ETIOLOGY OF INFECTIONS OF THE CENTRAL NERVOUS SYSTEM AMONG CHILDREN, MBARARA, UGANDA

Report

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Summary

Acute infections of the central nervous system (CNS) pose a real clinical problem due to the diagnostic difficulties and the severity of the illness. The organisms responsible for CNS infections cannot be differentiated with certainty based solely on clinical signs. Early aetiologic diagnosis is necessary for the proper treatment of these infections. In malaria endemic countries, cerebral malaria is one of the major causes of meningitis-like symptoms. However, in areas of high endemicity, having a positive malaria test does not imply that the symptoms are solely due to the parasite, and that bacterial and viral causes should not be excluded. Bacterial meningitis is a very serious condition and should be treated with antibiotics very urgently to avoid severe consequences or death. Although viral meningitis is generally undocumented and has a better prognosis than bacterial meningitis in the immunocompetent host, it is a major threat for the immunocompromised, such as HIV patients.

There is little exhaustive data on the different causes of CNS infections in developing countries due to lack of diagnostic capacity. The objective of this study was to describe the causative pathogens and the prevalence of acute CNS infections in children (2 months to 12 years old) hospitalized at the Mbarara Regional Referral hospital, Mbarara district, Uganda. Secondary objectives included determining antibiotic resistance profile of bacterial isolates, and evaluation of the diagnostic performances of biological markers and urine dipsticks on CSF for the identification of bacterial infections. The sample size for these objectives was 480 children. All children aged 2 months to 12 years presenting with fever or history of fever and at least one sign of infection of the central nervous system were included in the study if their parent accepted participation. A clinical examination was performed on inclusion and cerebrospinal fluid and blood were collected for systematic bacterial culture, PCR, malaria smear, complete blood count and biochemical examinations. Another clinical examination was recorded on day 3 after inclusion and at discharge. Children were followed up one and six months after discharge to assess their status and possible neurological sequelae.

Of the 480 children included in the study, 21 were excluded from the analysis since they were included more than 2 days after admission with a different diagnosis on admission and inclusion, suggesting possible nosocomial infection. Among the 459 children included in the analysis, 63% were males and the median age was 30 months. Prostration (79%), reduced consciousness (72%), and history of seizures (65%) were the most frequent inclusion criteria. Almost half of the children were in coma at inclusion.

The proportion of each laboratory-confirmed type of infection, after exclusion of mixed infections, is presented in the Table. The type of infection varied by age group and HIV status.

Table. Proportion of each type of infection after exclusion of mixed infection globally, per age group and HIV status.

	Total N=459	<1 year N=122	1-5 years N=221	> 5 years N=116	HIV + N=44	HIV - N=407
Cerebral malaria	24.2	9.8	34.4	19.8	6.8	26.5
Malaria	10.2	8.2	13.6	6	0	11.5
Bacterial meningitis	12.4	23	5.9	13.8	25	11.1
Viral infection	0.7	0	0.5	1.7	0	0.7
TB meningitis	0.9	0	0.9	1.7	0	1
Cryptococcal meningitis	0.4	0	0	1.7	4.6	0

Among bacterial meningitis cases, the most frequent bacteria found were *Streptococcus pneumoniae* (67%), followed by nontyphoidal *Salmonella* (156%) and *Haemophilus influenzae* type b (14%). Except for 1 ESBL *E.Coli* , the enterobacteriae were mostly sensitive to cephalosporin but around two thirds were resistant to amoxicillin and cotrimoxazole. *S. pneumoniae* were sensitive to ceftriaxone and amoxicillin, but showed intermediate resistance to penicillin G. Finally, most Hib isolates were sensitive to amoxicillin-clavulanic acid and ceftriaxone but resistant to amoxicillin.

Most patients had received a treatment before hospitalisation, mostly antimalarials. All but one child received some anti-infectious treatment during hospitalization, with more than half receiving a combination of antimalarials and antibiotics. In general, patients received appropriate treatment during hospitalization, although they did not always receive it immediately on admission (16.3%=38/233), and treatment was sometimes interrupted prematurely (8.6%=20/233) or given for too long (4.3%=10/213).

Eighty-three (18.1%) children died at the hospital, while an additional 23 died during follow up, leading to a total of 106 (23.1%) deaths. Overall 98 children were identified with sequelae during the whole study period; 12 died (12.2%), 38 recovered fully (38.8%), and 48 (49.0%) still had sequaelae the last time they were seen. TB meningitis showed the worst outcome overall with all 4 children dying over the period of follow up, followed by cryptococcal meningitis and mixed-viral other with half of the children dying during this period – although total numbers were small for all these categories. Around 50% and 30% of children with bacterial meningitis and cerebral malaria, respectively, had either died or long-term neurological sequelae at 6 months after discharge.

Differential diagnosis of CNS infections based on clinical signs only was not reliable, since many signs were common to different types of infections. Here, we found that the best individual marker for bacterial meningitis was leucocyte count. CSF lactate was elevated in bacterial meningitis (and TB meningitis) compared to all other groups, but the diagnostic performance, as measured by the area under the curve in the ROC curve analysis, was lower than that of CSF leucocytes. CRP and procalcitonin were elevated in bacterial meningitis, but also in malaria cases, as described previously, making them less useful for the diagnosis in a setting with a high malaria prevalence. In addition, their diagnostic performance remained low even after exclusion of malaria cases. Even when assessing combinations of markers, including scores that were reported to have good performance in Europe, we could not identify any markers that would be sufficiently performant to rule out bacterial meningitis in our study population.

In conclusion, this study showed that, despite decreasing prevalence here and elsewhere in Africa, malaria is the most common cause of CNS infection in this setting. Bacterial meningitis remains the second cause and the predominance of *S. pneumoniae* emphasizes the need for effective introduction of the pneumococcal vaccine. Viral infections of the CNS, which are often suspected when typical signs or markers of malaria or bacterial meningitis are absent, were less frequent than expected. Inadequate treatment was probably not responsible for most of the deaths and antibiotic resistant bacterial infections are still rare, although resistance to ceftriaxone is present and should be monitored closely. Most of the deaths might instead be due to the severity of illness, but also to delayed consultation or referral. Rapid diagnostic tools are needed to determine the type of infection; no good marker for bacterial infection could be identified here, but the diagnosis of TB meningitis could probably be improved by the use of scores or PCR.

Abbreviations

CFR	Case fatality rate
CMV	Cytomegalovirus
CNS	Central nervous system
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
HHV	Human herpes virus
HICH	Holy Innocent Children Hospital
HSV	Herpes simplex virus
MIC	Minimal inhibitory concentration
MMJAP	Mbarara-Mulago Joint AIDS Project
MRRH	Mbarara Regional Referral Hospital
MUST	Mbarara University of Science and Technology
NS	Neurological sequel
NTS	Non-typhoidal salmonella
PCT	Procalcitonine
PCR	Polymerase chain reaction
ROC	Receiver operating characteristic
RNA	Ribonucleic acid
VZV	Varicella zoster virus
WARN	World Antimalarial Resistance Network
WHO	World Health Organization

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1. Introduction

Acute infections of the central nervous system (CNS) pose a real clinical problem due to the diagnostic difficulties and the severity of the illness. The organisms responsible for CNS infections cannot be differentiated with certainty based solely on clinical signs. Early aetiologic diagnosis is necessary for the proper treatment of these infections. In malaria endemic countries, cerebral malaria is one of the major causes of meningitis-like symptoms. However, in areas of high endemicity, having a positive malaria test does not imply that the symptoms are solely due to the parasite, and that bacterial and viral causes should not be excluded. Bacterial meningitis is a very serious condition and should be treated with antibiotics very urgently to avoid severe consequences or death. Although viral meningitis is generally undocumented and has a better prognosis than bacterial meningitis in the immunocompetent host, it is a major threat for the immunocompromised, such as HIV patients.

1.1. Data on the etiology and prevalence of infections of the CNS

In developing countries, data on the etiology and epidemiology of infections of the CNS among children over two months of age are scarce, and most of the data available refer either to cerebral malaria or epidemic meningitis.

According to an extensive literature review, cerebral malaria would affect each year 575,000 children less than 5 years of age in sub-Saharan Africa, with an estimated incidence of 6.1 cases per 1,000 children aged less than 5 years and a mean case fatality ratio of 19.2% [1].

Despite the availability of rapid tests for the diagnosis of malaria, diagnosis of cerebral malaria is not straightforward, especially in areas of high endemicity. It is thought that up to 60% of fatal cases of cerebral malaria are either misdiagnosed as other conditions or receive a delayed diagnosis. A study in Kenya showed that in an area of high malaria transmission, the malaria attributable fraction in cerebral malaria-like illness with parasitaemia can be as low as 45% [2].

Most data available on bacterial meningitis refer to epidemic meningitis caused by *Neisseria meningitidis* in countries of the so-called meningitis belt [3]. Some research groups are also investigating invasive diseases caused by *Haemophilus influenzae* (Hib) and *Streptococcus pneumoniae*, in order to encourage the use of appropriate vaccines against these agents. The Maryland Bacterial Invasive Disease Surveillance Project has thus gathered data on burdens of disease in Mali [4, 5]. Pneumococcal serogrouping data from Malawi have been used to show that the 7-valent vaccine against *S. pneumoniae* used in developed countries is of low coverage potential for isolates from developing countries [6]. Outside of the meningitis belt, some data on bacterial meningitis are now accumulating through the pneumococcal surveillance system in Eastern Africa (www.netspear.org), and the Hib initiative (www.hibaction.org/index.php). These data show a variety of prevalence of *S. pneumoniae* and Hib according to the vaccine strategy used in different regions. Netspear data on antibiotic sensitivity of *S. pneumoniae* show that trends are changing over time with fluctuating resistance to chloramphenicol and an increasing resistance to cotrimoxazole. Data on the resistance of *H. influenzae* are worrying with up to 38% and 45% resistance to chloramphenicol and ampicillin, respectively, in two recent studies in Central Africa Republic and Mozambique [7, 8]. In Mbarara, a study done in the pediatric ward in 2001 showed very high levels of resistance of CSF isolates to penicillin and chloramphenicol [9].

The pattern of agents causing bacterial meningitis in children over two months of age has been changing recently, probably due to several factors. The introduction of Hib vaccine in some African countries has led to a clear reduction of Hib incidence in these countries. In Uganda, surveillance data pre- and post-vaccination against Hib has shown that Hib has practically been eliminated and that the most common causes of bacterial meningitis in children are now *S. pneumoniae* and *Salmonella* spp [10]. *Salmonella* spp., on the other hand, have increased in importance in bacterial meningitis in children. In a hospital in Malawi, 62 children were admitted with *Salmonella* meningitis between 1996 and 1998, while only 2 cases were recorded in the same hospital in 1982 [11]. This change was tentatively explained by the raise in HIV infections among children. However, a subsequent study on the effect of HIV infection on pediatric bacterial meningitis showed that HIV positive children had a higher incidence of *S. pneumoniae* infections but similar incidence of *Salmonella* meningitis as HIV negative children [12].

Very little is known about the etiology and prevalence of viral meningitis all over Africa. A group in Kilifi, Kenya, did PCR diagnosis for enterovirus and herpesvirus in 96 unconscious children and detected herpes simplex virus (HSV)-1 in 10 children, cytomegalovirus (CMV) in 1 child and a co-infection with varicella-zoster virus (VZV) and enterovirus in one [13]. This study also showed that a significant proportion of children who fulfill the WHO clinical definition of cerebral malaria may have viral encephalitis. The same might be true with bacterial meningitis, emphasizing the need for a thorough microbiological diagnosis of CNS infections [2, 14].

In contrast to Asia, where the role of flavivirus such as dengue or Japanese encephalitis in CNS infections has been shown, little is known about the role of flavivirus in Africa. No arbovirus has been found in patients with encephalitis in Kilifi, but this study was done on a small number of children [13].

1.2. Diagnosis of infections of the CNS

Traditional diagnosis of bacterial or viral infections of the CNS relies on the isolation of the pathogen by either bacterial or viral culture. Both of these techniques are very labour- and time-consuming, require sophisticated laboratory equipment, and do not allow timely diagnosis of the disease for proper care of the patient [15, 16].

Rapid dipstick tests and smear microscopy are being widely used for the diagnosis of malaria. However, a positive malaria test in an individual with signs of severe illness does not necessarily mean that the disease is severe malaria especially in high endemic areas. A group in Kenya tried to develop a case definition that would be more accurate than the WHO case definition for severe malaria. They showed that both a threshold level of parasite density and exclusion of other co-morbidities such as bacterial meningitis are needed to increase the accuracy of severe malaria prediction [2].

In this context, physicians involved in emergency medicine have been eager to develop rules and algorithms to predict the risks of bacterial, viral and other causes of CNS infections based on cerebrospinal and serum findings [15]. Standard textbooks describe typical cerebrospinal findings in bacterial versus viral meningitis consisting of cell count, cell type, protein levels, as well as CSF/blood glucose ratio. However, these host markers are not fully discriminatory and several studies have shown that >10% of patients with bacterial diseases have a low white blood cell count, whereas polymorphonuclear cells predominance,

considered to be associated with bacterial meningitis, was found in more than half of aseptic meningitis [17].

More recently, other biological markers have been suggested to distinguish between bacterial and aseptic meningitis. C-reactive protein (CRP) and procalcitonine (PCT), which increase after an acute inflammatory reaction, have been shown to be useful for the differentiation of bacterial and viral meningitis in children [18, 19], or adults [20]. However, a meta-analysis of studies on procalcitonine as a predictor of sepsis in critically ill patients has challenged the role of this protein as a reliable predictor [21].

PCR is now being used more widely for the rapid diagnosis of viral meningitis. A single multiplex PCR assay, detecting enterovirus RNA, and HSV and VZV DNA, is capable of diagnosing nearly all cases of viral infections of the CNS occurring in the population in the United Kingdom [22]. This technique is faster and estimated to be threefold to 1000-fold more sensitive than routine viral culture and has thus become the first line test for viral diagnosis [23].

Although bacterial culture remains the reference method for the diagnosis of bacterial meningitis, PCR can also be used to detect the pathogen, either more rapidly [24], or for surveillance in contexts where storage and transport conditions preclude growth of viable bacteria [25]. A single multiplex PCR assay is thus used in research centers in Niger and Burkina Faso for the diagnosis of *N. meningitidis*, *S. pneumoniae* and *H. influenzae* infections. However, all these techniques require trained personnel, sophisticated equipments and supplies and are not affordable in most resource poor countries outside a few research facilities.

1.3. Rationale of the study

Infections of the CNS can be life-threatening if they are not diagnosed and treated appropriately. In the absence of rapid and field-adapted diagnostic tests, these infections are treated presumptively with antibiotics and/or with anti-malarials. The process of re-evaluating such empirical approaches – to reduce antimicrobial overuse and minimize drug resistance in inpatient units – will involve well-conducted clinical studies. Data on the etiology and epidemiology of the different causes of meningitis syndromes would also be helpful in the revision of local guidelines for the management of CNS infections.

Rapid tests for the early diagnosis of meningitis are urgently needed and can only be developed based on solid knowledge of the local pathogens to be identified. Correlations between host markers and the type of infection can also be useful for early differential diagnosis if they are shown to be pertinent in the context where they are used. It is thus important to study the performance of these predictive factors in an African context.

Furthermore, several vaccines are currently deployed in developing countries or are under development. Most vaccines have been developed targeting serotypes or subtypes of several organisms mostly prevalent in industrialized countries, e.g. *S. pneumoniae*, rotavirus or *N. meningitidis*. A better knowledge of the specific serotypes/subtypes of organisms circulating in developing countries will guide further research and development of adapted vaccines.

2. Objectives

2.1. Principal objective

To describe the causative pathogens and the prevalence of acute CNS infections in children (2 months to 12 years old) hospitalized at the Mbarara Regional Referral hospital, Mbarara district, Uganda.

2.2. Secondary objectives

- To study the resistance profile of bacterial isolates against common antibiotics used to treat bacterial meningitis
- To study the genotypes and/or serotypes of the pathogens identified.
- To evaluate the validity of biologic markers (CSF leucocytes and proteins, CSF/blood glucose ratio, CSF lactate, serum CRP and PCT) for the differentiation of viral meningitis versus bacterial meningitis versus severe malaria
- To evaluate the validity of urine reagent strips for the measurement of CSF parameters and differentiation of viral, bacterial or parasitic infections of the CNS
- To provide recommendations on future diagnostic tests development
- To describe the pathogens identified according to age group, clinical symptoms at admission, HIV status, presence of sickle cell disease
- To improve the care of children presenting with CNS infection
- To provide capacity building for microbiological diagnosis in Mbarara.

3. Methods

3.1. Study design

This was a prospective descriptive cohort study.

3.2. Study site

3.2.1. Study site

The study took place in the Mbarara Regional Referral Hospital (MRRH) and Holy Innocent Children's Hospital (HICH) located in Mbarara Municipality, Mbarara District, Uganda. Mbarara Municipality is a town of 69,000 persons (2002 Population Census Provisional Results) located 300 km south-west of Kampala. Mbarara District has a population of approximately 600,000 inhabitants, and is mainly rural with a density of 98.6 inhabitants/km^2.

The main health facility is the MRRH that is also a teaching hospital for the Medical School of the Mbarara University of Science and Technology (MUST). This is the referral hospital for the Western region of Uganda. It has a bed capacity of 240 beds, OPD and IPD services, a pediatric unit that includes resuscitation equipment, incubators and oxygen for premature babies, and various diagnostic laboratories. The pediatric unit admitted 8800 patients in 2007/2008 including some patients from the Democratic Republic of Congo and Tanzania.

HICH is a private non-for-profit hospital which offers only paediatric services. It was established in 2009 by the Catholic Archdiocese of Mbarara and has a 60 bed capacity with a hope of expanding to 200 beds. It also offers outpatient's services, laboratory, X-ray and counselling services.

Inclusions were done in the paediatric ward of MRRH and HICH. The biological analysis of samples was done in the Epicentre laboratory, Mbarara and in La Timone Hospital in Marseille (France).

3.2.2. Epidemiological data

In Uganda, Hib vaccination was introduced in 2002. Surveillance data from 3 hospitals in Uganda, including MRRH, showed that Hib meningitis incidence dropped from 88 cases per 100 000 aged less than 5 years in the year before vaccine introduction to 13 within 4 years and to near zero in the fifth year [10]. Vaccine coverage in Mbarara district reached 88% in 2003 and then dropped to 75% in 2005. Vaccine co-financing from Uganda's own resources was to start in 2007.

In Mbarara district, the HIV prevalence based on diagnosis of pregnant mothers is 8%. Data on HIV prevalence among children under 5 years of age are available in Uganda, with a prevalence of 0.7%. Among children hospitalized at the MRRH pediatric ward and having a HIV-positive mother, the prevalence is about 3.7% (MRRH records of April-June 2013). There are seasonal variations of the incidence of malaria in Mbarara. There are two peaks of rainfall, the first peak usually occurs between March and May and the second between September and November. Malaria cases usually increase 6 - 8 weeks after the beginning of

the rainy season. Slide confirmed malaria in this season is approximately 70% among malaria suspects under 5 years, and between 40 – 60% in those above 5 years. In the low season, around 30% of malaria suspected cases have a positive slide overall. Approximately 95% of all infections are *Plasmodium falciparum* monoinfections, with the remaining 5% due to P. *malariae, ovale,* or *vivax* monoinfections and mixed infections with P. *falciparum* (Epicentre data).

3.3. Study population

Children admitted at the pediatric ward of MRRH were eligible for the study if they presented with signs of acute CNS infections at admission or at any time during hospitalization.

3.3.1. Inclusion criteria

- Children aged two months to 12 years of age, with
- Clinical suspicion of CNS infection, and
- Informed consent obtained from the parent or guardian

Clinical suspicion of CNS infection:

CNS infection were suspected in patients with fever or history of fever in the past 48 hours (except for children younger than 9 months who may present with fever, normal body temperature, or hypothermia)

AND recent onset of any of the following at inclusion:

- non traumatic reduced level of consciousness (Blantyre coma score <4 [26] for children <9 months and Blantyre coma score <5 for older (≥ 9 months, preverbal) children, Glasgow coma score <15 for verbal children) (Annex 1),
- prostration[1], hypotonia/hypertonia, irritability
- severe headache (severe enough to require hospitalization),
- photophobia,
- neck stiffness or bulging fontanel,
- prolonged, partial or multiple seizure(s)
- focal neurological signs
- in children older than 18 months: Kernig sign (flexion of the hip 90° with subsequent pain in legs extension), or Brudzinski sign (involuntary flexion of the knees and hips after passive flexion of the neck).
- purpura
- Cheyne Stokes breathing

[1] Inability to sit unassisted if age > 9 months or breastfeed if < 9 months

3.3.2. Exclusion criteria

There were no criteria for exclusion from the study.

However, some patients were excluded from the main analysis or analysed separately in parts of the analysis for the following reasons :

- Child included twice within one month
- Suspicion of nosocomial infection, ie patients developing clinical signs of CNS infections more than 48 hours after admission with different diagnoses at admission and inclusion
- Patients with no CSF collected due to persistent contra-indication for lumbar puncture, or failure of the lumbar puncture were not included in some analysis

3.3.3. Sample size and study duration

The prevalence of different types of infections can be determined with a precision of 0.05 and a confidence level of 95% using a sample size of 385, to which 20% were added to account for possible missing or inappropriate CSF sample or possible nosocomial infections, for a total sample size of 480 samples.

We estimated that around 8% to 10% of all inclusions would be due to bacterial infections and we could obtain 35 to 40 bacterial infections confirmed by culture or PCR. Using the hypothesis that viral and malarial CNS infections are more prevalent than bacterial infections, we would have a minimum of 40 samples confirmed for each type of infection. This would be sufficient to estimate a sensitivity of biological markers of 85% with a precision of 11%.

3.4. Study endpoints

The main study endpoint was the presence of a pathogen identified in the CSF by:

- positive bacterial culture, or
- a positive viral or bacterial PCR, or
- the presence of a parasite by direct microscopy examination

Secondary endpoints were:

- pathogen identified in blood (positive culture),
- positive microbiological rapid test (cryptococcal antigen),
- antibiotic sensitivity profile of the bacteria identified (defined as sensitive, intermediate or resistant, or as the MIC value when applicable),
- results of CSF examination (cell count, protein, glucose),
- results of biochemistry and haematology,
- results of HIV serology,
- results of haemoglobin electrophoresis,
- description of clinical signs and clinical evolution under treatment.

3.5. Study procedures

3.5.1. Inclusion and clinical examination

Inclusions were performed upon admission of the child in the pediatric ward or as soon as possible after occurrence of clinical signs justifying inclusion.

A study investigator explained the purpose and the process of the study to the patient and/or guardian and asked for written consent (Informed Consent Form in Annex 2). Written consent was obtained before sample collection. For urgent cases, inclusion (consent and sample collection) was delayed until first care had been provided to the patient.

Counseling for HIV was done separately by a nurse, as performed routinely in the ward. Written consent for HIV testing was obtained on a specific form (Annex 3).

The clinician recorded the patient's demographic and clinical characteristics in the patient file. The data was reported in the Case Report Form (CRF, Annex 4) by the clinician, and monitored by the study investigator.

3.5.2. Sample collection

Blood and CSF were systematically collected from all children included in the study, before antibiotherapy initiation when possible.

CSF collection

CSF was collected by a medical doctor following universal recommendations. Lumbar puncture could be delayed or not performed in case of contra-indications (signs or suspicion of raised intracranial pressure, focal neurological signs, local infection in the area of puncture, signs of bleeding disorders, cardio-respiratory compromise which may be exacerbated by the procedure, recent seizures, suspicion of meningococcal sepsis, purpura, acute trauma of the spinal cord).

In each child included in the study, we aimed to collect a total of 3 to 4 mL of CSF, into sterile tubes. One tube (1 mL) was sent to the MRRH bacteriology laboratory for the Netspear project (out of this study), and the other tube (2 to 3 mL) to the Epicentre laboratory for bacterial cultures, cytology, biochemistry, PCR, rapid tests and storage.

Aliquots of 200 µl were prepared and stored at -80°C for further investigations such as PCR confirmation, sequencing, virus isolation etc. Stored samples were sent to La Timone Hospital in Marseille, France every 6 months to a year.

Blood collection

Blood was collected using the ward's procedures.

The volume of blood collected in the initial blood collection was 3 mL for children <1 year of age, 4 mL for children > 1 year of age, distributed as follows:

- the first 1 mL (children < 1 year old) or 2 mL (children >1 year old) was used for blood culture
- 1 mL in an EDTA tube for thick and thin blood smears, blood count, CRP, PCT, haemoglobin electrophoresis and blood spots, and HIV serology if accepted
- 1 mL in a plain tube for serum storage

A second blood collection was performed about 30 minutes later. One (1) mL of blood was collected and used for blood culture. The main purpose of the second blood culture was to increase the chance of growing a possible pathogen. In addition, the second blood culture may help in differentiating real pathogens from contaminants.

One mL of blood was collected at discharge and at the one month follow-up visit in a plain tube for serum storage.

3.5.3. Laboratory procedures

The laboratory analyses were done under the supervision of the biologist in charge. The validity and quality of results as well as quality controls were supervised by the expert microbiologist referent for this study.

Results of CSF examination, blood and plasma analysis and bacterial culture were provided to the ward in a timely manner.

CSF examination

Examination of the CSF was done systematically upon admission and included:

- CSF aspect for gross appearance/turbidity
- Complete cell count
- Gram staining
- Measure of glucose, protein and lactate levels
- Latex agglutination for cryptococcal antigen detection, and, if positive direct smear examination (India ink)
- Urine reagent strips: leucocytes, glucose and protein patches

Blood and plasma analysis

Biochemistry and haematology analysis were performed systematically upon admission and included:

- Complete blood count (ABX Pentra 60 C+)
- Haemoglobin electrophoresis
- CRP (Roche Cobas Mira Plus)
- PCT (PCT-Q, Brahms)
- Glucose

In addition, thick and thin blood smears were prepared systematically for each patient and read according to WHO 1991 method. The SD Bioline Pan/Pf rapid test for diagnosis of malaria was performed by the clinical team at admission.

HIV serology was performed systematically for all patients for whom the guardian gave informed consent for the child to be tested (Annex 3), following the Ugandan National Guidelines, using Determine™ HIV-1/2 (Inverness Medical), Stat-Pak Dipstick (Chembio Diagnostic System, Inc) and Uni-Gold™ HIV-1/2 (Trinity Biotech).

Serum was prepared from plain tubes (at inclusion, discharge and follow-up at one month) and stored at –80°C. The tubes were sent to Marseille for serology (see below).

Bacterial culture

Bacterial culture was performed systematically from CSF and blood.

CSF was systematically inoculated onto chocolate agar and Schaedler liquid medium. In addition, if the Gram direct examination showed bacteria, CSF was inoculated onto the appropriate media, according to the type of bacteria seen on the Gram.

Blood was inoculated into Oxoid Signal liquid medium. Upon growth detection by the Signal detection system, a Gram examination was done and the bottle was subcultured onto appropriate media, according to the type of bacteria seen on the Gram.

Colonies were identified using standard methods. The antibiotic resistance profile of the pathogens identified was determined using the disk diffusion method and, when appropriate, Etests for determination of the minimum inhibitory concentrations.

