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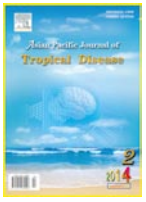

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Epidemiology and drug resistance profile of acute bacterial meningitis in children in Northern India: a university hospital perspective

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ABSTRACT

Objective: To assess epidemiology, trends in etiology and the antimicrobial sensitivity pattern of the pathogens.

Methods: Cerebrospinal fluid was collected from 150 patients who were suspected of meningitis and processed according to standard microbiological techniques. Cerebrospinal fluid samples were tested for antigen detection by latex agglutination test (LAT). Antimicrobial sensitivity test was done by Kirby-Bauer disc diffusion method.

Results: Fever, abnormal movements and altered sensorium were the most common presenting features. Etiological agents were identified in 79 (53%) cases. A total of 33 (42%) samples were cultured positive while 59 (75%) were positive by LAT. *Pneumococcus* followed by Gram-negative organisms were the most common pathogens. Mortality was 28 (19%). The aminoglycosides had the best spectrum of antimicrobial activity. An alarming rise of methicillin-resistant *Staphylococcus aureus* (75%) and extended-spectrum beta-lactamase (59%) was seen. No high-level aminoglycoside resistance, AmpC or mannose-binding lectin production was observed.

Conclusions: *Pneumococcus* and Gram-negative pathogens were the most common organisms. High prevalence of drug resistant pathogens is seen. Inclusion of LAT for antigen detection in routine diagnosis adds a valuable adjunct in the rapid and accurate diagnosis of pyogenic meningitis especially in partially treated cases.

1. Introduction

Despite the advances in vaccine development and chemoprophylaxis, bacterial meningitis remains a major cause of death and long-term neurological disabilities. Prior to the introduction of antibiotics in the 1940s, mortality for epidemic and endemic bacterial meningitis exceeded 70%[1]. Since the advent of antimicrobial agents, a profound change in the clinical course and prognosis of meningitis has been observed. Therefore, meningitis and its sequelae are best prevented by early diagnosis and appropriate treatment.

At present, it is estimated that there are 170 000 deaths annually worldwide with case fatality rate up to 50% if not treated[2]. In meningitis, the microbiological laboratory plays a critical role not only in the early identification of the causative bacterium for directing antimicrobial therapy but also in the establishment of guidelines for appropriate empirical treatment. Effective empirical therapy requires knowledge of the most frequent etiological agents of meningitis in the local population and the prevalent antibiotic sensitivity patterns. Cerebrospinal fluid (CSF) latex agglutination test (LAT) is of great promise. Various authors have suggested it as be simple with superior sensitivity and specificity and unaffected by previous antibiotics thereby[3]. There are several published studies regarding epidemiology, etiology and drug resistance in meningitis from the developed countries[4,5]. However, there is scarcity of such data from the Indian Subcontinent, especially in children.

The present study was undertaken in children from Aligarh Region of North India, with the aim to analyze the

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clinical and bacteriological profile as well as antimicrobial susceptibility pattern in cases of bacterial meningitis. The emphasis of this study was on rapid diagnosis of meningitis by LAT in order to initiate prompt and accurate therapy thus reducing morbidity and mortality.

2. Materials and methods

This prospective included 150 pediatric patients in the age group of 0-15 years, admitted to Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, from September 2009 to November 2010, with the clinical suspicion of acute bacterial meningitis (ABM). It is a 1500 bedded hospital with the total number of 13217 pediatric patients admitted the last year. The inclusion criterion was based on signs and symptoms of acute pyogenic meningitis as per a predesigned proforma. The patients with clinical, radiological, or microbiological examination suggestive of tubercular/viral/fungal meningitis were excluded from the study. Nosocomial infection was defined as a positive bacterial infection not present at the time of hospital admission or clinical evidence of an infection no sooner than 48 h after admission. Otherwise, the patient was considered to have community acquired infection.

