

Acute Meningoencephalitis in Hospitalised Children in Southern Bangladesh

Choudhury Habibur RASUL¹, Foiz MUHAMMAD², M Jahangir HOSSAIN³, Khayer Uddin AHMED¹, Mahmudur RAHMAN⁴

Submitted: 14 Apr 2011

Accepted: 1 Oct 2011

¹ Department of Paediatrics, Khulna Medical College and Hospital, Khulna-9000, Bangladesh

² Department of Microbiology, Khulna Medical College, Khulna-9000, Bangladesh

³ International Centre for Diarrhoeal Disease Research, Dhaka-1000, Bangladesh

⁴ Institute of Epidemiology, Disease Control and Research, Dhaka-1212, Bangladesh

Abstract

Background: Acute meningoencephalitis is an important cause of morbidity and mortality around the globe. The objective of this study was to examine the distribution of acute meningoencephalitis and its aetiological agents among children admitted to a tertiary hospital in southern Bangladesh.

Methods: This prospective study was carried out in Khulna Medical College Hospital from 2007 to 2009. All of the admitted children between 1 month and 12 years of age were enrolled over a 2-year period if they met the inclusion criteria of having an acute onset of fever (≤ 14 days) and any of the following 3 signs: neck stiffness, convulsion, or altered mental status. Cerebrospinal fluid (CSF) was collected within hours and sent to the laboratory for cytological and biochemical analyses. CSF was examined by Gram staining and a latex agglutination test to detect common bacteria. Serum and CSF were also tested for Japanese encephalitis virus antibodies.

Results: A total of 140 children were included in the study, which accounted for 2.5% of admissions between 2007 and 2009. The number of acute meningoencephalitis cases was relatively higher (37.9%) during the monsoon season. The CSF report revealed a pyogenic form in 24 (18.5%) and a viral form in 13 (10.0%) cases. Altered mental status was significantly less frequent ($P < 0.001$) in cases of pyogenic meningoencephalitis (62.5%) than in cases of non-pyogenic meningoencephalitis (93.4%). Bacterial causes were identified in 11 (8.5%) children; the causative agents included *Streptococcus pneumoniae* ($n = 8$), *Neisseria meningitidis* ($n = 2$), and *Haemophilus influenzae* ($n = 1$). Three (2.3%) patients were positive for Japanese encephalitis virus.

Conclusion: *S. pneumoniae* was the most common bacteria causing acute meningoencephalitis among the study subjects, and Japanese encephalitis virus was present in few patients.

Keywords: aetiology, Bangladesh, children, infectious diseases, meningoencephalitis, symptoms

Introduction

Acute meningitis and acute encephalitis constitute significant public health problems worldwide (1,2). Although bacteria are predominantly responsible for meningitis, viruses can cause both meningitis and encephalitis with equal frequency (3). It is often difficult to reliably differentiate meningitis and encephalitis clinically (4). Therefore, the term acute meningoencephalitis (AME) is used to denote both conditions. Despite advancements in diagnostic techniques and antimicrobial therapies, AME remains

an emergency infectious disease with a high fatality (5,6).

It has been estimated that 1–2 million cases of acute bacterial meningitis (ABM) occur annually worldwide (7). The problem is more acute in resource-poor countries in Latin America, sub-Saharan Africa, and South-East Asia (8). *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b are pathogens most commonly associated with bacterial meningitis globally, accounting for almost 90% of reported cases in patients between 2 months and 5 years of age (9). The overall

incidence of ABM in developed countries is 2–3 per 100 000, with peaks of incidence in infants and adolescents (10). The incidence of bacterial meningitis among children in developing countries is 10–20 per 100 000, a figure more than 10 times higher than that in Western Europe and the United States (11).