All bacteria identified during the study (pathogens or possible contaminants), except for mycobacteria, were stored and used for quality control and confirmation during the visit of the expert microbiologist.

Mycobacterial culture

In addition to regular bacterial culture, CSF was inoculated on Mycobacterial Growth Indicator Tubes (MGIT) to search for mycobacteria.

Initially, all patients with sufficient amount of CSF collected had 500 µL inoculated in MGIT tubes. After consultation of the scientific committee, a TB scoring system was introduced in December 2010 (from patient 189 on) using the case definition for tuberculous meningitis for clinical research defined by Marais et al. [27]. This scoring system relies on clinical and biological criteria to identify patients most at risk of TB (see Annex 6). After introduction of this score, only patients with a score of 6 or higher had TB culture performed, and the volume of CSF inoculated was increased to the maximum volume possible, considering all other analyses.

For mycobacterial culture, quality control will be performed by the Institute of Tropical Medicine, Antwerp, as part of the regular quality control of the mycobacteriology laboratory.

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed systematically on all CSF samples, regardless of results of other tests.

DNA and RNA were extracted from the CSF samples using the EZ1 system (Qiagen) and EZ1 Virus Mini Kit v2.0 kits.

Until April 2011, the following PCR were performed systematically at the Epicentre laboratory in Mbarara:

- Viral PCR: enterovirus, HSV, VZV, mumps, measles
- Bacterial PCR: *N. meningitidis*, *S. pneumoniae*, *H. influenzae* type b, *Salmonella*, *Listeria monocytogenes*, *M. tuberculosis*

After this date, due to a failure of the PCR instrument in Mbarara, these PCRs were performed at La Timone, Marseille.

In addition, the following PCR were done in Marseille on the first shipment of specimens (patients 1-122): pan-flavirvirus, dengue 1/2/3/4, Yellow fever, West Nile virus, influenza virus A, influenza virus B, influenza virus H1N1, Rift valley fever virus, Sicilian virus, Naples virus, Toscana virus, Leptospirosis, HHV6.

Considering the fact that all, except HHV-6, were negative on these specimens, only HHV6 was tested on all the following specimens. In addition, the specimens included in the second shipment (patients 137-348) were tested for parechovirus and nflebo and testing for pan-flavivirus and leptospirosis was resumed with the last shipment (patients 348-480).

Other analysis performed outside of Epicentre laboratory in Mbarara

The remaining CSF aliquots as well as frozen sera were sent to Hospital La Timone, Marseille, for further analysis.

On a case by case basis, serology was performed to either confirm the acute phase of infection by the virus identified in the patient's specimen, or investigate specimens for which no agent has been identified. The serology performed were for the same agents as those investigated by PCR. Whenever possible, serology was performed on paired acute (at inclusion) and convalescent (discharge and/or 1 month follow-up) sera.

3.5.4. Treatment

All the patients included in the study were treated according to the national guidelines and protocols of the paediatric ward of the MRRH. All children who tested positive for malaria were deemed to have severe malaria since on top of fever they had at least one sign of severe malaria. For the first part of the study from 2009 to early 2011 they were treated with intravenous quinine at a dose of 10mg/kg body weight 8 hourly until they were able to take oral medication. Thereafter treatment was switched to oral quinine for a total of 7 days. From September 2011 when national malaria treatment policy had changed, children deemed to have severe malaria were treated with intravenous artesunate 2.4mg/kg every 12 hours for the first day and there after once daily for a minimum of 48 hours or until the child was able to take oral medication. Thereafter treatment was switched to artemether/lumefantrine (20/120mg) for a total of 3 days. The dose depended on child's weight as shown in table below:

Table 1. Number of tablets of artemether/lumefantrine (20/120mg) according to body weight

Body weight of child	Number of tablets
5-<15kg	1 tablet
15-<25kg	2 tablets
25-<35kg	3 tablets
35kg and above	4 tablets

When meningitis was suspected, and in any case when malaria blood slide and rapid tests were negative, the child was empirically started on benzyl penicillin at 50,000 IU/kg every 6

hours and ceftriaxone at a dose of 100 mg/kg/day. If a bacterium was cultured from the CSF and shown to be resistant to the antibiotics given, the treatment was adapted in order to provide a better clinical care. Antibiotic treatment was also modified if the patient's clinical status did not improve. Antibiotics were stopped if there was no evidence of bacterial infection after extensive investigations. Acyclovir was given for suspected herpetic infection. Other medications were provided according to the final biological diagnosis.

All children received paracetamol for fever and phenobarbitone for seizures. Blood transfusion was given if the haemoglobin was below 5mg/dl or in case a severely anaemic child was in cardiac failure.

3.5.5. Follow-up and discharge

All the children included in the study were followed-up daily by the pediatric ward residents. Clinical data was reported in the hospital patient's file. At day 3, an update of the clinical status was reported in the case report form by a study investigator.

At discharge, a study investigator recorded the clinical information from the hospital patient file into the case report form. The patient was examined by a clinician to check for potential signs of neurological sequelae. In addition, 1 mL of blood was collected and serum was stored for serology.

The patients and their parent/guardian were asked to come back one month after discharge to check for sequelae, or possible relapses. In addition, 1 mL of blood was collected and serum was stored for serology. If they did not present to the appointment, a health visitor visited their home to find out the reasons why they missed the appointment and to bring them to the hospital, if they accepted it.

The patients and their parent/guardian were also asked to come back 6 months after discharge to check for neurological sequelae, which might have resolved after this period. Active tracing of patients not presenting to the 6-month appointment was performed for patients who presented neurological sequelae at exit and/or at one month follow-up.

3.6. Data collection and analysis

3.6.1. Data collection

Data were collected using a case report form for demographic and clinical data and a lab report form for laboratory results. These forms are presented in Annexes 5 and 6.

Clinical data were collected by the study nurses under the supervision of the clinical co-investigator. Laboratory data were collected by the biologist responsible for the study laboratory activity.

Data entry and management were done by the data management team in Mbarara. Data were double-entered into Epidata software (version 3.1), validated using Epidata and cleaned using Stata.

3.6.2. Definitions

The following definitions were used for the analysis:

Confirmed malaria case: patient with malaria trophozoites detected by smear microscopy

Probable malaria case: patient with a negative malaria smear result and a positive pLDH line on malaria RDT and previous antimalarial treatment OR patient with a positive pLDH line on malaria RDT and no smear microcopy result

Cerebral malaria: confirmed or probable malaria and coma on inclusion (Glasgow score<11 or Blantyre score<3)

Confirmed bacterial meningitis case: patient with a bacterial pathogen detected in the CSF by culture or PCR

Probable bacterial meningitis case: patient with bacteria detected in the CSF by gram staining, but negative CSF culture and PCR

Coma: Glasgow score less than 11 or Blantyre score less than 3

Bacterial meningitis score: score ranging from 0 to 6 based on the presence of bacteria on CSF Gram staining (2 points), CSF protein>=80 mg/dL (1 point), peripheral absolute neutrophil count>=10 000 cells/mm3 (1 point), seizure at or before presentation (1 point); CSF absolute neutrophil count>= 1000 cells/mm3. A score of 0 is used to exclude bacterial meningitis and a score of 2 to predict bacterial meningitis [28].

The score was then modified to classify patients with none of the 5 predictors used as “very low risk for bacterial meningitis” and those with one or more predictors as “not low risk” [29].

Meningitest: presence of at least one of the following criteria: seizures, toxic appearance, purpura, PCT level >=0.5 ng/mL, positive CSF Gram stain, or CSF protein level >=0.5 g/L.

3.6.3. Data analysis

Descriptive analysis of patients characteristics at admission

This analysis was performed on all patients included in the study.

Demographic and clinical characteristic of patients at inclusion were described using variables, mean, median, standard deviation, maximum and minimum for continuous and percentages and confidence intervals for categorical variables.

Microbial infections

The description of the causative pathogens and the prevalence of acute CNS infections was performed for all patients for whom bacteriological culture (except TB) and PCR on CSF were performed. In addition, prevalence of bacteria based on positive culture was described for all patients for whom bacteriological culture has been performed, regardless of PCR.

The following data were described by their proportions:

- type of pathogens identified globally, according to age group, clinical symptoms at admission, HIV status, presence of sickle cell disease

- children with more than one pathogen identified
- antibiotic resistance for each type of bacteria identified

Classification of types of infection

All patients were classified in one of the following categories (called “laboratory-confirmed diagnosis”) according to the definitions provided above:

- malaria: confirmed or probable malaria (with no co-infection)
- bacterial meningitis: confirmed or probable bacterial meningitis in the absence of any other infection
- bacteraemia : presence of bacteria in the blood but not in the CSF in the absence of any other confirmed infection
- viral infection: confirmed or probable viral infection in the absence of any other infection
- cryptococcal infection: cryptococcal infection confirmed by rapid test and Indian ink in the absence of any other infection
- TB meningitis: TB meningitis confirmed by CSF culture or PCR, in the absence of any other infection
- Mixed malaria- bacterial infection: confirmed malaria and confirmed bacterial meningitis or bacteraemia
- Mixed viral-other infection: confirmed viral infection with any other confirmed infection
- No laboratory-confirmed diagnosis: none of the above categories

Malaria cases was further divided as follows for the classification called “diagnosis”:

- Cerebral malaria: confirmed or probable malaria with coma
- Non-cerebral malaria: other cases

Correlation between biological markers and the type of infection

The results of biological markers were described per type of infection using the categories described above.

The diagnostic accuracy of quantitative markers (CSF leukocytes, CSF glucose, CSF proteins, CSF lactate, serum CRP) were assessed using a ROC curve analysis by comparing patients with confirmed or probable bacterial meningitis (with or without co-infection) with all other patients. The analysis was repeated after exclusion of all patients with confirmed or probable malaria. These markers were then classified as positive or negative using thresholds based on known normal values for leucocytes and glucose, or on the Youden index from the ROC analysis for CSF lactate.

Serum procalcitonin, for which the rapid test gave semi-quantitative results, was then classified as positive or negative using different cut-offs.

For dichotomous variables (including dichotomized quantitative markers and the bacterial meningitis score and meningitest), sensitivity was estimated by calculating the proportion of positive tests among the "true positive" group (probable or confirmed bacterial meningitis) and specificity by the proportion of negative tests among the "true negative" group (all others).

Definitions using different combinations of the independent predictors were assessed by calculating their sensitivity and specificity among the different groups.

Validity of urine reagent strips for measurement of CSF parameters

The median and IQR of reference values of CSF leucocytes, protein and glucose results were described for each category of urine

For the comparison between results of CSF leucocytes, protein and glucose results obtained with the urine reagent strips and concentrations determined on automated analysers, the median and IQR of reference the reference values were presented by urine strip category.

In addition, to measure correlation, the reference concentrations were grouped into categories using midpoints between the dipstick categories as cut-points. The results were then compared using unweighted and weighted kappa coefficients.

The performance of the strip results for the differentiation between types of infection was also analysed using difference cut-offs and different combinations of tests.

3.7. Ethics

The study was conducted following the principles defined by the World Health Assembly of 1975 with regard to the ethics principle of research involving human subjects as outlined in the Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm).

3.7.1. Ethics committees

The final protocol was submitted to the Mbarara University Faculty of Medicine Research and Ethics Committee, the Mbarara University Institutional Ethics Committee, the Uganda National Council for Science and Technology and the Comité de Protection des Personnes (CPP) Ile de France XI, Saint-Germain en Laye, France.

3.7.2. Confidentiality

No identifying data was recorded in the database or used in the analysis. The patients were identified by a numeric identifier.

A document including the name of the patient, name of the mother, inclusion number and precise address was kept locked and accessible only to investigators for the purpose of active follow-up.

3.7.3. Informed consent

The informed consent form (Annex 2) was translated in Runyankole. Each patient's legal representative (parent or guardian) had the study explained to them, including methods and

the potential risks and benefits of the study. The representative was asked to sign the informed consent form if they agree to participate.

3.7.4. Specimens management

Part of the specimens was sent to La Timone Hospital, Marseille, France to perform complementary testing and quality controls. All complementary testing were aimed at identifying or confirming the presence of infectious agents, or at including additional information that could have potential interest in the overall goal of this study.

4. Results

4.1. Study profile

4.1.1. Inclusions

Between August 2009 and October 2012, 613 children matching the inclusion criteria were screened for participation in the study. Of these, 133 were not included due to refusal to participate (n=92); death before enrolment (n=9); or because they were missed by the study team while symptomatic (n=32).

Overall, 480 patients were included in the study, with consistent peaks of inclusion in the months of June or July each year (Figure 1). Of these, 25 (5.2%) were included at HICH, and were analysed together with children included at MRRH.

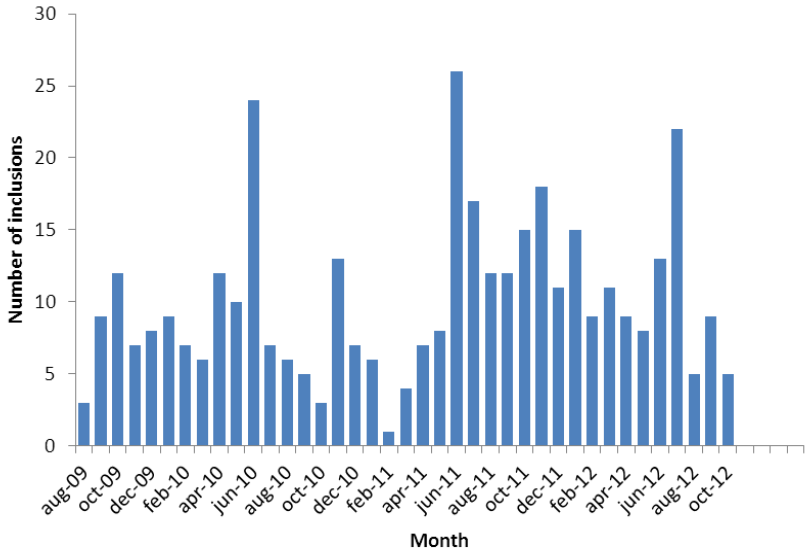


Figure 1. Number of inclusions per month

4.1.2. Exclusion criteria

Although there were no criteria to exclude patients from participating in this study, we describe here some special situations that might lead to exclusion from all or part of the analyses or to separate analyses.

Children included twice

Four children were included in the study twice. One was included a second time within one month of the first inclusion (8 days after) and the second episode was excluded from the analysis. The three other children had both episodes included in the study.

No CSF collected

Fifty six (14.7%) children had no CSF collected, of whom 35 had contra-indications to perform lumbar puncture (LP). The (non-exclusive) contra-indications were respiratory abnormalities (respiratory arrest, apnea, Cheyne Stokes respiration) in 24,followed by cardio-respiratory compromise which may be exacerbated by the LP in 19, decerebrate or decorticate posture in 10 and abnormalities in pupil size or reaction in 7. In addition, 9 children had a LP performed but with a dry or traumatic tap, 4 were unstable or moving too much, 2 had LP performed earlier, 1 refused LP, 1 child died before LP was performed and 4 had no reported reason.

Accordingly, the children with no CSF had significantly more chances of having Cheyne-Stokes breathing as inclusion criteria and less chances of having a reported headache at admission. There was no significant difference in all other inclusion criteria, or in other characteristics, such as age or sex.

Among the 56 children with no CSF collected, 26 (46.4%) had malaria proven by smear microscopy and/or rapid diagnostic test and one had *S. pneumonia* isolated from blood and clinical signs suggestive of bacterial meningitis. The other 29 had no final laboratory-proven diagnosis.

Finally, 19 (33.9%) of these children died, which was significantly higher than in children with CSF collected (16.3%, p=0.001).

Since excluding these children would cause a bias towards a less severe population, we decided to include these children in most of the descriptive analysis and exclude them for the estimation of proportions of the different types of infections (since not all infections could be diagnosed in the absence of CSF).

Possible nosocomial infections

As planned in the protocol, we investigated the characteristics of children with possible nosocomial infections including: (i) children included more than 2 days after admission at MRRH and with a different diagnosis at admission and inclusion (n=20, 4.2%); and (ii) children who had spent at least 2 days at another hospital during the week before reaching MRRH (n=13, 2.7%).

The characteristics of these children are presented in the table below. Only the inclusion criteria with significant differences are shown – other inclusion criteria were not significantly different among groups.

Although there were significant differences among groups, the profile of children hospitalized elsewhere for more than 2 days prior to admission at MRRH was globally similar to other children in terms of general characteristics and bacteria found. In addition, we have no information on the clinical presentation and diagnosis on admission in the first hospitalisation place for these patients.

In contrast, the higher proportion of bacteremia and different type of bacteria (*K. pneumoniae*, Brevibacterium spp., coagulase negative Staphylococcus) in the group with delayed inclusion and different diagnoses on admission and inclusion suggests that some of these might be nosocomial infections. To be noted also that the two *K. pneumoniae* isolated from children in this group showed an extended-spectrum beta-lactamase profile (ESBL; ie. resistant to ceftriaxone).

Table 2. Characteristics of children according to delay between admission and inclusions and hospitalization in the previous week

	No (N=447)	Clinical signs >48h after admission (N=20)	Hosp > 2 days in past week (N=13)	Fisher's exact p
Socio-demographic				
Sex, % males	282 (63.2)	9 (45.0)	9 (69.2)	0.23
Age, median (IQR)	30 (11-60)	10 (6.5-60.5)	28 (8-34)	
Inclusion criteria				
Reduced consciousness	124 (72.2)	8 (40.0)	7 (53.9)	0.004
Seizures on admission	234 (52.5)	5 (25.0)	4 (30.8)	0.054
History of seizures	395 (66.1)	5 (25.0)	4 (30.8)	0.001
Laboratory diagnosis				0.23
No laboratory diagnosis	206 (46.1)	11 (55.0)	6 (45.2)	
Malaria	156 (34.9)	2 (10.0)	2 (15.4)	
Bacterial meningitis	53 (11.9)	3* (15)	4£ (30.8)	
Bacteremia	11 (2.5)	3* (15)	1£ (7.7)	
Virus	3 (0.7)	0	0	
Crypto	2 (0.5)	0	0	
TB	4 (0.9)	0	0	
Mixed malaria -bacteria	7 (1.6)	1* (5)	0 (0)	
Mixed viral-other	5 (1.1)	0	0	
Death	82 (18.4)	5 (25.0)	1 (7.8)	0.51

* *S. pneumoniae* (2), *K. pneumoniae* (2), Hib (1), coagulase-neg *Staphylococcus* (1), *Brevibacterium* spp. (1)
£ Hib (2), *Salmonella* spp. (2), *S. pneumoniae* (1)

In conclusion, we decided to exclude the 20 children included more than 48 hours after admission with different diagnoses on admission and inclusion from the main analysis.

4.1.3. Study profile

The study profile is summarized in Figure 2.

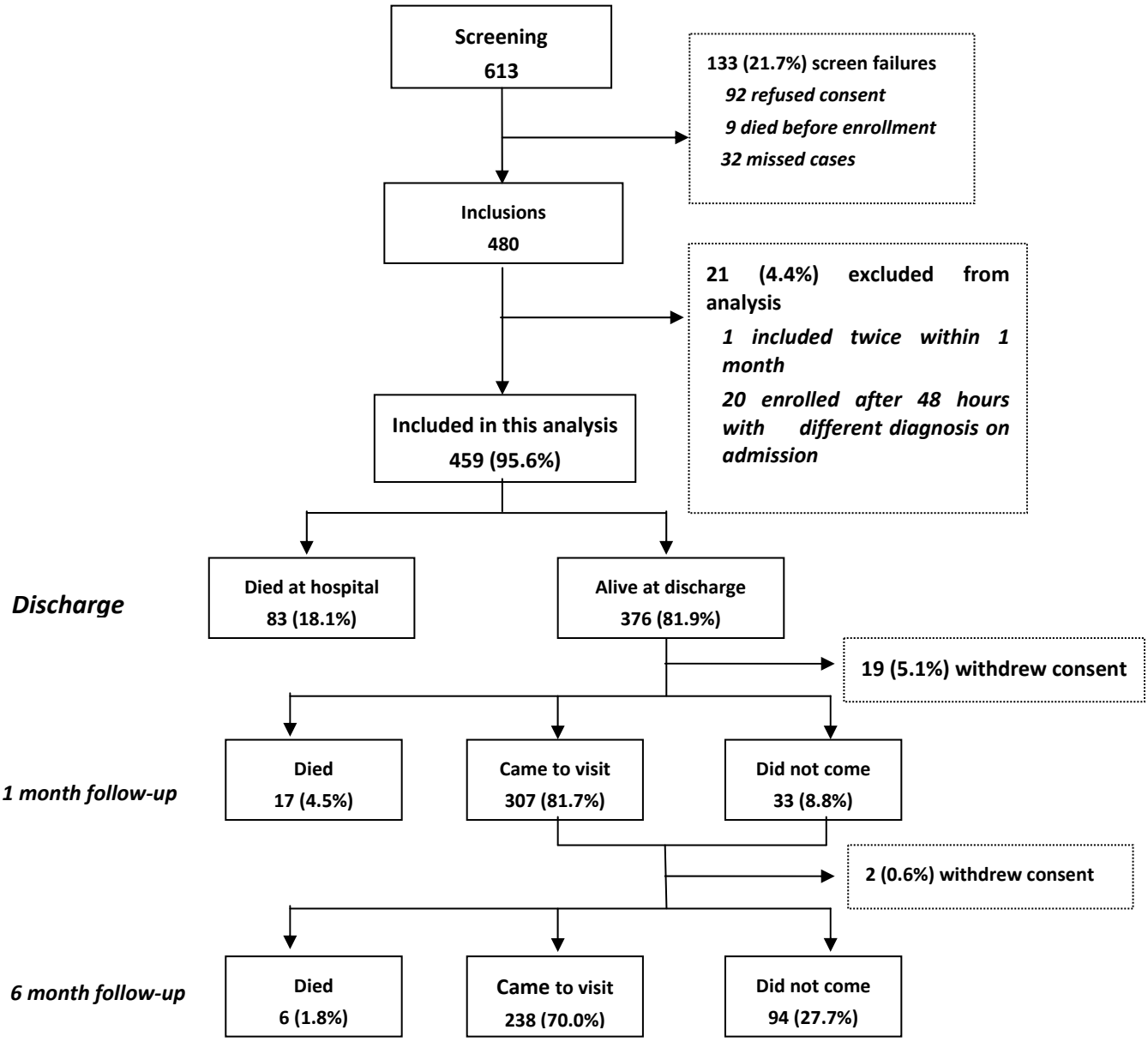


Figure 2. Study profile

4.2. General description

4.2.1. Socio-demographic and clinical history

Of the 459 children included in this analysis, 291 (63.4%) were boys. The median age was 30 months (IQR: 11-60), with 122 children (26.6%) less than one year of age, 221 (48.2%) between 1 and 5 years, and 116 (25.3%) more than 5 years.

Developmental disabilities were reported in 11 (2.4%) children, with hydrocephalus in 4, motor developmental delay in 2, global development delay and speech developmental delay in 1 each, and other disabilities in 3.

Of 451 patients who accepted to be tested for HIV, 44 (9.8%) were positive by serology. Among 297 children >= 18 months of age, 21 (7.1%) were thus considered HIV-positive. The final status of the 23/154 (14.9%) HIV-exposed children less than 18 months could not be assessed. Three children were on antiretroviral treatment before their admission at MRRH.

The sickle-cell trait was detected in 25 of 412 (6.1%) children tested but none was shown to be homozygote, potentially resulting from blood transfusion before testing.

The vaccination status of children included in the study is presented in Table 3. More than 80% of children were vaccinated against BCG, measles and Hib, although the vast majority did not show the vaccination card. Meningitis and pneumococcal vaccination were rare as they were not part of the EPI during the study period.

Table 3. Vaccination status in children above the recommended vaccination age

	No	Yes-card	Yes-verbal	Don't know
BCG (all) (N=459)	29 (6.3)	43 (9.4)	371 (80.8)	16 (3.5)
Measles (>9 months) (N=353)	47 (13.3)	25 (7.1)	267 (75.6)	14 (4.0)
Hib1 (>2 months) (N=445)	28 (6.3)	39 (8.8)	362 (81.4)	16 (3.6)
Hib2 (>3 months) (N=432)	44 (10.2)	32 (7.4)	340 (78.7)	16 (3.7)
Hib3 (>4 months) (N=416)	58 (13.9)	30 (7.2)	313 (75.2)	15 (3.6)
Meningococcus (>12 months) (N=332)	256 (77.1)	1 (0.3)	14 (4.2)	61 (18.4)
Pneumococcal (>4 months) (N=416)	326 (78.4)	1 (0.2)	13 (3.1)	76 (18.3)

Finally, concerning the episode of interest, more than half (n=254, 55.3%) of the children had received a treatment in the week before their admission at MRRH, mostly antimalarial (n=179) or antibiotic (n=107) treatments (more details provided in chapter 4.6). Fifty-eight (12.6%) of the children had been hospitalized elsewhere in the month before their admission, for a median duration of 2 days (IQR: 1-4, range: 0-48).

4.2.2. Clinical presentation at inclusion

Most children had several inclusion criteria in the study, with over half of the children with 3 or 4 inclusion criteria (n=239, 52.1%) or even 5 or more signs in a third of the children (n=156, 33.4%). The most frequent inclusion criteria were prostration, reduced consciousness, and seizures on admission or history of seizures (Figure 3).

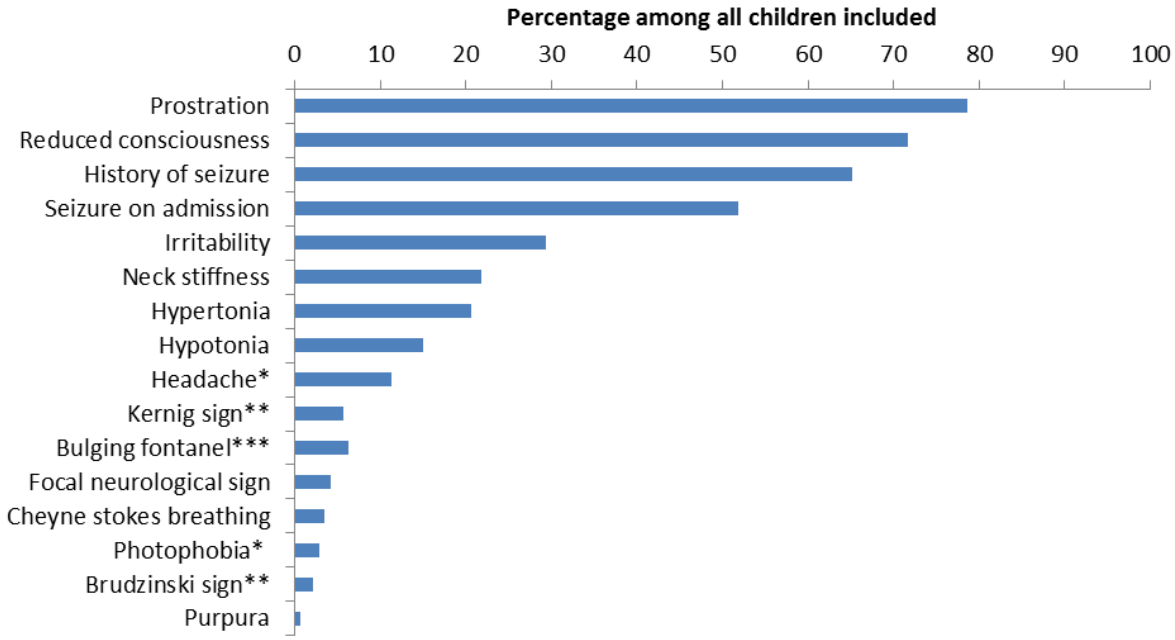


Figure 3. Proportion of each inclusion criteria reported among all children (N=459)

*Not applicable to all children
** Only for children > 18 months
*** Collected only for children <12 months

The most frequent clinical signs at inclusion were fever, coma (ie. Glasgow <11 or Blantyre <3) convulsions, hepatomegaly, respiratory distress and splenomegaly (Figure 4).