A detailed clinical history of fever, vomiting, headache, abnormal movements, head injury, immunization status, history of breast feeding, period of illness, antibiotic intake before admission were elicited. Socioeconomic status according to Kuppuswamy's socioeconomic status scales and demographic details like overcrowding, lack of proper health facilities were noted[6]. General and systemic examination was performed to assess level of consciousness, weight, pallor/icterus, papilledema, cutaneous signs like meningococcal rash, signs of meningeal irritation, and signs of raised intracranial tension like bulging anterior fontanel. Nutritional assessment was done by Gomez's classification to look for protein energy malnutrition (PEM)[7]. In this study, criteria for a definite diagnosis of bacterial meningitis were as follows: (i) identification of bacterial pathogens by culture and/or LAT; (ii) clinical features of meningitis including fever, consciousness disturbance, seizure or signs of meningeal irritation and (iii) purulent CSF feature including at least one of the following: leukocytosis with a leukocyte count>2.5×10^9/L and the lactate concentration of predominant polymorphonuclear cells>3.5 mmol/L, protein concentration>0.45 g/L, glucose ratio (CSF glucose/serum glucose)<0.4 mmol/L or glucose level<2.5 mmol/L[8].

After admission, 2-3 mL CSF was collected aseptically by lumbar puncture in two separate sterile test tubes. One of the two specimens from each patient was used for bacterial culture, Gram staining and LAT. The second was used for cytology and protein and sugar estimations. For bacterial analysis, each specimen was centrifuged at 1500 r/min for 5 min. The supernatant was removed aseptically into a separate tube and used for LAT for the detection of bacteriological antigens by Pastorex meningitis kit (Bio-Rad, France) for detection of soluble antigens of *Neisseria meningitides* A, B, C, W135 (*N. meningitides*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Streptococcus agalactiae* (*S. agalactiae*), *Escherichia coli* (*E. coli*), *Haemophilus influenzae* type B (*H. influenzae*) as per manufacturer's instructions. The sediment was cultured using standard microbiological techniques and was also used for Gram staining[9]. All isolates were identified on the basis of their colony morphology, culture characteristics, and their biochemical reactions were identified according to standard procedures[10]. Isolates were also tested for their antibiotic sensitivity by Kirby-Bauer disc diffusion technique using *Staphylococcus aureus* ATCC 25923 (*S. aureus*) and *E. coli* ATCC 25922 as control strains according to the Clinical and Laboratory Standards Institute[11] using the commercially available antibiotic discs from HiMedia (Mumbai, India). The antimicrobials used for Gram-negative bacilli were gentamicin, amikacin, ceftriaxone, cefoperazone sulbactam, imipenem, cefixime, cefoperazone, cefepime, gatifloxacin, ofloxacin, piperacillin and piperacillin-tazobactam. Screening of potential extended-spectrum beta-lactamase (ESBL) production was done by using ceftriaxone (30 μg) and cefoperazone (75 μg). Those isolates with zone diameters less than 25 mm for ceftriaxone and less than 22 mm for cefoperazone were subsequently confirmed for ESBL production. Confirmation was done by noting the potentiation of the activity of cefoperazone in the presence of cefoperazone-sulbactam[12]. Detection of AmpC beta-lactamase was done on isolates resistant to ceftriaxone, cefixime, cefoperazone and cefoperazone-sulbactam. Induction of AmpC synthesis was based on the disc approximation assay using imipenem as inducer. Detection of mannose-binding lectin was done by Hodge test and double disc synergy test using ethylene diamine tetraacetic acid as described by Lee *et al*[13].

The antibiotics used for the Gram-positive cocci were gentamicin, amikacin, ceftriaxone, erythromycin, ofloxacin, gatifloxacin, clindamycin, oxacillin, sparfloxacin, pristinomycin and vancomycin. Oxacillin (1 μg) for the detection of methicillin resistant *S. aureus* (MRSA) and 120 μg gentamycin and 300 μg streptomycin disc for the detection of high-level aminoglycoside resistance in *Enterococci* were also used[14].

Statistical analysis was performed by student's *t*-test and *Chi*-square test. Risk for mortality was also estimated by odds ratio (OR) and risk ratio with 95% confidence interval.

3. Results

In this prospective study, a number of 150 clinically suspicious cases of bacterial meningitis were studied.

