Original Article

Aetiology of Encephalitis and Meningitis in Children Aged 1-59 Months Admitted to the Children's Hospital, Lusaka, Zambia

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ABSTRACT

Background: Meningitis and encephalitis are important causes of admissions and mortality in Zambia. Apart from bacterial causes, no data is available on viral agents that cause disease at the Children's Hospital, Lusaka, Zambia. We conducted a prospective descriptive study to determine the viral and bacterial causes of encephalitis and meningitis in children aged 1-59 months.

Methods: From November 2016 to February 2018, we collected cerebrospinal fluid (CSF) samples and clinical details from children admitted to the inpatient wards with encephalitis and meningitis. Macroscopic examination, microscopy, bacterial culture and real-time (Multiplex) PCR were performed on the CSF samples.

Results: A total of 106 patients were enrolled. The median age was 10 months and 81 (76.4%) had meningitis while 25 (23.6%) had encephalitis. One (0.9%) participant had *Haemophilus influenzae* detected by both culture and PCR. Two (1.9%) had *Neisseria meningitidis* while 5 (4.7%) had *Streptococcus pneumoniae* detected only by PCR. Viruses were detected in 26.4% (28/106) and 64% had meningitis. The viral agents detected were: EBV (10%); Parvovirus B19, Human herpes virus type 6, Human herpes virus type 7 and CMV at 2.8% each. A raised CSF WBC was associated with the case definition (P=0.01) of meningitis. Patients with meningitis were more likely to be alive at discharge than those with encephalitis (OR = 3.6, CI = 1.96 – 6.68, P-value <0.001).

Conclusions: Viral infections of the central nervous system (CNS) are the commonest causes of both encephalitis and meningitis at the Children's Hospital, Lusaka, Zambia.

Disclosure: The authors declare no conflict of interest.

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INTRODUCTION

Meningitis and encephalitis are important causes of admissions, morbidity and mortality in children. The incidence and case fatality rates of the affected vary per region, country, pathogen and the affected age group.[1,2,3,4]

Worldwide, viruses are regarded as the commonest infectious causes of encephalitis. However, most of the infectious causes of encephalitis remain unknown; despite extensive investigations, in the California Encephalitis Project, about 6 in 10 cases of encephalitis had no known cause [5]. The common infectious causes of meningitis include *Streptococcus pneumoniae, Haemophilus influenza, Neisseria meningitidis* and group B Streptococcus.

However, these causes vary with immunisation trends, geographical location, age of the patient, socioeconomic factors and the climatic conditions among other factors.[6-9]

Although sub-Saharan Africa remains the most affected region in the world and the known variation in the causes, there is paucity of data on the causes of viral encephalitis and viral meningitis in children, Zambia included.[4]

In Zambia, *Haemophilus influenza* type b (Hib) and Pneumococcal conjugate (PCV) vaccines were introduced in the routine national immunization programme in 2004 and 2013 respectively.[10,11]

The study sought to identify the causes of encephalitis and meningitis in children aged 1-59 months presenting at the University Teaching Hospitals' (UTH) Children's Hospital. Furthermore, the pathogens' associated cerebrospinal fluid (CSF), biochemical and cellular changes, and the pattern of clinical features if identified could play a role in the clinical management of patients.

METHODS

Study location

The study was conducted at the UTH's Children Hospital, Lusaka, Zambia. In 2014, the hospital had a 352 bed spaces and attended to 33,706 patients seeking medical care in the Out-Patient Department (OPD) [12]. In 2013 and 2014, 16,191 and 16,440 children, respectively, were admitted. Approximately 2.9 percent of the under-five years children admitted had encephalitis and/or meningitis based on the clinical judgement of the paediatricians.[12]

Participants

A cumulative sample of children aged 1-59 months admitted at the Children's Hospital between November 2016 and February 2018 with acute encephalitis and meningitis were eligible for inclusion in this study. Those enrolled had given written informed consent by the parents or legal guardian.

The clinical diagnosis of acute encephalitis and/or meningitis was made by the admitting paediatrician and based on the case definitions of encephalitis and meningitis in children; based on the consensus statement of the International Encephalitis Consortium and a Word Health Organisation (WHO) case definition of acute bacterial meningitis (ABM) in under-five children.[13,14] Furthermore, the participant should have had a lumber puncture (LP) and the CSF successfully drawn by the attending physician as part of the clinical management of the disease.