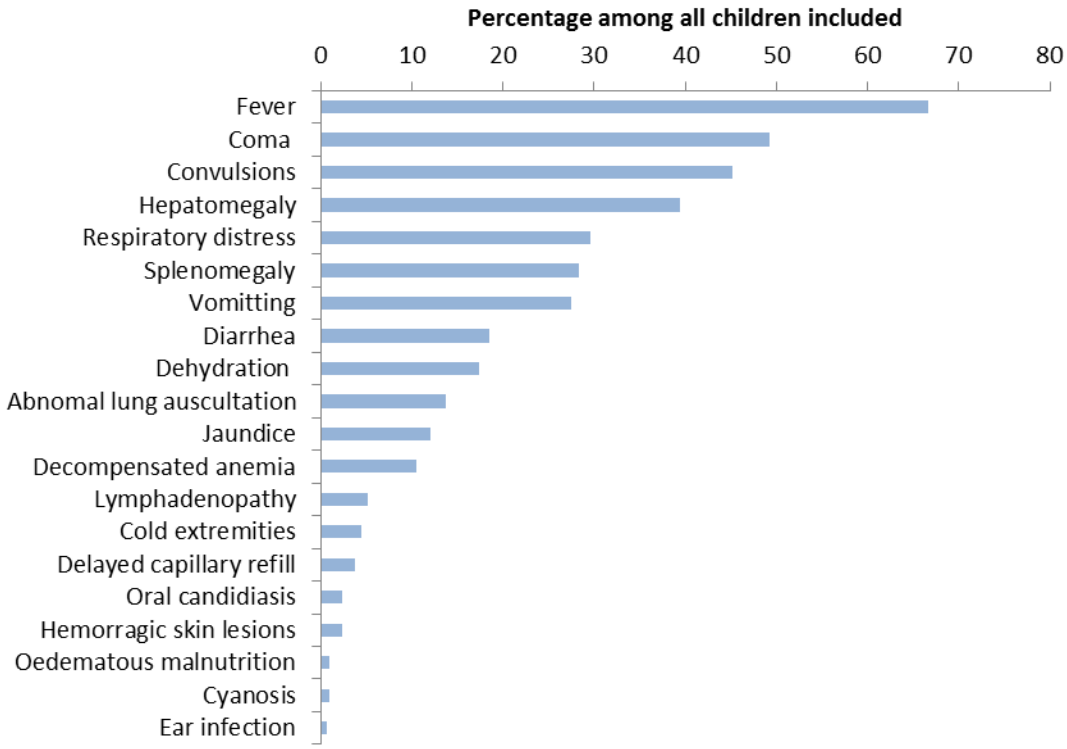


Figure 4. Proportion of clinical signs at inclusion among all children (N=459)

4.3. Main laboratory diagnoses

4.3.1. Malaria

Blood smear results

Blood smear results were available for 455 (99.1%) patients. Of these, 163 (35.8%) had asexual parasites seen on blood smear, and were considered as confirmed malaria cases. Of these, 7 (4.3%) had a mixed infection of *P. falciparum* and at least another species than *P. falciparum* (1 *P. vivax*, 2 *P. ovale* and 4 *P. malariae*).

The median parasiteamia among confirmed malaria cases was 20,792 parasites per microlitre of blood (range: 32-956,333, IQR; 1308 – 125,769).

Table 4. Level of parasitemia among patients with confirmed malaria (N=163)

Parasitemia	n	%
<1000	33	20.3
1000-10,000	36	22.1
10,000-100,000	44	27.0
>100,000	50	30.7

In addition, another 15 patients had no trophozoites but gametocytes seen on blood smear.

Globally, gametocytes were detected in 83/455 (18.2%) of the patients with blood smear results, schizonts in 44/455 (9.7%) and malaria pigments in 151/455 (33.2%).

Rapid diagnostic test results

The malaria rapid diagnostic test was done for 449 (97.8%) patients and was positive for 209 (46.6%). Of the 209 positive rapid tests, 186 were positive for Pf or Pf & Pan lines, indicating an infection with *P. falciparum* (and possible co-infection), while 20 (9.6%) were only *Pan* positive suggesting possible non-falciparum infections. However, the comparison with the microscopy results shows that the majority of positive RDT with the Pan line only were positive for *P. falciparum* by microscopy – suggesting that the differentiation by the RDT is probably not very reliable.

Table 5. Comparison between malaria RDT and smear microscopy results (N=459)

		Smear microsocopy				
			P. falciparum		Mixed	
		Neg	Trophozoites	Gametocytes	infections	Not done
RDT result	Not done	8	0	1	0	1
	Neg	231	5	2	0	2
	Pf	30	54	12	3	0
	Pan	4	16	0	0	0
	Pan & Pf	4	78	0	4	1
	Pos - no info	0	3	0	0	0

The HRP2 antigen used for the Pf line, as well as gametocytes, can persist for weeks after treatment. Forty-two with a Pf line only or gametocytes but no trophozoites seen by microscopy were thus not considered as malaria cases.

In contrast, the pLDH antigen used for the Pan line persists for only 3 days after treatment. We thus considered 3 patients with a positive Pan line (either Pan or Pan & Pf) and negative blood smear, and who had received malaria treatment in the previous week, as probable malaria cases. In addition, one patient with a positive RDT and no blood smear results was also considered as a probable malaria case.

Classification of malaria cases

Finally, of the 459 patients included in the analysis, 163 (35.5%) had confirmed malaria and 4 (0.9%) had probable malaria, for a total of 167 (36.4%) of the patients with confirmed or probable malaria. These were considered together as malaria cases in the following analysis.

4.3.2. Bacterial infections

Bacteria in the CSF

CSF was collected for 404/459 patients (88.0%). The reasons for not collecting CSF were contra-indications for lumbar puncture (n=35), lumbar puncture failed (dry or traumatic tap, n=9), refused or lumbar puncture had already done (n=3), child unstable or moving too much (n=4), and one child died before lumbar puncture. There was no reason recorded for 3 children.

Among patients in whom CSF was obtained, 48/404 (12.1%) had a positive CSF culture. Of these, 20 were considered contaminants and 28 were considered pathogenic. *Streptococcus pneumoniae* was the most commonly isolated pathogen by culture (17/30=56.7%), followed by *Haemophilus influenza* (n=6), *Salmonella* spp (n=3), *S.* Typhi (n=1). One uncommon/unexpected infection with *Brevibacterium* species was considered pathogenic. PCR detected another 17 *S. pneumoniae*, 1 Hib and 5 *Salmonella* spp. None of the other bacterial target, including *N. meningitidis* and *Listeria monocytogenes*, were detected by PCR.

Another 11 children had a probable bacterial meningitis based on bacteria seen on the CSF gram staining.

Finally, confirmed or probable bacterial infections were identified in the CSF of 62/404 (15.3%) of the patients with CSF analysed and the details of the mode of detection and bacteria distribution is summarized in the table below.

Table 6. Bacteria identified by culture , PCR and/or Gram staining in the CSF

	Detected by				Total (N=62)	
	Culture + PCR	Culture only	PCR only	Gram only	n	%
Confirmed					N=51	
S. pneumoniae	15	2	17	0	34	66.7
Hib	5	1	1	0	7	13.7
Salmonella spp.	3	0	5	0	8	15.7
S. Typhi	1	0	0	0	1	2.0
Brevibacterium spp.	0	1	0	0	1	2.0
Probable					N=11	
Gram positive cocci	0	0	0	10	10	90.9
Gram negative cocci	0	0	0	1	1	9.1

Blood culture

At least one specimen for blood culture was collected in 456 (99.3%) patients. Of these, 82 had at least one positive blood culture, which was considered as contamination in more than half of the cases (n=50, 61.0%), mostly with coagulase-negative *Staphylococcus* spp. Finally, 33 (7.0%) patients had a positive blood culture with 1 to 2 (in one case) pathogenic bacteria.

While 16 of these 33 had the same bacteria identified from CSF by culture or PCR (13 *S. pneumoniae*, 1 Hib, 1 *Salmonella* spp., 1 S. Typhi), the other 17 had bacteria isolated from blood only (Table 7).

Classification of bacterial infection

Finally, of the 459 patients included in the analysis 68 patients (14.6%) had a confirmed bacterial infection (including one infection with 2 bacterial pathogens) and another 11 had a probable bacterial infection.

Table 7. Bacteria identified in CSF and blood, CSF only or blood only

	Identified in			Total (N=69)	
	CSF and blood	CSF only	Blood only	n	%
S. pneumoniae	13	21	4	38	55.1
Salmonella spp.	1	7	4	12	17.4
Hib	1	6	0	7	10.1
S. Typhi	1	0	2	3	4.3
E. coli	0	0	3	3	4.3
S. aureus	0	0	1	1	1.4
Group B *Streptococcus*	0	0	1	1	1.4
Streptococcus spp.	0	0	1	1	1.4
Enterococcus spp.	0	0	1	1	1.4
S. epidermidis	0	0	1	1	1.4
Brevibacterium spp.	0	1	0	1	1.4

Antibiotic resistance

Except for 1 ESBL *E.Coli* , the enterobacteriae (7 *Salmonella* spp, 3 S. Typhi, 3 *E. coli*) tested for antibiotic sensitivity in the study were mostly sensitive to cephalosporin but around two thirds were resistant to amoxicillin and cotrimoxazole (Figure 5). *S. pneumoniae* were sensitive to ceftriaxone and amoxicillin, but showed intermediate resistance to penicillin G. Finally, the 6 Hib isolated in the study were sensitive to amoxicillin-clavulanic acid and ceftriaxone but 5 were resistant to amoxicillin. The details of the results are provided in Annex 7.

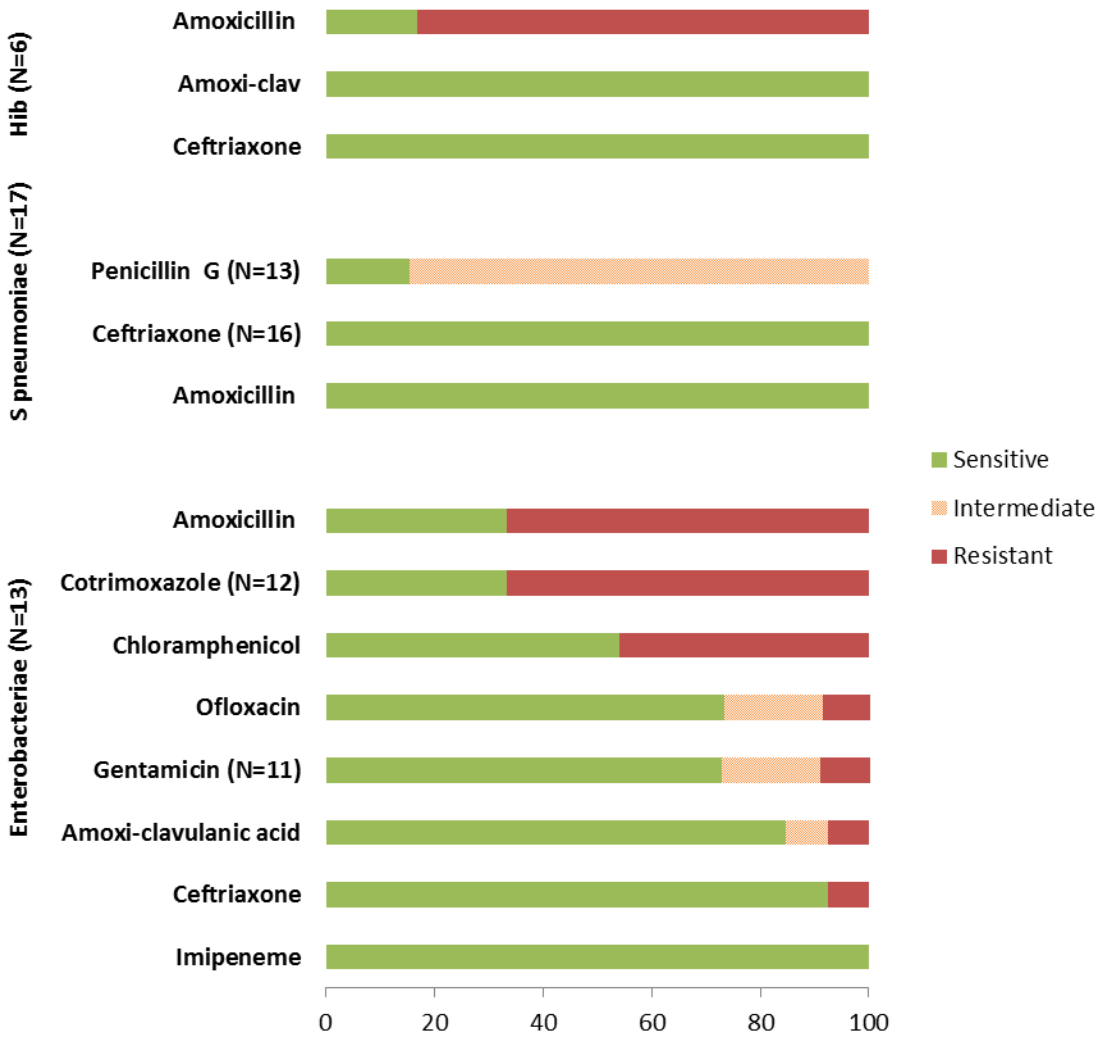


Figure 5. Proportion of sensitive, intermediate and resistant isolates among enterobacteriae, *S. pneumoniae* and Hib iolated in the study

4.3.3. Viral infections

PCR for mumps, measles, HSV, VZV, enterovirus and HHV-6 was performed on 390 patients with sufficient CSF specimens. PCR for dengue 1/2/3/4, Yellow fever, West Nile virus, influenza virus A, influenza virus B, influenza virus H1N1, Rift valley fever virus, Sicilian virus,

Naples virus, and Toscana virus was performed on the first 100 patients with sufficient specimens. PCR for pan-flavivirus was done on the first 100 patients with sufficient specimens and another 111 specimens from patients 347-480.

PCR for a viral agent in the CSF was positive in Mbarara or at La Timone in 29 patients. One specimen was positive for HSV in Mbarara but negative at La Timone and was thus considered as a false positive and is not included in the analysis below.

Of the 10 specimen positive for measles, all were positive in the same series. Of the 9 that could be tested by serology, all 9 had a negative serology profile (ie. no IgM positive at inclusion and/or discharge). We thus considered that all these specimen were false positive (possible contamination) and they are not included as viral infections below.

Of 4 patients positive for mumps, 3 could be tested by serology and 2 of these were confirmed, while 1 was IgM negative. The patient who could not be tested by serology was considered as a probable viral infection.

Of 11 patients positive for HHV-6, 6 could be tested by serology and 2 were confirmed, while 4 were IgM negative. Taking into account that the viral genome can integrate into the chromosome, we considered 5 patients who could not be tested by serology as possible old infections and did not consider them as viral infections in the analysis below.

Two patients were positive for VZV and one patient was positive for enterovirus. Due to the low added value of serology for these infections and the high probability of true viral infections when these pathogens are found in the CSF, these were considered as confirmed viral infections.

Finally we identified 8 viral confirmed or probable viral infections, 2 confirmed with HHV-6, 2 confirmed and one probable with mumps, 1 confirmed with enterovirus and 2 with VZV.

4.3.4. TB meningitis

CSF culture for TB was performed for 178 (39%) children. The mean volume of CSF inoculated for TB culture was 442.4 µl (100-900 µl).

Before use of the score to screen for TB meningitis (N=188), CSF culture for TB was performed in 110 children (58.2%). After, 32 did not have a TB score reported (mostly because CSF was not collected) and, among the 245 assessed by the score, 162 (66.1%) had a score below 6, while 80 (32.6%) had a score between 6 and 9 and only 3 (1.2%) patients had a score of 10 or higher. Of the 83 patients with a score of 6 and above, 66 (79.5%) were tested by culture and another two cultures were performed among children not scored during that period.

Overall, there were four positive TB cultures (2.2%; 2 among children with a score between 6 and 9, 1 in a child with a score of ten or more, 1 without score) and 17 contaminated cultures (9.6%).

TB PCR was performed only in Mbarara while PCR was available, and was not pursued in La Timone considering the low added value. Only 155 patients were tested by PCR for Mycobacterium tuberculosis, all of which were negative even though two of these were positive by culture.

4.3.5. Cryptococcal infections

Three-hundred and ninety-seven were tested for Cryptococcus infection and three children (0.8 %) were positive. All were detected by the RDT and confirmed with Indian ink. One of the three also grew in culture. All three cases were HIV positive.

4.3.6. Mixed infections

Mixed malaria – bacteria

Eight children had a confirmed bacterial infection in the CSF (4) or blood (4) with either confirmed (7) or probable (1) malaria. The one case of probable malaria had *S. pneumoniae* identified in the CSF, and was considered as simply bacterial meningitis. The other 7 cases of mixed infections had a co-infection with *Salmonella* spp. (3), *S. pneumoniae* (1), *S. epidermidis* (1), group B streptococcus (1) and *Brevibacterium* spp. (1).

Other mixed infections

All other mixed infections associated a viral infection and another type of infection. One patient with a probable HHV-6 infection had confirmed malaria and was considered as malaria only.

In total, 5 patients had other mixed infections: one with confirmed HHV-6 and confirmed malaria, one with confirmed HHV-6 and cryptococcal infection, one with confirmed mumps and *S. aureus* isolated from blood and one with confirmed mumps and probable bacterial meningitis (Gram positive cocci in the CSF). Finally, one patient had an enterovirus infection and *S. pneumoniae* identified in the CSF.

4.3.7. Final laboratory-confirmed diagnosis

Based on the results described above, and including both confirmed and probable infections, patients were classified in the following categories.

Table 8. Laboratory-based diagnosis among all patients and restricted to those with CSF collected.

	All (N=459)		With CSF (N=404)	
	n	%	n	%
Malaria	158	34.4	134	33.2
Bacterial meningitis	57	12.4	57	14.1
Bacteremia	11	2.4	10	2.5
Viral infection	3	0.7	3	0.7
Cryptococcal infection	2	0.4	2	0.5
TB meningitis	4	0.9	4	1.0
Mixed malaria-bacteria	7	1.5	7	1.7
Mixed viral -other	5	1.1	5	1.3
No lab diagnosis	212	46.2	182	45.0

4.4. Prevalence of the different types of infection

4.4.1. Final diagnosis

When taking into account clinical information, 111 malaria cases were classified as cerebral malaria (ie. malaria and coma on inclusion). Of the 47 non-cerebral malaria cases, 44 showed at least one sign of severe malaria. The table below shows the proportion of each type of infection.

Table 9. Prevalence of different types of infection (N=459)

	n	%	95% CI
Malaria (non-cerebral)	47	10.2	7.4-13.0
Cerebral malaria	111	24.2	20.3-28.1
Bacterial meningitis	57	12.4	9.4-15.4
Bacteremia	11	2.4	1.0-3.8
TB meningitis	4	0.9	0-1.7
Viral infection	3	0.7	0-1.4
Cryptococcal infection	2	0.4	0-1.0
Mixed malaria-bacteria	7	1.5	0.4-2.7
Mixed viral -other	5	1.1	0.1-2.0
No lab diagnosis	212	46.2	41.6-50.8

4.4.2. Diagnosis by age group

Bacterial meningitis were significantly more frequent among children less than one year ($p<0.001$) (Figure 6). In contrast, malaria was significantly more frequent among children more than one year of age ($p<0.001$), and particularly in children 1 to 5 years of age.

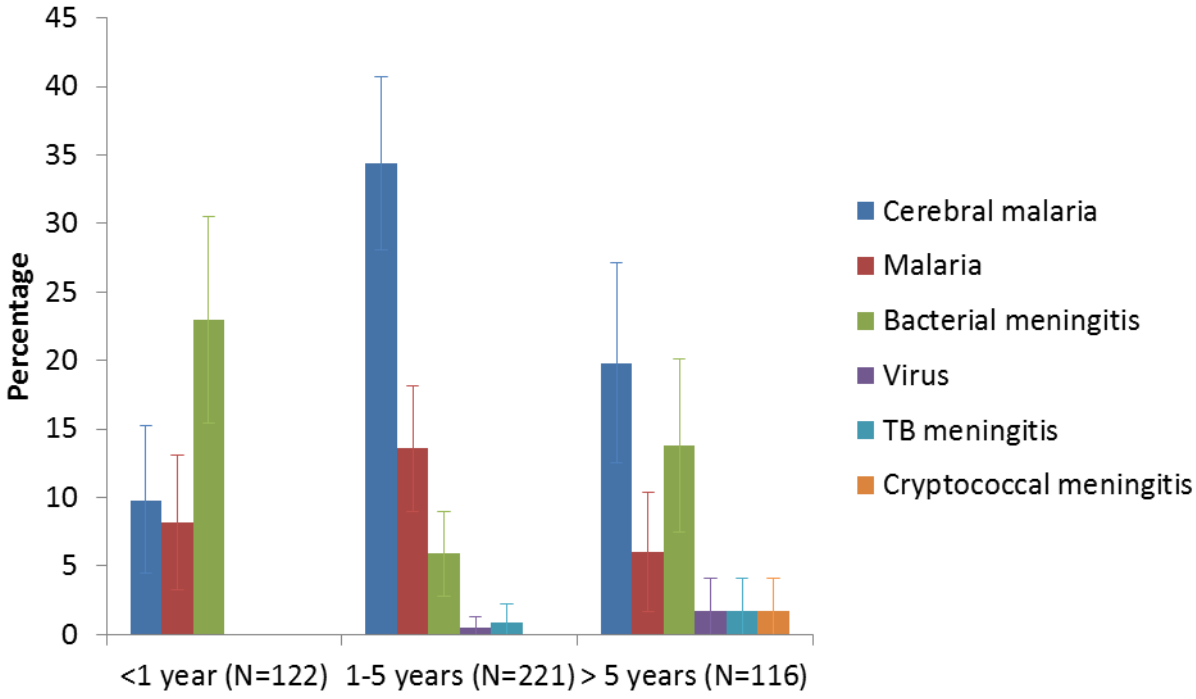


Figure 6. Final diagnosis by age group

4.4.3. Diagnosis by HIV status

Bacterial meningitis was more frequent in HIV-positive or HIV-exposed children (=p=0.019), while malaria was more frequent among HIV-negative patients (p<0.001) (Figure 7). All cases of cryptococcal meningitis were found in HIV-positive children, while all TB meningitis and viral infections were in HIV-negative children.

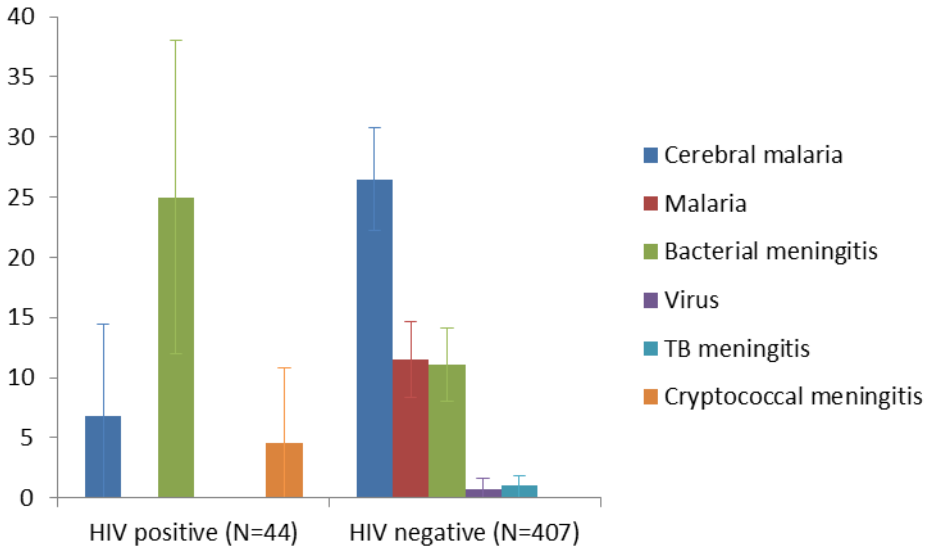


Figure 7. Final diagnosis by HIV negative or HIV-positive (including exposed) (N=459)

4.4.4. Seasonal distribution

The seasonal distribution showed peaks of cerebral malaria and malaria in June-July every year, while bacterial infections were more evenly distributed throughout the year, although they were also frequent in June (Figure 8).

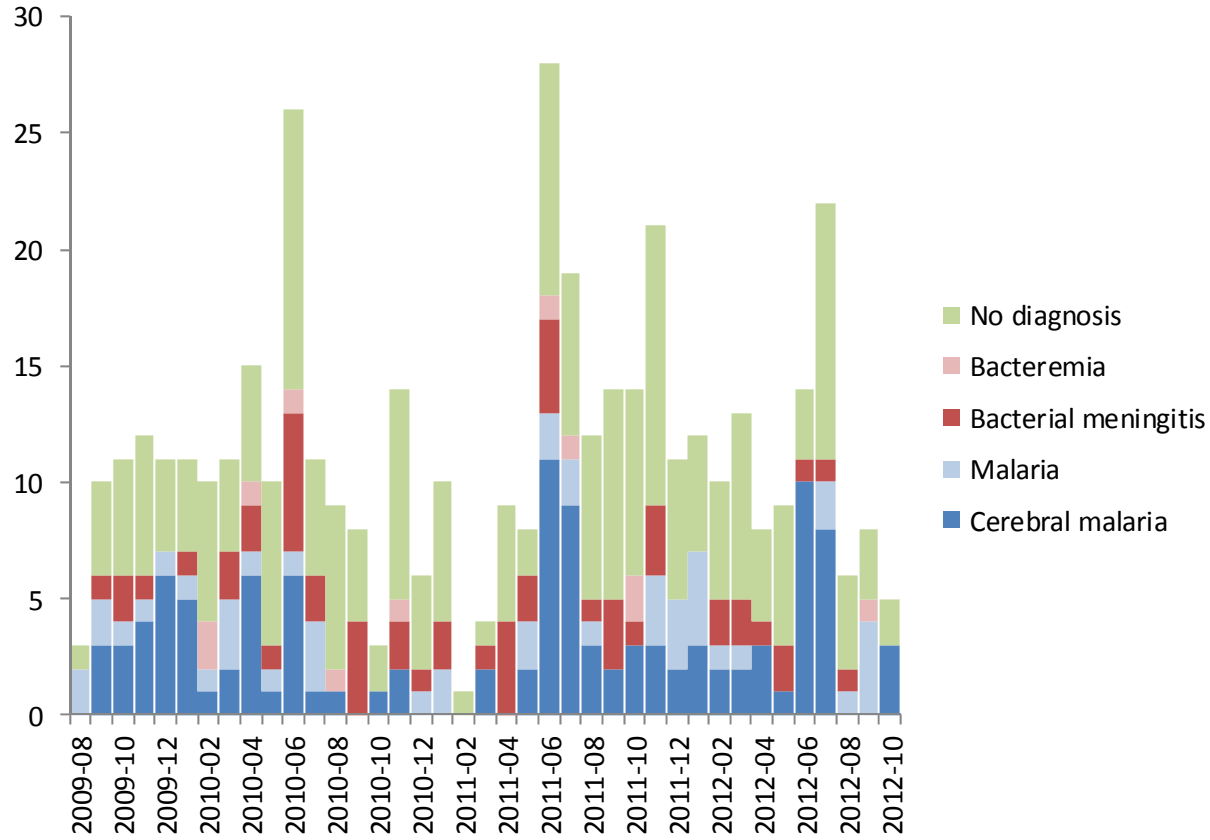


Figure 8. Seasonal distribution of the main types of infections

4.5. Clinical signs per laboratory diagnosis

Patients with a malaria diagnosis were more likely to show jaundice, hepatomegaly, splenomegaly, and decompensated anaemia compared with patients with other diagnoses. In terms of neurological signs, malaria patients were more likely to present with prostration and, for those with cerebral malaria, with seizures or history of prolonged or multiple seizures, particularly if considered cerebral malaria. Those with bacterial meningitis were more often irritable and showed signs of intracranial hypertension such as neck stiffness, bulging fontanelle, Kernig or Brudzinski sign. There was no difference in proportions of dehydration, lymphadenopathy, respiratory distress, abnormal lung auscultation, cyanoses, delayed capillary refill time, cold extremities, coma, hypotonia, hypertonia, photophobia, and focal neurological sign between the different categories of final diagnosis.