Viral meningoencephalitis is caused by a wide range of viruses, although most cases are caused by enterovirus, herpes simplex virus, and mumps virus (12). Japanese encephalitis (JE) virus is the main cause of meningoencephalitis in Asia, with 30 000–50 000 cases reported annually (13). Children between 5 and 15 years old are primarily affected, with a fatality rate of 20%–30%; in addition, one-third of survivors experience neuropsychiatric sequelae (13,14). The emerging Nipah virus causes severe febrile encephalitis, resulting in the death of 40%–75% of affected people, and in the recent past, several outbreaks of Nipah encephalitis have occurred in northwestern Bangladesh (15,16).

AME can be rapidly progressive, resulting in permanent sequelae in a relatively short period of time (6). The ability to treat the meningitis patients immediately depends upon the findings of the CSF examination. The emphasis is first not to miss bacterial meningitis and second not to treat viral meningitis with inappropriate antibiotics or steroids (17). Epidemiological and microbiological studies of AME are necessary to develop appropriate clinical management strategies and to implement effective preventive measures. However, national data on AME are extremely limited in Bangladesh. Therefore, this study was performed to determine the distribution of acute meningoencephalitis and the aetiological agents in children admitted to a tertiary care hospital in southern Bangladesh.

Subjects and Methods

This prospective study was carried out in the paediatrics ward of Khulna Medical College Hospital for a period of 2 years, from October 2007 to September 2009. Khulna Medical College Hospital is the only teaching hospital in southern Bangladesh and admits patients from all districts of the Khulna Division and from the neighbouring districts of other divisions. All of the admitted children between 1 month and 12 years of age who met the case definition of AME were included in this study. AME was defined as the acute onset of fever (1–14 days), followed by any of the followings: (a) signs of meningeal irritation, (b) convulsions, or (c) a change in mental status (18).

Children diagnosed later with febrile convulsions, cerebral palsy, stroke, or epilepsy were excluded from the study. Written permission was obtained from each parent after he or she was informed about the purpose of the study. Ethical permission was obtained from the ethical review committee of the International Centre for Diarrhoeal Disease Research, Bangladesh, and of the Khulna Medical College Hospital.

After a provisional diagnosis of AME, a trained doctor recorded each patient's history, examined the patient, collected specimens, and monitored the progress of the disease. All of the information was noted in a pre-designed form. In addition to taking a blood sample, cerebrospinal fluid (CSF) was collected within 2 hours from all patient unless contraindicated and was sent for biochemical and cytological analyses. In the microbiology laboratory, CSF was tested by Gram staining and latex agglutination test to detect the antigens of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*. The serum and CSF samples were aliquoted in the laboratory and stored in –20 °C freezer. The samples were transported in a cool box to the virology laboratory (Institute of Public Health) twice per month. The detection of JE virus IgM antibodies was carried out by enzyme-linked immunosorbent assays. Blood and CSF cultures could not be performed, and the serum was tested only for JE virus.

The CSF findings were categorised as viral or pyogenic based on the following criteria (17,19): (a) normal to high pressure, clear CSF, cell count of < 1000 cells/mm³, predominance of lymphocytes, CSF-to-plasma glucose ratio of > 0.3, and protein level of 0.5–1 g/L indicate viral AME, and (b) high pressure, cloudy CSF, cell count of > 1000 cells/mm³, predominance of neutrophils, CSF-to-plasma glucose ratio of < 0.3, and protein level of > 1 g/L indicate pyogenic AME. Based on physical characteristics of the CSF, treatment was started immediately. The treatment included parenteral antibiotics (ceftriaxone/cefotaxime), steroids, and supportive therapy for bacterial meningoencephalitis and was reassessed after receiving the bacteriology report (20). Cases of non-bacterial meningoencephalitis were managed symptomatically and by supportive therapy. Patients were observed twice daily, in the morning and evening, to monitor the recovery process or the development of complications.

The data were entered and analysed using SPSS version 12 (SPSS Inc., Chicago, IL, US). For continuous variables, the mean was calculated for symmetrical data, and the median was calculated for asymmetrical data. The categorical

variables were described using the frequency and percentages.