Those with pre-existing neurological problems were excluded from the study.

Clinical data and Laboratory specimen collection

A questionnaire was used to collect demographic and clinical data, including results of CSF microscopy, biochemistry, culture and sensitivity, and polymerase chain reaction (PCR).

Laboratory investigation

Standard bacteriological techniques were used to determine cell counts and culture of CSF samples.

Polymerase chain reaction techniques

Extraction of the nucleic acid

Before the samples were run, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) was extracted from 0.2ml of each clinical CSF sample using NucliSENS® easyMAG® (BioMérieux, Inc.) machine.[15,16]

Additionally, the CSF samples of enrolled patients were retrospectively analysed for bacterial infections using internally controlled real-time (Multiplex) PCR methods for detection of the bacterial organisms. The PCR reagents used were FTD Bacterial meningitis manufactured by Fast Tract Diagnostics, Luxembourg s.a.r.l.[16]

Viral investigations

The PCR reagents used were FTD Neuro9 manufactured by Fast Tract Diagnostics, Luxembourg s.a.r.l.[15] The real-time (multiplex) PCR

was used on the CSF samples for the identification of (i) HSV 1/2, (ii) VZV, (iii) Enteroviruses, (iv) Adenovirus (AV), (iv) Par echovirus, (vi) Epstein Bar virus, (vii) Human herpes virus 6 and 7, (viii) Parvovirus B19, and (ix) Cytomegalovirus (CMV). The CSF samples for PCR analysis were processed in batches of 30 or 62 per run using a real-time multiplex PCR (ABI® 7500) machine per the reagents and equipment manufacturer's instructions.[15,16]

Diagnostic interpretation of the PCR results

The presence of specific pathogen sequences in the reaction was detected by an increase in the fluorescence observed and reported as a cycle threshold by the real time thermocycler.[15,16] Any such detection in a patient with clinical diagnosis of encephalitis or meningitis was assumed to be the possible causes.[13,14]

Statistical analysis

IBM SPSS™ version 21.0 for Windows was used for statistical analysis and to produce some graphical output.

All statistical tests were done at 5% significance level. Independent samples t-test was used to compare mean values between groups. The Pearson's chi-squared test was used for comparison of proportions between groups and Fisher's exact test was used when one or more of the cells had an expected frequency of five or less.

Study variables were checked for evidence of co-linearity based on a correlation coefficient of >0.8. The relationship between study variables and case definitions was examined using backward logistic regression. The selections for entry into the logistic regression model was considered at level p<0.20 or known clinical significance from literature.

Ethical approval

Ethical approval was obtained from the Excellence in Research Ethics Science (ERES) Converge (IRB No. 00005948, FWA No. 00011697), Lusaka, Zambia. Written informed consent was obtained from the parents or legal guardians of each of the enrolled participants.

RESULTS

Patients

During the study period, November 2016 to February 2018, 270 children admitted at the hospital had a provisional diagnosis of meningitis, meningoencephalitis and/or encephalitis. Of these, 106 (106/270, 39.3%) were included into the study and the rest, 164 (60.7%), did not meet the inclusion criteria.

Clinical features

Fig 1 shows the age distribution of the patients. There were more patients diagnosed with meningitis than with encephalitis (P<0.001).