Table 10. Clinical signs by main laboratory diagnosis

	No diagnosis N=212		Malaria N=47		Cerebral malaria N=111		Bacterial meningitis N=57		Viral N=3		
	n	%	n	%	n	%	n	%	n	%	p-value
Jaundice	17	8.0	10	21.3	17	15.3	5	8.8	1	33.3	0.037
Hepatomegaly	60	28.3	25	53.2	73	65.8	14	24.6	0	0.0	<0.001
Splenomegaly	36	17.0	22	46.8	53	47.7	13	22.8	2	66.7	<0.001
Decompensated anemia	14	6.6	6	12.8	25	22.5	2	3.5	0	0.0	<0.001
Convulsions	82	38.7	19	40.4	67	60.4	28	49.1	2	66.7	0.004
Neurological signs at inclusion											
Prostration	153	72.2	39	83.0	106	95.9	43	75.4	1	33.3	<0.001
Irritability	67	31.6	19	40.4	7	6.3	30	52.6	1	33.3	<0.001
Headache	30	14.2	3	6.4	4	3.6	8	14.0	1	33.3	0.020
Neck stiffness	47	22.2	7	14.9	7	6.3	30	52.6	0	0.0	<0.001
Bulging fontanelle	16	7.6	1	2.1	0	0.0	11	19.3	0	0.0	<0.001
History of prolonged/multiple seizures	122	57.6	25	53.2	91	82.0	40	70.2	2	66.7	<0.001
If age > 18 months	N=128		N=27		N=89		N=25		N=3		
Kernig sign	10	7.8	1	3.7	2	2.3	9	36.0	0	0.0	<0.001
Brudzinski	3	2.3	0	0.0	0	0.0	6	24.0	0	0.0	<0.001

4.6. Treatment practices

4.6.1. Treatments received within 7 days prior admission

More than half (252/459=54.9%) had received some type of treatment prior admission at MRRH. Those who received antibiotics only were 42 (9.1%), 114 (24.8%) got antimalarials only, and 63 (13.7%) got both antimalarials and antibiotics. The main antibiotic received prior admission was benzyl-penicillin and quinine was the main antimalarial received. Of the 105 who received antibiotics, 24 received two different types of antibiotics while 3 received 3 different types of antibiotics (mean number of antibiotics 1.3). Of the 27 who received more than one antibiotic, 14 received benzyl penicillin and ceftriaxone, 6 received benzyl penicillin plus another antibiotic, 3 ceftriaxone plus another while 4 received other antibiotic combinations. Of the 105 who received antibiotics, 57.1% (n= 60) received intravenous and 9.5% (n=10) intramuscular antibiotic therapy.

Table 11. Frequency of antibiotics and antimalarial treatments received in the week prior admission

Type of treatment	Number (N=459)	Percentage (%)
Antibiotics		
Benzyl penicillin	44	9.6
Ceftriaxone	28	6.1
Chloramphenicol	10	2.2
Cotrimoxazole	18	3.9
Gentamycin	12	2.6
Amoxicillin	10	2.2
Others*	12	2.6
Antimalarial		
Quinine	129	28.1
Artemether/lumefantrine	42	9.2
Artemether	5	1.1
Sulfadoxine&Pyrazinamide	5	1.1
Chloroquine	3	0.7

* Azithromycin, amoxicillin/clavulanic acid, penicillin V, procaine penicillin (PPF),metronidazole, erythromycin

The median duration of antibiotics treatment prior admission was two days (interquartile range 1 to 4 days). More than 68% (n=72/105) of these were administered until day of admission.

4.6.2. Treatments received during hospitalisation

All but one[2] (n=458/459, 99.8%) children received some anti-infectious treatment during hospitalisation at MRRH or Holy Innocent hospital. More than half (n=245, 53.4%) received antibiotics and antimalarials, while 160 (34.9%) received antibiotics only, and 52 (11.3%) received antimalarial only. Finally, 18 (3.9%) received anti-viral treatment.

<u>Antibiotic treatments</u>

The most common antibiotics received were benzyl penicillin (n=333, 72.6%) and ceftriaxone (n=367, 80.0%) (Figure 9), usually as a combination (Table 12). The mean number of antibiotics received was 2.1 (SD 0.6). Almost half of the patients (n=204; 44.4%) had a change in antibiotic treatment after the first day of hospitalization, leading to different treatment regimens at admission and during hospitalization (Table 12). Ninety-five percent of antibiotics were given intravenously and 5% were given orally.

[2] Child with hydrocephalus, was transferred for neurosurgery

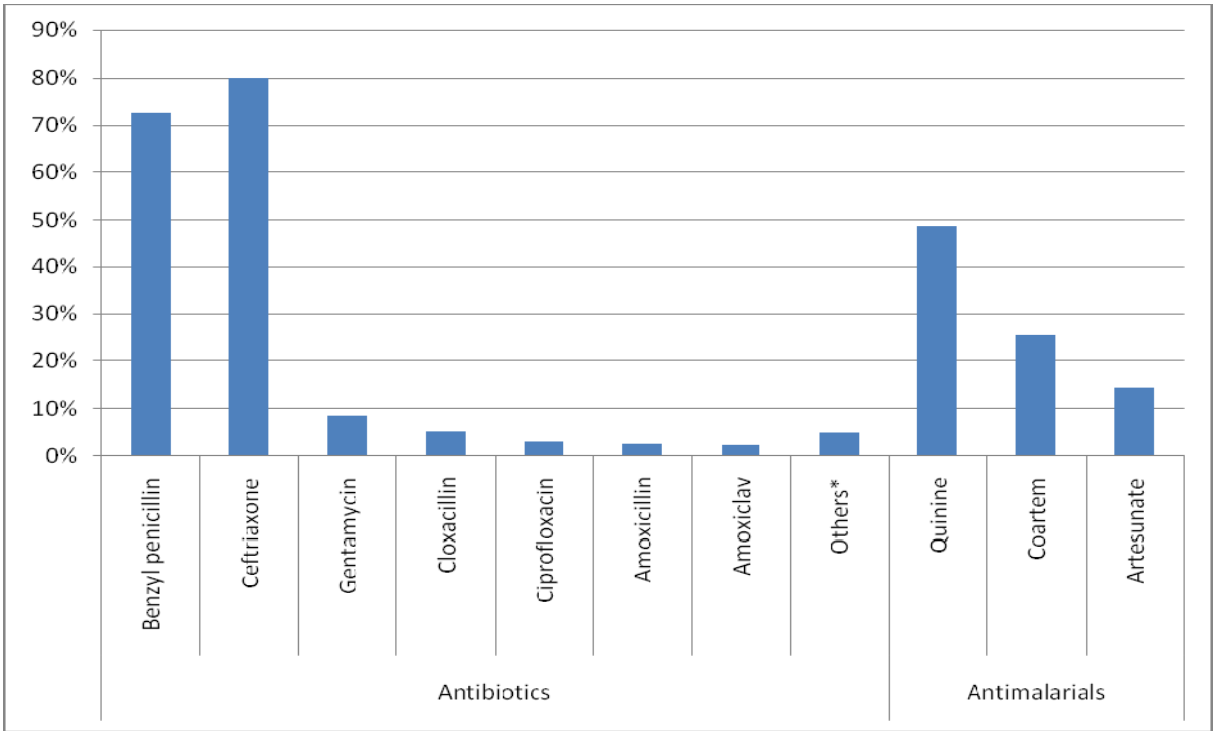


Figure 9. Types of antibiotics received during hospitalization

Table 12. Antibiotics regimen received on admission and during hospitalization (N=459)

	At admission		During hospitalization	
	n	%	n	%
Benzyl-penicillin + ceftriaxone	154	52.9	226	55.8
Benzyl-penicillin +ceftriaxone+ others*	17	5.8	82	20.2
Ceftriaxone only	52	17.9	40	9.9
Benzyl-penicillin only	34	11.7	13	3.2
Benzyl-penicillin + others*	12	4.1	12	3
Ceftriaxone + others*	4	1.4	20	4.9
Others*	18	6.2	12	3
Total	291	100	405	100

*Others: chloramphenicol, metronidazole, ampicillin, amoxiclav, erythromycin, antiTB, gentamycin, cloxacillin, cotrimoxazole and ciprofloxacin

Most children (247/405) received antibiotics for up to 7 days, median duration 6 days, (IQR 4-10 days;Table 13). The median duration of hospitalisation for these children was 7 days, (IQR 4-12). Of these, 158 (64.0%) had antibiotics until the day of discharge or death. For 9 of the 10 children who received antibiotics for more than 3 weeks, one regimen was given during this period (benzyl penicillin, cefriaxone or both) and the remaining child had a switch of the antibiotic regimen (benzyl penicillin and cefriaxone to gentamycin).

Table 13 : Duration of antibiotic treatment during hospitalization

Duration of antibiotic treatment	N= 405	%
up to 7days	247	61.0
8- 14 days	109	26.9
15 - 21 days	39	9.6
> 21 days	10	2.5

Antimalarial treatment

Of those given anti-malarial treatment, most of the children 224/297 (75.4%) were treated by quinine. The other common antimalarials used were artemether lumefantrine (Coartem) and artesunate. The median duration of all antimalarial treatment was 5 days (IQR 3 – 6; range 1 - 13) and 16 children received antimalarials for more than 8 days.

Table 14 Route and duration of antimalarial treatment

	First antimalarial given N= 297	Median duration in days(IQR)	Second antimalarial given N=183	Median duration in days(IQR)
Quinine				
Intravenous	204 (68.7)	3 (2-5)	8 (4.4)	3 (2- 4.5)
Intramuscular	11 (3.7)	2 (2-4)	0 (0.0)	
Oral	7 (2.4)	3 (2-4)	62 (33.9)	4.5 (3-6)
Artesunate, intravenous	58 (19.5)	3 (2-4)	9 (4.9)	4 (2-5)
Artemether intramuscularly	1 (0.3)	1	1 (0.6)	6
0ral	1 (0.3)	1		
Coartem, oral	15 (5.1)	3 (2-4)	103 (56.3)	3 (2-4)

Intravenous artesunate started to be used as first line antimalarial drug in the second half of 2011 but quinine was used throughout the study duration.

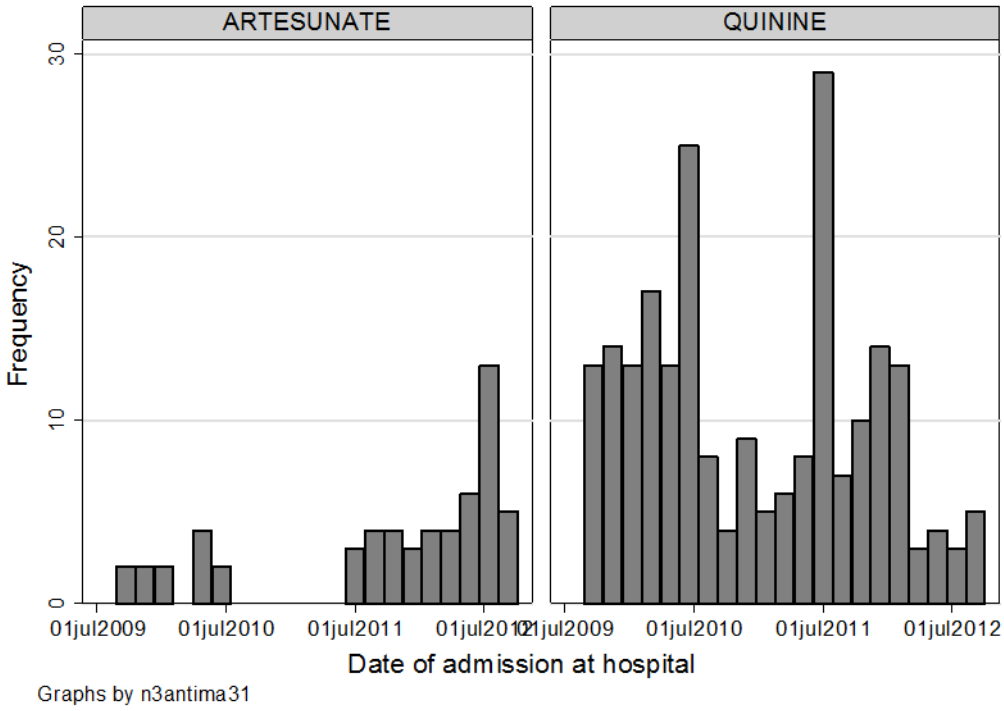


Figure 10. First line intravenous antimalarial used

Antiviral treatments

Of the 18 children (3.9%) who received antiviral treatment, 15 received acyclovir (13 oral, 1 intravenously, and one topical) and 3 had other antiretrovirals for HIV infection. All but one received antibiotics and 7 also received antimalarial treatment.

4.6.1. Per laboratory-confirmed diagnosis

During hospitalisation, out of the 75 with bacterial infection (including mixed infections) all but one[3] received antibiotics. Of the 27 who had antibiotics for less than 8 days, 21 received them until day of discharge or death. 19 had antibiotics for 8-14 days, 20 had for more than two weeks and 8 for more than 21 days. Median duration of treatment was 12 days (IQR 6-16). Only 26 of the 62 with bacterial meningitis received antibiotics for at least 14 days as recommended by national guidelines, of the remaining 36, 13 had died and 13 received antibiotics until the day of discharge.

Among those with a final diagnosis of malaria (N=158), 78 (49.4%) had prior antimalarials and 24 (15.2%) had antibiotics before hospitalisation. Among the 68 with bacterial infection 17 (25.0%) had received prior antibiotics and 29 (42.7%) had received prior antimalarials.

[3] One patient with mixed malaria and bacterial infection had cerebral malaria treated with quinine but died the following day of admission before receiving antibiotics. The positive blood culture results for salmonella came after death.

Overall, there was no difference in mortality among those with or without prior treatment (P>0.20).

In general, patients received appropriate treatment during hospitalization (antimalarial for malaria and antibiotics for bacterial infection; Table 15), although they did not always receive it immediately on admission (16.3%=38/233), and treatment was sometimes interrupted prematurely (8.6%=20/233) or given for too long (4.3%=10/213).

Table 15 Treatments received compared to laboratory-confirmed diagnosis

	malaria	bacterial infection	mixed malaria +bacterial infection
All , N	N=158	N=68	N=7
Appropriate treatment prior admission, n (%)	78(49.4%)	17 (25.0%)	0 (0.0%)
Appropriate treatment started on day of admission, n (%)	134 (85.4%)	57 (83.8%)	3 (42.9%)
Appropriate treatment prescribed over hospitalization, n(%)	158 (100%)	67 (98.5%)**	6 (85.7%)
*Treatment prematurely interrupted**	9 (5.7%)	9 (13.2%)	2 (28.6%)
Treatment given for too long#	8 (5.1%)	2 (2.9%)	0 (0.0%)
Deaths, N	N=22	N=16	N=1
Appropriate treatment prior admission, n (%)	10 (45.5%)	3 (18.8%)	0 (0%)
Appropriate treatment started on day of admission, n(%)	21 (95.5%)	14 (87.5%)	0 (0%)
Appropriate treatment prescribed over hospitalization, n(%)	22 (100%)	15 (93.8%)	0 (0%)
*Treatment prematurely interrupted**	3 (13.6%)	2 (12.5%)	1 (100.0%)
Treatment given for too long#	0 (0.0%)	0 (0.0%)	0 (0.0%)

Appropriate treatment for malaria: any antimalarial received

Appropriate treatment bacterial infections: antibiotic received, to which the organism was shown (or presumed) to be sensitive

**Treatment prematurely interrupted: Antimalarial received for less than 3 days if final diagnosis of malaria, antibiotic given for less than 8 days if final diagnosis of bacteremia or less than 15 days if final diagnosis of meningitis; treatment not given until day of discharge or death.*

 # Treatment given for too long: Antimalarial given for more than 8 days and second antimalarial not artemether-lumefantrine if final diagnosis of malaria, at least one same antibiotic given for more than 21 days if final diagnosis of bacteremia or meningitis

***1 ESBL (1 E. coli) did not receive appropriate antibiotic*

Of the 15 patients who received acyclovir, 2 had malaria, 2 had bacterial meninigitis, 1 had cryptococcal meningitis, 1 had TB meningitis, and 9 had no confirmed diagnosis.

4.7. Outcomes: deaths and neurological sequelae

4.7.1. Deaths: general description

Eighty-three (18.1%) children died at the hospital, while an additional 23 died during follow up, leading to a total of 106 (23.1%) deaths. Among the 83 children who died at the hospital, the main reported causes of death were meningitis in 29 (34.5%), cerebral malaria in 26 (31.0%), meningoencephalitis in 21 (25.3%) and other in 7 (8.4%): space occupying lesion (2), haemolytic uremic syndrome, respiratory distress, sepsis, sickle cell disease with coma, bleeding disorder.

Of the 23 children who died after discharge (during follow up), a cause of death was reported in only 3: meningitis in 1, meningoencaphalitis in 1, tuberculous meningitis in 1.

4.7.2. Neurological sequelae: general description

Overall 98 children were identified with sequelae during the whole study period; 86 at discharge time, an additional 7 at month 1 of follow-up and 5 more at month 6 of follow-up (Figure 11).

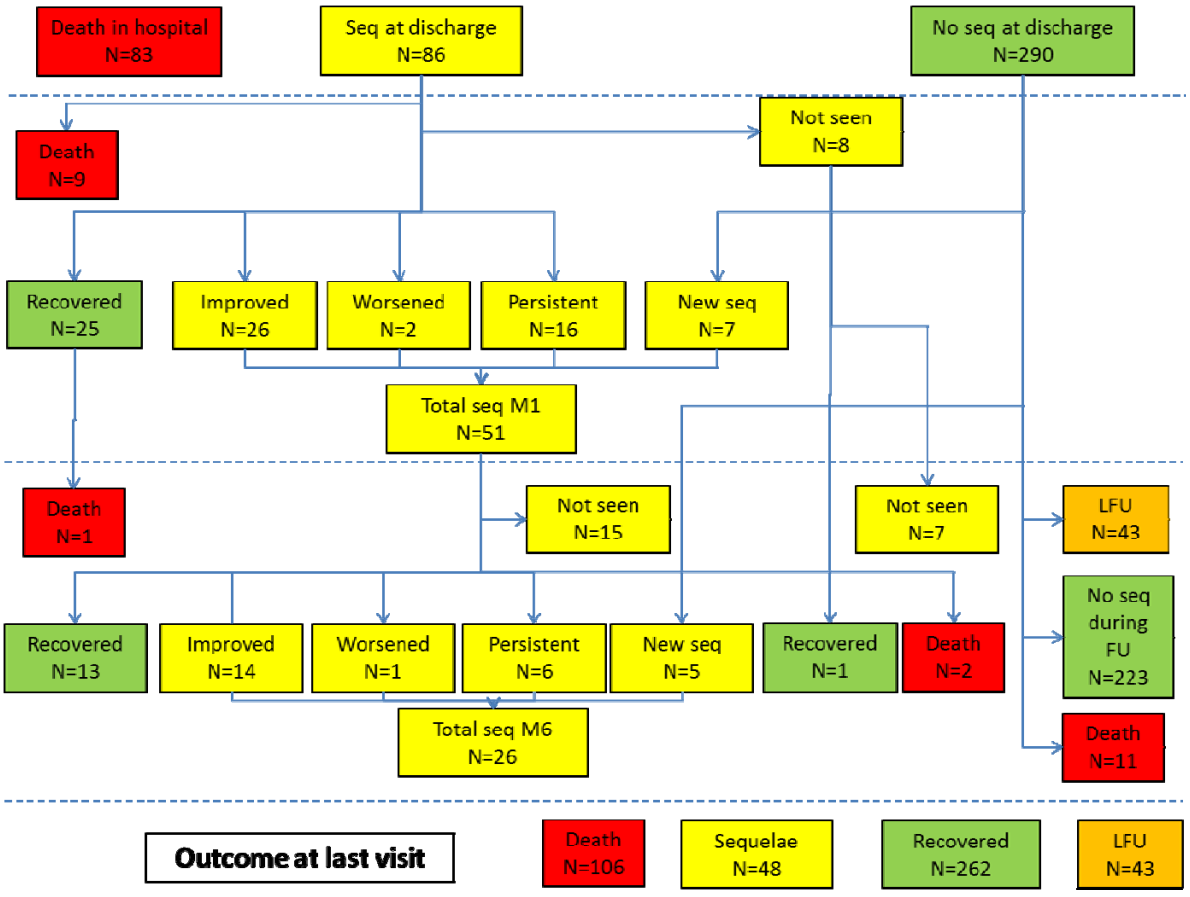


Figure 11: Profile of the neurological sequelae among children with CNS infection (N=459)

Of the 98 who were identified to have sequelae at discharge or during follow-up, 12 died (12.2%), 38 recovered fully (38.8%), and 48 (49.0%) still had sequaelae the last time they were seen ((Figure 13).

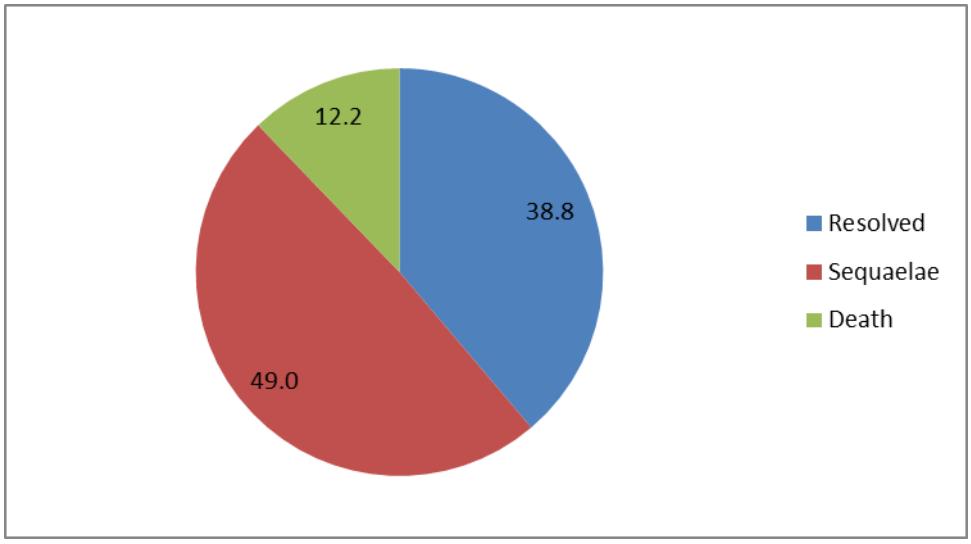


Figure 12. Outcome of patients with sequelae either at discharge or during follow up (N=98)

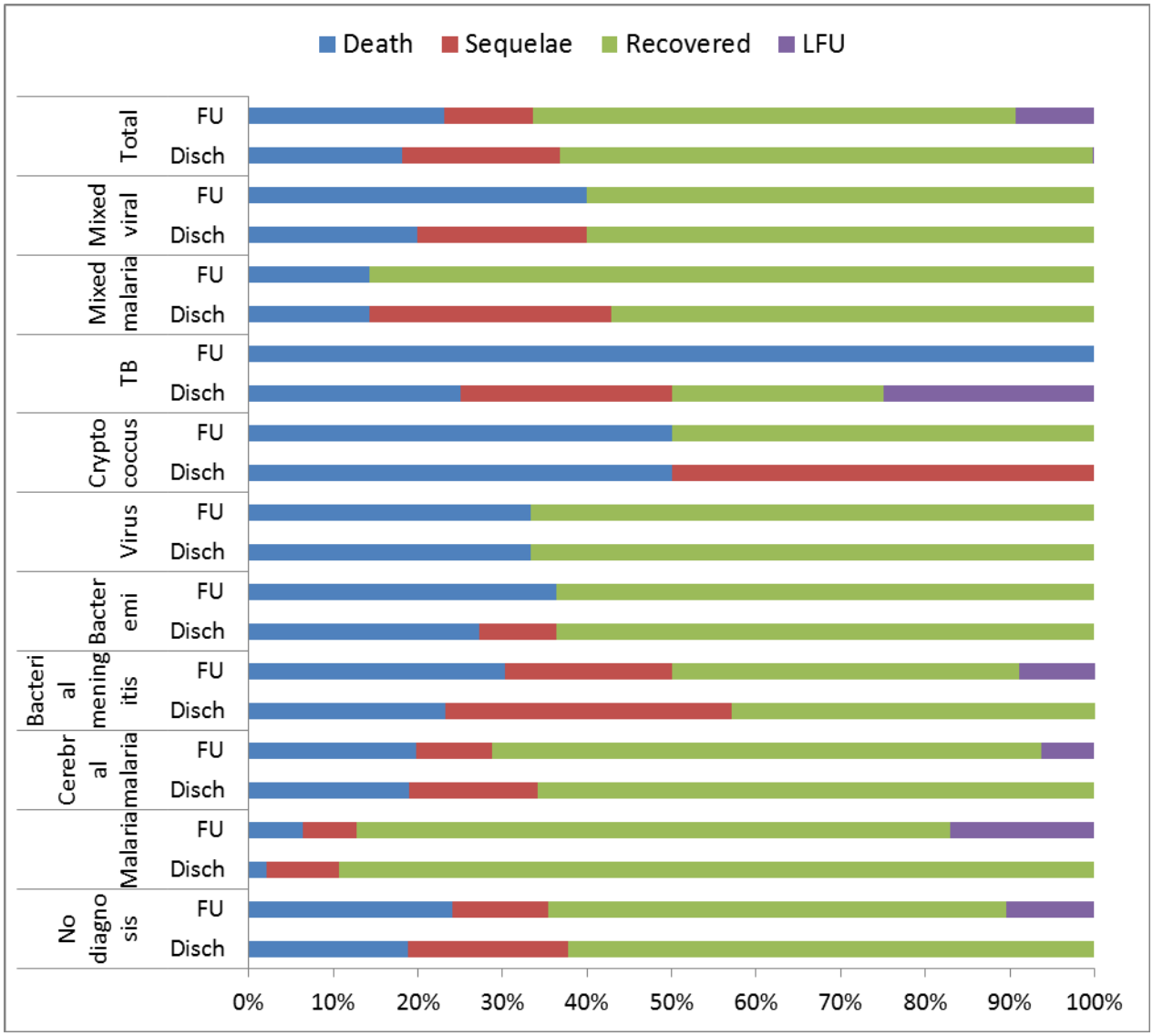
Most children had more than 1 type of sequelae (1.8 on average). The most frequent sequelae at the time of discharge were disorders of motor function (n=61/86, 70.9%); both tone and power deficits. Concerning the muscle tone hypertonia (47.7%) occurred more than hypotonia (25.6%) while considering the anatomical distribution of the disorder hemiplegia or hemiparesis was more common than quadriplegia or quadriparesis (Table 16). This was true even during the different points of follow-up (Table 16). Seizures were also a frequent sequelae (22.1%). The most common sensory impairments were blindness (19.8%) and hearing loss (12.8%).