Results

During the 2-year study period, 5605 children were admitted to the paediatric ward; 140 (2.5%) of the children met the inclusion criteria for AME. The mean (SD) age of AME cases was 60 (46) months. Sixty-four cases (45.7%) were identified in children younger than 48 months, and 41 patients (29.2%) were infants (≤ 12 months). The male-to-female ratio of the children was 1.4:1. One hundred and thirty-two (94.3%) children were Muslims (Table 1). Most of the admitted children (80, 57.1%) were from Khulna, followed by Jessore (45, 32.1%). AME cases were present throughout the year, with few increases and decreases in the incidence, but a consistent high number of cases (53, 37.9%) was observed between July and October, which is the monsoon period in Bangladesh (Figure 1). Of the 3 JE-positive cases, 2 occurred in October, and the other occurred in November.

The prodromal phase, calculated from the onset of fever to the onset of neurological features, ranged 1–14 days, and mean period was 4.3 days (SD 3.5). According to the laboratory report, the CSF was classified into 3 categories. Lumbar puncture could not be performed in 10 cases because of contraindications or refusal of the attendants. The reports were normal in 93 (71.5%) cases, pyogenic in 24 (18.5%) cases, and viral in 13 (10.0%) cases. The clinical

presentation of the cases was analysed in relation to the CSF results (Table 2). The majority of cases (59, 45.4%) presented with 2 features, both altered mental status and seizure. Altered mental status was significantly more frequent ($P < 0.001$) in non-pyogenic AME (99 out of 106, 93.4%) than in pyogenic AME (15 out of 24, 62.5%). In contrast,

Table 1: Demography of the study subjects

| Parameter | n | % |
|---------------|-----|------|
| Age | | |
| 1–48 months | 64 | 45.7 |
| 49–96 months | 43 | 30.7 |
| 97–144 months | 33 | 23.6 |
| Sex | | |
| Male | 82 | 58.6 |
| Female | 58 | 41.4 |
| Religion | | |
| Muslim | 132 | 94.3 |
| Hindu | 8 | 5.7 |
| Home district | | |
| Khulna | 80 | 57.1 |
| Jessore | 45 | 32.1 |
| Kushtia | 7 | 5.1 |
| Others | 8 | 5.7 |

The total number of subjects was 140.

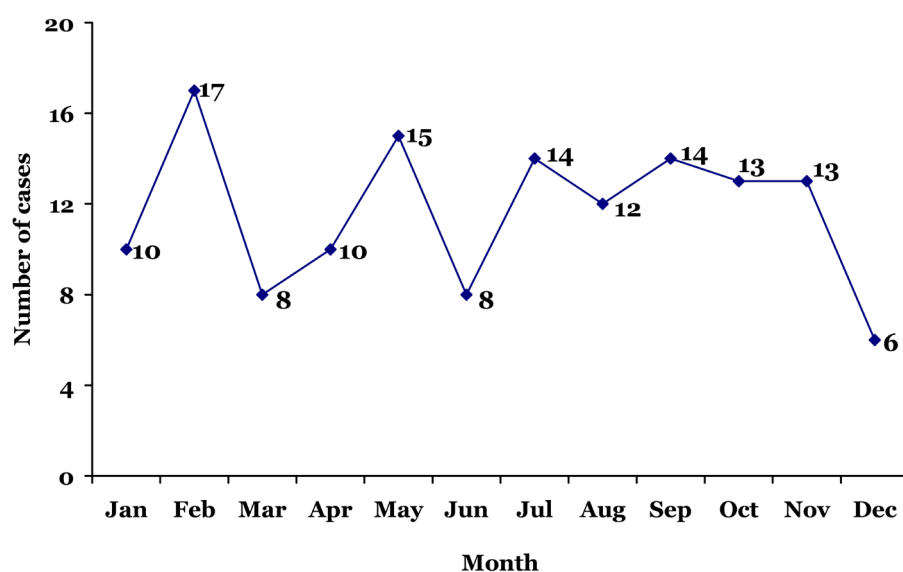


Figure 1: Monthly distribution of acute meningoencephalitis cases

the proportion of patients with neck stiffness was higher for pyogenic AME (13 out of 24, 54.2%) than non-pyogenic AME (41 out of 106, 38.7%), but this difference was not statistically significant ($P = 0.246$).

