:1 and least number (20.5%) of positive samples were from infants (1 month - 1 yrs) with M:F ratio of 1.33:1 Thus it may be noted that M:F ratio in different age groups was quite variable. Further, contrary to our findings a number of workers reported maximum cases from neonatal age group[19,24] or else from infant age group (62.5%).[23]

Abnormal body temperature, either fever or hypothermia, was the most frequent (25 cases) clinical presentation among neonates followed by convulsions in 22 cases and refusal to feed in 19 cases. Among infants convulsion was the most frequent symptom in 19 cases, followed by fever in 18, shrill cry in 17, irritability in 15, and vomiting in 15. While in children the most frequent symptom was convulsion in 42 cases followed by fever in 40, altered sensorium in 39, headache in 27, vomiting 24 and neck rigidity in 18 cases. It may be emphasized that one patient might have more than one clinical sign or symptom at the time of admission. Thus, 102 cases presented with 390 signs and symptoms at the time of admission. Our findings were in agreement with those of Salam[22] and Keshari et al[24] who held that children of all ages (newborn to 12 yrs) attended pediatric emergency with fever, convulsion and altered sensorium.

Maximum incidence (51 cases) was seen during the summer and rainy season i.e., from May to August. Our findings were contradictory to those of Farag et al[30] from Egypt who reported maximum incidence in cold seasons and in families with high crowding index. It may be argued that in tropical countries like India the incidence is usually more during summer - rainy season.

Of 102 culture positive cases only 36 (35.29%) were Gram negative. In contrast to our finding of predominance of Gram positive organisms, a number of workers in the field like Al khorasani et al[26], Keshari et al[24], Salam[22], and Sonavane et al[20] reported predominance of Gram negative isolates. We found 100% correlation between Gram staining results and culture. Our findings were in conformity with those of Sonavane et al[20] who also reported a correlation of 100% between Gram staining results and culture. Dunbar et al[31] reported that CSF Gram stain was 92% sensitive and observed that microscopic examination of Gram stained concentrated CSF is highly sensitive and specific in early diagnosis of bacterial or fungal meningitis. In contrast, Surinder et al[21] observed that Gram stain and culture showed 16.9% and 23.1% positivity respectively i.e., a correlation of 73.16%. This showed that Gram stain is a gold standard method to identify the causative agent of septic meningitis.

Institutional deliveries 56.86% exceeded home deliveries. Regarding mode of delivery, 79 (77.45%) were vaginal deliveries (including home or institutional) and 23 (22.55%) were caesareans. Amongst positive cases, 74 (72.55%) were immunized.

Of 36 Gram negative isolates maximum 9 cases were of *E. coli* followed by *Acinetobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. with 6 (16.67%) cases each. *Enterobacter* spp., *Neisseria* spp, and *Pseudomonas aeruginosa* were isolated in 3 (8.33%) cases each. Different workers reported a variable incidence of Gram negative organisms. Thus, O'neill et al[32] reported *Enterobacter* spp. 35%, *E. coli* 22.5%, and *Pseudomonas aeruginosa* 15%. Salam[22] reported *Klebsiella* spp. 43.76%, *H. influenzae* 31.25%, *E. coli* and *Pseudomonas* 25%. Al khorasani et al[26] reported *N. meningitidis* 77.11%, *H. influenzae* 21.87%, *E. coli* 1.02%. We did not report any case of *H. influenzae*. It may be mentioned that introduction of highly effective Hib vaccine led to decreased incidence of *H. influenzae* in many countries although this vaccine has yet not been included in National Immunization Programme of our country though physicians recommend its use.

All the Gram negative isolates were sensitive to meropenem (100%) followed by piperacillin-tazobactam, cefoperazone-sulbactam and cefepime these isolates showed 100% resistance to tetracycline and cotrimoxazole followed by amoxicillin, cefuroxime and chloramphenicol. Shah et al[33] also reported that Gram negative isolates were susceptible to meropenem. O'neill et al[32] reported that 25% of Gram negative isolates were resistant to third generation cephalosporins thus partly supporting our observations. Thomson et al[34] in a study to observe the threat of antibiotic resistance in Gram negative pathogenic bacteria, reported that most of these pathogens were able to evade killing by penicillins, cephalosporins and carbapenems thus their findings partly contradict ours.