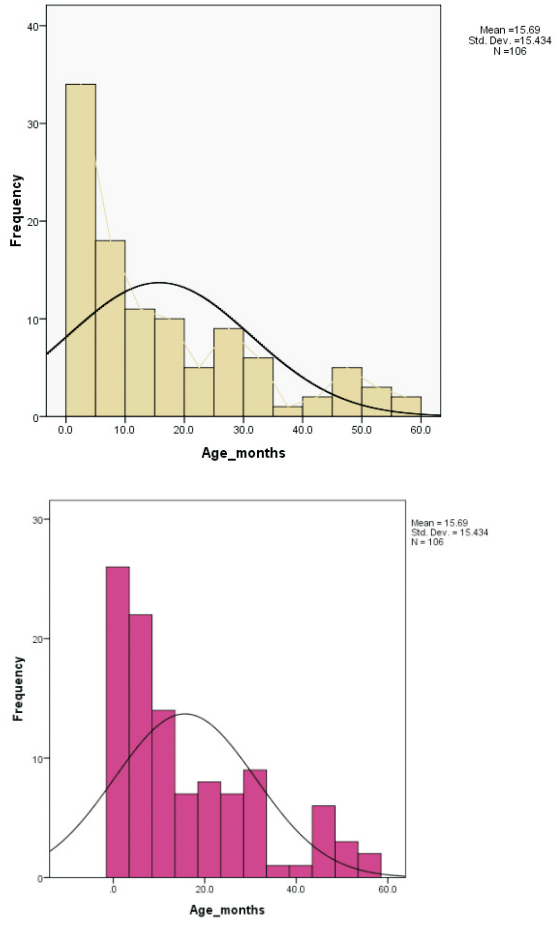

Figure 1. Study patient age distribution [minimum=1, maximum=58, median=10]

The median duration of symptoms was 3 days. About 50% of the participants had seizures on presentation. The majority (>90%) had fever [Table 1].

There were 63 (59.4%) participants that received antimicrobial drugs prior to admission while 21 (19.8%) did not [Table 1]. The proportional difference between participants who received antimicrobial drugs was statistically significant (P<0.001).

Table 1. Participants' demographic and clinical characteristics

Variable	Frequency	Percent
Sex		
Male	49	46.2
Female	57	53.8
Case definition		
Encephalitis	25	23.6
Meningitis	81	76.4
Related symptoms		
Seizures	57	53.8
Unable to feed or drink	39	36.8
Fever	99	93.4
Prostration	20	18.9
Petechial	2	1.9
Difficulties breathing	28	26.4
Stridor	4	3.8
Altered consciousness	26	24.5
Bulging fontanelle	10	9.4
Neck stiffness	28	26.4
Dehydration	17	16.0
Chest indrawing	6	5.7
Fast breathing	12	11.3
Antimicrobial drugs given		
Yes	63	59.4
No	21	19.8
Unknown	22	20.8
Patient outcome at discharge		
Alive	93	87.7
Died	13	12.3

Causative agents

There was one (0.9%) participant with *Haemophilus influenzae* bacteria detected by both microscopy and PCR. Two (1.9%) participants had *Neisseria meningitidis* while 5 (4.7%) had *Streptococcus pneumoniae* detected only by PCR.

Viruses were detected in 26.4% (28/106) of the cases. The most common viral agent detected by PCR was EBV (10%). Others were Parvovirus, Human herpes virus type 6, Human herpes virus type 7 and CMV at 2.8% each [Table 2].

Table 2. Viral organisms' distribution

Viral organisms	Frequency	Percent
HSV1	1	0.9
EBV	11	10.4
Parvovirus B19	3	2.8
HHV6	3	2.8
HHV7	3	2.8
CMV	3	2.8
Adenovirus	2	1.9

Bacterial and viral co-infections were established in 3.8% (4/106) of the participants with meningitis and 0.9% (1/106) with encephalitis. Due to the small number of organisms detected, analysis of the significance of these associations could not be done.