Table 16. Type of neurological sequelae at discharge and at months 1 and 6 of follow-up

Type of neurological sequelae	Discharge (N=86)		M1 (N=51)		M6 (N=26)	
	n	(%)	n	(%)	n	(%)
Seizures	19	(22.1)	7	(13.7)	4	(15.4)
Abnormal movements	1	(1.2)	0	(0)	0	(0)
Hemiplegia/hemiparesis	19	(22.1)	17	(33.3)	9	(34.6)
Quadriparesis	12	(14.0)	4	(7.8)	2	(7.7)
Extrapyramidal rigidity /hypertonia	41	(47.7)	14	(27.5)	10	(38.5)
Hypotonia	22	(25.6)	8	(15.7)	3	(11.5)
Blindness	17	(19.8)	7	(13.7)	2	(7.7)
Hearing loss	11	(12.8)	6	(11.8)	2	(7.7)
Speech impairment	6	(7.0)	2	(3.9)	1	(3.8)
Cranial nerve palsies	15	(17.4)	7	(13.7)	3	(11.5)
Neuropsychiatric	7	(8.1)	6	(11.8)	4	(15.4)
Foot drop	1	(1.2)	1	(2.9)	1	(3.8)
Cognitive impairment	1	(1.2)	2	(3.9)	1	(3.8)
Regression in developmental milestones	1	(1.2)	0	(0)	0	(0)

4.7.1. Outcome per laboratory-confirmed diagnosis

TB meningitis showed the worst outcome overall with all 4 children dying over the period of follow up, followed by cryptococcal meningitis and mixed-viral other with half of the children dying during this period (Figure 13) – although total numbers were small for all these categories. Around 50% and 30% of children with bacterial meningitis and cerebral malaria, respectively, had either died or long-term neurological sequelae at 6 months after discharge. Finally, those with no laboratory diagnosis were also severely affected, with over a third who had died or neurological sequelae at month 6.



LFU : lost to follow-up

Figure 13. Outcome at discharge and follow-up per laboratory diagnosis

Table 17. Most common sequelae for the 3 main laboratory confirmed diagnoses at discharge

Type of NS	Bacterial meningitis N=19 (%)	Cerebral malaria N=17 (%)	No confirmed laboratory diagnosis N=40 (%)	p-value
Seizures	2 (10.5)	2 (11.8)	14 (35.0)	0.057
Motor dysfunction	15 (79.0)	13 (76.5)	28 (70.0)	0.784
Blindness	5 (26.3)	5 (29.4)	4 (10.0)	0.110
Hearing impairment	2 (10.5)	5 (29.4)	3 (7.5)	0.085
Cranial nerve palsies	4 (21.0)	3 (17.6)	6 (15.0)	0.853
Speech impairment	0 (0)	4 (23.5)	0 (0)	0.002
Neuropsychiatric disorders	1 (5.3)	2 (11.8)	2 (5.0)	0.705

Apart from speech impairment which was significantly found more frequently among those with cerebral malaria (p-value 0.002) there was a trend towards seizures being more frequent among those with no laboratory diagnosis, and hearing impairment among cerebral malaria. Otherwise there was no difference in frequency of occurrence of the other forms of sequaelae among the different aetiologies (Table 17).

4.7.2. Risk factor analysis

HIV seropositivity, impaired consciousness, and neck stiffness were associated with increased risk of death during hospitalization in univariate analysis, while non-cerebral malaria was associated with lower risk. Significant factors in a multivariate analysis included HIV seropositivity, impaired consciousness and non-cerebral malaria.

Table 18. Univariate and multivariate analysis of the association between death during hospitalization and possible risk factors (N=459)

	n deaths (%)	Univariate analysis		Multivariate analysis		
		OR	p-value	OR	95% CI	p-value
Sex						
Masculine	58 (19.9)	Ref				
Feminine	25 (14.9)	0.7	0.2			
Age (months)						
2-5	12 (23.5)	1.3	0.5			
6-11	15 (21.1)	1.1	0.7			
12-23	12 (15.6)	0.8	0.5			
24-59	22 (15.3)	0.8	0.4			
>60	22 (19.0)	Ref				
HIV serology						
Negative	65 (16.0)	Ref				
Positive	16 (36.4)	3	**0.001**	2.6	1.2-5.6	0.013
Consciousness						
Normal	9 (6.9)	Ref				
Reduced	74 (22.5)	3.9	**<0.001**	5.2	2.3-11.8	<0.001
Neck stiffness						
Normal	55 (15.6)	Ref				
Stiff	25 (25)	1.8	**0.032**			
Diagnosis						
No lab diagnosis	40 (18.9)	Ref				
Malaria	1 (2.1)	0.09	**0.021**	0.11	0.01-0.9	0.038
Cerebral malaria	21 (18.9)	1	1			
Bacterial meningitis	13 (22.8)	1.3	0.51			
Bacteremia	3 (27.3)	1.6	0.5			
Virus	1 (33.3)	2.2	0.5			
Cryptococcus	1 (50)	4.3	0.3			
TB meningitis	1 (25)	1.4	0.8			
Mixed malaria - bacteria	1 (14.3)	0.7	0.8			
Mixed viral -other	1 (20.0)	1.1	0.9			

*Including age, sex, HIV serology, impaired consciousness and diagnosis

Among bacterial meningitis cases (excluding mixed infections), there was no significant association between death and the bacterial agent. The in-hospital case fatality rate (CFR) was 12.5% (n=4/32) for *S. pneumoniae* meningitis, 25% (n=2/8) for *Salmonella* spp.

(including 1 S. Typhi), and 28.6% (n=2/7) for Hib. When including also deaths during follow-up, mortality remained unchanged for *Salmonella* spp., and increased to 21.9% (n=7/32) for *S. pneumoniae*, and 42.9% (n=3/7) for Hib.

We also looked at potential risk factors for neurological sequaelae at the last visit among survivors and found that bacterial meningitis was the only associated factor in both univariate and multivariate analysis (adjusted on sex and age).

Table 19. Univariate and multivariate analysis of the association between neurological sequaelae and possible risk factors in survivors (N=353)

		Univariate analysis		Multivariate analysis*		
	n (%)	OR	p-value	OR	95% CI	p-value
Sex						
Masculine (N=218)	32 (14.7)	Ref				
Feminine (N=135)	17 (12.6)	0.84	0.6			
Age in months						
2-5 (N=36)	3 (8.3)	Ref				
6-11 (N=51)	7 (13.7)	1.8	0.4			
12-23 (N=64)	8 (12.5)	1.6	0.5			
24-59 (N=116)	16 (13.8)	1.8	0.4			
>60 (N=86)	15 (17.4)	2.3	0.2			
HIV serology						
Negative (N=323)	43 (13.3)	Ref				
Positive (N=24)	5 (20.8)	1.7	0.3			
Consciousness						
Normal (N=111)	13 (11.7)	Ref				
Reduced (N=242)	36 (15.9)	1.3	0.4			
Diagnosis						
No lab diagnosis	25 (15.5)	Ref				
Malaria	3 (6.8)	0.4	0.1			
Cerebral malaria	10 (11.2)	0.7	0.4			
Bacterial meningitis	11 (27.5)	2.1	0.08	2.4	1.04-5.7	**0.04**
Bacteremia	0					
Virus	0					
Cryptococcus	0					
TB meningitis	0					
Mixed malaria - bacteria	0					
Mixed viral -other	0					

* Including sex, age group and diagnosis

Neurological sequelae were found in half (n=14/28) of the *S. pneumoniae* meningitis survivors at discharge and 24% (n=6/25) at their last follow-up visit cases, in 2 of 5 Hib meningitis survivors at discharge and 2/4 at their last follow-up visit and in 3 of the 6 *Salmonella* spp. cases both at discharge and last follow-up visit.

4.8. Utility of biomarkers

4.8.1. Description

Cases of bacterial meningitis showed turbid or purulent CSF in 55% and 9% of cases, respectively, but it was crystal clear in 22%. The majority of bacterial meningitis cases showed high leucocyte count, low glucose, and high lactate, while protein level was often normal. Still, normal leucocyte, proteins, glucose and lactate levels were found in 18%, 71%, 30% and 26% of bacterial meningitis cases, respectively. Cases of viral infections showed often normal CSF findings, but numbers are very small (N=3). The few tuberculosis cases (N=4) had normal or moderately increased leucocyte count, low glucose and high lactate, and normal proteins. Most malaria cases, whether considered cerebral malaria or not, had normal CSF findings, although increased lactate was seen in 26% of cerebral malaria cases. Serum CRP and procalcitonin were generally elevated among patients with bacterial infections, but also malaria. Mixed infections are excluded from this analysis.

Table 20. CSF findings, CRP and procalcitonin by main laboratory diagnosis

	No diagnosis		Malaria		Cerebral malaria		Bacterial meningitis		Bacteremia		Virus		Tuberculosis		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	v
CSF appearance	N=181		N=39		N=94		N=55		N=10		N=3		N=4		<0
Crystal clear	145	80.1	30	76.9	83	88.3	12	21.8	6	60.0	2	66.7	2	50.0	
Yellow	7	3.9	1	2.6	4	4.3	2	3.6	0	0.0	0	0.0	1	25.0	
Hemorrhagic	25	13.8	7	18.0	5	5.3	6	10.9	4	40.0	1	33.3	0	0.0	
Turbid	4	2.2	1	2.6	2	2.1	30	54.6	0	0.0	0	0.0	1	25.0	
Purulent	0	0.0	0	0.0	0	0.0	5	9.1	0	0.0	0	0.0	0	0.0	
CSF leucocyte	N=180		N=39		N=94		N=56		N=10		N=3		N=4		<0
Median (IQR)	0 (0-0)		0 (0-0)		0 (0-0)		1150 (175-3460)		0 (0-0)		0 (0-70)		50 (0-23)		
0 cells/mm3	157	87.2	31	79.5	89	94.7	10	17.9	10	100.0	2	66.7	2	50.0	
<100 cells/mm3	11	6.1	4	10.3	4	4.3	2	3.6	0	0.0	1	33.3	0	0.0	
100-1000 cells/mm3	9	5.0	1	2.6	1	1.1	14	25.0	0	0.0	0	0.0	2	50.0	
>=1000 cells/mm3	3	1.7	3	7.7	0	0.0	30	53.6	0	0.0	0	0.0	0	0.0	
CSF proteins	N=173		N=39		N=92		N=52		N=7		N=3		N=4		0
Median (IQR)	0.1 (0-0.2)		0 (0-0.1)		0.1 (0-0.1)		0.2 (0.1-0.4)		0 (0-0)		0 (0-0.2)		0.2 (0.15-0.25)		
<0.4 g/L	153	88.4	34	87.2	88	95.7	37	71.2	7	100.0	3	100.0	4	100.0	
0.4 to 1.0 g/L	8	4.6	2	5.1	1	1.1	9	17.3	0	0.0	0	0.0	0	0.0	
>1.0 g/L	12	6.9	3	7.7	3	3.3	6	11.5	0	0.0	0	0.0	0	0.0	
CSF glucose	N=179		N=39		N=94		N=51		N=9		N=3		N=4		<0
Median (IQR)	64 (52-79)		69 (54-79)		73 (58-88)		16 (1-58)		72 (61-84)		52 (26-55)		29 (24-35)		
<50 mg/dL	41	22.9	8	20.5	11	11.7	36	70.6	1	11.1	1	33.3	4	100.0	
>50 mg/dL	138	77.1	31	79.5	83	88.3	15	29.4	8	88.9	2	66.7	0	0.0	
% CSF/blood glucose	N=176		N=39		N=94		N=51		N=9		N=3		N=4		<0
<40%	15	8.5	5	12.8	7	7.5	33	64.7	0	0.0	1	33.3	3	75.0	
40 to 59%	31	17.6	6	15.4	17	18.1	7	13.7	4	44.4	1	33.3	1	25.0	
>= 60%	130	73.9	28	71.8	70	74.5	11	21.6	5	55.6	1	33.3	0	0.0	
CSF lactate	N=179		N=40		N=94		N=55		N=9		N=3		N=4		<0
Median (IQR)	18 (14-24)		21 (16-28)		24 (19-35)		55 (23-74)		20 (17-24)		34 (15-83)		48 (44-54)		
0 to 35 mg/dL	156	87.2	35	87.5	70	74.5	15	27.3	8	88.9	2	66.7	0	0.0	
>35 mg/dL	23	12.8	5	12.5	24	25.5	40	72.7	1	11.1	1	33.3	4	100.0	
CRP	N=204		N=47		N=111		N=55		N=11		N=3		N=4		<0
Median (IQR)	39.5 (9-104)		103.0 (58-202)		159 (114-207)		176 (61-336)		195 (29-246)		119 (9-153)		49.5 (43.5-122.5)		
<20 mg/dL	79	38.7	8	17.0	0	0.0	4	7.3	1	9.1	1	33.3	0	0.0	
20-80 mg/dL	60	29.4	8	17.0	12	10.8	11	20.0	3	27.3	0	0.0	3	75.0	
80-120 mg/dL	25	12.3	8	17.0	23	20.7	5	9.1	0	0.0	1	33.3	0	0.0	
>=120 mg/dL	40	19.6	23	48.9	76	68.5	35	63.6	7	63.6	1	33.3	1	25.0	
Procalcitonin	N=205		N=47		N=109		N=57		N=11		N=3		N=4		<0
<0.5 ng/mL	70	34.1	3	6.4	3	2.8	10	17.5	0	0	0	0	2	50	
0.5-2 ng/mL	36	17.6	6	12.8	6	5.5	7	12.3	4	36.4	1	33.3	1	25.0	
2-10 ng/mL	43	21.0	13	27.6	26	23.8	10	17.5	3	27.3	0	0.0	0	0.0	
>10 ng/mL	56	27.3	25	53.2	74	67.9	30	52.6	4	36.4	2	66.7	1	25.0	
No lumbar puncture	29	13.6	6	12.8	17	15.3	0	0.0	1	9.1	0	0.0	0	0.0	0

4.8.2. ROC curve analysis

In the ROC curve analysis for the diagnosis of bacterial meningitis (including mixed infections), leucocytes numeration had the best performance, followed by lactate and glucose (Figure 14 and Table 21). The performance of CRP, as measured by area under the curve (AUC), was improved, when patients with malaria were excluded from the analysis. It did not improve, however, when all bacterial infections instead of only bacterial meningitis were considered (AUC: 0.77).

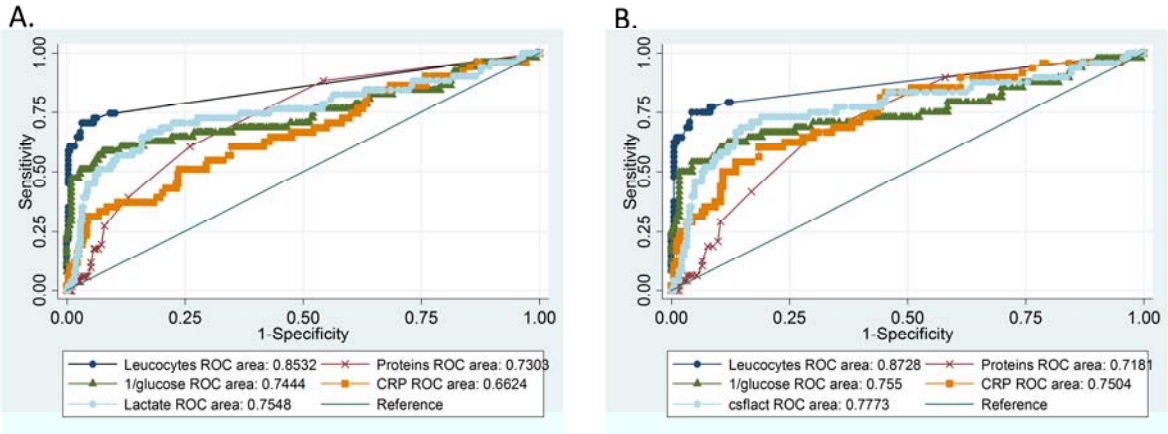


Figure 14. ROC curve analysis of CSF leucocytes, proteins, glucose, lactate and serum CRP for the diagnosis of bacterial meningitis in all (A) or malaria-negative patients (B)

Table 21. Areas under the curve and their 95% confidence intervals of CSF lactate and serum CRP for diagnosis of bacterial meningitis and bacterial infections in all or malaria-negative patients

	All (N=366)	Malaria excluded (N=231)
CSF leucocytes	0.85 (0.79-0.92)	0.87 (0.81-0.94)
CSF lactate	0.75 (0.67-0.84)	0.77 (0.69-0.86)
CSF glucose	0.74 (0.65-0.84)	0.75 (0.66-0.85)
CSF proteins	0.73 (0.66-0.80)	0.72 (0.64-0.79)
Serum CRP	0.66 (0.57-0.75)	0.78 (0.69-0.86)

4.8.3. Diagnostic performances

For continuous variables, the thresholds used for the analysis were based on known normal values for leucocytes and glucose, or on the Youden index from the ROC analysis for CSF lactate. The best performances for the diagnosis of bacterial meningitis (including mixed infections) were obtained with CSF leucocytes count with a threshold of 500 cells /µL or, for a better sensitivity, with a threshold of 100 cells/µL.

In order to increase sensitivity while keeping good specificity, we considered combinations of markers for parallel testing strategy. Only markers with good specificity (>90%) were considered since other such combinations would reduce specificity. The combination of CSF appearance and leucocyte count>100 cells/µL did not improve overall performance compared to leucocyte count>100 cells/µL only, while the combination of CSF appearance and leucocytes>500 cells/µL increased sensitivity while keeping a good specificity. However, 25% of bacterial meningitis would still be missed by this combination.

When patients who had received antibiotics prior to arrival at the hospital or for at least one day at the hospital prior to lumbar puncture were excluded, the sensitivity of CSF appearance and CSF lactate improved to 75% (53.3-90.2), while that of all combinations slightly increased to 79.2% (57.8-92.9).

Table 22. Performances of CSF markers for the diagnosis of bacterial meningitis among all patients

	Sensitivity		Specificity		PPV		NPV		LR+		LR-	
	%	95% CI	**%**	95% CI	**%**	95% CI	**%**	95% CI		95% CI		95% CI
CSF appearance												
Turbid or purulent	**61.7**	48.2-73.9	**97.6**	95.4-99.0	**82.2**	67.9-92.0	**93.5**	90.4-95.8	**26.2**	12.8-53.5	**0.39**	0.28-0.54
Leucocytes												
≥ 100 cells/µL	**75.4**	62.7-85.5	**95.8**	93.1-97.7	**76.7**	64.0-86.6	**95.5**	92.7-97.5	**18.0**	10.6-30.6	**0.26**	0.17-0.40
≥ 500 cells/µL	**63.9**	50.6-75.8	**99.7**	98.3-100	**97.5**	86.8-99.9	**93.8**	90.8-96.1	**214.0**	29.9-1525	**0.36**	0.26-0.50
CSF lactate												
>=35 mg/dL	**70.0**	56.8-81.2	**82.5**	78.1-86.4	**41.6**	31.9-51.8	**93.9**	90.6-96.4	**4.0**	3.0-5.3	**0.36**	0.25-0.54
Glucose												
< 50 mg/dL	**66.1**	52.2-78.2	**79.2**	74.5-83.4	**34.6**	25.6-44.4	**93.4**	89.8-96.0	**3.2**	2.4-4.2	**0.43**	0.30-0.62
< 40 mg/dL	**62.5**	48.5-75.1	**90.5**	86.9-93.4	**52.2**	39.7-64.6	**93.6**	90.3-96.0	**6.6**	4.5-9.7	**0.41**	0.30-0.58
Combinations												
Appearance or Leuco>100	**76.7**	64.0-86.6	**94.7**	91.8-96.8	**71.9**	59.2-82.4	**95.8**	93.1-97.7	**14.5**	9.0-23.2	**0.25**	0.16-0.39
Appearance or Leuco>500	**75.0**	62.1-85.3	**97.6**	95.4-99.0	**84.9**	72.4-93.3	**95.7**	93.0-97.6	**31.9**	15.8-64.2	**0.26**	0.17-0.40
Appearance or leuco>500 or glucose <40	**78.3**	65.8-87.9	**89.1**	85.3-92.2	**56.0**	44.7-66.8	**95.9**	93.1-97.8	**7.2**	5.2-10.0	**0.24**	0.15-0.39

We also assessed the performance of CRP and procalcitonin to diagnose all bacterial infections overall and after excluding patients with confirmed malaria. None of the biomarkers showed high diagnostic performances, as indicated by the moderate positive and negative likelihood ratios (LR+>10 or LR-<0.1 are generally considered as good diagnostic performances).

Table 23. Performances of serum markers for the diagnosis of bacterial infections among all and malaria-negative patients

	Sensitivity		Specificity		PPV		NPV		LR+		LR-	
	%	95% CI	**%**	95% CI	**%**	95% CI	**%**	95% CI	**%**	95% CI	**%**	95% CI
Invasive bacterial infection												
CRP ≥ 20 mg/L	**92.1**	83.6-97.0	**24.4**	20.1-29.1	**19.9**	15.8-24.4	**93.8**	87.0-97.7	**1.2**	1.1-1.3	**0.32**	0.15-0.71
CRP ≥ 120 mg/L	**63.2**	51.3-73.9	**61.7**	56.5-66.6	**25.1**	19.1-31.9	**89.1**	84.7-92.7	**1.6**	1.3-2.0	**0.60**	0.44-0.81
PCT ≥ 2 ng/ml	**70.5**	59.1-80.3	**35.5**	30.6-40.6	**18.6**	14.4-23.6	**85.2**	78.6-90.4	**1.1**	0.93-1.3	**0.83**	0.57-1.20
PCT ≥ 10 ng/ml	**50.0**	38.5-61.5	**57.5**	52.3-62.6	**19.8**	14.5-26.1	**84.6**	79.5-88.8	**1.2**	0.9-1.5	**0.87**	0.68-1.10
Invasive bacterial infection - malaria excluded												
CRP ≥ 20 mg/L	**91.3**	82.0-96.7	**38.8**	32.2-45.7	**32.5**	25.9-39.6	**93.3**	85.9-97.5	**1.5**	1.3-1.7	**0.22**	0.10-0.50
CRP ≥ 120 mg/L	**63.8**	51.3-75.0	**79.4**	73.4-84.6	**50.0**	39.1-60.9	**87.2**	81.7-91.5	**3.1**	2.3-4.3	**0.46**	0.33-0.63
PCT ≥ 2 ng/ml	**69.0**	56.9-79.5	**52.6**	45.7-59.4	**32.5**	25.1-40.5	**83.7**	76.4-89.5	**1.5**	1.2-1.8	**0.60**	0.41-0.85
PCT ≥ 10 ng/ml	**49.3**	37.2-61.4	**72.6**	66.1-78.4	**37.2**	27.5-47.8	**81.3**	75.0-86.5	**1.8**	1.3-2.5	**0.70**	0.55-0.89

4.8.4. Scores associating clinical and biological markers

We have assessed 2 scores based on a combination of clinical signs and biological markers that were developed to distinguish between bacterial and aseptic meningitis: the Bacterial Meningitis Score (BMS) and the Meningitest (see definitions in the method section) that both aim at good sensitivity. The score results by diagnostic category are presented in Annex. The BMS showed relatively good sensitivity with moderate specificity, while the Meningitis test was 100% sensitive but with very low specificity, which reduced its usefulness (Table 24).

Of the 7 bacterial meningitis cases missed by a BMS scored >=2 (one in co-infection), 4 had received antibiotics prior to sample collection, and 3 were identified by culture while the 4 others were identified by PCR only. The sensitivity of BMS slightly increased to 92% when only patients with no prior antibiotics were considered, while specificity decreased slightly to 57.7%.

Table 24. Performances of the BMS and Meningitiest criteria to diagnose bacterial meningitis

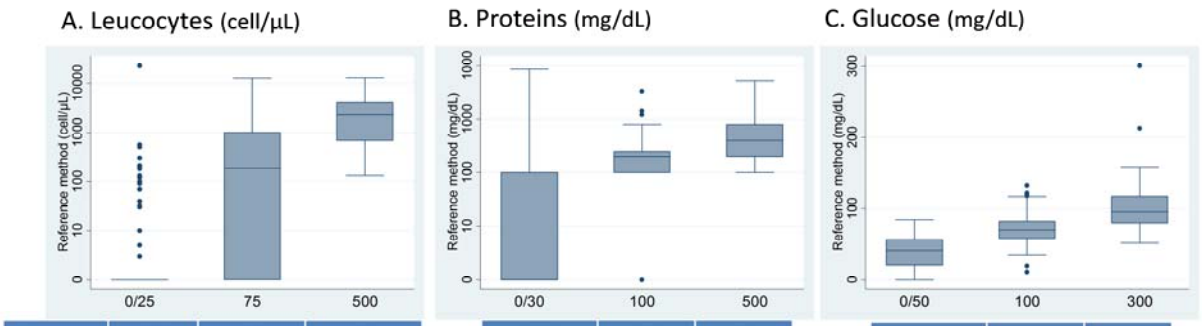
	Sensitivity		Specificity		PPV		NPV		LR+		LR-	
	%	95% CI	**%**	95% CI	**%**	95% CI	**%**	95% CI		95% CI		95% CI
BMS score >=2	**88.7**	78.1-95.3	**59.7**	54.7-64.6	**25.6**	19.9-32.0	**97.1**	94.2-98.8	**2.1**	1.8-2.5	**0.19**	0.09-0.39
BMS score >=1	**100.0**	94.2-100	**13.9**	10.6-17.6	**15.3**	12.0-19.2	**100.0**	93.5-100	**1.2**	1.1-1.2	**0.00**	
Meningitest	**100.0**	94.2-100	**7.3**	5.0-10.3	**14.4**	11.2-18.1	**100.0**	88.1-100	**1.1**	1.1-1.1	**0.00**	

4.8.5. Urine dipsticks

<u>Comparison with reference methods</u>

The reference values found by dipstick category for CSF leucocytes, proteins and glucose are shown and summarized in Figure 15. Although there was a clear trend of increasing reference values with increasing dipstick categories, the median and IQR of reference values were often substantially different from the value indicated on the dipstick.