Maximum clustering [129 (86%)] was observed in the 0-5 years age group. In this group, majority of the cases were infants [85 (57%)]. The male to female ratio was 2.7:1. Majority [125 (83.3%)] of the patients were from the lower socioeconomic class with incumbent problems of overcrowding and inadequate health facilities, which was 120 (80%) of them. Their per capita income was less than 3 000 INR which amounts to less than 27 000 INR per annum. There were 132 (88%) children with PEM. None of them were immunized for pneumococcal, meningococcal and *H. influenzae* type B vaccines. The most common presenting features were fever seen in 136 (90.7%) cases followed by altered sensorium [117 (78%)], and abnormal movements [90 (60%)] as evident in Table 1. Few patients also complained of excessive crying, vomiting, and blurred vision. None of them had a history of head trauma; there was just one patient who underwent a neurosurgical procedure. There were 108 (72%) patients who were partially treated and gave a history of prior antibiotic intake.

Table 1

Clinical profile of patients with meningitis (n=150). n (%).

Clinical presentation	Number of children
Fever	136 (90.7)
Altered sensorium	117 (78.0)
Abnormal movements	90 (60.0)
Low birth weight	90 (60.0)
Hydrocephalous	5 (8.0)
Signs of meningeal irritation	
Neck stiffness	51 (34.0)
Bulging anterior fontanelle	8 (12.0)
Kernig's and Brudzinski's	3 (5.0)
PEM	132 (88.0)
PEM grade I	9 (13.0)
PEM grade II	90 (60.0)
PEM grade III	13 (27.0)

Biochemical and cellular analysis revealed 117 (78%) patients with raised cell counts. There were increase in the protein level and decrease in sugar levels in the CSF of 135 (90%) cases. Normal biochemical and cellular counts in some patients could be attributed to the usage of prior antibiotics.

3.1. Etiology

The overall detection of pathogens causing meningitis in our study was positive in 79 (53%) patients. Out of these 79 cases, Gram stained smears were positive in 22 (28%) patients, culture was positive in 33 (42%) and LAT in 59 (75%) cases. Out of these 59 cases, 46 were only LAT positive while 13 were culture as well as LAT positive. The samples positive by Gram staining were also positive by culture. All organisms (*N. meningitides*, *S. pneumoniae*, *S. agalactiae*, *E. coli*, *H. influenzae* type B) which grew on culture were also positive for LAT. Gram-positive organisms were observed in 43 (54%) cases while Gram-negative organisms were seen in 36 (46%) cases. Table 2 shows that among Gram-positive organisms, *S. pneumoniae* was found in majority of the cases [32 (41%)] followed by 4 (5%) cases of *S. aureus*. Among Gram-negative organism, *E. coli* [14 (18%)] predominated followed by *N. meningitidis* [7 (9%)].

Age wise distribution of the different etiological agents of meningitis is presented in Table 3. Gram-negative pathogens were predominant in neonatal period while incidence of Gram-positive pathogens increased in the older age group.

Table 3

Age wise distribution of the different etiological agents of meningitis (n=150). n (%).

Organism	0-1 month	1-3 months	3 months-1 year	1-3 years	3-5 years	>5 years	Total
S. pneumoniae	5	10	11	4	2	0	32
E. coli	9	4	1	0	0	0	14
N. meningitidis	0	1	4	1	0	1	7
K. pneumonia	4	1	0	0	0	0	5
S. aureus	0	0	2	1	0	1	4
P. aeruginosa	3	1	0	0	0	0	4
S. agalactiae	3	0	0	0	0	0	3
E. faecalis	3	0	0	0	0	0	3
C. koseri	3	0	0	0	0	0	3
H. infleunzae type B	1	1	0	1	0	0	3
L. monocytogenes	1	0	0	0	0	0	1
Unidentified	None	5	12	19	16	19	71
Total	32 (22)	23 (15)	30 (20)	26 (17)	18 (12)	21 (14)	150

Table 2

Comparative analysis of different identification techniques for bacterial etiology of meningitis (n=79). n (%).

