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Causes of Meningitis in the era of HIV

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Declaration of authorship

I, Dr Praksha Ramjathan declare as follows:

1. That the work described in this dissertation has not been submitted to UKZN or any other institution for the purposes of an academic qualification, whether by myself or any other party.

2. That my contribution to the project is as follows: literature review, study concept, protocol writing, data collection, data analysis and writing of final dissertation.

3. That the contributions of others to the project are as follows:
   Dr Khine Swe Swe Han – supervision, assistance and editing of manuscript. Suggestions on improving study design and including more current data.

Dedication

I wish to dedicate

This work to

My parents, husband, Priyesh and children, Nikhil and Bhavya.

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Table of Contents

ABBREVIATIONS

BBB - blood brain barrier

CNS - Central nervous system

CSF - cerebrospinal fluid

DCs - dendritic cells

HIV – human immunodeficiency virus

IFN-γ - interferon gamma

KZN - KwaZulu-Natal

M.tb. - *Mycobacterium tuberculosis*

PLHIV - people living with HIV

TB - Tuberculosis

TBM - Tuberculous meningitis

Th1 - T-helper cells I

TNF-α - tumor necrosis factor alpha

CHAPTER 1

INTRODUCTION

Causes of meningitis in the Era of HIV

1.1 Clinical Problem

Meningitis is inflammation of the of the meninges or more specifically, infection within the subarachnoid space.[1] The subarachnoid space is the space located between the arachnoid mater and pia mater which are layers of connective tissue that cover the brain and spinal cord.[1] Meningitis is considered to be a medical emergency that has high mortality and morbidity. [2] Even with improvements in diagnostic methods, mortality is as high as 30% with pneumococcal meningitis.[3] In research settings, the acute mortality of cryptococcal meningitis is between 24% and 37%. [4,5] Lessells *et al*. suggest that, in routine settings, mortality rates associated with cryptococcal meningitis are higher, with up to 41% in-hospital mortality.[6] A retrospective study involving patients hospitalized with tuberculous meningitis between 2006 and 2015 found that the mortality was 30.4%. [7] In addition to this high mortality, there is an increase of neurologic sequelae in patients who survive meningitis.[8] The profile and susceptibility of microorganisms causing infectious syndromes varies over time and in different geographical locations and knowledge thereof will assist in the management of these patients.

1.2 Epidemiology

The estimated incidence of bacterial meningitis in South Africa is 4/100 000 in the general population, 40/100 000 in children < 1-year-old and 7/100 000 in children 1-4 years old, however this is most probably an underestimate as it excludes those patients with culture negative meningitis.[9] A large percentage of people living with HIV (PLHIV) continue to have advanced immune suppression that places them at risk for HIV-related infections. [10] In 2008 the number of HIV positive people in South Africa was 5.27 million and by 2018 this number had increased to 7,52 million people. [11] Central nervous system (CNS) infections remain a major reason for mortality in PLHIV in South Africa, with cryptococcal, tuberculous and pneumococcal meningitis the most common culture confirmed aetiologies.[12] Cryptococcal meningitis alone leads to approximately 15% of HIV-associated deaths worldwide, with almost 75% of these in sub-Saharan Africa. [13]

In April 2009 the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced into the South African Expanded Programme on Immunisation for children. In 2011 there was a gradual replacement of PCV7 by 13-valent pneumococcal conjugate vaccine PCV13. The World Health Organisation vaccine coverage estimated population percentages for receiving a third dose of the PCV vaccine in South Africa were 10% in 2008, 58% in 2009, 62% in 2011, 75% in 2012, 68% in 2017, 73% in 2018 and 76% in 2019.15 The increase in vaccine coverage would lead to decreased rates of pneumococcal meningitis in children; and in adults due to herd immunity offered by decreased carriage of pneumococcus in children. [14]

1.3 Study Rationale

Early diagnosis and prompt initiation of antimicrobials will improve clinical outcomes.[9] It is important that clinicians prioritize early investigations so that the diagnosis and treatment occur as soon as possible.[15] The clinical syndrome of meningitis may have viral, bacterial, fungal or mycobacterial aetiology and the laboratory examination of cerebrospinal fluid (CSF) can assist in determining the cause. Treatment of the different aetiological agents varies and establishing the cause is crucial in treating the patient. The classical triad of clinical symptoms that occur with meningitis include fever, altered mental state and neck stiffness, but relying on the presence of all three symptoms can result in more than 50% of cases of bacterial meningitis being missed. [16] Acute meningitis should be suspected in any adult with any two of the following: headache, neck stiffness, fever > 37.5^{o}C or altered mental status of < 7 days' duration.[17] The performance of a lumbar puncture is important, as CSF examination is needed to establish the diagnosis. [18] Laboratory analysis of CSF is crucial in suspected meningitis as the clinical features by themselves are insufficient to differentiate meningitis from other diagnoses, and to distinguish viral, bacterial, fungal and mycobacterial meningitis from each other. [19] The cell count, microscopy and biochemical parameters may be suggestive of a specific aetiology.[17] Major infectious aetiologies for meningitis in South Africa include, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Salmonella species*, *Mycobacterium tuberculosis* (M.tb.) and *Cryptococcus neoformans*. [20]