The aetiological causes of AME were analysed with respect to the CSF results (Table 3). Bacterial causes were identified only in 11 (8.5%) children, all of whom had pyogenic AME. *S. pneumoniae* was detected more frequently (8 cases) than *N. meningitides* (2 cases) and *H. influenzae* (1 case). JE virus-specific IgM was found in 3 (2.3%) patients: 2 had antibodies in the CSF, and 1 had antibodies in the serum. All JE-positive cases except 1 had CSF results indicative of a viral infection.

Discussion

The AME cases constituted 2.5% of all hospital admissions during the study period. Young children, particularly infants (29.2%), were the most affected. No difference among affected children was found with respect to sex,

religion, and location. In an Indian study (21), among all paediatric admissions, 1.5% were due to ABM. In Finland, the annual incidence of viral meningoencephalitis is 219, 27.8, and 7.6 per 100 000 for infants, children, and adult, respectively (12). Kabilan et al. (18) observed that the mean age of patients with JE was 5.5 years (SD 5.8), with a range from 6 months to 12 years. Nearly 70% of those patients were between 3 and 8 years old, and both the sexes were equally affected. The results of present study are, for the most part, in agreement with those of other reports.

The number of AME cases in this study was slightly higher during the monsoon period (37.9%), but the difference was not statistically significant. In contrast to this, two-third of the cases in Tamilnadu district of India were admitted during the monsoon period, coinciding with a high JE vector density during the period of cultivation (18). There were 8 human Nipah virus outbreaks in Bangladesh from 2001 to 2008, all occurring between December and May (22,23). Most cases of viral encephalitis in temperate climates occur

Table 2: Clinical features according to cerebrospinal fluid (CSF) categorical finding

| Symptom/sign | CSF finding | | | | | | Total | |
|--|-------------|--------|----------|--------|-------|--------|-------|---------|
| | Normal | | pyogenic | | Viral | | | |
| Seizure | 3 | (2.3) | 3 | (2.3) | 1 | (0.8) | 7 | (5.3) |
| Neck stiffness | 2 | (1.5) | 3 | (2.3) | 0 | (0.0) | 5 | (3.8) |
| Altered mental status | 7 | (5.3) | 1 | (0.8) | 2 | (1.5) | 10 | (7.7) |
| Seizure + neck stiffness | 1 | (0.8) | 3 | (2.3) | 0 | (0.0) | 4 | (3.1) |
| Seizure + Altered mental status | 48 | (36.9) | 7 | (5.3) | 4 | (3.1) | 59 | (45.4) |
| Neck stiffness + Altered mental status | 5 | (3.8) | 1 | (0.8) | 1 | (0.8) | 7 | (5.4) |
| Seizure + neck stiffness + altered mental status | 27 | (20.8) | 6 | (4.6) | 5 | (3.8) | 38 | (29.2) |
| Total | 93 | (71.5) | 24 | (18.5) | 13 | (10.0) | 130 | (100.0) |

The total number of samples was 130. Data are expressed in number of cases (percentage).

