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A descriptive study of meningeal pathogens cultured from the general paediatric wards in Chris Hani Baragwanath Hospital, 2012 to 2020

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The University of the Witwatersrand

Faculty of Health Sciences

A descriptive study of meningeal pathogens cultured from the general paediatric wards in Chris Hani Baragwanath Hospital, 2012 to 2020

By

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A Research Report submitted to the Faculty of Health Sciences,
University of the Witwatersrand, Johannesburg,
in partial fulfilment of the requirements for the degree of
Masters of Medicine in Paediatrics

December 2024

Declaration

I Sindiswa Charmaine Dlamini declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Masters of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of the candidate)

31 day of December 2024

Dedication

This work is dedicated to my wonderful mother Zevile Dlamini, who believed in me and my goals before I knew it was possible.

Presentations and Publications

The findings of this study were presented at the University of the Witwatersrand Paediatric Research Day on 8 December 2022. Title of presentation: "A descriptive study of meningeal pathogens cultured from the general paediatric wards in Chris Hani Baragwanath Hospital, 2012 to 2020".

Abstract

Background

Meningitis is a leading cause of childhood morbidity and mortality, with an incidence of 7/100,000 children aged 1-4 years and 40/100,000 in infants in South Africa. Since the incorporation of protein-polysaccharide vaccines to prevent infections due to *Haemophilus influenzae* type b and pneumococcus into the South African immunisation programme, the epidemiology of meningitis in South Africa is changing.

Methods

We reviewed the clinical and laboratory characteristics, and clinical outcomes of children aged 0 to 14 years of age that were hospitalised with meningitis in the general paediatric wards at a public sector teaching hospital in South Africa from 01 January 2012 through 31 December 2020.

Results

During the study period, 1,617 episodes of meningitis were diagnosed in 1,597 children. Median age at hospitalisation was 6.0 months (interquartile range (IQR), 1.2 to 38.6 months). Most (1,243/1,617, 78.9%) of the episodes occurred in HIV-uninfected children, 182 (11.2%) in HIV-infected children and 192 (11.8%) in children with undetermined HIV infection status. Two hundred, fifty-four (15.7%) episodes were microbiologically confirmed, and 1,268 (78.4%) were clinically diagnosed with no microbiological confirmation. Coagulase negative staphylococci (n=77), *Streptococcus pyogenes* (n=76) and pneumococcus (n=54) were the most frequently isolated organisms. The crude mortality rate was 8.5% (137/1,617). Using three multivariable logistic regression modelling approaches, HIV infection was consistently associated with mortality (adjusted odds ratios ranging from 3.658 (95% confidence interval (CI), 1.645-8.133) to 4.070 (95% CI, 1.474-11.235)). A composite measure of low cerebrospinal fluid (CSF) to plasma glucose ratio, raised CSF protein and raised C-reactive protein was

independently associated with a 3.413 (95% CI, 1.88-6.196) increased adjusted odds of death.

Conclusions

Most cases of meningitis are clinically diagnosed, and when microbiologically confirmed are caused predominantly by Gram positive organisms. HIV infection is consistently associated with worse outcomes. Biochemical parameters may be useful to prognosticate in children diagnosed with meningitis.

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Nomenclature

CCM	cryptococcal meningitis
CLWH	children living with HIV
CrAg	cryptococcal capsular polysaccharide antigen
CSF	cerebrospinal fluid
CHBAH	Chris Hani Baragwanath Academic Hospital
Hib	*Haemophilus influenzae* type b
HIV	human immunodeficiency virus type 1
IPD	invasive pneumococcal disease
LMIC	low- and middle-income country
PCV	pneumococcal conjugate vaccine
PCV-7	7-valent PCV
PCV-13	13-valent PCV
TAC	TaqMan Array Card
TB	tuberculosis
TBM	TB meningitis
WHO	World Health Organization

1 INTRODUCTION AND LITERATURE REVIEW

Meningitis, inflammation of the meninges and brain due to infection with bacterial or fungal pathogens[1], is a leading cause of childhood morbidity and mortality. In South Africa, the incidence of meningitis is 4 per 100,000 in the general population, 40 per 100,000 in infants <1 year and 7 per 100,000 in children aged 1 to 4 years[2].

The specific infectious aetiology of meningitis is generally age dependent. Organisms that cause meningitis usually arise from the mucosal surfaces of the upper respiratory tract or the blood stream and infiltrate the meninges by invading the blood brain barrier. In the neonatal period (age 0 to 28 days) the commonest organisms are *Streptococcus agalactiae*, *Listeria monocytogenes* and *Escherichia coli*[3]. In infants aged three months and older and children, the commonest bacterial organisms are *Neisseria meningitidis* (meningococcus), *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae* type b (Hib)[4]. In the context of human immunodeficiency virus type-1 (HIV) infection, important meningeal pathogens, in addition to those mentioned above, include *Mycobacterium tuberculosis* and *Cryptococcus neoformans*.

1.1 Impact of Vaccination on bacterial meningitis

South Africa introduced two protein-polysaccharide vaccines that target Hib and pneumococcus in 1998 and 2009, respectively[5,6]. As a consequence of the introduction of these vaccines, the epidemiology of bacterial meningitis in young children may be changing. While vaccines are available to prevent meningococcal meningitis[7], these have not been incorporated into the National Immunisation Programme in South Africa. The changing epidemiology of Hib and pneumococcal meningitis are presented below.

1.1.1 Pneumococcal meningitis

Streptococcus pneumoniae is the commonest cause of meningitis, it is a Gram-positive encapsulated diplococcus that is part of the normal flora of the nasopharynx[8]. It is also an

important cause of pneumonia and septicaemia. There are more than 90 serotypes of *S. pneumoniae*, approximately 23 of which are responsible for causing invasive pneumococcal disease (IPD) in children[9]. Pneumococcal serotypes are described according to their cell wall polysaccharides. Prior to vaccination with pneumococcal conjugate vaccine (PCV), serotypes 6 thorough 11 caused more than 70% of IPD in children less than 5 years of age[10]. In a survey done in Africa prior to widespread use of PCV, pneumococcal meningitis had a case fatality rate of 45% in children under the age of 5 years, and 50% of survivors had long term sequelae[8].

The introduction PCV into the Expanded Programme on Immunisation (EPI) in South Africa has resulted in a decline in morbidity and mortality associated with IPD. In 2009, South Africa introduced 7-valent PCV (PCV-7) into its EPI using a schedule of vaccination at 6 and 14 weeks, with a booster dose at 9 months. PCV-7 was replaced by the 13-valent PCV (PCV-13) in April 2011. PCV-13 is estimated to confer a further 20% protection against IPD compared to PCV-7[11]. The benefit of the introduction of PCV-7 and subsequently PCV-13 has been documented in South Africa using data from a nationwide laboratory-based IPD surveillance program[9,12]. PCV decreased the incidence of IPD by protecting both immunised and non-immunised persons through herd immunity, whereby persons that are non-immunised derive protection through residing in a highly vaccinated environment[8].

In a time-series analysis done at Chris Hani Baragwanath Academic Hospital (CHBAH), South Africa, there was a 4.7% reduction in the incidence of pneumococcal meningitis per annum prior to the inclusion of PCV into the EPI, which accelerated to an 18% reduction per annum post PCV introduction[8].

1.1.2 Hib meningitis

Haemophilus influenzae type b was the second commonest cause of meningitis in the pre-vaccination period. It is a Gram-negative coccobacillary, facultative anaerobe[13]. In the pre-vaccination era, Hib commonly affected children <5 years of age, with 59% of those infected developing meningitis[13]. In unvaccinated populations, Hib is the dominant cause

of non-epidemic bacterial meningitis in children <12 years age[13]. The organism commonly causes bacteraemia, pneumonia, and epiglottitis in unvaccinated communities[13].

Before the inclusion of Hib vaccine into vaccination programmes globally, Hib was responsible for at least 8.13 million cases of serious illness in children between the age of 1-5 years, causing an estimated 203,000 deaths in children <6 years[9]. Hib conjugate vaccine was introduced into the South African EPI in 1998. The absolute number of cases among children one year of age or younger decreased by 65% from 55 reported cases in 1999-2000, to 19 cases in 2003 and 2004[5].

1.2 Tuberculous meningitis and cryptococcal meningitis

Mycobacterium tuberculosis and *C. neoformans* account for a large burden of meningitis in children with immunodeficiency, including those with HIV.

Tuberculosis (TB) is caused by *M. tuberculosis*. It manifests primarily as pulmonary disease, however 20% of TB cases are due to extra-pulmonary tuberculosis (EPTB)[14]. EPTB is particularly common in young children and immunocompromised individuals. It is likely that early haematogenous spread to the brain occurs before a T-cell mediated immune response develops post primary exposure to *M. tuberculosis*. This mechanism could explain the vulnerability to TB meningitis (TBM) in conditions where T-cell mediated immunity is impaired, e.g. in persons infected with HIV and other immunocompromised persons.

The early clinical presentation of TBM is often non-specific, with symptoms such as cough, loss of weight, fever, vomiting and malaise. As the disease progresses, meningism, focal neurological signs, and a depressed level of consciousness can occur. The timing of initiation of anti-tuberculosis treatment is the most critical factor affecting morbidity, mortality, and healthcare costs, which emphasises the importance of early diagnosis of TBM. In a study of over 500 South African children with TBM, the majority (84%) presented with neurological manifestations of disease, including hearing loss, visual impairment, motor deficits and intellectual impairment[15].

Cryptococcal meningitis (CCM) is caused by *C. neoformans* and *C. gattii*. It commonly occurs in immunocompromised persons, but is an uncommon cause of meningitis even in older children and adolescents with untreated HIV infection. HIV infection is the main risk factor for CCM, accounting for 95% of cases in low- and middle-income countries (LMICs) and 80% of cases in high-income countries[16]. Other risk factors for CCM include bone marrow transplant, haematological malignancies, and receipt of immunosuppressive therapy including steroids. The outcomes can be devastating, with high mortality rates or severe morbidity such as irreversible blindness, deafness, and neurocognitive impairment[17].

1.3 Diagnosis of meningitis

Lumbar puncture with collection and analysis of cerebrospinal fluid (CSF) remains the gold standard for the diagnosis of meningitis. CSF is usually analysed for glucose (for comparison with a blood glucose level), total protein, cell count and differential, Gram stain and bacterial and fungal culture[18]. These investigations have incomplete sensitivity, especially if antibiotics have been administered prior to CSF collection, and further tests have been suggested for better identification of pathogens.

CSF lactate has been used to differentiate between viral and bacterial meningitis, and have been shown to be elevated in adult patients who subsequently die from TBM[18], but is rarely done as part of the routine clinical work-up. Latex agglutination assays have been recommended to further aid in detecting bacterial pathogens in CSF. These tests may be useful in patients that have received antimicrobial therapy prior to CSF collection. The Xpert-MTB/Rif assay has a good yield in suspected TBM[18], and the cryptococcal capsular polysaccharide antigen (CrAg) test together with fungal culture is useful in the diagnosis of CCM[18].

Molecular diagnostic techniques are useful in identifying CSF pathogens in children with meningitis in research studies, but are currently not widely used in clinical practice. In a study done in West Africa, a TaqMan Array Card (TAC) assay was used to identify the spectrum

of organisms causing meningitis in children[19]. The TAC assay increased the diagnostic yield and identified viruses, Gram negative bacteria and common meningeal pathogens as being important CSF pathogens in the era of access to vaccines which target pneumococcus, Hib, and meningococcus[19].

1.4 Treatment of meningitis

Successful treatment of meningitis to prevent morbidity and mortality depends on acting quickly based on clinical suspicion, instituting early empiric antimicrobial treatment, and directing management accordingly once culture results with antibiotic sensitivities are available. The definitive medical treatment of meningitis lies in treating the underlying causative agent using an antimicrobial agent to which it is susceptible. Many centres have developed treatment protocols, with striking similarities seen in them.

A standard treatment protocol published in South Africa's Western Cape Province recommends that empiric antibiotic treatment using drugs such as intravenous ceftriaxone at a dose of 50 mg/kg/dose 12 hourly, or 100 mg/kg/dose daily until CSF chemistry and culture are available, or until clinical improvement in patients who are culture negative but have neurological fallout[20]. In neonates, the use of cefotaxime and ampicillin are recommended to cover Group B streptococcus or *Listeria monocytogenes* meningitis.

TBM is treated using four drugs, namely rifampicin (RH), isoniazid (INH), pyrazinamide (PZA) and ethionamide for a period of six to nine months. High doses are used, for better CNS penetration. There is also addition of corticosteroids. CCM is managed using antifungal agents, including amphotericin B, flucytosine and fluconazole.