Jarvis *et al* found that in the 2006-2008-time period the top causes of meningitis were *Cryptococcal neoformans*, followed by *Mycobacterium tuberculosis* and then other bacteria. *S pneumoniae* constituted 90% of the non-mycobacterial bacteria cultured followed by *N. Neisseria meningitidis* which made up 3%. [21]

This study will describe the laboratory findings of patients with culture positive meningitis presenting to a referral hospital in KwaZulu-Natal with the aim of describing the aetiological profile over two different periods. It will also look at different tests for the laboratory diagnosis of tuberculous meningitis and the susceptibility profile of *Mycobacterium tuberculosis* and bacterial isolates, specifically in cerebrospinal fluid (CSF) samples.

1.4 Pathophysiology

1.4.1 Bacterial Meningitis

The majority of bacterial meningitis cases start with nasopharyngeal colonization of the host by a bacterial pathogen. After local invasion in the nasopharynx, bacteria enter the blood stream and cause a bacteremia. [22] Surface encapsulation of the bacteria is important in the bacteria surviving host defense mechanisms in the bloodstream.[22] The bacterial capsule plays a role in the inhibition of neutrophil phagocytosis. [23] The most common bacterial pathogens implicated in causing meningitis (***Streptococcus pneumoniae***, *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus agalactiae* and *Escherichia coli)*, are all encapsulated. Other structures, such as fimbriae and pili, are specific adhesins located on many bacteria that also enhance mucosal colonization by mediating adhesion of bacteria to host cells. Following the bacteraemia, there is invasion of the meninges but the exact mechanism is unknown.[22] It is thought that high grade bacteraemia, with large numbers of bacteria in the bloodstream, may result in invasion of the meninges. [24]

After a microorganism crosses the blood brain barrier (BBB), they multiply and produce an immune reaction through the release of pro inflammatory substances which cause migration of leucocytes, particularly neutrophils, across the BBB. This migration of leucocytes causes a condition known as pleocytosis which is the hallmark for diagnosis of meningitis. [25]

In the subarachnoid space there is an increase in exudate due to predominance of phagocytic polymorphonuclear leucocytes in early phase. In the later phase by lymphocytes histiocytes, fibrinogen and blood proteins further contributing to inflammatory exudate. [26]

1.4.2.1. Tuberculous meningitis

Tuberculosis (TB) infection is usually initiated by inhaling infectious droplets containing *Mycobacterium tuberculosis* (M. tb). The droplets end up in the lung and M. tb infects lung dendritic cells (DCs), neutrophils and alveolar macrophages. [27] The infected DCs are transported to lymph nodes and here they stimulate the differentiation of T-helper I (Th1) cells. The Th1 cells release interferon gamma (IFN-γ) and TNF-α at the site of infection, which in turn, activates macrophages and DCs to produce cytokines.[28] This process results in the formation of a granuloma, which encapsulates the infected cells and slows the replication of TB bacilli, leading to latent infection. In the immune compromised, e.g., HIV infected, however, granuloma formation may not occur and the patient may progress to active primary TB disease. [29] Active primary TB leads to pulmonary tissue destruction and spread of the TB bacillus to other organs.[30] Cerebral tuberculosis (TB) usually starts with respiratory infection via the lungs, followed by early blood spread to extra pulmonary sites, including the CNS.[31] The CNS is normally protected by the blood-brain barrier (BBB), which is composed of endothelial cells kept together by tight junctional complexes.[32] CNS tuberculosis is postulated to occur when *Mycobacterium tuberculosis* crosses the blood brain barrier, resulting in the formation of cortical and meningeal tuberculomas.[33] The early lesions, containing dormant TB bacilli, can be located in the subependymal surface, meninges, or the subpial parts of the brain. [31] Rupture of the tuberculomas into the subarachnoid space or into the ventricular system produces meningitis, the most common form of cerebral TB. [30]

There is a lack of data comparing the cerebral immune response to TBM in HIV-1 infected and uninfected hosts and more studies are needed. [27] Research, however, consistently shows that there is an association between HIV-1 infection and death in patients with TBM, as well as, decreased efficacy of treatment against M.tb. [34, 35]

Torok *et al* showed that there was a higher percentage of CSF neutrophils in HIV infected patients with TBM and suggested that this may be due to HIV-1 infection.[36]

Also, it has previously been shown in vitro, that numerous pro-inflammatory cytokines and chemokines are secreted by microglia, when infected by HIV-1, including the neutrophil chemotactic factor IL-8 and TNF which is a cytokine involved in BBB permeability. [37] TNF plays a critical role in the pathogenesis of M.tb. and this also indicates the effect of HIV-1 in the promotion of M.tb. spread in the CNS and consequently TBM.