Table 3: Detection of pathogens according to the cerebrospinal fluid (CSF) biochemistry results

| CSF results | Pathogen | | | | | Total | |
|-------------|------------|---------|---------|---------|---------|-------|---------|
| | None | SP | NM | HI | JE | | |
| Normal | 92 (70.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.8) | 93 | (71.5) |
| pyogenic | 13 (10.0) | 8 (6.2) | 2 (1.5) | 1 (0.8) | 0 (0.0) | 24 | (18.5) |
| Viral | 11 (8.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (1.5) | 13 | (10.0) |
| Total | 116 (89.2) | 8 (6.1) | 2 (1.5) | 1 (0.8) | 3 (2.3) | 130 | (100.0) |

The total number of samples was 130. Data are expressed in number of cases (percentage). Abbreviation: SP = *Streptococcus pneumoniae*, NM = *Neisseria meningitides* HI = *Haemophilus influenzae*, JE = *Japanese encephalitis virus*.

during the summer and fall (3). The only viral aetiological agent tested for in this study was JE virus, and the seasonality could not be ascertained due to small number of JE cases.

The mean length of the prodromal phase of the AME cases was 4.3 days (SD 3.4). Regarding clinical features, altered mental status (88%) was the most frequent presentation and was significantly more frequent in patients with non-pyogenic AME ($P < 0.001$). The majority of patients (45.4%) presented with both altered mental status and seizure. Neck stiffness was more frequent in patients with pyogenic AME (54.1%) than in those with non-pyogenic AME (38.6%), but the difference was not significant ($P = 0.246$). In Mexico, 21% of ABM patients presented on admission with the classic clinical triad of fever, altered mental status, and nuchal rigidity (1). Seizure disorder was present in 54% of patients and was most commonly observed in children aged 6 to 12 months of age. A study from Mozambique (20) suggested that the most evident clinical difference in ABM between children and adults was neck stiffness. Diagana et al. (14) noted that the first sign of JE appeared after 6–14 days and that convulsions were experienced by 85% of subjects. The higher frequency of altered mental status in the present study suggested that the majority of AME cases were of viral origin. However, the prodromal phase and clinical presentation of the 3 JE-positive cases were not notably different from that of other types of meningoencephalitis.

The overall bacterial detection rate in this series was very low (8.5%), but it was 45.8% among patients with pyogenic meningoencephalitis. The majority of bacterial cases were due to *S. pneumoniae* (6.1%), followed by *N. meningitides* (1.5%) and *H. influenzae* (0.8%). The pre-hospital use of antibiotics was a major factor hindering the isolation of bacteria. Although this practice can reduce the mortality of patients, it can increase the rate of emergence of resistant bacteria (24). Majed et al. (25) studied 160 CSF samples from patients with bacterial meningitis and found that 31 (19.4%) were culture positive, revealing the growth of *H. influenzae* (45%), *S. pneumoniae* (29%), and *N. meningitides* (19%); among children under 5 years old, *S. pneumoniae* was found in 80% of culture-positive cases. Another hospital-based study from Bangladesh (26) revealed that among 24% bacteria-positive cases of ABM, 18% were due to *N. meningitides*, 3% were due to *S. pneumoniae*, and 3% were due to *H. influenzae*. In contrast to this observation, *H. influenzae* (70%) was recorded as the main

agent, followed by *N. meningitides* (13%) and *S. pneumoniae* (10%), in the United States (27). Among the types of meningitis caused by the 3 important organisms, *H. influenzae* meningitis has decreased over time due to vaccination, but *S. pneumoniae* continues as persistent pathogen (28,29). Nearly 500 million people in the world harbour *N. meningitides* in their nasopharynx, but only a few develop meningococcaemia, and most of these cases occur in sub-Saharan Africa (29,30). Because the frequency of detection of bacteria in the CSF was low in this study and only 3 cases were positive for JE virus, other viruses such as herpes and Nipah could not be ruled out as causative agents.

The primary limitation of this epidemiological study was its hospital-based nature. The inability to isolate other viruses was also an obstacle in identifying the actual causative agent. Blood and CSF culturing, procedures not used in this study, could be very helpful, not only for detecting bacteria but also for choosing the proper antibiotic in the management of AME cases. An estimate of the cost of the care could better explain the burden of the problem.