The reference values were also classified in categories using midpoints between the dipstick categories as cut-points, in order to assess the kappa correlation coefficient (Tables in Annex 8). All kappa were low (leucocytes: 0.38; proteins: 0.31; glucose: 0.22), indicating poor agreement between dipstick categories and reference values.



N	326	30	24	278	60	34	106	252	21
Median	0	185	2300	0	200	400	41	70	96
IQR	0-0	0-1000	685-4050	0-100	100-250	200-800	21-56	58-82	80-118

Figure 15. Comparison between urine dipstick categories (X axis) and reference values (Y axis, median, IQR) for CSF leucocytes (A), proteins (B) and glucose (C)

Performance to diagnose bacterial meningitis

The individual or combinations of markers using the urine dipstick generally showed lower performances than their reference value counterpart.

Table 25. Performance of urine dipstick for the diagnosis of bacterial infections using different definitions

	Sensitivity		Specificity	
	%	95% CI	**%**	95% CI
Leucocytes >=75 cells/µL (2+)	**67.3**	53.3-79.3	**94.6**	91.5-96.7
Leucocytes >=500 cells/µL (3+)	**41.8**	28.7-55.9	**99.7**	98.3-100
Glucose <50 (normal)	**46.3**	32.6-60.4	**98.5**	96.5-99.5
Glucose <=50 (1+)	**63.0**	48.7-75.7	**76.7**	71.8-81.2
Proteins >=100 mg/dL (2+)	**74.5**	61.0-85.3	**82.2**	77.6-86.1
Combination				
leuco>=75 or glucose <50	**70.9**	57.1-82.4	**94.0**	90.8-96.3
leuco>=75 and glucose <=50 and proteins>=100	**49.2**	35.9-62.5	**98.5**	96.5-99.5

4.9. Description of less typical infections

Most of the pathogens sought and identified in the study have been described extensively. We aim to discuss here in more details only the less described pathogens.

4.9.1. Non-typhoidal Salmonella

We have identified 8 non-typhoidal salmonella (NTS), 3 by culture and 5 by PCR in the CSF of patients included in the study. One NTS meningitis case was in co-infection with malaria.

NTS meningitis affected all age groups, with ages ranging from 3 months to 9 years, although most children (n=7) were under 5 years of age. Two children had moderate malnutrition, and one 19-month old child was HIV-positive. Sickle-cell disease was not reported clinically for any child and haemoglobin electrophoresis showed the heterozygous trait in 1.

The most frequent signs of CNS infection in children with NTS meningitis were reduced consciousness (n=7), including 5 children in coma, prostration (n=7) and history of seizures (n=7) or seizures at admission (n=5). Three children had neck stiffness and one 3-month old child had bulging fontanel. Half of the children presented with hepatomegaly, 2 children were in respiratory distress and 2 children had cold extremities, compared to only 1 of 43 children with bacterial meningitis of other causes (p=0.061).

The CSF was clear in 5, yellow in 2 and turbid in only 1 child with NTS meningitis, although leucocyte count showed more than 500 cells/mm3 in 4 patients, with a majority of neutrophils in 3 and lymphocytes in 1. CSF proteins were elevated (> 100 mg/dL) in 6 and CSF glucose was low in 3 (<40 mg/dL). CSF lactate was elevated (>35 mg/dL) in 3. Serum CRP was lower than 30 mg/dL in all 8 patients with NTS meningitis, while 2 had procalcitonin > 10 ng/mL.

All children received ceftriaxone on admission, 6 in combination with benzyl-penicillin while the other 2 received benzyl-penicillin later on. Two children also received ciprofloxacin 5 and 9 days after admission. Only one child was prescribed benzyl-penicillin to be continued at home, while others received treatment only during admission.

While the majority of children with NTS meningitis had improved after 3 days (n=6), 2 remained in an unchanged clinical status, of which 1 child died after 5 days. Of the 7 other children, 5 were discharged after 5 to 17 days and 2 were transferred after 7 and 16 days to the surgical ward and a cure hospital in Mbale.

Of the 7 survivors at discharge or transfer, 3 had neurological sequelae at discharge: 2 with hemiplegia and 1 with blindness, hearing loss and extrapyramidal rigidity. Of these, two children had not evolved at the 1-month follow up, while one child with hemiplegia had worsened and became blind and deaf. This child also had a cerebral abscess, suggesting partial treatment or poor response to treatment. At 6 months, 2 of these 3 children were lost to follow-up, while the child with blindness, hearing loss and extrapyramidal rigidity had worsened, with spastic cerebral palsy in addition to other sequelae.

4.9.2. S. Typhi meningitis

The one patient with *S*. Typhi meningitis was an HIV-negative 11-month boy, who presented with reduced consciousness, prostration, irritability, neck stiffness, bulging fontanel, dehydration, and respiratory distress. The CSF was turbid, with 130 cells / mm3 and a majority of neutrophils. CSF proteins were elevated (200 mg/dL) and glucose was low (13 mg/dL). CRP was slightly elevated (32.5mg/dL) and PCT > 10 ng/mL.

The child received ceftriaxone and benzyl-penicillin on admission. At the day 3 follow-up visit, his condition had worsened, and he died 5 days after admission.

4.9.3. HHV-6

HHV-6 was detected by PCR in 11 patients, of whom 2 were IgM-positive at inclusion or discharge, 4 were IgM-negative in all specimens tested and 5 were not tested by serology.

One serology-confirmed case was an HIV-positive 8-year old boy with a cryptococcal infection, who was admitted with headache, photophobia, neck stiffness, history of seizure, Kernig and Brundziski signs. He was treated with cotrimoxazole, amphotericin B and

flucanozole during his 19-days stay at the hospital and then left against advice. He was reported death at the one-month follow-up visit.

The second serology-confirmed was an HIV-negative 31 months old girl who presented with coma, prostration, hypertonia, convulsions, jaundice and splenomegaly, who had microscopy-confirmed malaria. She was treated with intravenous quinine followed by coartem and was discharged after 5 days with no neurological sequelae, and had still no neurological sequelae at the 1-month follow-up.

4.9.4. Cryptococcal meningitis

The 3 cases of cryptococcal meningitis were 8, 9 and 10-year old HIV-positive patients. They presented with headache (n=3), photophobia (n=3), irritability (n=2), neck stiffness (n=2), Kernig sign (n=2), Brudzinski sign (n=1), seizures (n=1) and lymphadenopathy (n=1). The CSF was crystal clear with no leucocyte, and normal values for CSF glucose and proteins in 2. CRP and procalcitonin were low in all 3 patients.

All 3 patients received fluconazole and amphotericin B, although administration of fluconazole was delayed by one day in 2 patients, and that of amphotericin B was delayed of 1 day, 2 days and 9 days. One patient died after 2 days and the 2 survivors showed 6th nerve palsy at discharge, in addition to blindness and seizures in one. At 1 month follow up one patient was reported dead, while the other still showed palsies which had resolved at the month-6 follow up.

None of these 3 patients received antiretroviral therapy during hospitalization – 2 because they died shortly after admission, and one was on co-trimoxazol prophylaxis.

4.9.5. TB meningitis

The 4 children with culture-confirmed TB meningitis were 2, 3, 6 and 12 year old HIV-negative patients. One was severely malnourished. They presented with prostration (n=4), reduced consciousness (n=3), including coma in 2, neck stiffness (n=2), seizures (n=2), hypertonia (n=1), focal neurological signs (n=1), Kernig sign (n=1), and respiratory distress (n=1). The CSF was clear in 2, yellow in 1 and turbid in 1, and 2 patients had CSF leucocytes of 100 and 360 cells /mm3, respectively. CSF lactate was elevated and glucose was low in all.

All children received ceftriaxone and benzyl-penicillin during hospitalization but none was treated with anti-TB treatment, since culture results became available after death or discharge. One child died after 11 days of hospitalization while one was discharged after 8 days, and 2 left against advice after 1 and 3 days. Two children were reported dead at the month-1 follow up visit, before children were traced for TB treatment following positive culture results. One child was traced and started on TB treatment during follow-up, but died at month 5 of follow-up.

Another 2 patients with no other laboratory confirmed diagnosis had probable TB based on a TB score of >=10 (ref Marais). These were younger, 2 and 6 months old, did not have severe malnutrition, and one was HIV-positive. They presented with similar clinical signs including reduced consciousness (n=2), history of seizures (n=2), hypertonia (n=1), irritability (n=1), neck stiffness (n=1), bulging fontanel (n=1). The CSF was clear or hemorrhagic with no leucocytes, CSF glucose was reduced and proteins elevated and lactate was moderately elevated.

Both were discharged home after 13 and 15 days of hospitalization with no neurological sequelae and one was reported dead at the 1 month follow up visit while the other was lost to follow up at the 6th month visit.

5. Discussion

Despite the decrease in malaria prevalence in the district of Mbarara [30], cerebral malaria was the main cause of CNS infection in our cohort, representing around one fourth of all patients included in the study, and up to one third for severe - including cerebral - malaria. Bacterial meningitis was the second most common cause with around 12% of cases overall and up to 23% in children less than one year of age and 25% in HIV-positive or exposed children. Despite a large search for viral agents, we identified only 8 viral infections (2%), 5 of which were in association with another pathogen and might thus not be the cause of the CNS infections. Cryptococcal meningitis was found only in HIV-positive children, as expected, and represented 4.5% of cases in this population, and 9.6% when restricted to children >18 months of age with a confirmed diagnosis of HIV. In contrast, culture-confirmed TB meningitis was identified only in HIV-negative patients. Mixed infections were found in about 2.5% patients included in the study and could be grouped in 2 categories: mixed malarial and bacterial infections and mixed infections including a viral infection. Finally, despite exhaustive laboratory investigations, almost half of the patients were left without a laboratory-confirmed diagnosis.

5.1. Malaria

Cerebral malaria particularly affected children 1 to 5 years of age, with a prevalence of 35% in this age group. The prevalence was lower among HIV-positive or exposed patients. Non-cerebral malaria was responsible for another 10% of cases in our study population. The majority of these patients (44/47) had severe malaria with signs that fulfilled the relatively large inclusion criteria in this study. Finally around one third of the patients included in the study had cerebral or severe malaria, with an apparent peak in number of cases and proportion in June-July. Cerebral malaria was also the most common aetiology of coma in Africa in a recent literature review, except in one study in Nigeria and one in Egypt [31], representing up to 66% of children with impaired consciousness in a study in Kenya in 1994-1996 [14]. Since then, the incidence and proportion of cerebral malaria cases has declined in this Kenyan site, along with the decrease in malaria transmission, from 72% of coma cases in 2004 to 27% in 2009 [32].

The in-hospital CFR of cerebral malaria was 18.9%, similar to the mortality rate in the cohort overall. This was similar to the CFR among cerebral malaria cases in the recent multicentric study on treatment of severe malaria called AQUAMAT that included one site in Mbarara [33]. About one in ten survivors had neurological sequelae at their last follow-up visit, with motor dysfunction in three quarters of these and blindness and hearing loss in about one third. This figure is higher than 3.5% found in cerebral malaria cases at end of follow- up in the AQUAMAT study, but within the relatively large range (3-28%) reported in different studies in Africa [34].

Following the results of the AQUAMAT study, the treatment policy for severe malaria was changed from parenteral quinine to parenteral artesunate [35]. All children with malaria had received an appropriate treatment over hospitalization, although 15% did not receive anti-malarial treatment on the day of admission. Around two thirds received quinine while one third received artesunate. There was no significant difference in the outcome, in-hospital death, overall death, or neurological sequelae among those who received either treatment,

although numbers were too small to detect a relative difference in mortality of 22% as described in the multicentric study.

5.2. Bacterial meningitis

Bacterial meningitis was the second most common cause of CNS infection with around 12% of cases overall. This is slightly higher than reported in the few studies on the etiology of CNS infections or reduced consciousness outside of the meningitis belt [31, 36], probably due to the use of PCR in addition to culture and gram-staining, which allowed the identification of 23 additional confirmed bacterial meningitis cases compared to 28 detected by culture. However, in contrast to many other studies, we did not include any possible bacterial meningitis case based on CSF findings only, which might have led to an underestimate of the prevalence of bacterial meningitis considering the high consumption of antibiotics prior to enrolment. The large inclusion criteria not focussed only on bacterial meningitis might also explain the lower proportion of microbiologically confirmed cases compared to other studies, particularly in the pre-Hib era [37].

The in-hospital CFR of bacterial meningitis was 22.8%, slightly higher than the rest of the cohort, but the difference was not significant. Case fatality for bacterial meningitis is often very high, with an overall CFR of 45% and median CFR of 27% in reviews of African data from 2001 and 2009, respectively [37, 38]. The data here are slightly lower than these overall figures but within the range of reported CFRs.

Bacterial meningitis was significantly associated with the highest rate of neurological sequelae, with 48% of survivors at discharge and 27% of survivors at their last follow-up visit. This is within the relatively large range of values described in the 2009 review, from 4% to 57% at discharge. Motor impairment was found in around three quarters of children with NS and vision loss in around one quarter, which is higher than reported elsewhere, while hearing loss was less frequent [38].

Appropriate antibiotics were given to all children with bacterial meningitis over hospitalization, but 16% did not receive antibiotics on the day of admission. This delay was probably not the main reason for fatal outcome since the proportions were very similar in those who died and those who survived. One child with an ESBL *E. coli* found in blood was not treated with an appropriate treatment, since the bacterium was resistant to all antibiotics available, and died at the hospital.

5.2.1. S. pneumoniae

As reported in other countries outside the meningitis belt [31, 39] and in countries of the meningitis belt outside epidemic periods [40-42], *S. pneumoniae* was the main agent of bacterial meningitis, representing about two thirds of all confirmed cases.

The CFR for pneumococcal meningitis in our study, even when we included deaths during follow-up (22%), remained lower than those reported in a worldwide estimation of the burden of *S. pneumoniae*, with a CFR of 59% (27-80%) overall and up to 73% (18-94%) in Africa [43]. The proportion of NS among survivors of pneumococcal meningitis was high at discharge (50% of survivors), but decreased to 24% during follow-up (although 2 children with NS died during follow-up).

All *S. pneumoniae* tested were sensitive to ceftriaxone, the recommended first line treatment, as in other parts of Africa [44]. Although benzyl-penicillin is still active against isolates with intermediate resistance, the extent of this resistance and the lack of added value of benzyl penicillin in addition to ceftriaxone in the recommended treatment should encourage changing the first line treatment to ceftriaxone only.

5.2.2. Hib

Despite good coverage and high impact of the vaccine in Uganda [10], Hib was still found in around 10% of confirmed bacterial meningitis, but only in children less than one year of age and mostly unvaccinated children (n=5/7).

The 2 cases in children 8 and 9 months of age with complete vaccination reported orally were not investigated further, in particular to confirm vaccination status. Hib vaccine efficacy is reported to be lower in HIV-positive children [45], but these 2 children were HIV-negative, as were other cases of Hib in vaccinated children reported in Uganda previously [46].

5.2.3. Non-typhoidal Salmonella

NTS is becoming increasingly recognized as an important cause of bacterial meningitis in sub-Saharan Africa, is the second or third cause in several countries after the introduction of the Hib vaccine [9, 11, 42, 47], and might even become more predominant after effective introduction of the pneumococcal vaccines. NTS was the second cause of bacterial meningitis in our cohort – at levels similar to Hib in confirmed bacterial meningitis cases, and reaching 17% of bacterial infections when bacteria isolated from CSF or blood were included.

The children with salmonella meningitis in our study did not present with the described risk factors for salmonella invasive infections, since none had severe acute malnutrition and only one was HIV-positive. Risk factors are not very well described for salmonella meningitis due to the limited amount of reports so far, but studies in Malawi have shown that around 50% of cases were HIV-infected and generally malnourished, although the criteria used the to assess malnutrition, ie. weight for age compared to the NCHS standards, were different [48].

The clinical presentation of salmonella meningitis was generally severe, since more than half of the cases were in coma, which was however similar to the overall cohort, and presented classical signs of bacterial meningitis such as neck stiffness or bulging fontanel, as well as signs of shock in a significantly higher proportion than children with bacterial meningitis of other causes.

However the CFR was relatively low (25%) compared to what was reported elsewhere and we did not report any additional death at home during follow-up. However, one case with cerebral abscess at the 1 month follow up was lost to follow-up at 6 months. This child also had NS that had worsened between discharge and month 1, while another 2 children had NS that remained unchanged over the follow-up period – ie. 42% (n=3/7) of survivors with NS. Although based on small number, this proportion with NS is similar to the one in the Malawian cohort in the 2002-2006 period when treatment was changed from chloramphenicol (showing NS rates of 91.7%) to 14 days of IV ceftriaxone followed by 14 days of oral ciprofloxacin (showing NS rates of 41.9%) [48].

Treatment of salmonella meningitis is more challenging than other bacterial meningitis, due in particular to the intracellular location of the organism [49]. Most of the *Salmonella* isolates in this and other studies are resistant to previously used antibiotics, such as chloramphenicol, ampicillin and co-trimoxazole. The American Academy of Pediatrics recommends treatment with ceftriaxone or cefotaxime for 4 weeks or more, while some authors suggest a combination of ciprofloxacin and ceftriaxone for a minimum of 3 weeks after sterilisation of the CSF. Since ceftriaxone cannot be administered orally and prolonged hospital stay is often difficult in Africa, Molyneux et al. have opted for a pragmatic approach 14 days of 100mg/kg IV ceftriaxone followed by home-based 14 days of oral ciprofloxacin 10 mg/kg twice daily, with no effect on mortality but reduction of NS in survivors as mentioned above [48]. Here, patients were treated with IV ceftriaxone at a dose of 100 mg/kg/day during hospitalization, which lasted less than 14 days in 5 of 8 patients. Only one patient received a prescription for follow-up treatment with benzyl-penicillin at home. Although the outcome was reasonably good compared to what has been described in the literature except for one case with cerebral abscess during follow-up, prolonging treatment for salmonella meningitis for at least 4 weeks overall, including home-based oral ciprofloxacin, would be pragmatic. In addition, since *Salmonella* meningitis is becoming an important fraction of bacterial meningitis and might become even more predominant in the future with the introduction of vaccination against *S. pneumoniae*, empirical guidelines for the treatment of bacterial meningitis might need to be revised to include systematic prolonged treatment and/or oral fluoroquinolone relay.

5.2.4. Other bacteria

One confirmed case of *S*. Typhi meningitis was also found in this study, with typical signs of bacterial meningitis and a fatal outcome despite treatment with ceftriaxone. To our knowledge, meningitis due to *S*. Typhi is very rare and has only been reported in individual case reports [50, 51].

One patient also had *Brevibacterium* spp. isolated from the CSF, in co-infection with malaria. *Brevibacterium casei* have been involved in human infections and found in blood or CSF [52] and as a cause of brain abscess in an immunocompetent patient [53]. *Brevibacterium* spp. are found in raw milk (*B. casei*) and skin flora (*B. epidermidis*). Further characterization of the isolate found in the CSF in this study might help interpret whether this isolate represents contamination or a real pathogen.

Finally, although we were not expecting large number of cases since Mbarara district is located outside of the meningitis belt, the fact that *N. meningitidis* was not found in our study, either by culture or PCR, was surprising.

5.3. Viral infections

There is still very little information on the aetiology of viral encephalitis in children in sub-Saharan Africa. The only other study to our knowledge that used PCR to detect viral agent was conducted in Kilifi, Kenya, and identified HSV in 4 (9%) children with a clinical diagnosis of cerebral malaria and in 6 (12%) malaria-negative patients [13]. This latter group also included 1 patient positive for CMV and 1 for VZV and enterovirus. HSV was also the most suspected viral infection in our setting, as shown by the fact that acyclovir was prescribed to 3% of the children in the cohort. However, we never confirmed it by PCR performed on site

or in the reference laboratory, using a technique routinely use for diagnosis of HSV infection in France. Enterovirus, which is the most frequent cause of viral encephalitis in Europe and America, was also very rare in our study.

The most frequently identified virus in our study was HHV-6, but due to the possible integration of viral genome and detection of latent infection, we excluded cases that could not be confirmed by serology (by lack of serum, for example). HHV-6 is a causative agent of roseola infantum, generally in children less than 3 years. HHV-6 has been involved in infections of the central nervous system in both HIV-positive and negative patients [52, 54, 55], which is thought to be due to HHV-6 reactivation. Since the link between HHV-6 and meningoencephalitis or encephalitis is not yet very clearly established, HHV-6 is suspected to be the cause of the infection if no other pathogen is found in some reports [52, 54]. This was not the case here since the 2 serologically-confirmed cases described had co-infection with malaria and Cryptococcus, respectively. These 2 patients showed IgM at inclusion or discharge and only IgG later on, but an increase in IgM could be due to viral reactivation but does not guarantee that the virus is the cause of the symptoms. More investigations are needed to assess the role and prevalence of HHV-6 in CNS infections in Africa, as well as in other infections since HHV-6 was recently described as one of the most frequent viral agents detected in the blood of children with fever in Tanzania [56], where it could also be due to DNA persistence only [57].

Mumps and VZV were also detected in 3 and 2 patients, respectively. Mumps virus is known to cause meningitis and encephalitis, particularly in males and was one of the most important cause of viral encephalitis in the USA and China prior vaccine introduction [58]. Mumps vaccine is not available in the public sector in Uganda.

The search for less common viruses that we hypothesized could cause meningitis in sub-Saharan Africa, such as flavivirus or Rift Valley fever virus, did not lead to the identification of any such agent, although they were not tested systematically on all specimens but only on a sub-sample of 100 (Yellow fever, West Nile virus, Rift Valley fever virus) or 211 specimens (pan-flavivirus).

5.4. Cryptococcal meningitis

Cryptococcal meningitis was only found in HIV-positive children, as expected, and represented 4.5% of cases in this population, and 9.6% when restricted to children >18 months of age with a confirmed diagnosis of HIV – since all 3 cases were in older children (8, 9 and 10 years old). In this population, cryptococcal meningitis was the second diagnosis after bacterial meningitis and at the same level as cerebral malaria.

Clinical signs at inclusion, including headache, neck stiffness, reduced consciousness and convulsions were generally similar to those reported in other African children [59]. The outcome was severe, with death or NS in all children at discharge, although one fully recovered at 6 months. There is little data on the outcome of cryptococcal meningitis in children Africa and overall, with studies reporting a CFR of 46% in 13 children in Zimbabwe and 21.5% in 24 children in the United States [59, 60].

Recommended treatment for cryptococcal meningitis relies on amphotericin B deoxycholate, and fluconazole [61]. Here, all three patients with cryptococcal meningitis did receive appropriate treatment including fluconazole within one day of admission and amphotericin B, with a delay of 9 days for one patient.

5.5. TB meningitis

TB meningitis was confirmed by culture in 4 HIV-negative patients, which represented around 1% of the overall cohort and up to 1.7% in children over 5 years of age. This is likely to be an underestimate of the true burden of TB meningitis, since culture is not very sensitive and the volume of CSF cultured in the study was limited. In a retrospective study in South Africa, only 12% of patients diagnosed as TB meningitis were confirmed by culture [62]. PCR was negative in all 155 patients tested, probably also due to the very low volume of CSF used for PCR in our study, and was interrupted after considering the low added value. The TB score recommended for use in clinical research was used from patient 189 on and allowed the identification of another 2 probable cases (TB score >=10 and no other diagnosis) and 43 possible cases (TB score 6 to 9 and no other diagnosis).

TB meningitis is normally associated with young age, and risk of TB meningitis and severity of outcomes is increased in HIV-infected patients [62, 63], which was not reflected here in the confirmed cases, but numbers are too small to make any clear conclusion.

None of the children in this cohort received anti-mycobacterial treatment during hospitalization due to the delay to obtain culture results. Only one child received treatment during follow-up and died after 5 months. Overall, the outcome was very poor in all confirmed or probable cases since 5 out of 6 died during follow up and the last child was lost to follow-up. This high figure is consistent with poor outcomes of treatment of TB meningitis more so when treatment is delayed. This emphasizes further the need to have a high index of suspicion for TB meningitis and need for instituting treatment empirically until proven negative. It is therefore important to describe clinical features that make one highly suspect of TB meningitis.

5.6. Mixed infections

Mixed infections were found in about 2.5% patients included in the study. These were grouped in 2 categories: mixed malarial-bacterial infections and mixed infections including a viral infection. The importance of mixed infections with malaria and bacteria, and in particular with non-typhoidal *Salmonella* is recognized [14, 64]. Here, NTS were indeed the first bacteria found in co-infections. Other bacteria included less common pathogens, and it is not fully clear if they represented real mixed infection or possible contamination. Overall, the proportion of mixed bacterial and malarial infection was low in our setting, suggesting that empirical combination therapy with antibiotics and antimalarials might not be justified when good laboratory diagnostic capacity exists. However, the one child with mixed infection who did not receive antibiotics died, calling for good and early diagnostic tests for bacterial infections.

The fact that more than half of viral infections were found in co-infections with another confirmed pathogen, such as *Cryptococcus* or *P. falciparum* questions whether these might represent dual infections, superinfections or just viral re-activation, as is possible for HHV-6 in particular.

5.7. No diagnosis

Despite exhaustive laboratory investigations, almost half of the patients were left without a laboratory-confirmed diagnosis. Twenty-nine (14%) of these had no CSF collected, meaning

that their diagnosis was not complete. Overall, children in whom lumbar puncture was not performed had high case fatality rate (33.9%) compared to rest of the cohort. It is important to note that most children in this group already had risk factors for poor outcome such as cardiorespiratory compromise, or evidence of raised intracranial pressure.

An infectious cause might also have been missed in patients with CSF collected due to the lack of sensitivity of the techniques, small volumes used for certain investigations such as TB culture, or prior treatment. Indeed, patients with no laboratory diagnosis had significantly more chances of having received antibiotics prior to sample collection than patients with a confirmed diagnosis (66.5% versus 49.8%, $p<0.001$), although this might be due to the lower prescription of antibiotics in children with confirmed malaria.

Alternatively, some infectious causes of CNS infections might have been overlooked. Rabies, for example, was clinically diagnosed in one patient with no laboratory-confirmed diagnosis. Since viral causes of CNS infections in sub-Saharan Africa are not well characterized, it is also possible that some unrecognized virus could be responsible for part of these cases. Based on biochemistry results and traditional definitions for suspicion of bacterial, viral or TB infections, we identified another 5, 5 and 2 suspect cases, respectively, potentially explaining only 6% of patients with no laboratory confirmed diagnosis.

Metabolic disorders, which could also be a cause of coma and neurological symptoms, were not investigated here. Anemia and electrolyte imbalance were cited as the main diagnostic at discharge in 17 (8%) of patients with no laboratory-confirmed diagnosis.

5.8. Treatment practices

More than half of the children received treatments prior admission. The main treatments received were quinine and benzyl penicillin. Even though ceftriaxone is the recommended initial treatment for suspected meningitis prior to confirmation by culture (Uganda Clinical Guidelines, 2010), it was only received by 6% of the children. Most pre-referral antibiotics were administered parenterally. This a good practice since it is evidence that lower health units reffering the patients can identify severely ill children and offer the best available drug in the most efficacious route.