1.5 Justification for this Research Project

The last study to have evaluated the spectrum of meningitis pathogens in children hospitalised with meningitis at CHBAH, reviewed the time period from January 2006 to November 2011[8]. The proposed study aims to describe the bacterial and fungal pathogens isolated in CSF

samples of paediatric patients admitted to CHBAH from January 2012 through December 2020, and will thus give an updated evaluation of the meningeal pathogens that affect the childhood population served by the hospital.

1.6 Aim of the Study

The aim of the study is to describe the spectrum of meningitis pathogens in children hospitalised with meningitis at CHBAH.

1.7 Objectives of the Study

1. Appraisal of the certainty of diagnosis of bacterial or fungal meningitis:
   (a) Definite meningitis: children with bacterial or fungal pathogens detected on CSF specimens, either through culture or latex agglutination testing;
   (b) Clinically diagnosed meningitis: children whose CSF specimens yielded no growth, but who were discharged with a diagnosis of meningitis.
2. To evaluate and compare blood and urine culture, CSF parameters and HIV infection status among children with meningitis at CHBAH.
3. To assess clinical outcomes among children hospitalised with meningitis, as well as the outcomes associated with HIV co-infection.

The study hypothesis is that children with definite meningitis will constitute the minority of cases with a discharge diagnosis of meningitis over the study period, and that most cases of meningitis are diagnosed clinically, without microbiological confirmation. Furthermore, we anticipate that CSF parameters, blood culture and acute phase reactant measures, length of hospital stay, and clinical outcomes will be more deranged, longer, and more severe in children with definite meningitis compared to those with clinically diagnosed meningitis. We also hypothesise that children living with HIV (CLWH) will have worse outcomes than HIV-uninfected children.

2 ARTICLE IN SUBMISSIBLE FORMAT

Abstract

Introduction:

Meningitis is a leading cause of childhood morbidity and mortality, with an incidence of 7/100,000 children aged 1-4 years and 40/100,000 in infants in South Africa. Since the incorporation of protein-polysaccharide vaccines to prevent infections due to *Haemophilus influenzae* type b and pneumococcus into the South African immunisation programme, the epidemiology of meningitis in South Africa is changing.

Methods:

We reviewed the clinical and laboratory characteristics, and clinical outcomes of children aged 0 to 14 years of age that were hospitalised with meningitis in the general paediatric wards at a public sector teaching hospital in South Africa from 01 January 2012 through 31 December 2020.

Results:

During the study period, 1,617 episodes of meningitis were diagnosed in 1,597 children. Median age at hospitalisation was 6.0 months (interquartile range (IQR), 1.2 to 38.6 months). Most (1,243/1,617, 78.9%) of the episodes occurred in HIV-uninfected children, 182 (11.2%) in HIV-infected children and 192 (11.8%) in children with undetermined HIV infection status. Two hundred, fifty-four (15.7%) episodes were microbiologically confirmed, and 1,268 (78.4%) were clinically diagnosed with no microbiological confirmation. Coagulase negative staphylococci (n=77), *Streptococcus pyogenes* (n=76) and pneumococcus (n=54) were the most frequently isolated organisms. The crude mortality rate was 8.5% (137/1,617). Using three multivariable logistic regression modelling approaches, HIV infection was consistently associated with mortality (adjusted odds ratios ranging from 3.658 (95% confidence interval (CI), 1.645-8.133) to 4.070 (95% CI, 1.474-11.235)). A composite measure of low cerebrospinal fluid (CSF) to plasma glucose ratio, raised CSF protein and raised C-reactive protein was

independently associated with a 3.413 (95% CI, 1.88-6.196) increased adjusted odds of death.

Conclusions:

Most cases of meningitis are clinically diagnosed, and when microbiologically confirmed are caused predominantly by Gram positive organisms. HIV infection is consistently associated with worse outcomes. Biochemical parameters may be useful to prognosticate in children diagnosed with meningitis.

Introduction

Meningitis, inflammation of the meninges and brain due to infection with bacterial or fungal pathogens[1], is a leading cause of childhood morbidity and mortality. In South Africa, the incidence of meningitis is 4/100,000 in the general population, 40/100,000 in infants <1 year and 7/100,000 in children aged 1-4 years[2].

The specific infectious aetiology of meningitis is generally age dependent. Organisms that cause meningitis usually arise from the mucosal surfaces of the upper respiratory tract or the blood stream and infiltrate the meninges through invasion of the blood brain barrier. In the neonatal period (age 0 to 28 days) the commonest organisms are *Streptococcus agalactiae*, *Listeria monocytogenes* and *Escherichia coli*[3]. In infants aged three months and older and children, the commonest bacterial organisms are *Neisseria meningitidis* (meningococcus), *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae* type b (Hib)[4]. In the context of human immunodeficiency virus type-1 (HIV) infection, important meningeal pathogens in addition to those mentioned above include *Mycobacterium tuberculosis* and *Cryptococcus neoformans*.

South Africa introduced protein-polysaccharide vaccines targeting Hib and pneumococcus in 1998 and 2009, respectively[5,6]. As a consequence of the introduction of these vaccines, the epidemiology of bacterial meningitis in young children may be changing. While vaccines are available to prevent meningococcal meningitis[7], these have not been incorporated into the South African National Immunization Programme.

If not treated appropriately, bacterial and fungal meningitis can cause serious complications such as seizures, hearing loss, cortical blindness, cerebral oedema, brain damage and death. A quarter of patients who acquired meningitis in childhood stand a chance of developing epilepsy, intellectual impairment, or sensorineural deafness[21].

The gold standard for diagnosis of meningitis is by isolating pathogens in cerebrospinal fluid (CSF) culture[4]. Additionally, CSF biochemistry profiles may support a diagnosis of meningitis[18]. These investigations have incomplete sensitivity, especially if antibiotics have

been administered prior to CSF collection, because CSF sterilisation occurs rapidly after administration of antimicrobial therapy. Clearance of *N. meningitidis*, *S. pnemoniae* and *S. agalactiae* from the CSF is within 2, 4, and 8 hours respectively[4].

There have been significant reductions in the burden of pneumococcal and Hib meningitis in South African children since the incorporation of protein-polysaccharide pneumococcal vaccine (PCV) and Hib vaccine were introduced into the Expanded Programme of Immunisation (EPI). In this study, we describe the spectrum of bacterial and fungal pathogens identified in children hospitalised with meningitis at an academic public sector hospital in Johannesburg, South Africa.

Methods

We conducted a retrospective, observational study on meningeal pathogens in children <14 years admitted to the paediatric wards at Chris Hani Baragwanath Academic Hospital (CHBAH) from January 2012 to December 2020. CHBAH is a large secondary/tertiary level hospital in Gauteng Province, with approximately 5,000 general paediatric admissions annually. Children with meningitis are managed in the general paediatric wards, with clinical input from Paediatric Neurology, Paediatric Infectious Disease and Clinical Microbiology subspecialty units. Soweto primary health care clinics manage paediatric emergency cases according to Integrated Management of Childhood Illness (IMCI) guidelines[22], which recommends that children with suspected meningitis be administered a stat dose of intravenous or intramuscular ceftriaxone prior to referral to higher levels of care. Prior antibiotic therapy contributes to lower yield of CSF culture in suspected meningitis cases that are referred on for hospital care[23].

Children were eligible for inclusion in the study if they were aged 0 to 14 years, had a hospital discharge diagnosis of meningitis and were treated in the general paediatric units at the hospital during the study time period (01 January 2012 through 31 December 2020). Admission CSF, blood culture and urine culture specimens were be evaluated. Children with

missing CSF laboratory results were not included in the analysis.

Data obtained from the National Health Laboratory System (NHLS) database (for CSF biochemistry and culture, blood culture, urine culture, full blood count, C-reactive protein (CRP), and blood glucose results). For children living with HIV (CLWH), CD4 counts and HIV viral loads were obtained to establish the immunological and virologic status at the time of the meningitis episode. Date of ART initiation was obtained from the database of a large outpatient paediatric antiretroviral therapy (ART) Clinic treating children at CHBAH. Data were entered into an Excel database, prior to analysis.

A status of definite meningitis was assigned when bacterial or fungal pathogens were detected on CSF specimens, either through culture or latex agglutination testing. Clinical meningitis was assigned when CSF specimens remained negative in children with a discharge diagnosis of meningitis.

Statistical analysis

Characteristics of children with definite and clinically diagnosed meningitis were compared. Categorical variables are presented as percentages, and group comparisons were made using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were evaluated for normality, and means of normally distributed data was compared using the Student t-test. Medians of skewed continuous data was compared using the Kruskal-Wallis test. Factors associated with certainty of the meningitis diagnosis, and factors associated with in-hospital mortality were explored using logistic regression. In all analyses, two-sided P-values of <0.05 were considered significant.

Time-series analyses were conducted to evaluate the trend in prevalence of definite meningitis and suspected meningitis cases over the study time period; time series analysis evaluated trends in definite meningitis, stratified by pathogen type.

All statistical analyses were conducted using R version 4.4.0[24].

Ethical considerations

Ethical clearance for conduct of this study was obtained through the University of the Witwatersrand Human Research Ethics Committee (Medical) (clearance number M210970), and the Ethics Committee of the NHLS. As this was a retrospective study, a waiver of informed consent was applied for and granted by the respective Ethics Committees.

Results

From 03 January 2012 to 31 December 2020, 1617 episodes of meningitis were diagnosed in 1597 children and adolescents that were hospitalised at the general paediatric wards at CHBAH. Most (n=1243; 76.9%) meningitis episodes occurred in HIV uninfected children, 182 (11.3%) occurred in CLWH, and 192 (11.9%) occurred in children with unknown HIV status (Figure 2.1). The median age (in months) of the children and adolescents diagnosed with meningitis was 6.0 (IQR, 1.2 to 38.6 months) (Table 2.1).

The majority were clinically diagnosed (1268/1617, 78.4%). Neonates comprised almost half of the children with definite meningitis (Table 2.1). Children with definite meningitis had higher CRPs and CSF protein copared to those with clinically diagnosed meningitis, and those with possible CSF contaminants (Table 2.1). Furthermore, children with definite meningitis had low lymphocyte and monocyte counts on haematologic analysis, and lower CSF-to-blood glucose ratios (Table 2.1). Crude mortality rates were significantly higher in the definite meningitis group (Table 2.1).

In analysis of all age groups, there was a significant reduction in the burden of meningitis hospitalisations between March 2014 (46 of 661 hospitalisations; 7.0% (95%CI 5.1-9.2%)) and December 2020 (11 of 508 hospitalisations; 2.2% (95%CI 1.1-3.8%)); Odds Ratio 0.311 (95%CI, 0.144-0.618), P-value<0.001 (Supplementary Figure 3.1). Overall, there were trends towards lower rates of meningitis hospitalisations from mid-2016 onwards, which was also appreciable in HIV-uninfected children and those with undetermined HIV infection status (Figure 2.1).