Conclusion

AME remains as an important cause of emergency hospitalisation of children, particularly infants. It is difficult to differentiate bacterial and viral causes of AME by clinical features alone, but altered mental status is frequently associated with the non-pyogenic form of AME. Limited investigation of the aetiological agent revealed that *S. pneumoniae* tops the list of bacteria and that JE virus was present in a small number of patients. Further large-scale studies are required to explore the national picture of AME in Bangladesh.

Acknowledgement

We are grateful to the World Health Organization, the Centers for Disease Control and Prevention, as well as the Institute of Public Health and Institute of Epidemiology, Disease Control and Research for their technical assistance and logistic support in carrying out this study. We are also grateful to Stephen Luby and Emily Gurley of International Centre for Diarrhoeal Research, Bangladesh, for their suggestions in writing this manuscript. We would like to thank the paediatrics consultants of Khulna Medical College Hospital and laboratory staffs of Khulna Medical College and Institute of Public Health for their cooperation in this study.

Authors' Contributions

Conception and design: MR

Analysis and interpretation of the data, CHR, FM, MJH, KUA

Drafting of the article: CHR, MJH

Critical revision and final approval of the article: CHR, FM, MJH, KUA, MR

Correspondence

Dr Choudhury Habibur Rasul
FCPS (Bang), MMed (Dundee), FRCP (Edin)
Department of Paediatrics
Khulna Medical College and Hospital
Khulna-9000, Bangladesh
Tel: 88-041-813679
Fax: 88-041-760350
Email: rasulch@gmail.com,
chrasul@btcl.net.bd