Organisms identified		Culture/Gram stain/LAT	Culture as well as Gram stain positive	LAT positive	LAT and culture both positive
Gram-positive	*S. pneumoniae*	32 (41)	8 (10)	24 (30)	32 (41)
	S. agalactiae	3 (4)	0 (0)	3 (4)	3 (4)
	S. aureus	4 (5)	4 (5)	NP	NP
	E. faecalis	3 (4)	3 (4)	NP	NP
	L. monocytogenes	1 (1)	1 (1)	NP	NP
Gram-negative	*E. coli*	14 (14)	5 (6)	9 (11)	14 (14)
	N. meningitidis	7 (9)	0 (0)	7 (9)	7 (9)
	K. pneumoniae	5 (6)	5 (6)	NP	NP
	P. aeruginosa	4 (5)	4 (5)	NP	NP
	H. influenzae type B	3 (4)	0 (0)	3 (4)	3 (4)
	C. koseri	3 (4)	3 (4)	NP	NP
Total		79 (53)	33 (42)	46 (58)	59 (75)

E. faecalis: *Enterococcus faecalis*; *L. monocytogenes*: *Listeria monocytogenes*; *K. pneumoniae*: *Klebsiella pneumoniae*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *C. koseri*: *Citrobacter koseri*; NP: Not provided with the kit.

3.2. Antibiotic resistance profile

Antimicrobial susceptibility pattern to different groups of antibiotics is shown in Table 4. Among Gram-positive isolates, aminoglycosides were shown to have the highest spectrum of activity. *S. pneumoniae* was 100% sensitive to amoxicillin and vancomycin and majority of them were sensitive to gentamicin and amikacin. Three isolates (75%) of *S. aureus* were MRSA which were also multidrug resistant. There were no cases of vancomycin resistant *Enterococcus* and vancomycin resistant *S. aureus*. Out of 17 Gram-negative isolates, 10 (59%) were ESBL producers (four *E. coli*, three *K. pneumoniae*, two *P. aeruginosa*, and one strain of *C. koseri*). One *E. coli* ESBL producer was of nosocomial origin. No AmpC and mannose-binding lectin were detected.

3.3. Mortality

The mortality among the 150 cases was 28 (19%) despite treatment. Patients with Gram-negative organisms as etiological agents had higher mortality than those with Gram-positive organisms. Mortality was 71% with Gram-negative pathogens and it was 29% with Gram-positive pathogens. Protein energy malnutrition and multi drug resistant pathogens appear to be mortality associated factors. The strength of association (OR) of mortality on infection with Gram-negative pathogens was 9.84 times higher than with Gram-positive pathogens. A strong association of PEM was noticed with mortality. Children with PEM had poorer prognosis with 15 deaths in this group. Mortality was directly proportional to the MRSA and ESBL producing bacteria. Out of 28 mortalities, 10 (36%) were ESBL producers and 2 (7%) were MRSA. Only 1 ESBL producing *E. coli* was of nosocomial origin.

4. Discussion

One hundred and fifty children suspected of meningitis were screened. The majority of our cases were children of less than one year old (70%), $P<0.01$, which correlates well with other studies[4]. Meningitis predominated among boys. Other authors have made similar observations[4]. Majority of the cases belonged to the lower socioeconomic class. The poverty, malnutrition, overcrowding, and lack of proper health facilities appear to be strong determinants of meningitis. Farag *et al*. proposed that patients from low socioeconomic groups were more prone to the carriage and transmission of the causative pathogens of meningitis[15].

The main presenting complaint of the patients at the time of admission was fever which was in accordance with Abdulrab *et al*[16]. In this study, there were 78% patients with altered sensorium in varying grades of coma. This finding is consistent with Charles who reported 75% of patients with altered sensorium[8].

Few patients with normal biochemical and cellular counts could be attributed to the usage of prior antibiotics

Table 4

Antimicrobial susceptibility profile of bacterial isolates from meningitis cases.