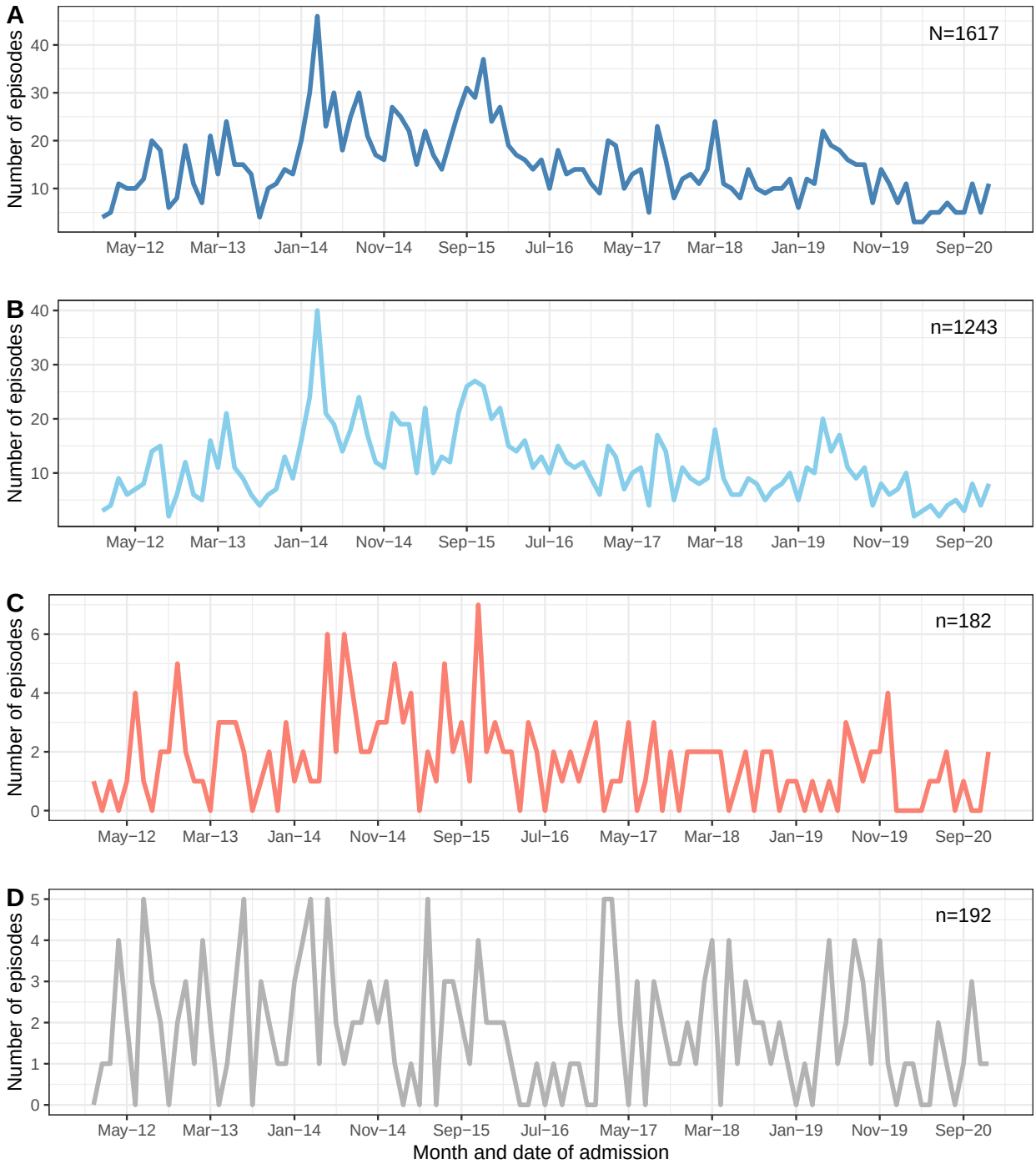


Figure 2.1: Timeseries plots of number of meningitis episodes per month during the study time period

A = All cases; B = HIV negative cases; C = HIV positive cases;
D = Cases with undetermined HIV status.

Table 2.1: Comparison of clinical and laboratory indicators, stratified by certainty of meningitis diagnosis

	Overall	Clinically diagnosed	Possible CSF contaminant	Definite meningitis	p
n	1617	1268	95	254	
Female (%)	694 (43.2)	541 (42.9)	41 (43.6)	112 (44.3)	0.923
Age category (%)					<0.001
Neonate	353 (21.8)	210 (16.6)	25 (26.3)	118 (46.5)	
Infant	588 (36.4)	447 (35.3)	42 (44.2)	99 (39.0)	
Preschool	388 (24.0)	351 (27.7)	15 (15.8)	22 (8.7)	
Older children	288 (17.8)	260 (20.5)	13 (13.7)	15 (5.9)	
Median age (months) [IQR]	6.03 [1.17, 38.56]	9.89 [1.69, 46.49]	3.16 [0.92, 17.88]	1.16 [0.39, 4.66]	<0.001
Median Weight-for-age Z-score [IQR]	-1.21 [-2.39, -0.22]	-1.23 [-2.40, -0.18]	-0.95 [-2.22, -0.37]	-1.26 [-2.39, -0.49]	0.673
Median Height-for-age Z-score [IQR]	-1.41 [-2.83, -0.12]	-1.37 [-2.81, -0.11]	-1.29 [-2.33, 0.00]	-1.69 [-2.88, -0.33]	0.494
Median Weight-for-height Z-score [IQR]	-0.40 [-1.67, 0.76]	-0.44 [-1.74, 0.79]	-0.61 [-1.80, 0.54]	-0.15 [-1.26, 0.69]	0.491
Median BMI Z-score [IQR]	-0.65 [-1.88, 0.42]	-0.65 [-1.91, 0.50]	-0.77 [-1.80, 0.35]	-0.62 [-1.70, 0.26]	0.932
HIV Status (%)					0.003
Negative	1243 (76.9)	966 (76.2)	78 (82.1)	199 (78.3)	
Positive	182 (11.3)	161 (12.7)	2 (2.1)	19 (7.5)	
Unknown	192 (11.9)	141 (11.1)	15 (15.8)	36 (14.2)	
White cell count	11.45 [8.36, 15.75]	11.47 [8.44, 15.76]	11.62 [9.02, 14.76]	11.22 [7.53, 16.25]	0.597
Haemoglobin	11.50 [9.90, 13.10]	11.50 [10.00, 12.90]	11.70 [10.30, 13.30]	11.20 [9.60, 13.80]	0.589
MCV	82.10 [75.40, 91.50]	80.80 [74.70, 89.40]	85.80 [76.75, 93.80]	89.50 [79.60, 97.50]	<0.001
Platelet count	356.00 [258.00, 455.00]	360.00 [266.50, 455.00]	340.00 [269.50, 443.00]	325.00 [217.00, 455.50]	0.136
Lymphocytes	4.05 [2.37, 6.34]	4.15 [2.55, 6.38]	4.30 [2.58, 6.84]	3.47 [1.80, 5.88]	0.010
Neutrophils	5.18 [2.86, 9.84]	5.24 [2.98, 10.12]	4.63 [2.84, 7.98]	5.15 [2.54, 9.53]	0.240
Monocytes	1.22 [0.74, 1.98]	1.23 [0.78, 1.98]	1.31 [0.86, 1.78]	1.09 [0.55, 2.01]	0.018
CRP	19.00 [4.00, 77.00]	17.00 [4.00, 63.98]	6.00 [1.00, 29.00]	57.00 [6.00, 177.00]	<0.001
CD4 count	456.00 [180.00, 816.00]	421.00 [171.00, 801.00]	1204.00 [962.00, 1446.00]	534.00 [259.75, 865.75]	0.311
Log10 HIV viral load	5.20 [4.10, 6.04]	5.20 [3.99, 6.04]	6.52 [6.32, 6.71]	4.80 [4.56, 5.24]	0.226
Blood glucose	5.40 [4.70, 6.40]	5.50 [4.70, 6.40]	5.00 [4.55, 5.50]	5.40 [4.35, 6.40]	0.076
CSF glucose	3.10 [2.40, 3.90]	3.20 [2.60, 3.90]	3.30 [2.60, 4.10]	2.45 [1.40, 3.30]	<0.001
CSF-to-Blood glucose ratio	0.58 [0.43, 0.70]	0.59 [0.48, 0.71]	0.64 [0.49, 0.78]	0.43 [0.21, 0.63]	<0.001
CSF protein	0.60 [0.30, 1.40]	0.49 [0.26, 1.02]	0.71 [0.29, 1.08]	1.52 [0.61, 2.72]	<0.001
CSF ADA	1.10 [0.50, 4.00]	1.00 [0.40, 3.20]	2.30 [0.95, 3.72]	1.90 [1.00, 12.60]	0.072
Died (%)	137 (8.5)	92 (7.3)	3 (3.2)	42 (16.5)	<0.001

Note:
CRP = C-reactive protein; BMI = body mass index; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; IQR = interquartile range. P-values derived using the Kruskal-Wallis test for skewed continuous data.

Table 2.2 illustrates the characteristics of the study participants, stratified by HIV infection status. CLWH tended to be older at the time of their meningitis admission (median age 42.8 months, compared to 5.1 months in HIV-uninfected children), were significantly underweight for age, and were more likely to have clinically diagnosed meningitis (161/182, 88.5%). Most of the laboratory parameters were significantly different in CLWH, including the CRP (median 41 mg/L in CLWH, compared to 15 mg/L in HIV-uninfected children) (Table 2.2). Crude mortality rates were significantly higher in CLWH (38/182, 20.9%) compared to HIV-uninfecteed children and those with unknown HIV status (Table 2.2).

Receiver operating characteristic (ROC) analyses of blood and CSF parameters to distinguish between definite and clinically dagnosed meningitis cases indicated that the best area under the curve (AUC) was for CSF protein (0.738), while other commonly utilised biomarkers - CSF-to-blood glucose ratio, and CRP - had AUC of 0.666 and 0.608, respectively (Table 2.3). Laboratory cut-offs for CSF protein, CSF-to-blood ratio and CRP which discriminated best between definite and clinically-diagnosed meningitis were 0.81 g/L, 0.353 and 90 mg/L, respectively (Table 2.3).

Among 746 children with available CSF protein, CSF-to-blood glucose ratio and CRP results, children with at least one of these laboratory indices in the "abnormal" range were significantly younger than those in whom none of these indicators was abnormal (2.39 vs 7.61 months), and were more likely to have a diagnosis of definite meningitis (32.4% vs 11.1%) (Supplementary Table 3.1). Crude mortality rates were also significantly greater in the group of children with at least one abnormal laboratory result (11.8% vs 1.7%) (Supplementary Table 3.1). Death occurred in seven (21.9%) of the 32 children with all three laboratory indices in the "abnormal" range, and almost two thirds of them (20/32, 62.5%) had definite meningitis (Supplementary Table 3.2).

Table 2.2: Comparison of clinical and laboratory indicators, stratified by HIV status

	HIV Negative	HIV Positive	HIV status unknown	p
n	1243	182	192	
Female (%)	538 (43.6)	83 (45.6)	73 (38.4)	0.322
Age category (%)				<0.001
Neonate	289 (23.3)	14 (7.7)	50 (26.0)	
Infant	468 (37.7)	45 (24.7)	75 (39.1)	
Preschool	304 (24.5)	47 (25.8)	37 (19.3)	
Older children	182 (14.6)	76 (41.8)	30 (15.6)	
Median age (months) [IQR]	5.13 [1.10, 29.40]	42.76 [7.09, 110.92]	4.65 [0.82, 27.90]	<0.001
Median Weight-for-age Z-score [IQR]	-1.15 [-2.27, -0.20]	-2.22 [-3.11, -1.06]	-0.93 [-1.81, -0.03]	<0.001
Median Height-for-age Z-score [IQR]	-1.33 [-2.72, -0.06]	-2.09 [-3.56, -0.55]	-1.32 [-2.39, -0.01]	0.007
Median Weight-for-height Z-score [IQR]	-0.41 [-1.65, 0.71]	-0.84 [-2.34, 0.31]	0.48 [-1.10, 1.47]	0.004
Median BMI Z-score [IQR]	-0.62 [-1.86, 0.41]	-1.26 [-2.59, -0.11]	-0.06 [-1.38, 0.99]	0.001
Meningitis classification (%)				0.003
Clinically diagnosed	966 (77.7)	161 (88.5)	141 (73.4)	
Possible contaminant	78 (6.3)	2 (1.1)	15 (7.8)	
Definite	199 (16.0)	19 (10.4)	36 (18.8)	
White cell count	11.58 [8.53, 15.77]	9.56 [6.21, 14.73]	11.88 [8.91, 15.91]	<0.001
Haemoglobin	11.60 [10.10, 13.20]	10.50 [8.70, 12.22]	11.60 [10.20, 13.20]	<0.001
MCV	82.60 [75.50, 91.70]	79.55 [73.80, 87.57]	82.95 [77.60, 93.55]	0.002
Platelet count	363.00 [269.00, 455.00]	300.00 [181.00, 443.25]	359.50 [254.25, 462.50]	<0.001
Lymphocytes	4.22 [2.59, 6.60]	2.66 [1.49, 4.40]	4.30 [2.38, 6.24]	<0.001
Neutrophils	5.24 [2.93, 9.55]	4.73 [2.40, 10.02]	5.25 [2.97, 11.47]	0.387
Monocytes	1.24 [0.75, 1.98]	0.94 [0.60, 1.59]	1.31 [0.96, 2.16]	<0.001
CRP	15.00 [3.00, 66.00]	41.00 [8.65, 98.75]	23.00 [3.00, 102.00]	0.005
CD4 count	-	456.00 [180.00, 816.00]	-	-
Log10 HIV viral load	-	5.20 [4.10, 6.04]	-	-
Blood glucose	5.40 [4.70, 6.30]	5.68 [4.80, 6.75]	5.50 [4.60, 6.25]	0.238
CSF glucose	3.10 [2.40, 3.80]	2.90 [2.30, 3.90]	3.30 [2.68, 4.03]	0.398
CSF-to-Blood glucose ratio	0.58 [0.44, 0.71]	0.51 [0.36, 0.69]	0.59 [0.45, 0.68]	0.071
CSF protein	0.62 [0.30, 1.45]	0.49 [0.29, 1.48]	0.55 [0.30, 1.04]	0.527
CSF ADA	1.10 [0.40, 4.45]	1.10 [0.83, 3.20]	1.00 [0.50, 3.90]	0.899
Died (%)	81 (6.5)	38 (20.9)	18 (9.4)	<0.001

Note:
CRP = C-reactive protein; BMI = body mass index; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; IQR = interquartile range. P-values derived using the Kruskal-Wallis test for skewed continuous data.