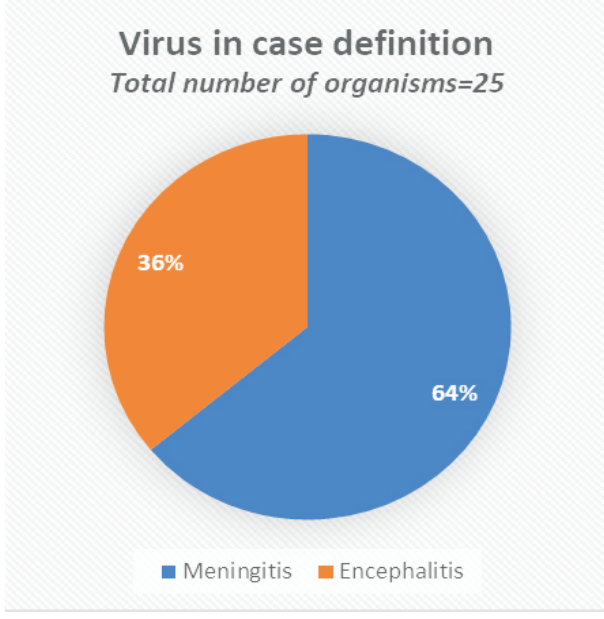

Figure 2. Viral organisms' association with case definition

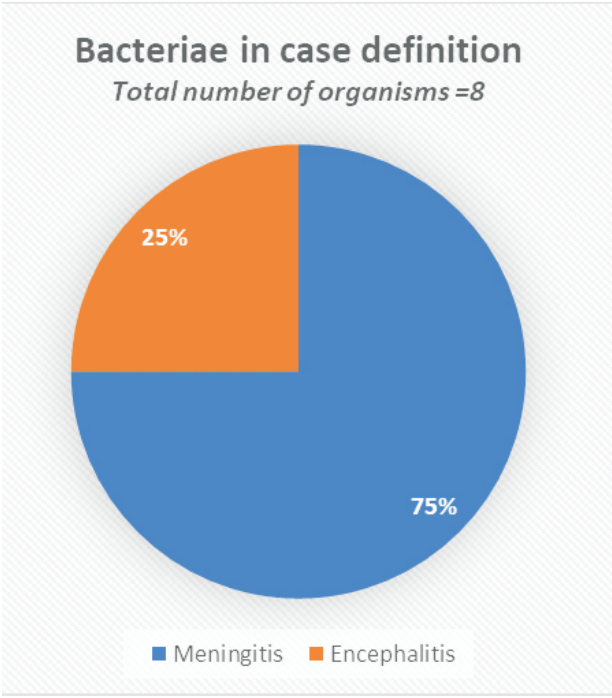

Figure 3. Bacterial organisms' association with case definition

Pathogen detection per clinical case definition

Viral and bacterial agents' detection per case definitions are as shown in figures 2 and 3, respectively. The distribution of organisms between encephalitis and meningitis was similar for both bacteria and viruses.

Antibiotic sensitivity of the bacterial isolates

This could not be done on the bacterial isolates as only one sample yielded an organism (*Haemophilus influenza*) by culture.

Bivariate analysis for case definition association

At 5% significance level, only the WBC was significantly associated with the case definition of ABM (P=0.01) [Table 3].

Table 3. Bivariate analysis for case definition association (Laboratory findings)

Variable	Encephalitis		Meningitis		P-value
	n	%	n	%	
CSF Appearance					
Clear	16	64.0%	39	48.1%	0.59
Xanthochromic	1	4.0%	9	11.1%	
Cloudy	1	4.0%	8	9.9%	
Bloody stained	1	4.0%	8	9.9%	
Other	6	24.0%	17	21.0%	
EBV					
No	23	92.0%	72	88.9%	0.99
Yes	2	8.0%	9	11.1%	
HSV1					
No	25	100.0%	80	98.8%	0.99
Yes	0	0.0%	1	1.2%	
Parvovirus B19					
No	25	100.0%	78	96.3%	0.99
Yes	0	0.0%	3	3.7%	
HHV6					
No	24	96.0%	79	97.5%	0.56
Yes	1	4.0%	2	2.5%	
HHV7					
No	23	92.0%	80	98.8%	0.14
Yes	2	8.0%	1	1.2%	
CMV					
No	24	96.0%	79	97.5%	0.56
Yes	1	4.0%	2	2.5%	
Age					
(mean, SD)	19.8 (20.24)		14.4 (13.52)		0.22
WBC					
(mean, SD)	3.9 (14.88)		91.5 (303.42)		0.01
Protein CSF					
(mean, SD)	0.85 (0.87)		0.70 (1.09)		0.67

Logistic regression analysis results predicting meningitis

Patients with meningitis were more likely to be alive at discharge than those with encephalitis (OR = 3.6, CI = 1.96 – 6.68, P-value <0.001) [Table 4].