Antibiotic	Gram-positive organism				Gram-negative organism			
	S. pneumoniae *n*=8	*S. aureus* *n*=4	*E. faecalis* *n*=3	*L. monocytogenes* *n*=1	*E. coli* *n*=5	*K. pneumoniae* *n*=5	*P. aeruginosa* *n*=4	*C. koseri* *n*=3
Amikacin	8	4	3	1	3	3	2	2
Ampicillin	8	2	2	1	2	2	2	1
Clindamycin	7	2	2	0	NT	NT	NT	NT
Erythromycin	6	3	3	1	NT	NT	NT	NT
Gentamycin	8	4	3	1	3	3	2	2
Gatifloxacin	6	2	2	0	2	3	2	2
Imipenem	NT	NT	NT	NT	5	5	4	3
Ofloxacin	8	3	3	1	3	4	3	2
Oxacillin	NT	1	NT	1	NT	NT	NT	NT
Cefoperazone	NT	NT	NT	0	1	2	2	2
Ceft/Sulbactam	NT	NT	NT	NT	5	5	4	3
Ceftriaxone	8	3	2	1	1	2	2	2
Cefixime	NT	NT	NT	NT	2	3	2	2
Cefepime	NT	NT	NT	NT	2	2	2	2
Piperacillin	NT	NT	NT	NT	1	2	2	2
Cefop/Sulbactam	NT	NT	NT	NT	5	5	4	2
Sparfloxacin	7	–	3	–	NT	NT	NT	NT
Piper/Tazobactam	NT	NT	NT	NT	5	5	4	2
Pristinomycin	NT	NT	NT	1	NT	NT	NT	NT
Vancomycin	8	4	3	1	NT	NT	NT	NT

NT: Not tested.

as has also been concluded in the study by Devlin and Devlin[17].

The overall detection of pathogens causing meningitis in our study was 79 (53%). Gram stained smears identified the causative agent in lesser number of patients in comparison to culture and LAT. Chávez-Bueno and McCracken reported that the Gram stained smear of CSF has a lower detection limit of about 10^5 colony-forming units/mL[18]. Prior use of antibiotics and low count of CFU/mL of CSF might be the reason for the low sensitivity of Gram staining which is also supported by Kabra *et al*. who suggested in their study that the probability of visualizing bacteria on Gram stained CSF preparations was dependent on the number of organisms present: 25% of smears are positive with <1 000 CFU/mL of CSF, 60% with 1 000 to 10 000 CFU/mL, and 97% with>10 000 CFU/mL[19]. Most Indian studies reported only culture findings or low smear have positivity[20]. Culture positivity was inversely proportional to previous antibiotic intake and the results were correlate well with Bloch[21]. The correlation between Gram staining and culture was 100%.

LAT for antigen detection was the most sensitive diagnostic method as it was able to identify the etiological agents in patients with negative culture and Gram staining report. LAT identified 59 etiological agents; out of which 13 were also positive by culture but rest of the 46 patients were LAT positive only. It also gave superior results in patients who were partially treated with prior antibiotics. Lack of precise bacteriological etiology in such cases may result in delayed and improper treatment, ultimately leading to mortality and morbidity in the patient. This study and several other studies advocate the usefulness of latex agglutination, especially in pretreated cases[20,22]. The limitation of LAT is that it cannot detect beyond a battery of a limited number of pathogens which is circumvented by the conventional culture method. Moreover, antimicrobial susceptibility and profiling of drug resistance are possible only if the organism is cultured.

In our study, *S. pneumoniae* and Gram-negative bacilli were the most commonly occurring organisms. The incidence of *S. pneumoniae* (the most predominant pathogen) was more common in children of less than one year old. Gram-negative bacilli in general and *E. coli* in particular were most frequently identified pathogens in neonates. Seven cases of *N. meningitides* and three cases of *H. influenzae* type B were identified by LAT but yielded no result on culture. This could be due to the lack of viable organisms in the CSF due to prior treatment. Pathogens like *S. aureus*, *E. faecalis*, *C. koseri*, and *K. pneumoniae* were the other organisms isolated in this study. They have been reported as frequent etiological agents of meningitis in children, especially neonates[23].

It is worth noting that 47% of bacterial meningitis cases had an unidentified etiology, which has also been observed by other workers. A Singapore study reported an even lower rate of pathogen detection (42%) probably explained by the administration of antibiotics prior to hospitalization[24]. Some of these cases may be caused by *N. meningitides*, which is known in some cases to show a lack of organisms in smears, CSF and blood cultures, and even in antigen detection tests[25].