Table 2.3: ROC analysis of laboratory indications, comparing clinically diagnosed and definite meningitis cases

Predictor	Optimal cutpoint	Youden	Accuracy	Sensitivity	Specificity	AUC
CSF Lymphocyte count	2.000	0.160	0.625	0.686	0.473	0.546
CSF Neutrophil count	39.000	0.277	0.707	0.479	0.798	0.621
CSF Neut-to-lymph ratio	2.333	0.310	0.669	0.624	0.686	0.652
Total CSF white cell count	Inf	0.000	0.833	0.000	1.000	0.500
CSF Chloride	119.000	0.314	0.759	0.805	0.509	0.628
CSF protein	0.810	0.370	0.693	0.670	0.700	0.738
CSF glucose	2.400	0.320	0.760	0.831	0.489	0.697
CSF ADA	1.600	0.276	0.636	0.640	0.636	0.636
CSF-to-Blood glucose ratio	0.353	0.320	0.791	0.894	0.426	0.666
WCC	5.850	0.096	0.761	0.894	0.202	0.521
Lymph	1.490	0.118	0.761	0.901	0.217	0.563
Neut	3.090	0.068	0.655	0.733	0.335	0.519
Blood Neut-to-lymph ratio	0.574	0.064	0.365	0.814	0.250	0.531
CRP	90.000	0.244	0.739	0.423	0.821	0.608

In 105 children with definite meningitis and available CSF protein and CSF-to-serum glucose ratio results, 57 (54.3%) had high CSF protein and low CSF-to-serum glucose ratios (Supplementary Table 3.3 and Supplementary Figure 3.2). In contrast only 40 (10.1%) of 397 clinically diagnosed cases and 3 (11.1%) of 27 with possible CSF contaminants had high CSF protein and low CSF-to-serum glucose ratios.

The most commonly isolated organisms were *Streptococcus agalactiae*, *Streptococcus pneumoniae* and coagulase negative staphylococcus (CNS) (Figure 2.2). This pattern of microbiological isolates was seen in HIV-uninfected children, and children with unknown HIV infection status (Figure 2.2). Pneumococcus was the most frequently isolated pathogen among CLWH (Figure 2.2). Most of the CNS isolates were cultured in children that were designated as having CSF contamination (Supplementary Figure 3.3).

Children who died during their hospitalisation had significantly lower weight-for-age Z-scores (-1.99; 95% CI, -2.84 to -1.28) compared to those who survived (-1.22; 95% CI, -2.40 to -0.29); P=0.004 (Supplementary Table 3.4). They were also more likely to be HIV-infected (34.0% vs 10.0%), to have higher CRP results (69.0 mg/L vs 12.0 mg/L), and to have lower CSF glucose, lower CSF-to-blood glucose levels, and higher CSF protein levels compared to

those who survived (Supplementary Table 3.3).

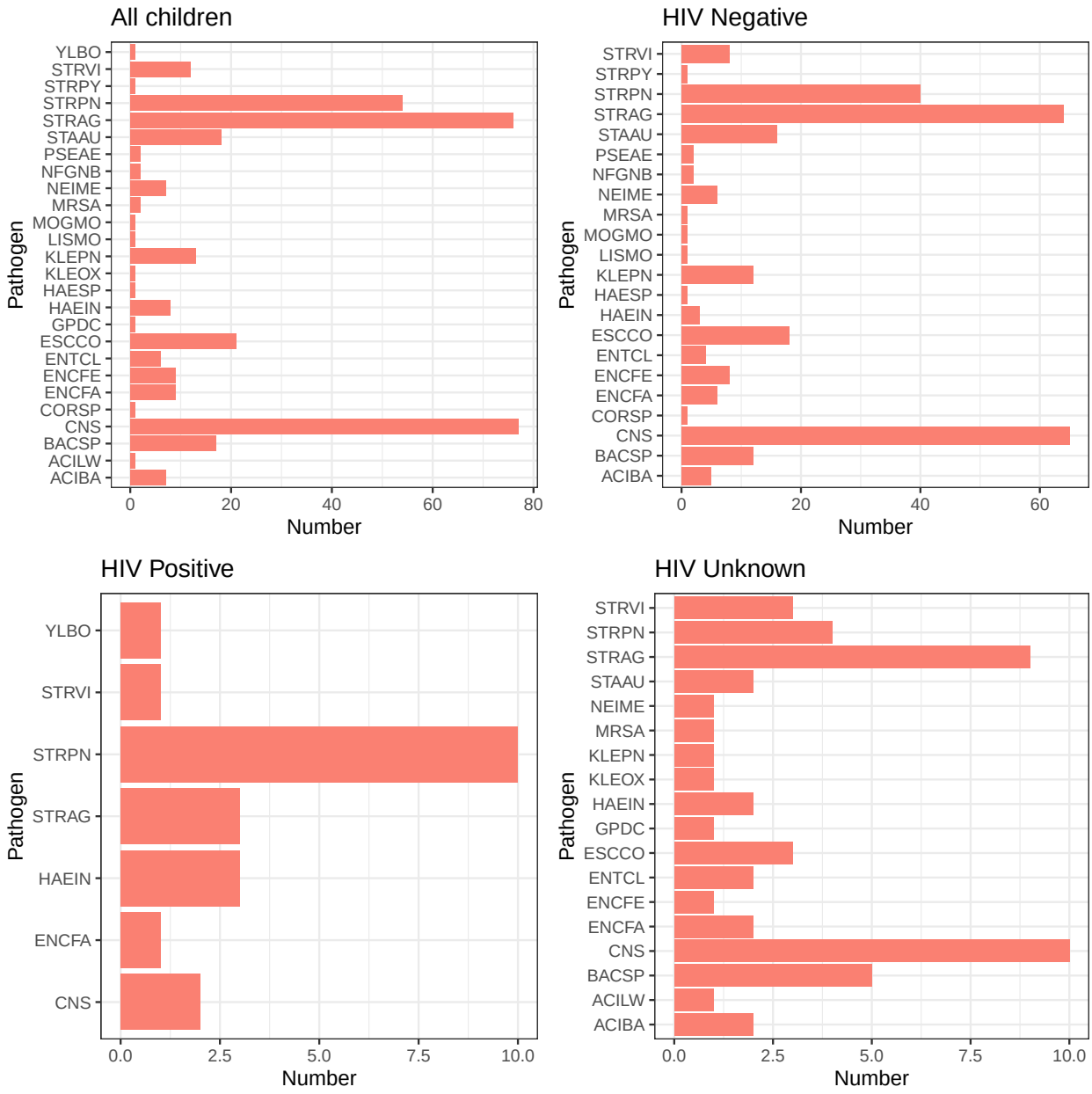


Figure 2.2: First CSF isolate organisms in children with culture-positive specimens, stratified by HIV status

CNS = Coagulase-negative staphylococcus; ENCFA = *E. faecium*; HAEIN = *H. influenzae*; STAAU = *S. aureus*; STRAG = *S. agalactiae*; STRPN = *S. pneumoniae*; STRVI = Viridans streptococci; YLBO = Yeast-like bodies; Please refer to Supplementary Materials for organism identification of less prevalent pathogens.

Being HIV-infected was consistently associated with death, with adjusted Odds Ratios ranging from 4.07 (95% CI, 1.47 to 11.24) to 4.92 (95% CI, 2.01 to 12.03) (Table 2.4). Children with a combined composite measure of low CSF-to-serum glucose, elevated CSF protein and elevated CRP had a 3.50-fold (95% CI, 1.86 to 6.56) greater adjusted odds of death (Table 2.4).

Table 2.4: Univariate and multivariate logistic regression outputs for factors associated with death

	Univariate Analyses				Multivariate Model 1				Multivariable Model 2				Multivariable Model 3			
Variable	Estimate	Lower	Upper	p	Estimate	Lower	Upper	p	Estimate	Lower	Upper	p	Estimate	Lower	Upper	p
Sex	1.042	0.731	1.486	0.819												
1 to 11 months	1.271	0.786	2.055	0.327												
12 to 59 months	0.903	0.519	1.573	0.718												
>= 60 months	1.249	0.714	2.183	0.435												
Age (months)	1.001	0.997	1.005	0.522												
Wt-for-age Z-score	0.831	0.748	0.924	0.001	1.174	0.215	6.425	0.853	2.489	0.654	9.477	0.181	1.77	0.443	7.068	0.419
Ht-for-age Z-score	0.952	0.884	1.026	0.188												
Wt-for-ht Z-score	0.909	0.801	1.033	0.143												
BMI Z-score	0.885	0.784	1.000	0.052												
HIV Positive	3.785	2.478	5.780	0.000	4.07	1.474	11.235	0.007	4.915	2.009	12.028	0	4.466	1.825	10.928	0.001
HIV Unknown	1.484	0.869	2.535	0.148	0.872	0.688	1.105	0.256	0.855	0.702	1.042	0.121	0.849	0.693	1.041	0.116
WCC	0.953	0.922	0.985	0.004												
Hb	0.841	0.778	0.910	0.000	0.917	0.778	1.08	0.297	0.847	0.736	1.000	0.02	0.848	0.735	0.978	0.023
MCV	1.008	0.992	1.024	0.307												
Plt	0.996	0.994	0.998	0.000	0.999	0.996	1.001	0.322	0.998	0.996	1.000	0.138	0.999	0.996	1.001	0.205
Lymph	0.826	0.758	0.901	0.000	0.971	0.861	1.095	0.63	0.966	0.874	1.067	0.492	0.972	0.878	1.075	0.574
Neut	0.998	0.982	1.014	0.758												
Mono	0.867	0.763	0.985	0.029												
CRP	1.007	1.005	1.009	0.000	1.006	1.002	1.01	0.007								
CD4 count	0.999	0.999	0.999	0.121												
Log10 HIVVL	1.539	1.022	2.318	0.039												
Blood glucose	1.197	1.090	1.315	0.000												
CSF glucose	1.138	1.004	1.290	0.045												
CSF-to-blood glucose ratio	0.069	0.019	0.252	0.000	0.696	0.177	2.729	0.603								
CSF protein	1.486	1.347	1.639	0.000	1.3	1.078	1.567	0.006								
CSF ADA	1.036	0.982	1.092	0.200												
Composite measure	7.599	3.356	17.207	0.000					6.065	2.33	15.787	<0.001				
Combined composite measure	4.195	2.560	6.875	0.000									3.497	1.863	6.563	<0.001

Note:
ADA = adenosine deaminase; BMI = body mass index; CRP = C-reactive protein; CSF = cerebrospinal fluid; CSF = cerebrospinal fluid; Hb = haemoglobin; HIV = human immunodeficiency virus; HIVVL = HIV viral load; Lymph = lymphocytes; MCV = mean cell volume; Mono = monocytes; Neut = neutrophils; Plt = platelets; WCC = white cell count. Composite measure defined as CSF-to-blood glucose ratio less than 0.353, and/or CSF protein greater than 0.810 g/L, and/or CRP greater than 90 mg/L. Combined composite measure assigned if all three components of the composite measure were present in the same patient. Multivariable model 1 = 328 degrees of freedom, Akaike Information Criterion (AIC) = 174.82; Multivariable model 2 = 549 degrees of freedom, AIC = 288.23; Multivariable model 3 = 531 degrees of freedom, AIC = 255.13.

Discussion

Meningitis remains an important health concern in children, causing significant morbidity and mortality despite new developments in treatment and prevention. The introduction of vaccines has caused a shift in organisms commonly responsible for bacterial meningitis in the paediatric population. There has been a decline in the number of cases caused by *Streptococcus pneumoniae* and Hib. Other organisms, including *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli*, are also important contributors to the burden of childhood meningitis. In this analysis of bacterial meningitis admissions over a 9-year period at an academic hospital in Soweto, South Africa, the predominant organisms associated with meningitis included pneumococcus, Group B streptococcus (GBS) and coagulase negative staphylococcus (CNS).