In the present study *E. coli* were 100% susceptible to meropenem, piperacillin-tazobactam and cefoperazone-sulbactam. Shah et al[33] reported that *E. coli* were susceptible to meropenem supporting our observations. Gram negative organisms were 100% resistant to ceftazidime, tetracycline and cotrimoxazole and showed a high degree of resistance to amoxicillin (88.89%) and to cefotaxime, ceftriaxone, gatifloxacin, levofloxacin and chloramphenicol 66.67% in each case. Sharma et al[35] reported that *E. coli* were 100% resistant to ampicillin followed by ciprofloxacin (89.8%), cefotaxime 88.6%, gentamicin 65.8% cotrimoxazole 64.5%, amikacin 46.8% and netilmicin 37.9%. Thus, these findings are in agreement with those of our observations. O'neill et al[32] also reported that *E. coli* were resistant to third generation cephalosporins thus supporting our observations.

All strains of *Acinetobactor* spp. were 100% sensitive to fourth generations cephalosporin-cefepime, meropenem and piperacillin-tazobactam and these were 100% resistant to gentamicin, amoxicillin, cefuroxime, levofloxacin, cotrimoxazole and tetracycline. In contrast, Prashant et al[36] reported that *A. baumannii* was susceptible only to amikacin, netilmicin, and ceftazidime while poorly sensitive to ciprofloxacin. Rao et al[37] observed that imipenem-resistant *A. baumannii* showed 100% resistance to imipenem, 89% resistance to cefotaxime, 80% to amikacin and 73% to ciprofloxacin, while cefoperazone and norfloxacin were found to be more effective against most of these isolates. Thus, different workers in the field observed different patterns of susceptibility based on different geographical distribution.

All the strains of *Citrobacter* spp. were 100% resistant to levofloxacin, chloramphenicol, tetracycline and cotrimoxazole, and a high degree of resistance 83.33% each to amoxicillin, ceftriaxone and gentamicin. O'neill et al[32] supported our findings by reporting that 25% of Gram negative isolates were resistant to third generation cephalosporins, while Thomson et al[34] observed that these isolates were resistant to penicillin, cephalosporins and carbapenems.

In the present study all the strains of *Klebsiella* spp. were 100% susceptible to meropenem, cefoperazone-sulbactam, piperacillin-tazobactam, gatifloxacin and levofloxacin. All the strains were 100% resistant to cefuroxime, and cotrimoxazole, and 83.33% resistant each to amoxicillin, cefotaxime and ceftriaxone. Sonavane et al[20] observed that *Klebsiella pneumoniae* showed maximum sensitivity to netilmicin (66%) followed by chloramphenicol, amikacin and ciprofloxacin 44% each, while Thomson et al[34] reported that these pathogens evade killing by penicillins, cephalosporins and carbapenems. Tallur et al[38] reported that among *Klebsiella* spp. multidrug resistance was common.

According to the present study all strains of *Enterobacter* spp. showed 100% sensitivity to meropenem and piperacillin-tazobactam. They were 100% resistant to amoxicillin, gentamicin, cefuroxime, cotrimoxazole, chlormphenicol and tetracycline. Marothi et al[39] reported that *Enterobacter* showed resistance to many commonly used antimicrobial agents such as aminoglycosides, cephalosporins, aztreonam, semisynthetic penicillin and cotrimoxazole. Thus, their findings are in line with those of our observations. O'neill et al[32] further support our findings by stating that *Enterobacter* isolates were resistant to third generation cephalosporins.

All the strains of *Neisseria* spp. were 100% susceptible to amikacin, cefepime, meropenem, piperacillin-tazobactam, cefoperazone-sulbactam, gatifloxacin and pristinamycin. The resistance to cotrimoxazole and tetracycline was 100%. Manchanda et al[40] reported that all the isolates were resistance to cotrimoxazole and two third of the isolates were non- susceptible to ciprofloxacin thus supporting our finding. We observed pristinamycin to be 100% effective against meningococci hence may be used if patient has meningococcal infection.

Pseudomonas aeruginosa showed 100% susceptibility to meropenem, followed by cefepime, piperacillin-tazobactam and gatifloxacin. They were 100% resistant to cefuroxime, ceftazidime, chloramphenicol and cotrimoxazole. Thomson et al[34] reported that these pathogens were resistant to penicillins, cephalosporins, and carbapenems, thus supporting our observations. Sonavane et al[20] observed that *P. aeruginosa* was 70% sensitive to piperacillin, 60% to chloramphenicol and netilmicin, and 40% sensitive to ceftazidime. These findings contradict ours.