All Gram-positive isolates were sensitive to gentamicin and amikacin and most of them were also sensitive to ceftriaxone. Gatifloxacin and clindamycin were the least effective. *S. pneumoniae* was 100% sensitive to amoxicillin and vancomycin. An alarmimg rise of MRSA and ESBL producing bacteria was observed. Other authors have recorded an alarming rise in ESBL production from 10.00% to 16.67%[23].

We recommend ceftriaxone (cefotaxime in neonates) with a combination of aminoglycosides as the empirical therapy for ABM in this region. Apart from having a broad spectrum of activity, ceftriaxone has excellent penetrability into the CSF.

The mortality was high despite of the treatment. Although lower mortality rates have been reported in industrialized countries, higher rates (12%-50%) have been reported in non-industrialized countries[26]. Patients with Gram-negative etiology had a higher mortality than those with Gram-positive etiology which has also been mentioned in by Pomar *et al*[27]. The strength of association (OR) of mortality on infection with Gram-negative bacilli was 9.84 times higher than with Gram-positive bacilli. Children with PEM were also shown to have poor prognosis. Mortality was directly proportional to the MRSA and ESBL producing bacteria. One *E. coli* ESBL producer was of nosocomial origin.

Prior antibiotic treatment precludes the determination of the true bacterial etiology prevalent in our region which could be the reason for low or no detection of *H. influenzae* tpye B and *N. meningitides* by culture. Diagnostic sensitivity and specificity could have been improved with multiplex polymerase chain reaction. It has a very promising role in detecting the etiology of meningitis in lesser time.

In conclusion, this study demonstrates that ABM still remains a serious infection. In India, low socioeconomic conditions and insufficient healthcare system contribute to the problem. Early diagnosis and treatment may reduce fatal outcome and improve the course of the disease. In patients with a history of antibiotic intake, antigen detection should be the investigation of choice. Inclusion of LAT for detection of bacterial antigen in the routine diagnosis adds a valuable adjunct in the rapid and accurate diagnosis of pyogenic meningitis.

The high prevalence of drug resistant pathogens is a cause for worry and should be dealt with by rational use of antimicrobials. Timely revisions in drug policy may be

necessitated for optimum management of patients.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Neuman HB, Wald ER. Bacterial meningitis in childhood at the Children's Hospital of Pittsburgh: 1988-1998. *Clin Pediatr (Phila)* 2001; **40**(11): 595-600.

[2] Agrawal S, Nadel S. Acute bacterial meningitis in infants and children: epidemiology and management. *Paediatr Drugs* 2011; **13**(6): 385-400.

[3] Adhikari S, Gauchan E, BK G, Rao KS. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with acute bacterial meningitis. *Nepal J Med Sci* 2013; **2**(2): 135-139.

[4] Pellegrino P, Carnovale C, Perrone V, Salvati D, Gentili M, Brusadelli T. Epidemiological analysis on two decades of hospitalisations for meningitis in the United States. *Eur J Clin Microbiol Infecti Dis* 2014; doi: 10.1007/s10096-014-2102-2.

[5] Cabellos C, Verdaguer R, Olmo M, Fernández-Sabé N, Cisnal M, Ariza J, et al. Community-acquired bacterial meningitis in elderly patients: experience over 30 years. *Medicine (Baltimore)* 2009; **88**(2): 115-119.

[6] Kumar N, Shekhar C, Kumar P, Kundu AS. Kuppuswamy's socioeconomic status scale-updating for 2007. *Indian J Pediatr* 2007; **74**(12): 1131-1132.

[7] Indu, Kumar N, Kumar A, Kumari M. Prevalence of malnutrition among pre-school children of Khagaria District of Bihar. *Food Sci Res J* 2013; **4**(2): 164-166.

[8] Charles GP. Chapter 595: central nervous system infections. In: Kliegman RM, Stanton BMD, St. Geme J, Schor NF, Behrman RE, editors. *Nelson textbook of pediatrics*. 19th ed. Philadelphia: Saunders; 2011, p. 2086-2097.