Many of the meningitis cases attributable to CNS may have been due to CSF contamination through inoculation of skin commensals into the specimen at the time of specimen collection, and were assigned as being "possible CSF contaminant" cases rather than "definite meningitis" in our analysis. CSF biochemical parameters and serum CRP levels were significantly lower in the "possible CSF contaminant" group compared to the "definite meningitis" group, and their mortality rate was lower further indicating that these isolates may have been CSF contaminants rather than true pathogens.

Most of the children that were hospitalised with a diagnosis of meningitis had "clinically diagnosed" meningitis, without microbiological confirmation. While a proportion of these cases may have sterilised CSF, following administration of antibiotics prior to referral to hospital, or in hospital casualty prior to referral for admission, a proportion of these cases may have represented children without meningitis. This indicates that clinicians may have a low threshold to diagnose and treat suspected meningitis in children for fear of the severe morbidity and mortality rates associated with untreated CNS infection. Concerning in this regard is the fact that many children may be over-treated for meningitis, and that antibiotic therapy may be unjustifiably over-used which impacts on the accumulation of antibiotic

resistance.

Although there were some similarities between the groups, those with "definite meningitis" had marked derangement in microbiological markers as evidenced by elevated serum CRP and CSF protein, and low CSF-to-blood glucose ratios. The triad of elevated CRP, elevated CSF protein and low CSF-to-blood glucose was associated with an almost 4-fold increased adjusted odds of death. Biochemical parameters in the "clinically diagnosed" group were normal or minimally deranged, further supporting our premise that many of these cases may not in fact have had meningitis. Alamarat et al[4] described features of bacterial meningitis as the presence of polymorphonuclear pleocytosis, hypoglycorrachia (low CSF glucose) and elevated CSF protein. Bacterial meningitis was highly associated with neurological morbidity and mortality, despite vaccinations and other preventative measures[4].

HIV infection was consistently associated with an increased adjusted odds of death. A diagnosis of bacterial meningitis in HIV positive children was associated with high mortality and high rates of recurrent illness in a double blind randomised study in Kenya, conducted in the pre-ART era[25].

As untreated meningitis has high rates of morbidity and mortality[26], most children in whom clinicians consider a diagnosis of the condition are administered antibiotic therapy. In the era of widening resistance to antimicrobial therapy, a more cautious approach to antimicrobial prescribing is warranted. Clinicians should consider the biochemical and microbiological results within the first 72 hours of management, and stop unnecessary antimicrobial therapy if baseline tests indicate a low probability of active CNS infection. In a study done in the UK, empirical antibiotic use for a period of at least 48 to 72 hours is recommended for the management of suspected cases of meningitis with discontinuation of therapy if there is no microbiological growth and clinical improvement[27].

Study limitations

This study has limitations. The retrospective, observational design of the study may have given rise to gaps in available data; however this would probably not have affected the cohort in a systematic manner. Clinical data relating to antibiotic therapies prescribed, duration of therapy and changes to antibiotic regimens were not available. Furthermore, underlying co-morbidities, other than HIV infection, were not available to us. A strength of the study lies in the large sample size and extended time period. Few other studies on paediatric meningitis include over 1,000 patients[28].

Conclusions

In our setting, the majority of the suspected cases of meningitis are clinically diagnosed without any laboratory correlation.

Streptococcus pneumoniae persists as an important cause of bacterial meningitis, despite improvements in our EPI schedule.

CLWH presented with severe disease, cultured unusual organisms like fungi and had high mortality rates.

3 CONCLUDING CHAPTER

The aim of the study was to investigate the characteristics of children with a discharge diagnosis of meningitis, hospitalised at an academic hospital in Johannesburg, South Africa, and their laboratory results. The minority of cases (254/1,617, 15.7%) were microbiologically confirmed. Coagulase-negative staphylococcus (CNS), *Streptococcus agalactiae*, and pneumococcus were the most frequently isolated species, with the majority of CNS isolates representing likely contamination.

Through this study, we underline the pivotal role of laboratory investigations in facilitating the accurate diagnosis of meningitis. An accurate diagnosis is instrumental in guiding antimicrobial therapy, selection of empiric therapy, and determining treatment duration for patients.

This study holds practical implications, particularly in instances where microbiological evidence of meningitis is lacking, prompting clinicians to explore alternative diagnoses, to reconsider the clinical diagnosis of meningitis where necessary, and to rationalise the use of antibiotic therapy in the management of children with CSF parameters (including a negative culture) suggestive of alternative diagnoses. Given the observed high rate of specimen contamination, this study underscores the importance of employing sterile technique during procedural CSF specimen collection.

Children with a combined composite measure of elevated CSF protein, low CSF-to-serum glucose ratio, and elevated CRP had a 3.5-fold higher odds of death compared to children without those features. This triad of biomarkers may assist in prognosticating disease severity in children with suspected meningeal infection. Furthermore, CLWH had consistently greater adjusted odds of death compared to HIV-uninfected children. Expedited access to antiretroviral therapy, in order to preserve immune function in CLWH, would be anticipated to prevent meningitis in these vulnerable children.

Future research should be done to explore the empiric and targeted antibiotic choices utilised in suspected cases of meningitis admitted to CHBAH paediatric ward. Analysis of

the timing of initial antibiotic administration in relation to the first CSF specimen collection, which can influence CSF culture positivity, could provide valuable insights. Additionally, the impact of antibiotic receipt prior to transfer to hospital on CSF culture yields would be important. Studies interrogating antimicrobial usage would best be undertaken using prospective experimental designs. Documentation of complications occurring in patients with microbiologically-confirmed meningitis would further enhance our understanding of the disease's clinical course.

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Appendix 1

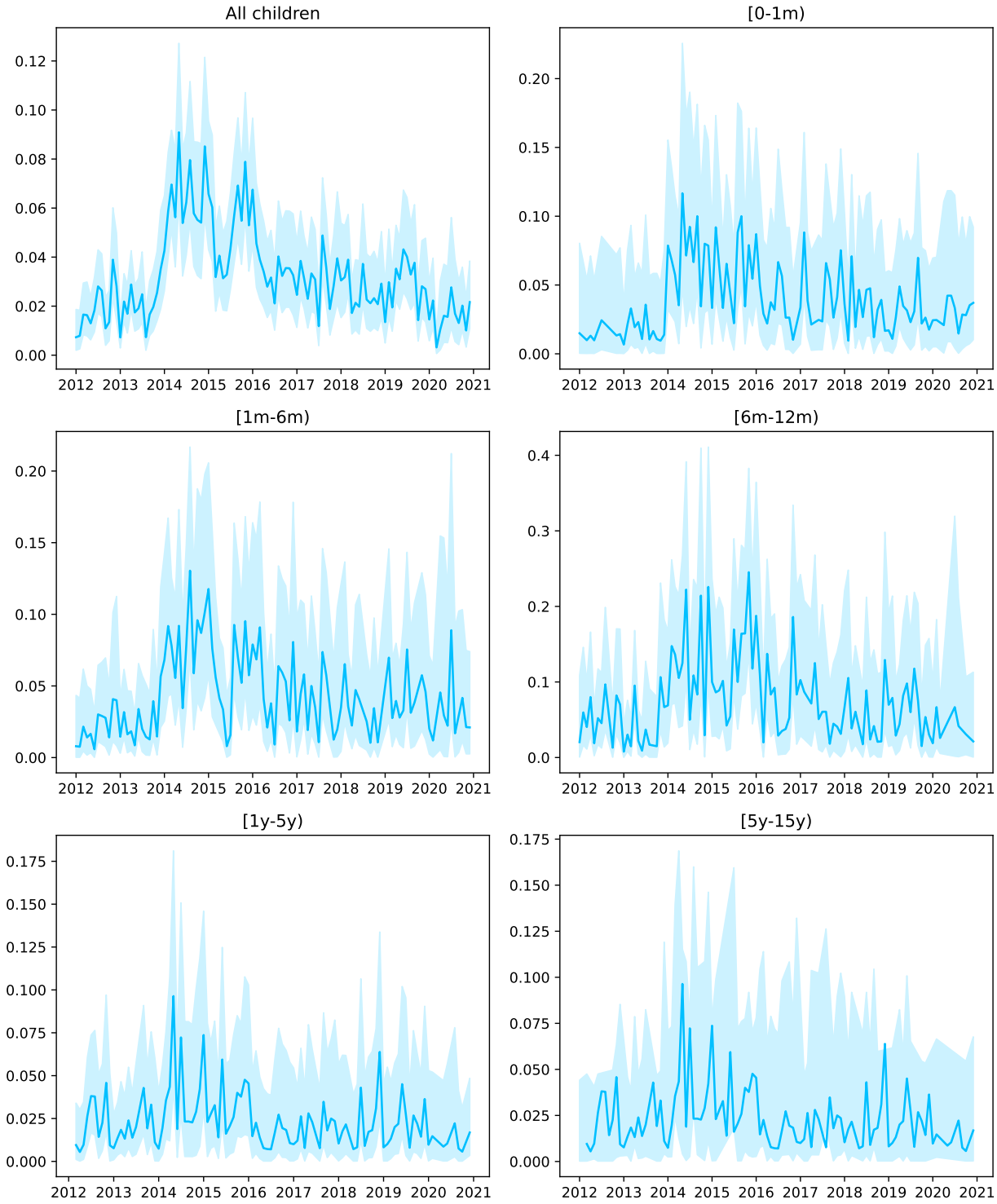


Figure 3.1: Time series plots of proportion of all paediatric admissions diagnosed with meningitis at the general paediatric wards, CHBAH 2012 to 2021 - stratified by age group

X-axis = year; Y-axis = proportion with exact binomial 95% confidence interval.

Table 3.1: Comparison of clinical and laboratory indicators, stratified by crude laboratory composite measure

	No lab indicators abnormal	At least one lab indicator abnormal	p
n	406	340	
Female (%)	157 (39.0)	141 (41.6)	0.513
Age category (%)			<0.001
Neonate	74 (18.2)	107 (31.5)	
Infant	152 (37.4)	142 (41.8)	
Preschool	108 (26.6)	56 (16.5)	
Older children	72 (17.7)	35 (10.3)	
Median age (months) [IQR]	7.61 [1.46, 40.21]	2.39 [0.63, 15.28]	<0.001
Median Weight-for-age Z-score [IQR]	-1.15 [-2.22, -0.25]	-1.44 [-2.62, -0.45]	0.027
Median Height-for-age Z-score [IQR]	-1.46 [-2.73, -0.16]	-1.60 [-3.17, -0.27]	0.231
Median Weight-for-height Z-score [IQR]	-0.42 [-1.56, 0.76]	-0.45 [-1.84, 0.85]	0.541
Median BMI Z-score [IQR]	-0.82 [-1.95, 0.46]	-0.92 [-2.13, 0.54]	0.678
HIV Status (%)			0.529
Negative	314 (77.3)	270 (79.4)	
Positive	46 (11.3)	40 (11.8)	
Unknown	46 (11.3)	30 (8.8)	
Meningitis classification (%)			<0.001
Clinically diagnosed	337 (83.0)	210 (61.8)	
Possible contaminant	24 (5.9)	20 (5.9)	
Definite	45 (11.1)	110 (32.4)	
White cell count	11.96 [9.17, 15.43]	11.69 [8.49, 16.99]	0.886
Haemoglobin	11.70 [10.30, 13.10]	11.30 [9.40, 13.10]	0.032
MCV	81.20 [74.88, 88.50]	85.60 [77.80, 94.50]	<0.001
Platelet count	362.00 [273.50, 444.50]	356.00 [244.00, 461.00]	0.425
Lymphocytes	4.61 [2.72, 7.01]	3.74 [2.09, 6.40]	0.003
Neutrophils	4.96 [2.71, 9.26]	6.08 [3.08, 10.84]	0.050
Monocytes	1.28 [0.84, 1.95]	1.23 [0.68, 2.15]	0.208
CRP	7.00 [2.00, 22.75]	45.00 [6.00, 150.00]	<0.001
CD4 count	619.50 [237.25, 1237.25]	380.00 [176.50, 722.75]	0.120
Log10 HIV viral load	5.47 [4.21, 6.29]	4.79 [3.37, 5.66]	0.121
Blood glucose	5.35 [4.70, 6.20]	5.60 [4.70, 6.70]	0.052
CSF glucose	3.30 [2.90, 3.90]	2.50 [1.50, 3.30]	<0.001
CSF-to-Blood glucose ratio	0.63 [0.53, 0.73]	0.47 [0.27, 0.65]	<0.001
CSF protein	0.36 [0.21, 0.54]	1.45 [0.91, 2.52]	<0.001
CSF ADA	0.90 [0.20, 1.40]	2.55 [1.00, 8.80]	<0.001
Died (%)	7 (1.7)	40 (11.8)	<0.001

Note:
CRP = C-reactive protein; BMI = body mass index; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; IQR = interquartile range. P-values derived using the Kruskal-Wallis test for skewed continuous data. Composite measure defined as CSF-to-blood glucose ratio less than 0.353, and/or CSF protein greater than 0.810 g/L, and/or CRP greater than 90 mg/L.