CONCLUSION

In conclusion, it may noted that susceptibility pattern of different pathogens was subjected to change with geographical variations as well as with the variety of antimicrobials used at different centers. It is also concluded that most isolates depicted high level of resistance to examined antibiotics. There are many reasons for this alarming phenomena including inappropriate and incorrect administration of antimicrobial agents in empirical therapies. Hence the clinicians should keep themselves abreast with current problems concerned with antimicrobial resistance. It is recommended that clinicians should opt for rational prescribing based on culture and sensitivity.

REFERENCES

1. Sáez-Llorens X, McCracken GH Jr. Bacterial meningitis in children. *Lancet*. 2003 Jun;361(9375):2139-48.
2. Namani SA, Koci RA, Dedushi KH, et al. Causative pathogens of bacterial meningitis in children and their susceptibility to antibiotics. *Internet J Infect Dis*. 2010;9(1):1-6.
3. Prober CG. Central Nervous System Infections. In Nelson Textbook of Pediatrics. 18th ed. Kliegman RM, Behrman RE, Jenson HB, Stanton BF editors, Elsevier, New Delhi 2008;2512-21.
4. Puopolo KM. Bacterial and fungal infections. In Manual of Neonatal Care 4th ed. Cloherty JP, Eichenwald EC, Stark AR editors, Lippincott-Raven, Philadelphia 2008;274-93.
5. Berkley JA, Mwangi 1, Mellington F, et al. Cerebral malaria versus bacterial meningitis in children with impaired consciousness. *QJM*. 1999 Mar;92(3):151-7.
6. Lin AL, Safdieh JE. The evaluation and management of bacterial meningitis: current practice and emerging developments. *Neurologist*. 2010 May:16(3):143-51.
7. El Bashir H, Laundy M, Booy R. Diagnosis and treatment of bacterial meningitis. *Arch Dis Child*. 2003 Jul;88(7):615-20.
8. Hoffman O, Weber JR. Pathophysiology and treatment of bacterial meningitis. Ther Adv Neurol Disord. 2009 Nov;2(6):1-7.
9. Moon SY, Chung DR, Kim SW, et al. Changing etiology of commonly-acquired bacterial meningitis in adults: a nationwide multicenter study in Korea. *Eur J Clin Microbiol Infect Dis*. 2010 Jul;29(7):793-800.
10. Aneja S, Agarwal A. Acute Bacterial Meningitis. In Principles of Pediatric & Neonatal Emergencies. 2nd ed. Sachdev HPS, Choudhury P, Bagga A, Chugh K, Ramji S, Puri RK editors, Jaypee Brothers, New Delhi 2006;203-14.
11. Tunkel AR. Bacterial Meningitis. Philadelphia: Lippincott, Williams and Wilkins. 2001.
12. Theodoridou MN, Vasilopoulou VA, Atsali EE, et al. Meningitis registry of hospitalized cases in children: epidemiological patterns of acute bacterial meningitis throughout a 32-year period. *BMC Infect Dis*. 2007 Aug:7:101.