[9] Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory strategy in the diagnosis of infective syndromes. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackey & McCartney practical medical microbiology*. 14th ed. London: Churchill Livingstone; 1996, p. 53-94.

[10] Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie & McCartney practical medical microbiology*. 14th ed. London: Churchill Livingstone; 1996, p. 131-149.

[11] Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial disc susceptibility tests. Approved standards-10th ed.* Pennsylvania: Clinical and Laboratory Standards Institute; 2009 Jan; CLSI document M2-A10. 2009, p. 27-33.

[12] Rizvi M, Fatima N, Rashid M, Shukla I, Malik A, Usman A, et al. Extended spectrum AmpC and metallo-beta-lactamases in *Serratia* and *Citrobacter* spp. in a disc approximation assay. *J Infect Dev Ctries* 2009; **3**(4): 285-294.

[13] Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yun JH. Modified Hodge test and EDTA-disc synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 2001; **7**(2): 88-91.

[14] Swenson JM, Hindler JF, Jorgensen JH. Special phenotypic methods for detecting antibacterial resistance. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. *Manual of clinical microbiology*. 8th ed. Washington, D.C.: ASM Press; 2003, p. 1178-1195.

[15] Farag HF, Abdel-Fattah MM, Youssri AM. Epidemiological, clinical and prognostic profile of acute bacterial meningitis among children in Alexandria, Egypt. *Indian J Med Microbiol* 2005; **23**(2): 95-101.

[16] Abdulrab A, Algobaty F, Salem AK, Mohammed YA. Acute bacterial meningitis in adults: a hospital based study in Yemen. *Jpn J Infect Dis* 2010; **63**(2): 128-131.

[17] Devlin SB, Devlin JJ. Clinical prediction rules for meningitis in children pretreated with antibiotics. *Pediatrics* 2009; **123**(2): 365-366.

[18] Chávez-Bueno S, McCracken GH Jr. Bacterial meningitis in children. *Pediatr Clin North Am* 2005; **52**: 795-810.

[19] Kabra SK, Kumar P, Verma IC, Mukherjee D, Chowdhary BH, Sengupta S, et al. Bacterial meningitis in India: an IJP survey. *Indain J Pediatr* 1991; **58**(4): 505-511.

[20] Surinder K, Bineeta K, Megha M. Latex particle agglutination test as an adjunct to the diagnosis of bacterial meningitis. *Indian J Med Microbiol* 2014; **25**(4): 395-397.

[21] Bloch KC. Do delays in performing lumbar puncture after administration of antibiotics alter the results of CSF cultures? *Curr Infect Dis Rep* 2011; **13**(4): 305-307.

[22] Mohammadi SF, Patil AB, Nadagir SD, Nandihal N, Lakshminarayana SA. Diagnostic value of latex agglutination test in diagnosis of acute bacterial meningitis. *Ann Indian Acad Neurol* 2013; **16**(4): 645-649.

[23] Khan F, Rizvi M, Fatima N, Shukla I, Malik A, Khatoon R. Bacterial meningitis in North India: trends over a period of eight years. *Neurol Asia* 2011; **16**(1): 47-56.

[24] Chan YC, Wilder-Smith A, Ong BK, Kumarasinghe G, Wilder-Simth E. Adult community acquired bacterial meningitis in a Singaporean teaching hospital. A seven-year overview (1993-2000). *Singapore Med J* 2002; **43**(12): 632-636.

[25] Paterson PY. Central nervous system infections: general considerations. In: Youmans GP, Paterson PY, Sommers HM, editors. *The biological and clinical basis of infectious diseases*. 3rd ed. Philadelphia: W.B. Saunders; 1985, p. 552-567.

[26] Pelkonen T, Roine I, Monteiro L, Correia M, Pitkäranta A, Bernardino L, et al. Risk factors for death and severe neurological sequelae in childhood bacterial meningitis in Sub-Saharan Africa. *Clin Infect Dis* 2009; **48**(8): 1107-1110.

[27] Pomar V, Benito N, López-Contreras J, Coll P, Gurguí M, Domingo P. Spontaneous Gram-negative bacillary meningitis in adult patients: characteristics and outcome. *BMC Infect Dis* 2013; **13**: 451-459.