Almost all children received some form of anti-infectious treatment during hospitalization, and more than half received both antibiotics and antimalarials. Most antibiotics were received as combination therapy, the most common regimen being benzyl penicillin and ceftriaxone, received by three quarters of those on antibiotics. This practice is contrary to the sensitivity tests we carried out as well as current guidelines that suggest use of ceftriaxone alone as appropriate treatment for meningitis (UCG, 2010). We believe that some of the reasons for this practice is delay or even failure to receive laboratory results on time outside the study setting but also difficulty in changing practices learned over a long time regarding treatment despite change in guidelines. This also results from inadequate sensitization on new treatment guidelines on the side of the policy makers.

The median duration of antibiotics given was 7 days, with a few children having antibiotics given for more than 3 weeks. This was mainly due to fact that some diagnoses changed during the course of hospitalization probably including nosocomial infections. Some organisms, such as *Salmonella* spp., too need longer periods of treatment because of blood-brain barrier but also because the organisms are intracellular and thus difficult to clearing if

treatment is given for shorter periods. About 13% with confirmed bacterial infection had premature interruption of treatment of less than 5 days despite the children being admitted for a longer.

Parenteral quinine was the most common antimalarial used. At the beginning of our study the Uganda government policy for treatment of malaria had not changed from quinine to artesunate even though WHO had had advocated for change in policy of treatment of severe malaria following the AQUAMAT study. Artesunate was introduced 1 year in the recruitment period. Median duration of antimalarial treatment was 5 days, but some children received antimalarial treatment for more than 8 days. The prolonged administration of antimalarials or antibiotics may lead to drug wastage and increase resistance, this emphasizes need to follow treatment guidelines.

With regard to laboratory-confirmed malaria or bacterial infections, appropriate treatment was received by most children during hospitalization, although about 15% with confirmed malaria or bacterial infection did not get appropriate treatment on the day of admission, but they eventually all received antimalarials and antibiotics. This calls for rapid point-of-care tests which can be used for screening and as point of reference to initiate lifesaving medications to severely ill children. Only two children with malaria and/or bacterial meningitis did not receive appropriate treatment over hospitalization, both of whom died. One child had a mixed bacterial-malaria infection and was treated for malaria only. This supports the need for further discussion as to whether it is appropriate to administer both antimalarials and antibiotics to severely ill children at lower health units or at admission prior to laboratory confirmation and to develop rapid tests for identifying life-threatening bacterial infections. The other child had an ESBL infection which was resistant to all available antibiotics. Although most of the deaths of children with malaria and/or bacterial infection were more likely to be caused by severity of the underlying illness and delays in initiating appropriate treatment than to inappropriate treatment, these 2 cases highlight the need for increased diagnostic capacity to be able to diagnose bacterial co-infections in malaria cases and to study antimicrobial resistance. Although still limited in Africa, the spread of ESBL bacteria, which are generally also resistant to most classes of antibiotics available in Africa, represents an important threat [65]. Measures should be taken to prevent their spread, including close monitoring, increasing diagnostic capacity, isolation of patients with ESBL infection, and reduction in antibiotic overuse.

Thanks to the availability of a rapid test to diagnose cryptococcal meningitis, their treatment was mostly appropriate. In contrast, TB meningitis was mostly left untreated and the only child who received treatment did so with a long delay, emphasizing the need to use faster diagnostic method, such as the Xpert MTB/RIF using appropriate amounts of CSF, or scores mixing clinical and biological parameters, and starting empiric treatment without delay.

Finally, none of the putative HSV infections treated using acyclovir was confirmed by PCR. The low prevalence of HSV infection – for which a treatment is available – and more generally for viral infections, may question the use of empiric acyclovir treatment. More data is needed from Uganda and other African countries to confirm this low prevalence and identify the cause of aseptic meningitis.

5.9. Differential diagnosis of CNS infections

As expected, differential diagnosis of CNS infections based on clinical signs only was not reliable, since many signs were common to different types of infections. Although signs of elevated intracranial pressure such as neck stiffness, bulging fontanel, Kernig or Brudzinski signs were significantly associated to bacterial meningitis, these signs were not present in all cases of bacterial meningitis and were also found in other types of infection, in particular TB meningitis, for which the recommended treatment is very different.

Rapid diagnostic tests for malaria are a great aid in the diagnosis of severe or cerebral malaria. However, HRP2 tests remain positive for very long periods and their specificity is thus reduced, particularly in areas of high transmission {Grandesso, 2014 117 /id}, and good malaria microscopy should be preferred whenever available. Possible bacterial co-infections should also be kept in mind even in case of confirmed malaria, but biomarkers associated to severe bacterial infections such as CRP and PCT are unfortunately not useful to detect these co-infections since their levels are already elevated in patients with malaria.

CSF characteristics are also widely used for the differential diagnosis of bacterial and other types of infections of the central nervous system. Apart from the traditional parameters (CSF appearance, leucocyte count, protein and glucose), other markers, in particular CSF lactate, and serum CRP and procalcitonin, have been described to be better predictors of bacterial meningitis [18, 66-69], although their value as individual marker is controversial [70-72]. Here, we found that the best individual marker for bacterial meningitis was leucocyte count. CSF lactate was elevated in bacterial meningitis (and TB meningitis) compared to all other groups, but the diagnostic performance, as measured by the area under the curve in the ROC curve analysis, was lower than that of CSF leucocytes. CRP and procalcitonin were elevated in bacterial meningitis, but also in malaria cases, as described previously [73], making them less useful for the diagnosis in a setting with a high malaria prevalence. In addition, their diagnostic performance remained low even after exclusion of malaria cases.

Decision rules combining clinical criteria and biological markers have been developed to overcome the insufficient performances of individual markers. Based on data from the literature, an expert panel on the use of biological markers for initiation of antibiotherapy have recommended the use of the Bacterial Meningitis Score (BMS) or the Meningitest for the differential diagnosis of bacterial and aseptic meningitis [71, 74, 75]. The BMS score has been evaluated in different settings and show very high sensitivity >99% with moderate specificity of 62% [29]. The Meningitest was developed as a refinement of the BMS in order to avoid false negative results and had a sensitivity of 100% in the initial evaluation, as well as in a subsequent analysis in 6 pediatric intensive care units in 5 European countries, where, however it showed lower specificity compared to BMS [74, 76]. To our knowledge, there has been no evaluation of these scores in an African setting. Here the modified BMS (ie. presence of any sign or score >=1) and Meningitest did also show a sensitivity of 100%, but with the specificity was so low (13% and 8%) that there would be no added value of using this test to discriminate between bacterial meningitis and other types of infections. In contrast, the specificity of the initial BMS (score >=2) (60%) was in the range described in the literature, but the sensitivity (88%) was lower. Although this might be partly due to the fact that our study population, representative of children attending a referral hospital, included a relatively large proportion of patients who had received antibiotics prior to sample collection, the sensitivity when considering only patients with no prior antibiotics (92%) was still lower than the very high sensitivity reported in Europe. In conclusion, we could not

identify any markers that would be sufficiently performant to rule out bacterial meningitis in our study population.

However, there is still room for improvement, in particular for the diagnosis of TB meningitis which was mostly overlooked in our study population. The TB meningitis score proposed by Marais et al. for the purposes of clinical research could probably be used also for diagnosis purposes [27]. Here, out of 3 children with a score >=10, one was confirmed by culture and the 2 others seemed compatible with this condition. This threshold could probably be recommended to start empirical meningitis TB treatment. However, based on this threshold, 2 patients with scores of 6 and 7 would have been missed. The number of children with scores between 6 and 9 is probably too high to start empiric treatment based on this score only, but it could also be used to recommend additional testing. Although PCR was not very useful in our study, probably due to the low volume of CSF used as TB meningitis was not the main focus, Xpert MTB/RIF has been shown to detect some TB meningitis cases when a volume of > 2.5 mL of CSF was used [77]. Non-improvement on antibiotics treatment could also be an indication to start empiric TB meningitis treatment in patients with a TB score between 6 and 9.

Although PCR is not currently available as a routine test, simplified PCR systems, such as the Xpert system, could be a good way forward for the diagnosis of bacterial meningitis in this type of context. Indeed, in a referral hospital where patients might have received antibiotics prior to their admission, PCR allows the detection of cases that would be missed by culture, as shown here by the fact that around 45% of bacterial meningitis cases were detected by PCR only.

5.10. Limitations

The study had several limitations or issues that need to be taken into account when extrapolating or comparing with other studies. First, it should be kept in mind that inclusion criteria were quite wide and not very specific of infections of the central nervous system. This explains, for example, the fact that some cases of non-cerebral malaria or bacteraemia were included in the study. It might also be responsible for the lower proportion of bacterial meningitis found here compared to other studies on bacterial meningitis where more than half of the cases were bacteriologically confirmed [37]. Finally, it could also explain the high proportion of patients with no laboratory-confirmed diagnosis.

Another limitation of the study is that not all tests could be performed on all patients. Around 15% of children had no CSF collected due to contra-indication, most of which were in severe conditions, as indicated by the fact that one third eventually died. In addition, PCR for the most common bacteria and viruses were performed for all patients with sufficient CSF, but other agents such as dengue 1/2/3/4, Yellow fever, West Nile virus, influenza virus A, influenza virus B, influenza virus H1N1, Rift valley fever virus, Sicilian virus, Naples virus, Toscana virus were tested only on the first 100 CSF specimens.

Although data collection on drug prescription was quite comprehensive, the final interpretation of was made difficult by lack of standardization in its completion (individual dose instead of daily dose reported, etc…). In addition, we were not able to determine correct drugs and doses received before admission.

5.11. Conclusions

In conclusion, this study showed that, despite decreasing prevalence here and elsewhere in Africa, malaria is the most common cause of CNS infection in this setting. Bacterial meningitis remains the second cause and the predominance of *S. pneumoniae* emphasizes the need for effective introduction of the pneumococcal vaccine, which is currently delayed in Uganda due to supply issues. With the introduction of this vaccine, *Salmonella* meningitis might become the first cause of bacterial meningitis, which might necessitate changes in treatment of bacterial meningitis since this infection requires prolonged treatment. Viral infections of the CNS, which are often suspected when typical signs or markers of malaria or bacterial meningitis are absent, were less frequent than expected, despite an exhaustive range of common and uncommon viruses investigated. Whether HHV-6, the most frequently identified virus, is truly responsible for the symptoms of CNS infections remains to be investigated. Finally, although the prevalence of TB and cryptococcal meningitis were low, their high toll in terms of death and sequelae should be considered with attention.

Although treatment practices did not always strictly follow the national guidelines in terms of duration in particular, inadequate etiologic treatment was probably not responsible for most of the deaths, except maybe for TB meningitis, although this disease is often fatal. Overall, antibiotic resistant bacterial infections do not yet play a major role in children admitted with febrile syndromes with neurological signs. Most of the deaths might instead be due to the severity of illness, but also to delayed consultation or referral. Lower level health centers should be encouraged and empowered to give IV ceftriaxone and IV artesunate as pre-referral treatment and to refer severely ill children as soon as possible. However, resistance to ceftriaxone, the first line antibiotic recommended for meningitis in Uganda, was present in one case of community-acquired and two possible nosocomial infections (excluded from the main analysis), and should be monitored closely. Randomized clinical trials could help better define the role of empirical treatment with broad spectrum treatments such as ceftriaxone versus penicillin for proven bacterial meningitis in this setting. More data is also needed on the assess the risks and benefits of using empirical antibiotics and antimalarials considering the low prevalence of mixed infection, but fatal outcome when inappropriate treatment is given.

Although improved and fast diagnosis would be of great support at each level of care, we did not identify clinical signs or biological markers that could identify different types of infection with high accuracy, either individually or in combination. For bacterial meningitis, the bacterial meningitis score showed very good sensitivity and negative predictive value, as required for a rule-out test, but its very low specificity and positive predictive value in our setting may question its usefulness in practice since it would only slightly reduce overuse of antibiotics. In contrast, the TB meningitis score proposed for clinical research, even with limited sensitivity and specificity, might be very useful to start an empiric treatment that is currently never or very rarely provided.

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7. Annex

7.1. Annex 1– Coma scales

Glascow coma scale

	Adult		**Child (<4 y-o)**	
	Response	Score	Response	Score
Eye opening	Spontaneously	4	Spontaneously	4
	To verbal stimuli	3	To verbal stimuli	3
	To pain	2	To pain	2
	No response to pain	1	No response to pain	1
Motor response	Obeys verbal command	6	Spontaneous or obeys verbal command	6
	Localizes to pain	5	Withdraws from touch	5
	Withdraws from pain	4	Withdraws from pain	4
	Abnormal flexion to pain (decorticate)	3	Abnormal flexion to pain (decorticate)	3
	Abnormal extension to pain, (decerebrate)	2	Abnormal extension to pain (decerebrate)	2
	No response to pain	1	No response to pain	1
Verbal response	Oriented and converses	5	Alert, babbles, coos, words to usual ability	5
	Disorientated and converses	4	Less than usual words / spontaneous irritable cry	4
	Inappropriate words	3	Cries only to pain	3
	Incomprehensible sounds	2	Moans to pain	2
	No verbal response to pain	1	No verbal response to pain	1

Blantyre coma scale (preverbal children)

	Response	Score
Eye movement	Watches or follows	1
	Fails to watch or follow	0
Best motor response	Localizes painful stimulus	2
	Withdraws limb from painful stimulus	1
	No response or inappropriate response	0
Best verbal response	Cries appropriately with painful stimulus	2
	Moan or abnormal cry with painful stimulus	1
	No vocal response	0

7.2. Annex 2 – Informed consent

INFORMED CONSENT FORM

Etiology of infections of the central nervous system among children in Mbarara, Uganda

Madam, Sir,

Your child (or the child under your care) has been hospitalized because he/she was diagnosed with a possible meningitis or brain infection. The investigators from Epicentre and the Mbarara University of Science and Technology would like to study the causes of meningitis or brain infection in Mbarara. You are being asked to allow your child (or the child under your care) to participate in this study. Before you decide, it is important for you to understand why this study is being done and what it will involve.

Purpose of the study

Meningitis and brain infection are very serious diseases in children. Many times, clinicians don't know what is the infectious agent causing the disease and this makes the treatment difficult. The goal of this study is to better understand what the infectious agents causing meningitis or brain infection are in the region of Mbarara, in order to improve their diagnosis and treatment.

How the study is done

The study will last for about 2 years and will include approximately 500 children presenting with possible meningitis or brain infection at the pediatric ward of the Mbarara Regional Referral Hospital (MRRH).

If you accept that your child participates in this study, he/she will be hospitalized and treated by the MRRH medical officers and nurses according to the usual procedures and treatment guidelines of the hospital.

In addition, today, a study medical officer will collect some specimens of cerebrospinal fluid (that is the liquid surrounding the brain) using a procedure called a lumbar puncture. Lumbar puncture is a usual procedure part of the management of patients presenting possible meningitis or brain infection. In this procedure, the cerebrospinal fluid is collected with a needle inserted in the bottom part of child's back. Before doing the lumbar puncture, a medical officer will carefully examine your child to rule out any possible contraindication to this procedure. Also, an experienced study nurse will draw 2 small amount of blood from venous puncture *at 30 minutes interval*. Finally, a study member will ask you a few questions about the health of your child.

The cerebrospinal fluid and the blood of you child will be analysed in the Mbarara Epicentre laboratory. The laboratory study team will conduct several biological analyses in order to identify the infectious agent causing your child's disease. The specimens collected from your child will also be sent to a laboratory in France/Europe for additional laboratory analyses. We will also ask you if you allow some of the sample to be saved for possible future laboratory tests, even though the exact nature of these tests is not known at this time.

As part of the usual care provided from the hospital, you will be offered an HIV counseling and an opportunity to perform an HIV testing for your child. If you accept this test, you will be asked to share this confidential result for the purpose of the study.

At discharge, your child will have a complete neurological examination to check for possible neurological sequelae. Also, a third small amount of blood will be collected by venous puncture to help confirm the infectious diagnosis.

You will be asked to come back to the hospital with your child one month and six months after discharge for a short visit. The goal of these visits will be to assess the possible sequelae or relapse of the disease. At the on month visit, a fourth amount of blood will be collected by venous puncture to help confirm the infectious diagnosis earlier discussed. In case of any disease, your child will be treated according to the standard practice at the MRRH. If you don't show up to the appointment, a home health visitor might visit your child at your home to find out why you missed the appointment and to bring your child to the clinic for assessment, if you accept it.

Confidentiality

All the information you will give us and all the data collected for this study will be kept confidential. The name of your child and your name will not be used for analysis and will never appear in the communication related to the results of this study.

Risks and discomfort

The potential risk of your child participation in this research include temporary discomfort from drawing blood from his/her vein. Blood collection may cause pain from the needle stick and small bruising around the puncture site. The amount of blood removed will be too small to affect your child's health.

Lumbar puncture is a normal procedure for the case management of patients with possible meningitis or brain infection. It can cause pain and headaches. Sometimes, the procedure has to be repeated if the medical doctor has failed to obtain cerebrospinal fluid at the first attempt. The amount of fluid removed will be too small to affect your child's health. Although very uncommon, severe complications (respiratory distress, cardiac arrest or even death) may occur after lumbar puncture. However, those complications are very unlikely in absence of contraindications to the procedure. The medical officer will make sure that your child does not present any of those contraindications before doing the lumbar puncture.

Benefits

Your child will benefit from the complete laboratory analysis for free. This analysis will help the medical officer to find the best treatment for him/her. Treatment against the infectious agent(s) identified as responsible for your child's disease will be provided for free.

The knowledge gained from this study will help Uganda to determine the way forward in diagnosing and treating meningitis and brain infections. Therefore the results of this study will benefit the community.

Cost/payment

There will be no financial expenses for your child to participate in this study.

You will not be paid for your child to participate in this study, but you will receive transport reimbursement for the study visit you will be asked to make one month after discharge.

Voluntary participation

The participation of your child in the study is completely voluntary. If you decide that you do not want your child to participate in the study or if you would like to withdraw your consent of participation at any time, and for any reason, this will not affect the medical care your child would benefit in the pediatric ward in any way.

Treatment and compensation for injury

If your child is injured or if you have questions about any unexpected injuries as a result of being in this study, please contact the clinicians in the pediatric ward of the hospital.

Implication of your signature or thumbprint

If you have any questions, you may ask them now or if you have questions later, please contact any study members working at the hospital (in particular Dr Margaret Nansumba, Dr Juliet Mwanga) or the Director of Epicentre in Mbarara. You may also call the following number 0772-519445 (Dr Juliet Mwanga).

If you give your consent for your child to participate in this study, you should sign or place your thumbprint in the consent form. Your signature or thumbprint below means that you understand the information given to you about your child's participation in the study and in the consent form. You will be asked to sign two copies of this informed consent form, one for our documentation and the other that you should keep.

After the HIV counseling, if you do agree to test your child for HIV and if you do agree to share your child's HIV results for the purpose of the study, you will be invited to sign or place your thumbprint on a separate consent form.

CONSENT PAGE

Etiology of infections of the central nervous system among children in Mbarara, Uganda

Principal investigators: Dr Juliet Mwanga, MUST, Mbarara
Dr Anne-Laure Page, Epicentre, Paris

Contact number: 0772-519445 (Dr Juliet Mwanga)

I, _____,
mother |_| father |_| legal representative |_|
of _____

declare that I have understood the objectives and purposes of this study, as well the as the risks and benefits of participating in the study. I agree that my child participates in this study. I am aware that I can withdraw my consent at any time and without any consequences to me or my child.

Signature/thumbprint: _____ Date: _____

In case of witnessed consent:

I, …….. declare that I have understood the objectives and purposes of this study and that I have witnessed the above named person(s) understand the objectives and purposes of the study and freely give their consent to participate.

Name of witness: _____

Signature/thumbprint: _____ Date: _____

My child's specimens may be saved for future research, even though the purpose of the future research is not known at this time.

☐ I consent to have my child's specimen(s) saved for future research studies

☐ I do not consent to have my child's specimen(s) saved for future research studies.

Name of Investigator: _____

Signature: _____ Date: _____

7.3. Annex 3 – HIV informed consent page

HIV TESTING CONSENT PAGE

Etiology of infections of the central nervous system among children in Mbarara, Uganda

Principal investigators: Dr Juliet Mwanga, MUST, Mbarara
Dr Anne-Laure Page, Epicentre, Paris

Contact number: 0772-519445 (Dr Juliet Mwanga)

I, _____

Mother |_| father |_| legal representative |_|

of _____

declare that :

- I have received explanations concerning HIV/AIDS: the virus causing the disease, its transmission modes, the clinical signs of the disease and their consequences.
- I have been informed of the possibility for my child to accept or refuse the screening.
- I have been informed that the result of the screening test will remain confidential. The confidential information regarding the diagnosis of HIV infection will only be given to the persons to whom I have given written consent, or to the staff who need this information to provide medical care and services.
- I have been informed about the medical care that may be provided by the HIV clinic to my child, myself and my family if I wish.

I have obtained answers to my questions concerning HIV screening and I accept that my child be tested for HIV infection.

Signature/thumbprint: _____ Date: _____

In case of witnessed consent:

I, …….. declare that I have understood the purposes of HIV screening and that I have witnessed the above named person(s) understand and freely give their consent to be tested for HIV infection.

Name of witness: _____

Signature/thumbprint: _____ Date: _____

Name of Investigator: _____

Signature: _____ Date: _____

7.4. Annex 4– Case report form

ETIOLOGY OF INFECTIONS

OF THE CENTRAL NERVOUS SYSTEM

AMONG CHILDREN

MRRH

MBARARA, UGANDA

CASE REPORT FORM

Name and surname of the Resident:

|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|

Patient Registration Number |__|__|__|__|__|

Inclusion Number |__|__|__|__|__|

Patient's initials |__|__|__|

Mother's initials |__|__|__|

1. INCLUSION

Inclusion criteria (ALL of the 3 point following should be YES or NA)	**YES**	*NO*	*NA*
1. Males and Females aged between 2 months and 12 years inclusive.	☐	☐	
2. Fever (axillary temperature at ≥ 37.5°C) or history of fever (subjective) in the past 48 hours (For children < 9 months, not applicable NA)	☐	☐	☐
3. Suspicion of CNS infections (see below)	☐	☐	

Suspect a CNS infection if recent onset of ANY of the following signs:	**YES**	*NO*	*DK*	*NA*
1. Non traumatic reduced level of consciousness: **children <9 months:** Blantyre coma score <4* If Yes, specify: E___ M ___ V ___ *See scale on page 8*	☐	☐	☐	☐
2. Non traumatic reduced level of consciousness: **children ≥ 9 months, preverba**l: Blantyre coma score <5* If Yes, specify: E___ M____ V___ *See scale on page 8*	☐	☐	☐	☐
3. Non traumatic reduced level of consciousness: **verbal children:** Glasgow coma score <15* If Yes, specify: E___ M___ V___ *See scale on page 8*	☐	☐	☐	☐
4. Prostration (Inability to breastfeed if < 9 mo or sit unassisted if > 9 mo)	☐	☐	☐	☐
5. Hypotonia	☐	☐	☐	☐
6. Hypertonia	☐	☐	☐	☐
7. Irritability	☐	☐	☐	☐
8. Headache (severe enough to require hospitalization)	☐	☐	☐	☐
9. Photophobia	☐	☐	☐	☐
10. Neck stiffness	☐	☐	☐	☐
11. Bulging fontanel	☐	☐	☐	☐
12. Focal neurological sign (if yes, specify): _____ _____	☐	☐	☐	☐
13. Prolonged, multiple or partial seizure on admission	☐	☐	☐	☐
14. History of prolonged, multiple or partial seizure (last 48 h)	☐	☐	☐	☐
15. Kernig sign (**children > 18 months:** flexion of the hip 90° with subsequent pain in legs extension)	☐	☐	☐	☐
16. Brudzinski sign (**children > 18 months:** involuntary flexion of the knees and hips after passive flexion of the neck).	☐	☐	☐	☐
17. Purpura	☐	☐	☐	☐
18. Cheyne stokes breathing	☐	☐	☐	☐
19. Other reason for suspecting a CNS infection (if yes, specify): _____ _____	☐	☐	☐	☐

* Complete coma score on the Medical examination form

Inclusion for the study (If any NO, patient should not be included)	**YES**	*NO*
Has the patient's representative provided the written informed consent?	☐	☐
Does the patient meet all inclusion criteria?	☐	☐

If both answers are YES, assign a number to the patient:

Inclusion number |___|___|___|___|___|

Date of inclusion in the study |___|___| |___|___| |___|___|___|___|
Day Month Year

Time of inclusion |___|___| |___|___| 24 hours clock
Hour Minutes

DATE of admission at MRRH |___|___| |___|___| |___|___|___|___|
Day Month Year

Time of admission at MRRH |___|___| |___|___| 24 hours clock
Hour Minutes

	YES	*NO*
Was the child included in the CNS infection study in the previous year?	☐	☐

If YES,

DATE of inclusion |___|___| |___|___| |___|___|___|___|
Day Month Year

Inclusion number at previous inclusion |___|___|___|___|___|

HIV testing	**YES**	*NO*
Has the patient's representative accepted HIV testing for the child and provided written consent?	☐	☐

2. LUMBAR PUNCTURE

Contraindication to Lumbar puncture AT INCLUSION: (if any YES, delay the LP and re-evaluate the situation)	**YES**	**NO**
1. Abnormalities of pupil size and reaction	☐	☐
2. Absence of oculocephalic reflex, fixed oculomotor deviation of the eyes	☐	☐
3. Abnormal tone (decerebrate/decorticate posture)	☐	☐
4. Focal neurological signs	☐	☐
5. Respiratory abnormalities (Hyperventilation, respiratory arrest, apnea, Cheyne Stokes respiration)	☐	☐
6. Papilledema (if fundoscopy done)	☐	☐
7. Local infection in the area through which the needle will have to pass	☐	☐
8. Signs of bleeding disorders; coagulopathy/thrombocytopenia	☐	☐
9. Cardio-respiratory compromise which may be exacerbated by the LP	☐	☐
10. Recent seizures (within 30 minutes)	☐	☐
11. Suspicion of meningococcal septicaemia; purpura	☐	☐
12. Acute trauma of the spinal cord	☐	☐
13. Other contraindication to LP (if yes, specify): _____ _____ _____	☐	☐

Has the lumbar puncture been delayed for the patient? YES ☐ NO ☐

If yes, specify why? _____
_____

Has the lumbar puncture been performed for the patient? YES ☐ NO ☐

If YES, the LP has been performed

1. LP Date |__|__| Day |__|__| Month |__|__|__|__| Year
2. LP Time |__|__| Hour |__|__| Minutes 24 hours clock
3. Result ☐ ≥ 3mL CSF ☐ < 3mL CSF ☐ Traumatic tap
4. Complications NO ☐ YES ☐

   If yes, specify:_____
   _____

Anti-infective Medication received BEFORE the Lumbar Puncture

A) BEFORE Admission at MRRH

Has the patient taken any medication in the previous 7 days? Yes ☐ No ☐ DK ☐

If 'Yes', please record all medication taken in the last week whether or not continuing.