References

1. Franco-Paredes C, Lammoglia L, Hernandez I, Santos-Preciado JI. Epidemiology and outcome of bacterial meningitis in Mexican children: 10-year experience (1993–2003). *Int J Inf Dis*. 2008;**12**(4):380–386.
2. Mwangi I, Berkley J, Lowe B, Peshu N, Marsh K, Newton CR. Acute bacterial meningitis in children admitted to a rural Kenyan Hospital: Increasing antibiotic resistance and outcome. *Pediatr Infec Dis J*. 2002;**21**(11):1042–1048.
3. Prober CG. Acute bacterial meningitis and viral meningoencephalitis. In: Behrman RE, Kliegman R, Jenson HB, editors. *Nelson textbook of pediatrics*. 17th ed. Philadelphia (PA): Saunders; 2004. p. 2038–2047.
4. Logan SA, Macmohan E. Viral meningitis. *BMJ*. 2008;**336**(7634):36–40.
5. Quagliarello VJ, Scheld WM. Treatment of bacterial meningitis. *N Engl J Med*. 2001;**336**(10):708–716.
6. Robbins JB, Schneerson R, Gotschlich EC. Surveillance for bacterial meningitis by means of polymerase chain reaction. *Clin Inf Dis*. 2005;**40**(1):26–27.
7. Bennet JV, Platonov AE, Slack MPE, Mala P, Burton H, Robertson SE. *Haemophilus influenzae type b (Hib) meningitis in the pre-vaccine era: A global review of incidence, age distribution, and case fatality rate*. Geneva (CH): World Health Organization; 2002.
8. Van de Beek D, de Gans J, Tunkel AR, Wijdicks EF. Community-acquired bacterial meningitis in adult. *N Engl J Med*. 2006;**354**(1):44–53.
9. Tzanakaki G, Mastrantonio P. Aetiology of bacterial meningitis and resistance to antibiotic of causative pathogens in Europe and in Mediterranean region. *Int J Antimicrob Agents*. 2007;**29**(6):621–629.
10. Ramakrishnan KA, Levin M, Faust SN. Bacterial meningitis and brain abscess. *Medicine*. 2009;**37**(4):567–573.
11. Kabani A, Jadavji T. Sequelae of bacterial meningitis in children. *Pediatr Infect Dis*. 1992;**45**(6):209–217.
12. Rice P. Viral meningitis and encephalitis. *Medicine*. 2009;**37**(11):574–578.
13. World Health Organization. Japanese encephalitis vaccines. *Wkly Epidemiol Rec*. 2006;**81**:331–340.
14. Diagana M, Preuex PM, Dumas M. Japanese encephalitis revisited. *J Neurol Sci*. 2007;**262**(1–2):165–170.
15. Epstein JH, Field HE, Luby S, Pullium JR, Daszak P. Nipah virus: Impact, origins, and causes of emergence. *Curr Inf Dis Rep*. 2006;**8**(1):59–65.
16. Luby SP, Rahman M, Hossain MJ, Blum LS, Hussain MM, Gurley E et al. Food borne transmission of Nipah virus, Bangladesh. *Emerg Infec Dis*. 2006;**12**(12):1988–1994.
17. Chavanet P, Schaller C, Levy C, Flores-Cordero J, Arens M, Piroth L, et al. Performance of a predictive rule to distinguish bacterial and viral meningitis. *J Inf*. 2007;**54**(4):328–336.
18. Kabilan L, Vrati S, Ramesh S, Srinivasan S, Appaiahgari MB, Arunachalam N, et al. Japanese encephalitis virus (JEV) is an important cause of encephalitis in children of Cuddalore district, Tamil Nadu, India. *J Clin Virol*. 2004;**31**(2):153–159.
19. Kneen R, Solomon T. Management and outcome of viral encephalitis in children. *Paediatr Child Health*. 2007;**18**(1):7–16.
20. Sigaque B, Roca A, Sanz S, Oliveiras I, Martinez M, Mandomando I, et al. Acute bacterial meningitis among children, in Manhica, a rural area in southern Mozambique. *Acta Trop*. 2008;**105**(1):21–27.
21. Anjela S, Aggarwal A. Acute bacterial meningitis. *Indian Pediatr*. 1997;**34**:1097–1099.
22. International Centre for Diarrhoeal Disease Research, Bangladesh. Person-to-person transmission of Nipah infection in Bangladesh, 2007. *Health Sci Bull*. 2007;**5**(4):1–6.
23. Luby SP, Gurley ES, Hossain MJ. Transmission of human infection with Nipah virus. *Clin Inf Dis*. 2009;**49**(11):1743–1748.
24. Youssef FG, El-Sakk H, Azab A, Eloun S, Chapman GD, Ismail T, et al. Etiology, antimicrobial susceptibility profiles, and mortality associated with bacterial meningitis among children in Egypt. *Ann Epidemiol*. 2004;**14**(1):44–48.
25. Mazed MA, Tarafdar S, Miah RAM. Antibacterial sensitivity pattern of bacteria causing meningitis in paediatric patients. *Bang J Child Health*. 2003;**27**(1):1–5.

26. Gurley ES, Hossain MJ, Montgomery SP, Petersen LR, Sejvar JJ, Mayer LW, et al. Etiologies of bacterial meningitis in Bangladesh: Results from a hospital-based study. *Am J Trop Med Hyg.* 2009;**81**(3): 475–483.
27. Gray LD, Fedorku DP. Laboratory diagnosis of bacterial meningitis. *Clin Microbiol Rev.* 1992;**5**(2):130–95.
28. Singhi P, Bansal A, Geeta P, Singhi S. Predictor of long term neurological outcome in bacterial meningitis. *Ind J Pediatr.* 2007;**74**(4):369–374.
29. Tzeng YL, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microbes Infect.* 2000;**2**(6):687–700.
30. Berkley JA, Mwangi I, Ngetsa CJ, Mwarumba S, Lowe BS, Marsh K, et al. Diagnosis of acute bacterial meningitis in children at a district hospital in sub-Saharan Africa. *Lancet.* 2001;**357**(9270): 1753–1757.