Table 3.2: Comparison of clinical and laboratory indicators, stratified by final laboratory composite measure

	No lab indicators abnormal	At least one lab indicator abnormal	Three lab indicators abnormal	p
n	406	308	32	
Female (%)	157 (39.0)	128 (41.6)	13 (41.9)	0.766
Age category (%)				<0.001
Neonate	74 (18.2)	95 (30.8)	12 (37.5)	
Infant	152 (37.4)	132 (42.9)	10 (31.2)	
Preschool	108 (26.6)	51 (16.6)	5 (15.6)	
Older children	72 (17.7)	30 (9.7)	5 (15.6)	
Median age (months) [IQR]	7.61 [1.46, 40.21]	2.15 [0.65, 14.97]	4.58 [0.56, 42.15]	<0.001
Median Weight-for-age Z-score [IQR]	-1.15 [-2.22, -0.25]	-1.42 [-2.63, -0.43]	-1.56 [-2.30, -0.52]	0.086
Median Height-for-age Z-score [IQR]	-1.46 [-2.73, -0.16]	-1.59 [-3.12, -0.27]	-1.73 [-3.41, -0.91]	0.343
Median Weight-for-height Z-score [IQR]	-0.42 [-1.56, 0.76]	-0.50 [-1.92, 0.71]	0.31 [-1.08, 0.90]	0.156
Median BMI Z-score [IQR]	-0.82 [-1.95, 0.46]	-0.97 [-2.18, 0.43]	-0.26 [-1.08, 0.76]	0.164
HIV Status (%)				0.266
Negative	314 (77.3)	248 (80.5)	22 (68.8)	
Positive	46 (11.3)	36 (11.7)	4 (12.5)	
Unknown	46 (11.3)	24 (7.8)	6 (18.8)	
Meningitis classification (%)				<0.001
Clinically diagnosed	337 (83.0)	198 (64.3)	12 (37.5)	
Possible contaminant	24 (5.9)	20 (6.5)	0 (0.0)	
Definite	45 (11.1)	90 (29.2)	20 (62.5)	
White cell count	11.96 [9.17, 15.43]	11.58 [8.50, 16.99]	12.68 [8.48, 16.41]	0.990
Haemoglobin	11.70 [10.30, 13.10]	11.30 [9.40, 13.20]	10.80 [9.00, 12.40]	0.051
MCV	81.20 [74.88, 88.50]	86.00 [78.30, 94.60]	80.50 [76.17, 90.12]	<0.001
Platelet count	362.00 [273.50, 444.50]	361.00 [250.00, 467.00]	300.00 [117.50, 404.25]	0.030
Lymphocytes	4.61 [2.72, 7.01]	3.87 [2.20, 6.50]	2.99 [0.85, 5.24]	0.003
Neutrophils	4.96 [2.71, 9.26]	5.80 [3.13, 10.79]	7.23 [2.67, 11.73]	0.145
Monocytes	1.28 [0.84, 1.95]	1.27 [0.69, 2.14]	0.94 [0.24, 2.31]	0.168
CRP	7.00 [2.00, 22.75]	29.00 [4.00, 102.00]	188.20 [145.50, 251.00]	<0.001
CD4 count	619.50 [237.25, 1237.25]	452.50 [176.50, 722.75]	351.50 [244.50, 985.50]	0.298
Log10 HIV viral load	5.47 [4.21, 6.29]	4.74 [2.87, 5.54]	4.80 [4.79, 5.30]	0.262
Blood glucose	5.35 [4.70, 6.20]	5.50 [4.68, 6.60]	6.00 [5.40, 6.82]	0.031
CSF glucose	3.30 [2.90, 3.90]	2.60 [1.70, 3.42]	0.95 [0.50, 1.65]	<0.001
CSF-to-Blood glucose ratio	0.63 [0.53, 0.73]	0.49 [0.32, 0.65]	0.17 [0.07, 0.26]	<0.001
CSF protein	0.36 [0.21, 0.54]	1.36 [0.87, 2.30]	2.59 [1.33, 3.20]	<0.001
CSF ADA	0.90 [0.20, 1.40]	2.50 [1.00, 8.58]	6.40 [1.75, 8.80]	<0.001
Died (%)	7 (1.7)	33 (10.7)	7 (21.9)	<0.001

Note:
CRP = C-reactive protein; BMI = body mass index; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; IQR = interquartile range. P-values derived using the Kruskal-Wallis test for skewed continuous data. Laboratory composite measure of high CSF protein, low CSF-to-serum glucose, and high serum CRP.

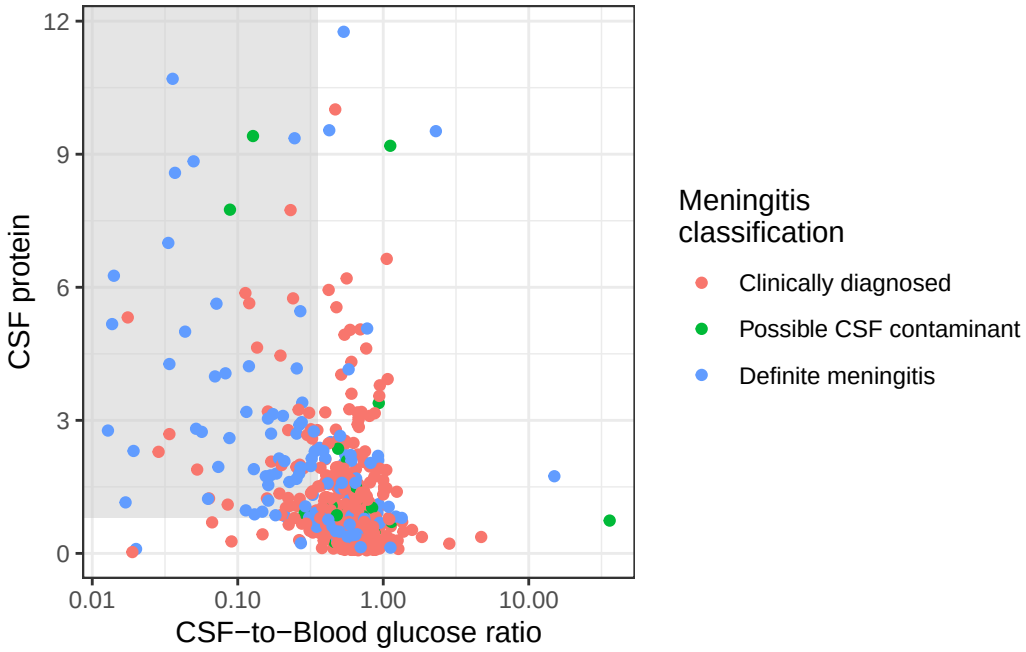


Figure 3.2: CSF protein and CSF-to-blood glucose ratio correlates with the identified CSF isolates in children, stratified by certainty of meningitis diagnosis

The gray rectangle highlights the sector with CSF protein >0.81 g/L and glucose ratio <0.353.

Table 3.3: Certainty of meningitis diagnosis, stratified by whether or not there was a high CSF protein and low CSF-to-serum glucose ratio in initial CSF specimens

Meningitis classification	No	Yes
Clinically diagnosed	89.9% (357)	10.1% (40)
Possible CSF contaminant	88.9% (24)	11.1% (3)
Definite meningitis	45.7% (48)	54.3% (57)
Total	81.1% (429)	18.9% (100)

Table 3.4: Comparison of clinical and laboratory indicators, stratified by death as outcome

	Survived	Died	p
n	699	47	
Female (%)	277 (39.9)	21 (44.7)	0.618
Age category (%)			0.280
Neonate	175 (25.0)	6 (12.8)	
Infant	273 (39.1)	21 (44.7)	
Preschool	151 (21.6)	13 (27.7)	
Older children	100 (14.3)	7 (14.9)	
Median age (months) [IQR]	4.19 [1.00, 28.02]	8.10 [1.84, 25.26]	0.161
Median Weight-for-age Z-score [IQR]	-1.22 [-2.40, -0.29]	-1.99 [-2.84, -1.29]	0.004
Median Height-for-age Z-score [IQR]	-1.51 [-2.94, -0.18]	-2.22 [-3.58, -1.25]	0.066
Median Weight-for-height Z-score [IQR]	-0.36 [-1.64, 0.88]	-0.86 [-1.93, -0.16]	0.111
Median BMI Z-score [IQR]	-0.82 [-1.96, 0.51]	-1.48 [-2.62, -0.21]	0.092
HIV Status (%)			<0.001
Negative	556 (79.5)	28 (59.6)	
Positive	70 (10.0)	16 (34.0)	
Unknown	73 (10.4)	3 (6.4)	
White cell count	11.87 [8.98, 15.85]	11.58 [6.57, 18.03]	0.817
Haemoglobin	11.60 [10.00, 13.20]	9.60 [8.50, 11.60]	<0.001
MCV	82.90 [76.05, 91.60]	83.30 [77.93, 91.17]	0.740
Platelet count	363.00 [269.00, 454.00]	294.00 [142.25, 411.00]	0.011
Lymphocytes	4.25 [2.61, 6.78]	2.66 [1.23, 4.75]	0.002
Neutrophils	5.31 [2.95, 9.70]	6.21 [2.35, 10.03]	0.528
Monocytes	1.27 [0.78, 2.13]	0.99 [0.48, 1.75]	0.037
CRP	12.00 [2.25, 60.00]	69.00 [21.00, 190.00]	<0.001
CD4 count	543.00 [236.00, 1139.00]	323.00 [133.00, 720.00]	0.146
Log10 HIV viral load	4.81 [3.09, 5.75]	5.85 [4.67, 6.58]	0.093
Blood glucose	5.40 [4.70, 6.30]	5.70 [5.00, 8.05]	0.042
CSF glucose	3.10 [2.40, 3.80]	2.50 [1.05, 3.85]	0.011
CSF-to-Blood glucose ratio	0.58 [0.45, 0.70]	0.37 [0.16, 0.56]	<0.001
CSF protein	0.60 [0.30, 1.19]	1.47 [0.48, 3.04]	<0.001
CSF ADA	1.10 [0.55, 3.85]	5.05 [1.10, 12.93]	0.088
Laboratory composite measure (Yes, %)	300 (42.9)	40 (85.1)	<0.001

Note:
CRP = C-reactive protein; BMI = body mass index; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; IQR = interquartile range. P-values derived using the Kruskal-Wallis test for skewed continuous data.

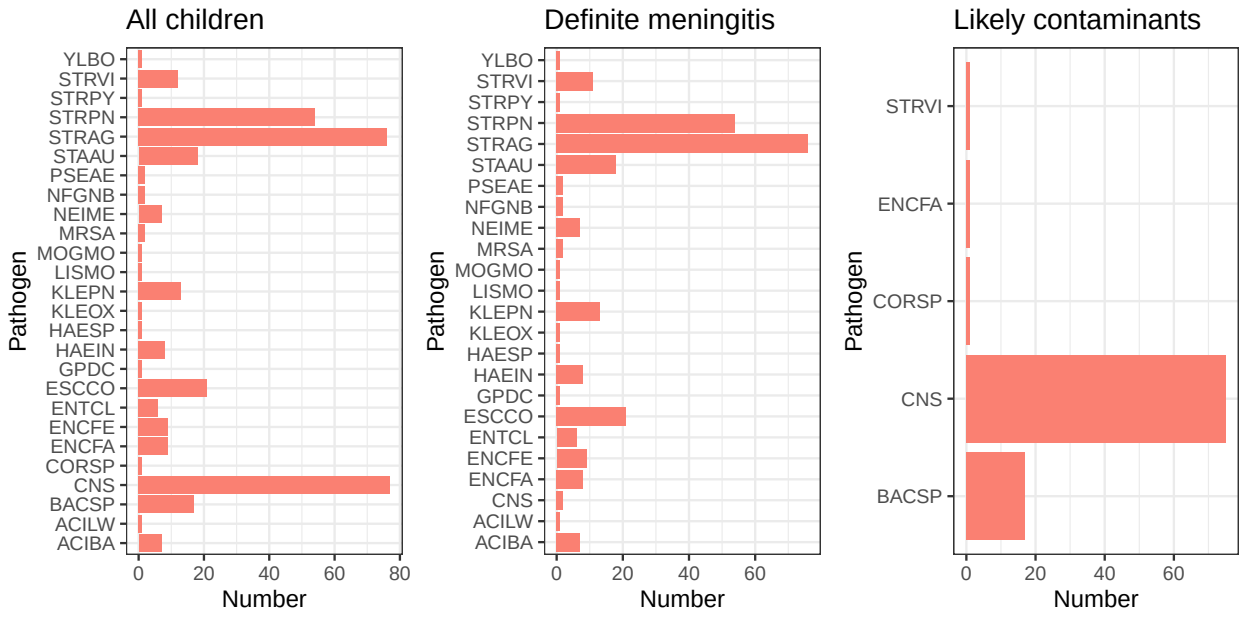


Figure 3.3: First CSF isolate organisms in children with culture-positive specimens, stratified by CSF contaminant status

CNS = Coagulase-negative staphylococcus; ENCFA = *E. faecium*; HAEIN = *H. influenzae*; STAAU = *S. aureus*; STRAG = *S. agalactiae*; STRPN = *S. pneumoniae*; STRVI = Viridans streptococci; YLBO = Yeast-like bodies; Please refer to Supplementary Materials for organism identification of less prevalent pathogens.