Table 4. Logistic regression predicting meningitis case definition

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age_months	-.015	.020	.544	1	.461	.985	.947	1.025
	Sex(1)	-.450	.671	.449	1	.503	.638	.171	2.377
	Seizures(1)	-.991	.865	1.313	1	.252	.371	.068	2.022
	AlteredConsciouness(1)	.232	.653	.127	1	.722	1.262	.351	4.535
	Outcome(1)	2.140	.859	6.212	1	.013	8.501	1.580	45.748
	WBCcellsmm3	.020	.023	.720	1	.396	1.020	.974	1.068
Step 6[a]	Outcome(1)	1.285	.313	16.820	1	.000	3.615	1.956	6.682

a. Variable(s) entered on step 1: Age_months, Sex, Seizures, AlteredConsciouness, Outcome, WBCcellsmm3.

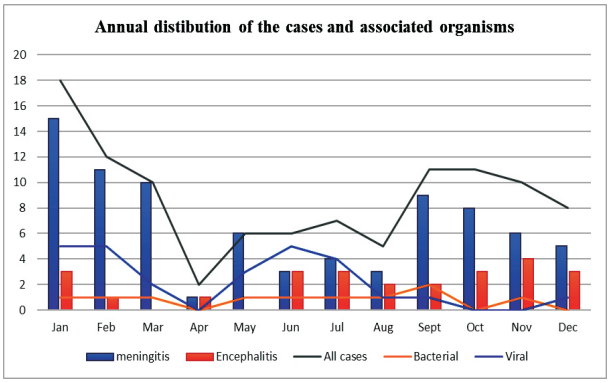

Figure 4. Annual distribution of the cases and organisms

DISCUSSION

This study is the first to utilise molecular techniques to facilitate diagnosis of central nervous system (CNS) infections in children in Zambia. It was expected that the proportion of identifiable aetiologies would be higher than in previous studies which relied only on culture and latex agglutination tests. The current study used real-time PCR which has a higher detection rate of organisms than culture and antigen agglutination tests.[14]

The study period is 16 months which is much longer than previous studies in this region.[17-19] It therefore potentially lent itself to assessing the impact of season on incidence of meningitis and encephalitis at our institution (Fig 4).

High incidences of viral infection were seen in January to February and May to July (Fig 4). This was similar to findings in the United States of America (USA) where the occurrence of CNS infections where higher in winter and summer months.[1,20]

Bacterial pathogens were identified in 7.5% of the participants which is significantly less than what was previously described at the Children's Hospital in Lusaka, Zambia.[17-19] These previous studies showed higher presence of bacterial organisms of up to around 59%.[17,19] Two of these studies were done in the pre-vaccine era and one was in the post Hib vaccine era.[18,19,17]

Though the prevalence of the bacterial causes of meningitis and/or encephalitis is significantly lower in this study compared to the previous ones, the range of causative bacterial organisms has remained the same.[17-19] The reduction in overall numbers of bacteria detected could be attributed to the introduction of *Haemophilus influenza type b* (Hib) vaccine in 2004 and *Pneumococcal conjugate vaccine* (PCV) in 2013 into the EPI program.[10,11] The ability of vaccines to avert disease was noted in a WHO study which showed that despite the low coverage of PCV, the vaccine was able to significantly reduce disease and deaths from *Streptococcus pneumonia.*[10]

Viral aetiological agents were established in 26.4% (28/106) and the most common viruses detected in the children with encephalitis and meningitis, was EBV (10%). This was similar to the study in the USA which showed that about 25% of the admissions were due to a viral aetiology.[20] However, viruses accounted for a higher proportion of cases in a study done in Vietnam (41%) and the age group was between 0-16 years.[21] There is paucity of data in sub-Saharan Africa on the causes of viral encephalitis in children. The study in Vietnam used serological methods in the CSF; and viral and bacterial culture in both blood and CSF.[21] This could have contributed to the higher yield.

Bivariate analysis to determine whether viral agents were associated with either encephalitis or meningitis did not show statistical significance for either clinical diagnosis. This was peculiar and requires more studies as most literature associates viral agents with encephalitis.[20-23]

The study highlighted the challenge of establishing the aetiology of meningitis and encephalitis. Despite the use of molecular methods in this study, the likely aetiology was not established in the over 70% of the cases. In Zambia, Integrated Management of Childhood Illnesses (IMCI) is widely implemented and any child who presents to a health facility with suspected meningitis receives a parenteral dose of

antibiotics before being referred. This could have contributed to the negative CSF culture results. As used in the study in Vietnam, viral and bacterial blood and CSF culture could have improved the yield.[21]

There is a documented association of clear CSF with viral meningitis and/or encephalitis. CSF is more commonly turbid in ABM than in viral CNS infections. It is thought that turbidity of the CSF is to a large extent due to CSF leucocyte counts exceeding 200-400 cells/mm^3.[22,24,25] Bivariate analysis did not suggest a correlation between CSF appearance and case definition of either encephalitis or meningitis [Table 2].