1. Antibacterials (including antimycobacterial) **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐

2. Antivirals **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐

3. Antimalarials **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐

4. Other anti-infectives (Antimycotics, antihelmintics…) **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Stop at MRRH ☐

Abbreviation: Capsule=CAP; Cream=CR; Don't Know=DK; Drops=DRO; Intramuscular=IM; Intravenous=IV; Micrograms= µG; Milligrams = MG; Ointment=OI; Oral=O; Other=OTH; Packet=PAC; Spoon=SP; Subcutaneous=SC; Suppository=SU; Syrup=SYR; Tablet=TAB; Topical=T; Vial=V. **Dose** = total dose per day

B) At the MRRH BEFORE the Lumbar Puncture

1. Antibacterials (including antimycobacterial) **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY) TIME (HH MM)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐

2. Antivirals **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY) TIME (HH MM)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐

3. Antimalarials **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY) TIME (HH MM)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP☐

4. Other anti-infectives (Antimycotics, antihelmintics…) NO ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY) TIME (HH MM)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP ☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP ☐
					|__|__| |__|__| |__|__|__|__| |__|__| |__|__|	|__|__| |__|__| |__|__|__|__| Ongoing at LP ☐

Abbreviation: Capsule=CAP; Cream=CR; Don't Know=DK; Drops=DRO; Intramuscular=IM; Intravenous=IV; Micrograms= µG; Milligrams = MG; Ointment=OI; Oral=O; Other=OTH; Packet=PAC; Spoon=SP; Subcutaneous=SC; Suppository=SU; Syrup=SYR; Tablet=TAB; Topical=T; Vial=V. **Dose** = total dose per day

3. MEDICAL HISTORY AND DEMOGRAPHIC DATA

1. Sex Male ☐ Female ☐

2. Date of birth |__|__| |__|__| |__|__|__|__| DK ☐
   Day Month Year

If DK, specify:

3. Age |__|__| |__|__|
   Years Months

MEDICAL HISTORY Please record all significant medical history	**YES**	**NO**	**DK**	If YES: Onset date	If YES: End date (DD MM YYYY)
1. Hospitalization in the previous month	☐	☐	☐	|__|__| |__|__| |__|__|__|__| (DD MM YYYY)	|__|__| |__|__| |__|__|__|__| Up to transfert to MRRH ☐
2. Significant developmental disability (Specify:_____ _____)	☐	☐	☐	|__|__| |__|__|__|__| (MM YYYY)	|__|__| |__|__|__|__| (MM YYYY) Ongoing ☐
3. Sickle cell disease	☐	☐	☐		
4. Chronic illness (Specify:_____ _____)	☐	☐	☐	|__|__| |__|__|__|__| (MM YYYY)	|__|__| |__|__|__|__| (MM YYYY) Ongoing ☐
5. Other (Specify:_____ _____ _____)	☐	☐	☐	|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Ongoing ☐
6. Other (Specify:_____ _____ _____)	☐	☐	☐	|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| Ongoing ☐

Vaccine history before admission at MRRH	**Yes, card**	**Yes, verbal**	*NO*	**DK**
1) BCG	☐	☐	☐	☐
2) Measles	☐	☐	☐	☐
3) Haemophilus Influenzae type B (1st dose)	☐	☐	☐	☐
4) Haemophilus Influenzae type B (2nd dose)	☐	☐	☐	☐
5) Meningitis vaccine	☐	☐	☐	☐
6) Pneumoccocal vaccine	☐	☐	☐	☐

4. CLINICAL EXAMINATION AND DIAGNOSTIC AT **INCLUSION IN THE STUDY**

Vital signs

1.	Temperature	|___|___|.|___|	°C Oral ☐ Axillary ☐ Tympanic ☐ Rectal ☐
2.	Heart rate	|___|___|___|	b.p.m
3.	Respiratory rate	|___|___|___|	p.m
4.	Systolic blood pressure	|___|___|___|	mm Hg
5.	Diastolic blood pressure	|___|___|___|	mm Hg

Anthropometric measurements

6.	Weight	|___|___|.|___|___|	kg
7.	Height	|___|___|___|	cm
8.	Mid upper arm circumference	|___|___|,|___|	cm

Coma Scores

9. Glasgow coma scale for verbal children

Eye opening : _____
Motor response: _____
Verbal response: _____
Total : _____

Adult or child ≥ 4 y		Child <4 y	
Spontaneously	4	Spontaneously	4
To verbal stimuli	3	To verbal stimuli	3
To pain	2	To pain	2
No response to pain	1	No response to pain	1
Obeys verbal command	6	Spontaneous or obeys verbal command	6
Localizes to pain	5	Withdraws from touch	5
Withdraws from pain	4	Withdraws from pain	4
Abnormal flexion to pain (decorticate)	3	Abnormal flexion to pain (decorticate)	3
Abnormal extension to pain, (decerebrate)	2	Abnormal extension to pain (decerebrate)	2
No response to pain	1	No response to pain	1
Oriented and converses	5	Alert, babbles, coos, words to usual ability	5
Disorientated and converses	4	Less than usual words / spontaneous irritable cry	4
Inappropriate words	3	Cries only to pain	3
Incomprehensible sounds	2	Moans to pain	2
No verbal response to pain	1	No verbal response to pain	1

10. Blantyre coma scale for preverbal children

Eye opening : _____
Motor response: _____
Verbal response: _____
Total : _____

Watches or follows	1
Fails to watch or follow	0
Localizes painful stimulus	2
Withdraws limb from painful stimulus	1
No response or inappropriate response	0
Cries appropriately with painful stimulus	2
Moan or abnormal cry with painful stimulus	1
No vocal response	0

Physical and Clinical Examination at inclusion

	No	Yes	DK or NA
Abdominal pain	☐	☐	☐
Jaundice	☐	☐	
Anorexia	☐	☐	
Nausea	☐	☐	☐
Vomiting (> 2 episodes in 24h)	☐	☐	
Diarrhoea (> 3 stools in 24h)	☐	☐	
Hepatomegaly	☐	☐	
Slenomegaly	☐	☐	
Weakness	☐	☐	☐
Dizziness	☐	☐	☐
Headache	☐	☐	☐
Muscle/joint ache	☐	☐	☐
Prurit	☐	☐	☐
Convulsions	☐	☐	
Dehydration	☐	☐	
Oedematous malnutrition	☐	☐	
Lymphadenopathy	☐	☐	
Cough	☐	☐	
Respiratory distress (costal indrawing)	☐	☐	
Abnormal lung auscultation	☐	☐	
Cyanosis	☐	☐	
Delayed capillary refill (≥3 sec)	☐	☐	
Cold extremities	☐	☐	
Ear infection	☐	☐	
Symptomatic anemia	☐	☐	
Tonsillitis	☐	☐	
Oral candidiasis	☐	☐	
Skin lesions	☐	☐	
Eye infection	☐	☐	
Clinicial suspicion of AIDS	☐	☐	
Other 1_____	☐	☐	
Other 2_____	☐	☐	

Reason of hospitalization (from mother)	

Diagnosis at INCLUSION

Main clinical diagnosis at inclusion (one only)	Bacterial meningitis ☐		Viral meningoencephalitis ☐	
	Cerebral malaria ☐		Other ☐	
If other, specify:				
Other diagnosis at admission:	Gastroenteritis	YES ☐	NO ☐	DK ☐
	AIDS	YES ☐	NO ☐	DK ☐
	Pneumonia	YES ☐	NO ☐	DK ☐
	Sepsis	YES ☐	NO ☐	DK ☐
	Severe malnutrition	YES ☐	NO ☐	DK ☐
	Other	YES ☐	NO ☐	DK ☐
If other, specify:				

Diagnosis at ADMISSION at MRRH (if different)

Main clinical diagnosis at admission (one only)	Bacterial meningitis ☐		Viral meningoencephalitis ☐	
	Cerebral malaria ☐		Other ☐	
If other, specify:				
Other diagnosis at inclusion:	Gastroenteritis	YES ☐	NO ☐	DK ☐
	AIDS	YES ☐	NO ☐	DK ☐
	Pneumonia	YES ☐	NO ☐	DK ☐
	Sepsis	YES ☐	NO ☐	DK ☐
	Severe malnutrition	YES ☐	NO ☐	DK ☐
	Other	YES ☐	NO ☐	DK ☐
If other, specify:				

5. FOLLOW-UP AT DAY 3 AFTER INCLUSION

Date of follow-up (Day 3 after inclusion) |__|__| Day |__|__| Month |__|__|__|__| Year

Clinical signs at day 3	Fever	YES ☐	NO ☐	
	Convulsions	YES ☐	NO ☐	
	Consciousness	Coma ☐	Altered ☐	Normal ☐
Clinical evolution of initial signs		Improved ☐	Unchanged ☐	
		Gradual worsening ☐	Improvement followed by worsening ☐	
		Don't know ☐		
Onset of other clinical signs		YES ☐	NO ☐	DK ☐
If YES, specify				

6. DISCHARGE : TREATMENTS RECEIVED DURING HOSPITALISATION

1. Antibacterials (including antimycobacterial) **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐

2. Antivirals **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐

3. Antimalarials **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐

4. Other anti-infectives (Antimycotics, antihelmintics…) **NO** ☐ **DK** ☐ **YES** ☐ (if YES, specify)

Name	Indication	Dose	Unit	Route	Start (DD MM YYYY)	Stop (DD MM YYYY)
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐
					|__|__| |__|__| |__|__|__|__|	|__|__| |__|__| |__|__|__|__| To be continued at home ☐

Capsule=CAP; Cream=CR; Don't Know=DK; Drops=DRO; Intramuscular=IM; Intravenous=IV; Micrograms=µG; Milligrams=MG; Ointment= OI; Oral=O; Other=OTH; Packet=PAC; Spoon=SP; Subcutaneous=SC; Suppository=SU; Syrup=SYR; Tablet=TAB; Topical=T; Vial=V. **Dose** = total dose per day

Other treatments	Blood transfusion	Yes ☐	NO ☐
	IV rehydration	Yes ☐	NO ☐

7. DISCHARGE: OUTCOME AND DIAGNOSIS AT EXIT

Outcome

Cured ☐ Date of discharge |__|__| |__|__| |__|__|__|__| Day Month Year

Death ☐ Date of death |__|__| |__|__| |__|__|__|__| Day Month Year

Withdrawal of consent ☐ Date of withdrawal |__|__| |__|__| |__|__|__|__| Day Month Year

Discharge against advice ☐ Date of discharge |__|__| |__|__| |__|__|__|__| Day Month Year

Transferred ☐ Date of transfer |__|__| |__|__| |__|__|__|__| Day Month Year

If transfered, specify where to: _____

If cured, withdrawal, transferred or discharge against advice: diagnosis at discharge/ transfer / withdrawal

Main clinical diagnosis* at discharge/ transfer / withdrawal: Bacterial meningitis ☐ Viral meningoencephalitis ☐ Cerebral malaria ☐ Other ☐

If other, specify:

Other diagnosis:								
	Gastroenteritis	YES ☐	NO ☐	DK ☐	Sepsis	YES ☐	NO ☐	DK ☐
	Pneumonia	YES ☐	NO ☐	DK ☐	Other	YES ☐	NO ☐	DK ☐
	AIDS	YES ☐	NO ☐	DK ☐				

If other, specify:

Neurological sequelae not present at admission								
	Seizures	YES ☐	NO ☐	DK ☐	Blindness	YES ☐	NO ☐	DK ☐
	Hemiplegia/ paresia	YES ☐	NO ☐	DK ☐	Hearing loss	YES ☐	NO ☐	DK ☐
	Neuropsychiatric	YES ☐	NO ☐	DK ☐	Hypotonia	YES ☐	NO ☐	DK ☐
	Extrapyramidal rigidity	YES ☐	NO ☐	DK ☐	Cerebral ataxia	YES ☐	NO ☐	DK ☐
	Cranial nerve plasies	YES ☐	NO ☐	DK ☐	Other	YES ☐	NO ☐	DK ☐

If any YES, specify

* Main clinical diagnosis: diagnosis leading to hospitalisation or requiring the highest level of care

If death:				
Main cause of death:				
Other cause(s) of death:				
Time of death 24 hours clock		___|___| Hour	___|___| Minutes	
New clinical signs before death	Fever	YES ☐	NO ☐	DK ☐
	Convulsions	YES ☐	NO ☐	DK ☐
	Conscience	Coma ☐	Altered ☐	Normal ☐
Clinical evolution of initial clinical signs before death		Gradual worsening ☐ Unchanged ☐	Improvement followed by worsening ☐ Don't know ☐	
Onset of new clinical signs since inclusion If YES, specify		YES ☐	NO ☐	DK ☐

7. FOLLOW-UP AT 1 MONTH

Planned visit date	|___|___| |___|___| |___|___|___|___| D D M M Y Y Y Y
Actual date	|___|___| |___|___| |___|___|___|___| D D M M Y Y Y Y Child never showed up ☐ (Specify Why: _____) Child was reported dead ☐ (Date of death: |__|__| |__|__| |__|__||__|__|
Is the child sick?	YES ☐ NO ☐

If the child is sick	
Diagnosis	
Treatment prescribed	
Did the child have neurological sequelae AT DISCHARGE?	NO ☐ YES ☐ DK ☐
If YES, has the sequelae	Resolved ☐ Improved ☐ Worsened ☐ Persistent, No change ☐ If improved or worsened, specify:

If death:	
Reported cause of death:	

8. FOLLOW-UP AT 6 MONTHS

Planned visit date	|___|___| |___|___| |___|___|___|___| D D M M Y Y Y Y
Actual date	|___|___| |___|___| |___|___|___|___| D D M M Y Y Y Y Child never showed up ☐ (Specify Why: _____) Child was reported dead ☐ (Date of death: |__|__| |__|__| |__|__||__|__|
Is the child sick?	YES ☐ NO ☐

If the child is sick	
Diagnosis	
Treatment prescribed	
Did the child have neurological sequelae AT DISCHARGE?	NO ☐ YES ☐ DK ☐
If YES, has the sequelae	Resolved ☐ Improved ☐ Worsened ☐ Persistent, No change ☐ If improved or worsened, specify:

If death:	
Reported cause of death:	

To be completed by the investigator at the end of the study participation of this child

I certify that I have reviewed all the information recorded in the pages of this CRF and that they are a complete, accurate and consistent reflection of the patient's original data.

Name of the resident _____

Signature_____ Date (DD/MM/YYY)

Monitoring by the co-investigator :

Signature_____ Date (DD/MM/YYY)

Tick for data entry 1 ☐ 2 ☐

7.5. Annex 5 – Laboratory report form

LAB TEST REQUEST FORM

ETIOLOGY OF INFECTION OF CENTRAL NERVOUS SYSTEM AMONG CHILDREN, MBARARA, UGANDA

Requested by: Clinician's Initials : |___|___|___| Clinician's Signature : _____

Sample: Blood ☐ CSF Tube A ☐ Tube B ☐.

Lumbar Puncture Date : ____/____/_____(DD/MM/YYYY) Time: |___|___| |___|___| (Hour/Minutes)

PATIENT STUDY N°: |___|___|___|___|___| **Initials:** |___|___|___| **Sex: M ☐ F ☐** **Age:** |___|___| **Year** |___|___| **Month**

COMMENTS:

_____

MALARIA DIAGNOSTIC (RDT): ☐ Negative ☐ Positive Initials: |___|___|___| Sign:_____

1. SAMPLE RECEPTION: Date : ____/____/_____(DD/MM/YYYY) Time: |___|___| |___|___| (Hour/Minutes)

Blood ☐ CSF Tube A ☐ Tube B ☐. **COMMENTS:** _____ _____

_____**Initials: |___|___|___| Sign:_____**

2. CYTOLOGY ON CSF (KOVACSLIDES) Analyse Time: |___|___| |___|___| Initials: |___|___|___| Sign:_____

Aspect: Crystal clear ☐ Yellow ☐ Hemorrhagic ☐ Trouble ☐ Purulent ☐ .

Germs : **Y** ☐ **N** ☐ . Hématies : _____/ mm^3 Leucocytes : _____/ mm^3 >10 **Y** ☐ **N** ☐ .

3. GRAM (MICROSCOPY) **DONE: Y** ☐ **N** ☐ **Initials: |___|___|___| Sign:_____**

BGN	Rare	Few	Many	Numerous	Very Numerous	(circle the right answer)
BGP	Rare	Few	Many	Numerous	Very Numerous	
CGN	Rare	Few	Many	Numerous	Very Numerous	
CGP	Rare	Few	Many	Numerous	Very Numerous	**NTR** (Nothing to Report) ☐.

4. CYTOLOGY ON CSF (MICROSCOPY) DONE: Y ☐ N ☐ Initials: |___|___|___| Sign:_____

Polymorphonucleocytes : _____% Lymphocytes : _____%

Monocytes : _____% Others cells : _____%

5. REAL-TIME PCR RUN TIME: |___|___| |___|___| Initials: |___|___|___| Sign:_____

PCR 1 ☐.		**PCR 2 ☐.**		
☐ *Herpes Simplex virus*	☐ *Neisseria Meningitidis*	☐ *Mycobacterium tuberculosis*	☐ *Measles virus*	☐ NTR

☐ *Varicella zoster virus*	☐ *Streptococcus pneumoniae*	☐ *Salmonella enterica*	☐ *Mumps virus*	
☐ *Enterovirus*	☐ NTR (Nothing to report)	☐ *Haemophilus influenzae b*	☐ *L. monocytogenes*	

6. BIOCHEMISTRY DONE: Y ☐ N ☐ **Initials: |___|___|___| Sign_____**

METHOD	COBAS MIRA				Brahms RDT
Item	Blood-CRP	Blood-Glucose	CSF-Glucose	CSF- Protein	Blood-PCT
Result					<2 ☐ 0.5-2 ☐ 2-10 ☐ >10 ☐.
Unit	g/L	g/L	g/L	g/L	ng/ml
Normal (Y/N)					

7. HAEMATOLOGY (ABX PENTRA 60) DONE: Y ☐ N ☐ **Initials: |___|___|___| Sign:_____**

Item	HGB	RBC	HCT	WBC	GRA	NEU	EOS	BAS	LYM	MON	PLT
Result											
Unit	g/dL	x 10^{12}/L	%	x 10^{9}/L	x 10^{9}/L	x 10^{9}/L	x 10^{9}/L	x 10^{9}/L	x 10^{9}/L	x 10^{9}/L	x 10^{9}/L
Normal (Y/N)											

8. HB ELECTROPHORESIS ☐ Normal (Hb AA) ☐ Other (Hb |___/___|)

☐ Sickle Cell Trait (Hb AS) ☐ Sickle Cell Anaemia (Hb SS) **Initials: |___|___|___| Sign_____**

9. CRYPTOCOCCUS ANTIGEN LATEX SYSTEM (RDT) **Initials: |___|___|___| Sign_____**

- ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐ .

_____

10. MALARIA DIAGNOSTIC (BLOOD SMEAR) **Initials: |___|___|___| Sign_____**

		☐ *P. falciparum*	☐ *P. vivax*	☐ *P. ovale*	☐ *P. malariae*
☐ **NTR**	**Trophozoites / µl**	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__|__|__|__|__|
(Nothing to Report)	**Gametocytes / µl**	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__|__|__|__|__|
	Schizonts	|___| +	|___| +	|___| +	|___| +
	Malaria Pigment	**Y** ☐ **N** ☐ **.**			

11. GERMS IDENTIFICATION (BLOOD CULTURE)

☐ *N. meningitidis*	☐ *S. pneumoniae*	☐ *H. influenzae b*	☐ *Salmonella*
☐ *M. tuberculosis*	☐ *L.monocytogenes*	☐ *M. tuberculosis*	☐ _____

☐ NTR (Nothing to report)

Initials: |___|___|___| Sign:_____

12. GERMS IDENTIFICATION (CSF CULTURE)

☐ *N. meningitidis*	☐ *S. pneumoniae*	☐ *H. influenzae b*	☐ *Salmonella*
☐ *M. tuberculosis*	☐ *L.monocytogenes*	☐ *M. tuberculosis*	☐ _____

☐ NTR (Nothing to report)

Initials: |___|___|___| Sign:_____

13. DRUG SUSCEPTIBILITY TEST (CULTURE)

DONE: Y ☐ **N** ☐ **.**

Species	Antibiotic	DST 1		DST 2	
		Inhibition size (mm)	S/I/R	Inhibition size (mm)	S/I/R

S = Sensible I = intermediary R = Resistant

Initials: |___|___|___| Sign:_____

14. TB CULTURE DONE: Y ☐ N ☐ **Initials: |___|___|___| Sign:_____**

☐ NTR (Nothing to report)

☐ *M. tb complex*	☐ *Nontuberculous mycobacteria*

15. COMBUR 4 (RDT) DONE: Y ☐ N ☐ **Initials: |___|___|___| Sign:_____**

Item	Leucocytes	Protein	Glucose	Blood
Result				Hb Erythocytes
Unit		**g /L**	**g/L**	
Normal (Y/N)				

COMMENTS:

7.6. Annex 6 – TB culture form

LABORATORY REQUEST AND RESULTS TB

ETIOLOGY OF INFECTION OF CENTRAL NERVOUS SYSTEM AMONG CHILDREN, MBARARA, UGANDA

Requested by: Clinician's Initials : |___|___|___|

CSF: Yes ☐ **No** ☐ **If Yes,** Date : ____/____/_____(DD/MM/YYYY) Time: |___|___| |___|___|
(Hour/Minutes)

PATIENT STUDY N°: CNS |___|___|___| **Initials: |___|___|___|** **Sex: M** ☐ **F** ☐

Age: |___|___| Year |___|___| Month

Received by: Nurse's Initials : |___|___|___| Nurse's Signature : _____

Received by: Clinician's Initials : |___|___|___| Clinician's Signature : _____

16. TB REQUEST

CRITERIA	Score	If yes write the score and if NO write 0
Clinical criteria (PD Ward) - Maximum score = 6		**Total=_____/6**
Symptom duration more than 5 days	**4**	
Systemic symptoms suggestive of TB (one or more of the followings): weight loss, night sweats, persistent cough for more than 2 weeks	**2**	
History of recent (within 1 year) close contact with PTB patient or a positive TST or IGRA (only for children < 10 years of age)	**2**	
Focal neurological deficit (excluding cranial palsies)	**1**	
Cranial nerve palsy	**1**	
Altered consciousness	**1**	
Evidence of TB elsewhere – Maximum score = 4		**Total = _____/4**
Chest radiograph suggestive of active tuberculosis	**2**	
Chest radiograph suggestive of miliary tuberculosis	**4**	

CSF criteria (Laboratory) –Maximum score = 4		Total = _____/4
Clear appearance	1	
Cells: 10-500 per ul	1	
Lymphocytic predominance (> 50%)	1	
Protein concentration > 1g/L	1	
CSF to plasma glucose ratio <50% or CSF glucose < 2.2 mmol/L	1	
TOTAL SCORE of Clinical and CSF criteria score		

If the TOTAL SCORE is < 6:

- **Store the CSF in as many Aliquots of 200 µl**

If the TOTAL SCORE is ≥ 6:

- **Store 1 aliquot of 200 µl of CSF in -80 C Ultra freezer**
- **Perform the TB culture in MGIT with the CSF remaining according to the study flow chart**

17. TUBERCULOSIS CULTURE **DONE** ☐ **NOT DONE** ☐

Date : ____/____/_____(DD/MM/YYYY) **Initials: |___|___|___|**

Sign:_____

Volume inoculated: |___|___|___| µl

Result: **Negative after 56 days** ☐ **Positive** ☐ **Contaminated** ☐

If positive, specify : **MTBC** ☐ **NTM** ☐

Name: _____

Date : ____/____/_____(DD/MM/YYYY)

Signature :_____

7.7. Annex 7. Antibiotic resistance results

Table 26. Proportion of susceptible isolates among enterobacteria isolated in the study

	All (N=13)		S. Typhi (N=3)		Salmonella spp. (N=7)		E. coli (N=3)	
	n	%	n	%	n	%	n	%
Amoxicillin	4	30.8	2	66.7	1	14.3	1	33.3
Amoxi-clavulanic acid	11	84.6	3	100.0	6	85.7	2	66.7
Cefotaxime	12	92.3	3	100.0	7	100.0	2	66.7
Ceftazidime	12	92..3	3	100.0	7	100.0	2*	66.7
Imipeneme	13	100.0	3	100.0	7	100.0	3	100.0
Gentamicin (10 µg)	8/11	72.7	3	100.0	4/6	66.7	1/2	50.0
Amikacin	12/12	100.0	2/2	100.0	7	100.0	3	100.0
Ofloxacin	9	69.2	0	0.0	7	100.0	2	66.7
Cotrimoxazole	4	30.8	2	66.7	2	28.6	0	0.0

* 1 resistant isolate with ESBL detected

Table 27. Resistance profile of *S. pneumoniae* isolated in the study (N=18)

	S n (%)	I n (%)	R n (%)	Mean MIC (mg/L) among intermediate
E-test				
Penicillin G (N=13)	2 (15.4)	11 (84.6)	0	0.34
Amoxicillin (N=17)	17 (100)	0	0	0
Ceftriaxone (N=16)	16 (100)	0	0	0
Ciprofloxacine (N=14)	0	14 (100)	0	0.61
Disc-diffusion test				
Erythromycin (N=18)	14 (77.8)	3 (16.7)	1 (5.6)	
Cotrimoxazole (N=15)	1 (6.7)	0	14 (93.3)	
Chloramphenicol (N=15)	15 (100)	0	0	
Tetracycline (N=17)	16 (94.1)	0	1 (5.9)	

Table 28. Resistance profile of Hib isolated in the study (N=6)

	S n (%)	R n (%)	Mean MIC (mg/L) among resistant isolates
E-test			
Amoxicillin	1 (16.7)	5 (83.3)	4.8
Ceftriaxone	6 (100)	0	-
Ciprofloxacine	6 (100)	0	-
Disc-diffusion test			
Amoxi-clav	6 (100)	0	
Chloramphenicol (N=5)	1 (20.0)	4 (80.0)	
Tetracycline	1 (16.7)	5 (83.3)	

7.8. Annex 8. Results of BMS and Meningitis scores by diagnostic category

Table 29. BMS and Meningitis Scores by main diagnostic categories

	No diagnosis		Malaria		Cerebral malaria		Bacterial meningitis		Bacteremia		Virus		Tuberculosis	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
BMS score														
0 or 1	127	59.9	33	70.2	58	52.3	6	10.5	10	90.9	2	66.7	1	25.0
>=2	85	40.1	14	29.8	53	47.7	51	89.5	1	9.1	1	33.3	3	75.0
Meningitest														
Negative	22	10.4	2	4.3	3	2.7	0	0.0	0	0	0	0	0	0.0
Positive	190	89.6	45	95.7	108	97.3	57	100.0	11	100	3	100	4	100.0

7.9. Annex 8. Comparison between dipstick categories and reference values

Table 30. Comparison between urine dipstick categories and reference method for leucocytes measurement

		Reference method			
		<50 cells/µL	50-212 cells/µL	>=213 cells/µL	Total
Dipstick	0/25 cells/µL	314	8	4	326
	75 cells/µL	10	7	13	30
	500 cells/µL	0	3	21	24
	Total	324	18	38	380

Table 31. Comparison between urine dipstick categories and reference method for protein measurement

		Reference method			
		0-74 mg/dL	75- 299 mg/dL	>=300 mg/dL	Total
Dipstick	0/30 mg/dL	151	105	22	278
	100 mg/dL	4	41	15	60
	500 mg/dL	0	10	24	34
	Total	155	156	61	372

Table 32. Comparison between urine dipstick categories and reference method for glucose measurement

		Reference method			
		0-74 mg/dL	75-199 mg/dL	>=200 mg/dL	Total
Dipstick	0/50 mg/dL	102	4	0	106
	100 mg/dL	156	96	0	252
	300 mg/dL	3	16	2	21
	Total	261	116	2	379