Appendix 2

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Department of Paediatrics and Child Health
Metabolic Unit
Chris Hani Baragwanath Academic Hospital
P. O. Bertsham
2013

28 April 2021

The Research Protocol Review Committee
Chris Hani Baragwanath Academic Hospital
Soweto
Johannesburg
South Africa

Dear Madam/ Sir

I would like to inform you that **Sindiswa Dlamini**has been given a permission to conduct her research study in the Department of Paediatrics at Chris Hani Baragwanath Academic Hospital. The title of her study is: **"A descriptive observational study of meningeal pathogens cultured from the general paediatric wards in Chris Hani Baragwanath Hospital, 2012 to 2020."**

While she has been given permission to conduct this study, she cannot start with data collection until she has provided the Department with Ethics Committee Clearance Certificate.

Yours Sincerely

Professor SC Velaphi
Head of Department of Paediatrics
Chris Hani Baragwanath Academic Hospital

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GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 26th May 2021

TITLE OF PROJECT:

A descriptive observational study of meningeal pathogens cultures from the general paediatric wards in Chris Hani Baragwanath Hospital, 2021-2020.

UNIVERSITY: Witwatersrand

Principal Investigator: Dr Sindiswa Dlamini

Department: Paediatrics

Supervisor : Prof D Moore

NHRD Number: -

Permission Head Department (where research conducted): Yes

NHRD No.

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- **Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.**
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

.....
Recommended
(On behalf of the MAC)
Date: 26/05/2021

.....
Approved/~~Not Approved~~
Hospital Management
Date: 01/06/2021

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Dr S Dlamini
School of Clinical Medicine
Department of Paediatrics and Child Health
Medical School
University

E-mail: Ntoyakhe27@gmail.com

CC: Supervisor: Professor DP Moore
<David.Moore@wits.ac.za>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2022/02/04

REF: R14/49

PROTOCOL NO: **M210970** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Descriptive study of meningeal pathogens cultured from the general paediatric wards in Chris Hani Baragwanath Academic Hospital, 2012 to 2020*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.



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Academic Affairs and Research
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01 February 2022

Applicant: Sindiswa Dlamini
Institution: University of the Witwatersrand
Department: Paediatrics
Email: ntoyakhe27@gmail.com
Tel: 011 960 3250 **Cell:** 061 926 0509

CC: Jeanette Wadula, David Moore

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"A descriptive study of meningeal pathogens cultured from the general wards in Chris Hani Baragwanath Hospital, 2012 to 2020" Ref No: PR2222650** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- Jeanette Wadula is noted as NHLS collaborator for this study.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za



Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix 3

A descriptive study of meningeal pathogens cultured from the general paediatric wards in Chris Hani Baragwanath Hospital, 2012 to 2020

Candidate: Sindiswa Dlamini

Student Number: 361398

Supervisor: Prof David Moore

Co-supervisor: Dr Marc Hauptfleisch

Collaborator from NHLS: Dr Jeannette Wadula

Nomenclature

ART	antiretroviral therapy
CCM	cryptococcal meningitis
CLWH	children living with HIV
CrAg	cryptococcal capsular polysaccharide antigen
CRP	C-reactive protein
CSF	cerebrospinal fluid
EPI	Expanded Programme on Immunization
EPTB	extrapulmonary tuberculosis
Hib	*Haemophilus influenzae* type b
HIV	human immunodeficiency virus type 1
IMCI	Integrated Management of Childhood Illness
IPD	invasive pneumococcal disease
LMIC	low- and middle-income countries
NHLS	National Health Laboratory Service
PCV	pneumococcal conjugate vaccine
PCV-7	7-valent pneumococcal conjugate vaccine
PCV-13	13-valent pneumococcal conjugate vaccine
TAC	TaqMan array card assay
TB	tuberculosis
TBM	TB meningitis

Introduction

Meningitis, inflammation of the meninges and brain due to infection with bacterial or fungal pathogens [1], is a leading cause of childhood morbidity and mortality. In South Africa, the incidence of meningitis is 4 per 100 000 in the general population, 40/100 000 in infants <1 year and 7/100 000 in children aged 1-4 years [2].

The specific infectious aetiology of meningitis is generally age dependent. Organisms that cause meningitis usually arise from the mucosal surfaces of the upper respiratory tract or the blood stream and infiltrate the meninges through invasion of the blood brain barrier. In the neonatal period (age 0 to 28 days) the commonest organisms are *Streptococcus agalactiae*, *Listeria monocytogenes* and *Escherichia coli* [3]. In infants aged three months and older, and children, the commonest bacterial organisms are *Neisseria meningitidis* (meningococcus), *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae* type b (Hib)[4]. In the context of human immunodeficiency virus type-1 (HIV) infection, important meningeal pathogens, in addition to those mentioned above, include *Mycobacterium tuberculosis* and *Cryptococcus neoformans*.

Impact of Vaccination on bacterial meningitis

South Africa introduced two protein-polysaccharide vaccines that target Hib and *S. pneumoniae* in 1998 and 2009, respectively[5] [6]. As a consequence of the introduction of these vaccines, the epidemiology of bacterial meningitis in young children may be changing. While vaccines are available to prevent meningococcal meningitis [7], these have not been incorporated into the National Immunization Programme in South Africa. The changing epidemiology of Hib and pneumococcal meningitis are presented below.

Pneumococcal meningitis

Streptococcus pneumoniae is the commonest cause of meningitis, it is a gram-positive encapsulated diplococcus that is part of the normal flora of the nasopharynx [8]. It is also an important cause of pneumonia and septicaemia. There are more than 90 serotypes of *S. pneumoniae*, about 23 of which are responsible for causing invasive pneumococcal disease (IPD) in children [9]. The serotypes are described according to their varying polysaccharides which are found in the bacterial cell wall. Prior to vaccination with pneumococcal conjugate vaccine (PCV), serotypes 6-11 caused more than 70% of IPD in children less than 5 years of age. In a survey done in Africa prior to widespread use of PCV, pneumococcal meningitis had a case fatality rate of 45% in children under the age of 5 years, and 50% of survivors had long term sequelae[8].

The introduction PCV into the Expanded Programme on Immunization (EPI) in South Africa has resulted in a decline in morbidity and mortality associated with IPD. In 2009, South Africa introduced the 7-valent PCV (PCV-7) into its routine infant immunization program using a schedule of vaccination at 6 and 14 weeks, with a booster dose at 9 months. PCV-7 was replaced by the 13-valent PCV (PCV-13) in April 2011. PCV-13 is estimated to confer a further 20% protection against IPD compared to PCV-7 [10]. The benefit of the introduction of PCV-7 and subsequently PCV-13 has been documented in South Africa using data from a nationwide laboratory-based IPD surveillance program [9][11]. PCV has decreased the incidence of IPD by protecting both immunised and non-immunised persons through herd immunity, whereby the non-immunized get protection from residing in a highly vaccinated environment [8].

In a time-series analysis done at Chris Hani Baragwanath Academic Hospital (CHBAH), there was a 4.7% reduction in the incidence of pneumococcal meningitis per annum prior to the inclusion of PCV into the EPI, which accelerated to an 18% reduction per annum post PCV introduction [8].

Hib meningitis

Haemophilus influenzae type b is the second commonest cause of meningitis. It is a gram negative coccobacillary, facultative anaerobe [12]. In the pre-vaccination era, Hib commonly affected children <5 years of age, with 59% of those infected developing meningitis [12]. In unvaccinated populations, Hib is the dominant cause of non-epidemic bacterial meningitis in children <12 years age. The organism also commonly causes bacteraemia, pneumonia, and epiglottitis in unvaccinated communities.

Before the adoption of Hib vaccination into vaccination programmes globally, Hib was responsible for at least 8.13 million cases of serious illness in children between the age of 1-5 years, causing an estimated 203 000 deaths in children <6 years [9]. The Hib vaccine was introduced into the South African EPI in 1998. The absolute number of cases amongst children one year of age or younger decreased by 65% from 55 reported cases in 1999-2000, to 19 cases in 2003-04 [6].

Tuberculous meningitis and cryptococcal meningitis

Mycobacterium tuberculosis and *Cryptococcus neoformans* account for a large burden of meningitis in children with immunodeficiency, particularly those with HIV.

Tuberculosis (TB) is caused by *M. tuberculosis*. It primarily manifests as pulmonary disease, however 20% of TB cases are due to extra-pulmonary tuberculosis (EPTB) [13]. EPTB is particularly common in young children and immunocompromised individuals. It is likely that early haematogenous spread to the brain occurs before a T-cell mediated immune response is activated, this mechanism could explain the vulnerability to TB meningitis (TBM) when T-cell mediated immunity is sub-optimal in persons infected with HIV and other immunocompromised persons.

The early clinical presentation of TBM is often non-specific, with symptoms such as cough, loss of weight, fever, vomiting and malaise. As the disease progresses, meningism, focal neurological signs, and a depressed level of consciousness can occur. The timing of initiation of treatment is the most critical factor affecting morbidity, mortality, and healthcare costs, which emphasises the importance of early diagnosis of TBM. In a study of over 500 South African children with TBM, the majority (84%) presented with neurological manifestations of disease, including hearing loss, visual impairment, motor deficits and intellectual impairment [14].

Cryptococcal meningitis (CCM) is caused by *C. neoformans* and *C. gattii*. It commonly occurs in immunocompromised patients but there are few cases that have been reported in immune competent children. HIV infection is the main risk factor, accounting for 95% of cases in low- and middle-income countries (LMICs) and 80% of cases in high-income countries [15]. Other risk factors for CCM include bone marrow transplant, haematological malignancies, and receipt of immunosuppressive therapy including steroids. The outcomes can be devastating, with high mortality rates or severe morbidity such as irreversible blindness, deafness, and neurocognitive impairment.

Diagnosis of meningitis

A lumbar puncture and analysis of cerebrospinal fluid (CSF) remains the gold standard for diagnosis and management of a meningitis. CSF is usually analysed for glucose (for comparison with a blood glucose level), total protein, cell count and differential, gram stain and bacterial culture [16]. These investigations have incomplete sensitivity, especially if antibiotics have been administered prior to CSF collection, and further tests have been suggested for better identification of pathogens. CSF lactate has been used to differentiate between viral and bacterial meningitis, and have been shown to be elevated in adult patients who subsequently die from TBM[16], but is rarely done as part of the

routine clinical work-up. Latex agglutination assays have been recommended to further aid in detecting bacterial pathogens in CSF: these tests may be useful in patients that have received antimicrobial therapy prior to CSF collection. The Xpert-MTB/Rif assay has a good yield in suspected TBM [16], and the cryptococcal capsular polysaccharide antigen (CrAg) test together with fungal culture is useful in the diagnosis of cryptococcal meningitis [16].

Molecular diagnostic techniques are useful in identifying CSF pathogens in children with meningitis in research studies but are not widely used in clinical practice at the current time. In a study done in West Africa, a TaqMan Array Card (TAC) assay was used to identify the spectrum of organisms causing meningitis in children [17]. The TAC assay increased the diagnostic yield and identified viruses, gram negative bacteria and common meningeal pathogens as being important CSF pathogens in the era of access to vaccines which target pneumococcus, Hib, and meningococcus [17].