Patients with meningitis were more likely to be alive at discharge than those with encephalitis (OR = 3.6, CI = 1.96 – 6.68, P-value <0.001) [Table 3]. The prognosis of meningitis and/or encephalitis is known to vary with causative organism, the severity of the clinical illness, and the age of the child.[1,21,22,24] In this study, EBV is the most prevalent viral agent at 10%. There is no specific antiviral agent effective for EBV infection [Fig 2].

The limitations in this study are that only patients whom clinicians at the lower level facilities felt needed tertiary services were captured. A surveillance study over a longer period, with a larger sample size, in Zambia are urgently needed to capture more participants and further understand the aetiology of viral encephalitis and meningitis in children.

CONCLUSION

With the help of molecular testing by PCR, we were able to detect viruses as important and more frequent causes of CNS infections in children presenting to a tertiary care hospital in Lusaka, Zambia.

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AUTHOR CONTRIBUTIONS

Wrote the paper/Designed the study: Akakambama Imamba, Evans M Mpabalwani, Pota Kalima, Kate Templeton and James Chipeta. Performed the experiments: Geoffrey Kwenda and Ruth Nakazwe. Recruited participants: Akakambama Imamba.

REFERENCES

1. Falchek J S. Encephalitis in the paediatric population. pediatrics in review Vol.33 No.3 March 2012. Available at: http://pedsinreview.aappublications.org/ [Downloaded: 25 September 2015]
2. World Health Organisation (2011). Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitides, streptococcus pneumoniae and Haemophilus influenzae. 2nd Edition. Geneva, Switzerland. Available at: http://apps.who.int/iris/bitstream/10665/70765/1/WHO_IVB_11.09_eng.pdf [Downloaded: 25 September 2015]
3. Jmor F, Emsley H C, Fischer M, Solomon T, and Lewthwaite P. The Incidence of acute encephalitis syndrome in western industrialised and tropical countries. Virology Journal 2008, 5:134. Available at; http://www.virologyj.com/content/5/1/134 [Downloaded: 25 September 2015]
4. Luksic I, Mulic R, Falconer R, Orban M, Sidhu S and Rudan I. Estimating global and regional morbidity from acute bacterial meningitis in children: assessment of the evidence. Croat Med Journal. 2013;54:510-8 doi: 10.3325/cmj.2013.54.510. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3893986/ [Downloaded: 04 November 2015]
5. Glaser C A, Honarmand S, Anderson L J, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. Clinical Infectious Disease. 2006; 43:1565–1577. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17109290 [Downloaded: 14 October 2015]