13. Collee JG, Duguid JP, Fraser AG, et al. Laboratory strategy in the diagnosis of infective syndromes. In Mackie & Mc Cartney Practical Medical Microbiology 14th ed. Colle JG, Fraser AG, Marmion BP, Simmons A editors, Churchill Livingstone, Edinburgh 1996;53-94.
14. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility test, 7th ed. Approved standards, NCCLS Document M2-A7. Wayne, PA: NCCLS, 2000.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 16th Informational Supplement (M100-S16). Wayne PA: CLSI. 2006.
16. Rodloff A, Bauer T, Ewig S, et al. Susceptible, intermediate, and resistant - the intensity of antibiotic action. *Dtsch Arztebl Int*. 2008 Sep;105(39):657-62.
17. Miles RS, Amyes SGB. Laboratory control of antimicrobial therapy. In Mackie and Mc Cartney: Practical medical Microbiology 14th ed. Collee JG, Fraser AG, Marmion BP and Simmons A editors, Churchill Livingstone, Edinburg 1996;151-58.
18. Kalghatgi AT, Praharaj AK, Sahni AK, et al. Detection of bacterial pathogens in cerebrospinal fluid using restriction fragment length polymorphism. *MJAFI*. 2008;64(1):29-32.
19. Agnihotri N. Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. *Jpn J Infect Dis*. 2004 Dec;57(6):273-5.
20. Sonavane A, Baradkar VP, Mathur M. Bacteriological profile of pyogenic meningitis in adults. *Bombay Hospital J*. 2008;50(3):452-55.
21. Surinder K, Bineeta K, Megha M. Latex particle agglutination test as an adjunct to the diagnosis of bacterial meningitis. *Indian J Med Microbiol*. 2007 Oct;25(4):395-7.
22. Salam AK. Etiology and presentation of acute bacterial meningitis in children at Al-Thawrah Hospital, Sana'a, Yemen. J *Ayub Med Coll Abbottabad*. 2004 Oct-Dec;16(4):40-3.
23. Singhi P, Bansal A, Geeta P, et al. Predictors of long term neurological outcome in bacterial meningitis. *Indian J Pediatr*. 2007 Apr;74(4): 369-74.
24. Keshari SS, Kapoor AK, Kastury N, et al. Antibiotic sensitivity pattern among Gram-nigative bacterial isolates in septicemic neonates in a tertiary care hospital. *Biomedicine*. 2008;28(4):292-7.
25. Roca A, Bassat O, Morais L, et al. Surveillance of acute bacterial meningitis among children admitted to district hospital in rural Mozambique. *Clin Infect Dis*. 2009 Mar;48 Suppl 2:S172-80.
26. Al Khorasani A, Banajeh S. Bacterial profile and clinical outcome of childhood meningitis in rural Yemen: a 2-year hospital-based study. *J Infect*. 2006 Oct;53(4):228-34.
27. Mani R, Pradhan S, Nagarathna S, et al. Bacteriological profile of community acquired acute bacterial meningitis: a ten-year retrospective study in a tertiary neurocare centre in South India. *Indian J Med Microbiol*. 2007 Apr;25(2):108-14.
28. Guerina NG. Bacterial and fungal infections. In Cloharty JP, Stark AR editors, Manual of Neonatal Care 4th ed. Philadelphia: Lippincott - Raven;1998:271-99.
29. Dutta DC. Preterm labour, preterm rupture of membranes, post maturity, intrauterine death of the fetus. In: Konar H editor Text Book of Obstetrics 5th ed. Calcutta: New Central Book Agency; 2001: 334-47.
30. Farag HF, Abdel-Fattah MM, Youssri AM. et al. Epidemiological, clinical and prognostic profile of acute bacterial meningitis among children in Alexandria, Egypt. *Indian J Med Microbiol*. 2005 Apr;23(2):95-101.
31. Dunbar SA, Eason RA, Musher DM, et al. Microscopic examination and broth culture of cerebrospinal fluid in diagnosis of meningitis. *J Clin Microbiol*. 1998 Jun;36(6):1617-20.
32. O' neill E, Humphreys H, Phillips J, Smyth EG. Third-generation cephalosporin resistance among Gram-negative bacilli causing meningitis in neurosurgical patients: significant challenges in ensuring effective antibiotic therapy. *J Antimicrob chemother*. 2006 Feb;57(2):356-9.
33. Shah D, Narang M. Meropenem. *Indian Pediatr*. 2005;42:443–50.
34. Thomson JM, Bonomo RA. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: Beta-lactams in peril! *Curr Opin Microbiol*. 2005 Oct;8(5):518-24.
35. Sharma S, Bhat GK, Shenoy S. Virulence factors and drug resistance in Escherichia coli isolates from extraintestinal infections. *Indian J Med Microbiol*. 2007 Oct;25(4):369-73.
36. Prashanth K, Badrinath S. In Vitro susceptibility pattern of Acinetobacter species to commonly used cephalosporins, quinolones, and aminoglycosides. *Indian J Med Microbiol*. 2004 Apr-Jun;22(2):97-103.
37. Rao RS, Karthika RU, Singh SP, et al. Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of Acinetobacter baumannii. *Indian J Med Microbiol*. 2008 Oct-Dec;26(4):333-7.
38. Tallur SS, Kasturi AV, Nadgir SD, et al. Clinico-bacteriological study of neonatal

septicemia in Hubli. *Indian J Pediatr*. 2000 Mar;67(3):169-74.
39. Marothi YA, Agnihotri H, Dubey D, Enterococcal resistance--an overview. *Indian J Med Microbiol*. 2005 Oct;23(4):214-9.
40. Manchanda V, Gupta S, Bhalla P. Menigococcal disease: history, epidemiology, pathogenesis, clinical manifestations, diagnosis, antimicrobial susceptibility and preventions. *Indian J Med Microbiol*. 2006 Jan;24(1):7-19.