Justification for this study

The last study to have evaluated the spectrum of meningitis pathogens in children hospitalised with meningitis at CHBAH, reviewed the time period from January 2006 to November 2011[8]. The proposed study aims to describe the commonest bacterial and fungal pathogens isolated in CSF samples of paediatric patients admitted to CHBAH from January 2012 through December 2020 and will thus give an updated evaluation of the meningeal pathogens that affect the childhood population served by the hospital.

AIM AND OBJECTIVES

The aim of the study is to describe the spectrum of meningitis pathogens in children hospitalised with meningitis at Chris Hani Baragwanath paediatric wards.

Objectives

1. Appraisal of the certainty of diagnosis of bacterial or fungal meningitis:
    a. Definite meningitis: children with bacterial or fungal pathogens detected on CSF specimens, either through culture or latex agglutination testing.
    b. Clinically diagnosed meningitis: children whose CSF specimens yielded no growth, but who were discharged with a diagnosis of meningitis.
2. To evaluate and compare blood and urine culture, CSF parameters and HIV status among children with meningitis at Chris Hani Baragwanath Hospital.
3. To assess the outcome among children hospitalized with meningitis as well as the outcome in association with HIV.

The study hypothesis is that children with definite meningitis will constitute the minority of cases with a discharge diagnosis of meningitis over the study period, and that most cases of meningitis are diagnosed clinically, without microbiological confirmation. Furthermore, we anticipate that CSF parameters, blood culture and acute phase reactant measures, length of hospital stay, and clinical outcomes will be more deranged, longer, and more severe in children with definite meningitis compared to those with clinically diagnosed meningitis. We also hypothesise that children living with HIV (CLWH) will have worse outcomes than HIV-uninfected children.

METHODS

Study site

CHBAH is a large secondary/tertiary level hospital in Soweto, Gauteng Province. Soweto has a population of 1.5 to 2.0 million persons. There are approximately 5000 general paediatric admissions annually, to four general paediatric units which admit patients in a 1-in-4 rota system. The hospital provides training for undergraduate and postgraduate medical students, paediatric registrars and paediatric subspecialties. Children with meningitis are managed in the general paediatric wards, with clinical input from Paediatric Neurology and Paediatric Infectious Disease subspecialty units.

Study population

The population of Soweto comprises mostly black South Africans, most of whom have no access to private medical insurance and therefore depend on the public health system for their health care needs. CHBAH is one of two public sector hospitals serving the population of Soweto, but is a referral centre for complicated paediatric patients from the other public sector hospital (Bheki Mlangeni Hospital), as well as from hospitals in the wider catchment area.

The Soweto primary health care clinics manage paediatric emergency cases according to the Integrated Management of Childhood Illness (IMCI) guidelines [18]. The IMCI management algorithm requires that children with suspected meningitis be treated with an intravenous or intramuscular dose of ceftriaxone prior to referral for ongoing care at the referral hospitals. Prior antibiotic therapy likely contributes to lower yield of CSF culture in suspected meningitis cases that are referred on for hospital care.

Inclusion criteria

1. Children aged 0 days to 14 years.
2. Children hospitalised at CHBAH with a discharge diagnosis of meningitis.
3. Children treated in the general paediatric wards at CHBAH: wards 17, 18, 19 and 33 (some CSF specimens may have been submitted from Wards 31 and 36, and these wards will also be considered for retrieval of CSF specimens).
4. Time period: 01 January 2012 through 31 December 2020.
5. Only admission CSF, blood culture and urine culture specimens will be evaluated in this study.

Exclusion criterion

1. Patients with inadequate CSF specimen results will not be considered in this analysis. All included patients will need to have a minimum complete set of:
    a. CSF biochemistry (protein, glucose);
    b. CSF microscopy (appearance, white cell count, red cell count, gram stain), and;
    c. CSF culture results (positive or negative for bacterial or fungal pathogens).

Data collection form

The Data collection chart looks at different variables important for extracting specific information to meet our study requirement, such as patient demographics, CSF perimeters, HIV status, blood culture and urine culture results. Refer to Appendix A.

Data sources

Data will be collected, with permission from the Head of the Department of Paediatrics at CHBAH, the CHBAH Medical Advisory Board, the NHLS Ethics Commitee, the Director of the VIDA Research Unit and the head of the Harriet Shezi Childrens Clinic, using the CHBAH Paediatric Admissions Database (to identify all children with a discharge diagnosis of meningitis) and the National Health

Laboratory System (NHLS) database (to access CSF, blood culture, urine culture, full blood count, CRP, and blood glucose results). For CLWH, CD4 counts and HIV viral loads will be obtained to establish the immunological and virologic status of the child at the time of the meningitis episode. Date of ART initiation will be obtained from the Harriet Shezi Children's Clinic. Data will be entered into an Excel spreadsheet, and thereafter will be analysed using Statistica and R version 4.0.4.

Data Analysis Plan

Characteristics of children with definite and clinically diagnosed meningitis will be compared. Categorical variables will be presented as percentages, and group comparisons will be made using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables will be evaluated for normality, and means of normally distributed data will be compared using the Student t-test. Medians of skewed continuous data will be compared using the Kruskal-Wallis test. Factors associated with certainty of the menigitis diagnosis, and factors associated with in-hospital mortality will be explored using logistic regression. In all analyses, two-sided P-values of <0.05 will be considered significant.

Time-series analysis will be conducted to evaluate the trend in prevalence of definite meningitis and suspected meningitis cases over the study time period; time series analysis will also evaluate trends in definite meningitis, stratified by pathogen type.

Ethics statement

This is a retrospective, descriptive study of available routinely collected data, for which we request a waiver of informed consent from study participants and their parents. Permissions will be sought from the Medical Advisory Committee at CHBAH, the Head of Department of Paediatrics and Child Health at CHBAH, and the Director of the Vaccines and Infectious Diseases Analtics (VIDA) Research Unit to conduct the study. The NHLS Ethics Committee will be approached for permission to access the NHLS database. The Harriet Shezi Children's Clinic database will be accessed, with permission of the head of the clinic, to access the date of initiation of ART for CLWH that were diagnosed with meningitis.

All data will be deidentified prior to analysis. Patient names, surnames and hospital numbers as well as dates of birth will be required to link between the CHBAH Paediatric Database and the NHLS database: all identifying information will be removed from the cleaned dataset prior to analysis. The dataset that will be compiled for the analysis will be kept in a password protected computer, accessible only to the MMed candidate and her supervisors.

This protocol will be submitted for approval to the Human Research Ethics Committee at the University of Witwatersrand, as well as to the Paediatric Protocol Assessor Committee.

Dr Jeannette Wadula has been identified as the NHLS Microbiologist who will contribute to this study from the laboratory aspect, and who will co-author the scientific manuscript that will emanate from this study.

Limitations

As this is a retrospective study, reliance on routinely collected data, it will not be possible to interrogate the types of antimicrobial therapy used in the management of the children diagnosed with meningitis at CHBAH during the study period as, as these data are not routinely captured in hospital discharge summaries from which the available clinical data will be extracted. Data quality and missingness will impact on the data analysis and some information may not be thoroughly

interpreted secondary to poor documentation. This will be dealt with to some extent, through the evaluation of children with a "complete" set of CSF results (as mentioned in the exclusion criteria).

Study timeline DEC 2020-AUG 2021

	DEC	JAN	FEB	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPT
LITERATURE REVIEW										
PROTOCOL PREPARATION										
SUBMISSIONS TO ETHICS										
ETHICS APPROVAL										
DATA COLLECTION										
DATA ANALYSIS										
PROTOCOL WRITE UP										

Budget

The student will be responsible for funding the study. Estimated costs of stationary, printing of the dissertation, and miscellaneous costs will be in the region of R 2500. Assistance with publication costs for any manuscripts produced through this research will be sought through the University of the Witwatersrand Faculty of Health Sciences.

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Data Collection Sheet

Variable	Variable code		
Study no	patid		
Sex	sex		F=female; M=male
Date of birth	dob	dd/mm/yyyy	
Date of admission	doa	dd/mm/yyyy	
Ward	ward		17, 18, 19, 33, 31, 36
PCV immunization	pcv_imm		0=unvaccinated; 1=incomplete; 2=complete; 3=unknown
Hib immunization	hib_imm		
BCG immunization	bcg_imm		
Anthropometry			
Admission weight	wt		
Admission length	lt		
MUAC	muac		
HIV status	hiv		0=neg; 1=pos; 2=unknown
If HIV negative:			
HIV exposure	hiv_exp		0=no; 1=yes; 2=unknown
If HIV positive:			
CD4 count	cd4_num		
CD4 percent	cd4_perc		
CD4 date	cd4_dt	dd/mm/yyyy	
HIV VL	hivvl		
HIV VL date	hivvl_dt	dd/mm/yyyy	
On ART?	on_art		
ART start date	art_dt	dd/mm/yyyy	
CSF Results			
CSF date	csf_d	dd/mm/yyyy	
CSF protein	csf_prot		
CSF glucose	csf_gluc		CSF Appearance: 0=clear; 1=turbid; 3=bloodstained; 4=xanthochromic; 5=other
CSF ADA	csf_ada		
CSF appearance	csf_app		
CSF Gram stain	csf_gram		0=no bacteria observed; 1=Gram pos; 2=Gram neg
Gram positive shape	gp_shape		Gram stain organism shape: 1=cocci; 2=diplococci; 3=coccobacilli; 4=bacilli; 5=other
Gram negative shape	gn_shape		
CSF lymphocytes	csf_l		
CSF neutrophils	csf_n		
CSF red cells	csf_r		
Latex done	ltx_done		0=No; 1=Yes
Latex positive	ltx_pos		0=No; 1=Yes
Latex organism	ltx_org		Organism codes: 1=pneumococcus; 2=Hib; 3=*S. agalactiae* ; 4=*E. coli* ; 5=*N. meningitidis* ; 6=*L. monocytogenes;* 7=*S. aureus* ; 8=*A. baumannii* ; 9=Klebsiella spp; 10=Candida spp; 11=Cryptococcus spp; 12=*M. tuberculosis* ; 13=Other (if "other", write free text)
CSF culture positive?	csf_pos		
CSF organism 1	csf_org1		
CSF organism 1 growth	csf_org1_growth	light; heavy; other	
CSF organism 2	csf_org2		
CSF organism 2 growth	csf_org2_growth	light; heavy; other	
CSF organism 3	csf_org3		
CSF organism 3 growth	csf_org3_growth	light; heavy; other	

Data Collection Sheet

Variable	Variable code		
Organism 1 medium	org1_med		1=agar plate; 2=broth (subculture)
CSF TB Culture	csf_tb_cult		0=neg; 1=pos; 2=not done
Blood and Urine Results			
WCC	wcc		
Neutrophil count	neut		
Lymphocyte count	lymp		
Monocyte count	mono		
Platelet count	plt		
Date of FBC	fbc_dt	dd/mm/yyyy	
CRP	crp		
Date of CRP	crp_dt	dd/mm/yyyy	
Blood glucose	bl_gl		
Blood glucose date	bl_gl_dt	dd/mm/yyyy	
Serum chlorise	cl		
Serum chloride date	cl_dt	dd/mm/yyyy	
Admission blood culture positive?	bc_pos		0=no; 1=yes; 2=not done
BC date	bc_dt	dd/mm/yyyy	
Admission urine cuture positive?	ur_pos		0=no; 1=yes; 2=not done
Urine culture date	ur_dt	dd/mm/yyyy	
Blood culture results			Organism codes: 1=pneumococcus; 2=Hib; 3=*S. agalactiae* ; 4=*E. coli* ; 5=*N. meningitidis* ; 6=*L. monocytogenes;* 7=*S. aureus* ; 8=*A. baumannii* ; 9=Klebsiella spp; 10=Candida spp; 11=Cryptococcus spp; 12=*M. tuberculosis* ; 13=Other (if "other", write free text)
Blood cult organism 1	bc_org1		
Blood cult organism 2	bc_org2		
Blood cult organism 3	bc_org3		
Urine culture results			
Urine cult organism 1	ur_org_1		
Urine cult organism 2	ur_org_2		
Urine cult organism 3	ur_org_3		
Urine antimicrobial substances			
Urine antimicrobial substances?	ur_abic		0=no; 1=yes; 2=not done
Outcome			
Outcome date	dod	dd/mm/yyyy	Outcome codes: 1=discharged; 2=died; 3=transferred out; 4=refused hospital treatment; 5=other
Outcome	outcome		
Form administration			
Date form completed	form_dt	dd/mm/yyyy	
Completed by initials	form_init		

Appendix 4

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