6. Kliegman R M, Stanton F B, St. Geme W J, Schor F N and Behrman E R (2011). Nelson textbook of paediatrics, 19th Edition. Elsevier. Philadelphia, United States of America. Part XVII. Chapters 175,184 and 186. Pages 910-943.
7. World Health Organisation (2015). Meningococcal meningitis. Available at: http://www.who.int/mediacentre/factsheets/fs141/en/ [Accessed: 22 November 2015]
8. World Health Organisation (2015). Sentinel surveillance for pediatric bacterial meningitis in World Health Organisation African region. Available at: http://www.afro.who.int/index.php?option=com_docman&task=doc_download&gid=5804 [Downloaded: 30 November 2015]
9. Centers for Disease Control and Prevention (2015). Epidemiology and prevention of vaccine-preventable diseases. Hamborsky J, Kroger A, Wolfe S, eds. 13th ed. Washington D.C. Public Health Foundation. Page 279. Available at: http://www.cdc.gov/vaccines/pubs/pinkbook/index.html [Downloaded: 05 December 2015]
10. World Health Organisation (2015). Immunisation coverage. Available at: http://www.who.int/mediacentre/factsheets/fs378/en/ [Accessed: 22 November 2015]
11. United Nations Children's Fund (2014). Pneumococcal conjugate vaccine: supply and demand update. Available at: http://www.unicef.org/supply/files/PCV_Update_Note_July_2014.pdf [15 January 2016]
12. University Teaching Hospital, Lusaka, Zambia: 2015 Hospital information system and planning (HISAP)
13. Venkatesan A, Tunkel A R, Lauring A S, et al (2013). Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the international encephalitis consortium. Available at: http://www.macpeds.com/documents/06Encephalitisconcensusstatementofinternationalencephalitisconsortium.pdf [Downloaded: 25 November 2015]
14. Nhantumbo A A, Cantarelli V V, Caireão J, et al (2015) Frequency of pathogenic paediatric bacterial meningitis in Mozambique: the critical role of multiplex real-time polymerase chain reaction to estimate the burden of disease. Available at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0138249 [Downloaded: 19 March 2016]
15. Fast-track DIAGNOSTICS Manual: FTD Neuro9. FTD 60.3 – 32_64 – MANUAL- v2 – 2016_03 EN Available at: http://www.fast-trackdiagnostics.com/products/ftd-neuro-9/ [Downloaded: 07 May 2016]
16. Fast-track DIAGNOSTICS Manual: FTD Bacterial meningitis. FTD 28– 32_64 – MANUAL- v4 – 2016_03 EN Available at: http://www.fast-trackdiagnostics.com/products/ftd-bacterial-meningitis/ [Downloaded: 07 May 2016]
17. Kabamba J D B (2004). Profile of acute bacterial meningitis in children aged between 1 and 59 months admitted to the paediatric wards at the UTH, Lusaka. Dissertation submitted for the award of a Master's degree in Paediatrics and Child Health. University of Zambia Library. Available at: http://dspace.unza.zm:8080/xmlui/handle/123456789/1561 [Accessed: 25th September 2015]
18. Kankasa C (1997) Acute bacterial meningitis in Zambian children: highlighting the changing pattern in the aetiology of bacterial meningitis in Zambia. Dissertation submitted for the award of a Master Medicine in Paediatrics and Child Health. University of Zambia, School of Medicine. University of Zambia, Library. Available at: [Accessed: 25 September 2015]
19. Chintu C and Bathirunathan N. Bacterial meningitis in infancy and childhood in Lusaka (One-year prospective sturdy). Medical Journal of Zambia. 1975 Dec-1976 Jan;9(6):150-7. PubMed PMID: 5834.
20. George B P, Schneider E B, and Venkatesan A (2014). Encephalitis hospitalization rates and

inpatient mortality in the united states, 2000-2010. Available at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0104169 [Downloaded: 25 September 2015]

21. Tan L V, Qui P T, Ha D Q, et al (2010). Viral etiology of encephalitis in children in southern Vietnam: results of a one-year prospective descriptive study. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21049060 [Downloaded: 25 September 2015].
22. Ford-Jones E L, MacGregor D, Richardson S, Jamieson F, Blaser S, and Artsob H. (1998). Acute childhood encephalitis and meningoencephalitis: diagnosis and management. Available at: http://www.researchgate.net/publication/43183733_Acute_childhood_encephalitis_and_meningoencephalitis_Diagnosis_and_management [Accessed: 29 November 2015]
23. Kelly T A, O'Lorcain P, Moran J, Garvey P, McKeown P, Connell J, and Cotter S. (2013). Underreporting of viral encephalitis and viral meningitis, Ireland, 2005-2008. Emerging Infectious Diseases Vol. 19, No. 9. Available at: http://dx.doi.org/10.3201/ eid1909.130201 [Downloaded: 24 November 2015]
24. Prober C G and Dyner L. Central Nervous System Infections. In: Nelson textbook of paediatrics, 19th edition, Kliegman R M, Behrman R E, Jenson H B and Stanton B F, Elsevier. Philadelphia 2011. Chapter 595. Pages 2086-2097
25. Venkatesan A and Geocadin R G (2014). Diagnosis and management of acute encephalitis: a practical approach. Available at: http://cp.neurology.org/content/4/3/206 [Downloaded: 24 November 2015.