1.4.2.2 Drug Resistance in M. tb.

There is a paucity of data on drug resistant TBM in South Africa. The Xpert MTB/RIF Ultra was introduced in KZN in 2018 and was used on CSF samples from that year. It detects *M. tuberculosis* and resistance to Rifampicin. The last study on drug resistant adult TBM in Kwa-Zulu Natal was done between 1999-2002 and showed that 8.6% of M.tb. isolates from CSF samples were multidrug resistant i.e., resistant to both rifampicin and isoniazid. [38] No further published studies have looked at drug resistance in adult CSF samples in KZN. Treatment of drug resistant TBM is problematic and mortality is high. [38] In children the diagnosis is often more difficult. A retrospective analysis of paediatric records by Padayatchi N *et al*, in King George V Hospital, found 8 children with resistant TBM between 1992 and 2003. 7/8 children died, highlighting the high mortality. In almost all children the diagnosis was made posthumously, reinforcing the need for more rapid diagnostic methods. [39]

1.4.3 Cryptococcal meningitis

Cryptococcal disease is one of the most important opportunistic infections among people living with advanced HIV disease and is a major contributor to mortality. [40]

Despite the advances made in diagnosis and treatment of HIV, many people still die from HIV-related opportunistic infections. [41]

Cryptococcus species, the fungal causative agent of cryptococcal disease, is found in the environment globally. The organism is inhaled, but rarely results in significant invasive disease in most immunocompetent people. [42] Significant, invasive diseases are believed to be triggered by reactivation of latent infection among immunocompromised individuals, such as people living with HIV (PLHIV), sometime after initial exposure. [43] Immunocompromised individuals have impaired T cell immunity, leading to multiplication and dissemination of the fungus to other parts of the body, but especially to the central nervous system, causing cryptococcal meningitis. One of the main reasons for the high mortality is the delay in diagnosis of this serious medical condition. Other contributing factors are the limited access and high cost of first-line antifungal drugs. [44]

1.4.4 Viral Meningitis

Viral meningitis is a consideration in culture negative meningitis, especially with a lymphocytic pleocytosis. Common viruses include enteroviruses, herpes simplex 1 and 2, varicella –zoster virus, cytomegalovirus, adenovirus, measles virus, Epstein - Barr virus, human immunodeficiency virus and human herpes viruses 6,7 and 8. [45] Specific polymerase chain reaction tests need to be requested and performed for these viruses after routine microscopy and culture and they were not included in this study.

1.5 Clinical presentation

1.5.1 Acute Meningitis

Patients with acute meningitis present over the space of hours or days with rapid clinical deterioration. The causes are usually bacterial or viral. The clinical presentation of bacterial meningitis differs depending on the age of the patient. In the paediatric population the signs and symptoms are subtle and atypical. The classical triad of neck stiffness, fever and altered mental status seen in adults, is often absent in infants and younger children. [46]

In neonates, meningitis presents with fever, poor feeding, irritability, vomiting, lethargy and seizures. Physical examination findings are also nonspecific and can include hyper reflexia and a bulging fontanel. Older children may have nonspecific signs such as anorexia, headache, myalgia, arthralgia or haemodynamic alterations with hypotension and tachycardia. [46] With meningococcal infection there may be dermatological alterations such as petechial purpura or macular erythematous rash together with to meningeal signs. [46] Clinical signs of meningeal irritation include nuchal rigidity, back pain, Kerning sign and Brudzinski sign. Studies in adults have shown that nuchal rigidity, headache and fever are only seen in 35 - 40% of adults with bacterial meningitis. [47] Children with bacterial meningitis often present with seizures that can be focal or generalized. Children with first presentation of seizures should undergo a lumbar puncture to exclude the diagnosis of bacterial meningitis. [46] Due to the non-specific presentation in children it is difficult to make a diagnosis of meningitis on clinical features alone and lumbar puncture is essential. [48]

1.5.2 Chronic Meningitis

Chronic meningitis presents over weeks and the commonest culture positive causes in the South African setting are *Cryptococcus* species and M.tb.

Other infective causes include *Treponema pallidum, Taenia solium, Nocardia* species, *Histoplasma* species, *Brucella* species and Echo virus. [45] Chronic meningitis may present with a normal physical examination and absence of fever. There may be nonspecific signs such as confusion, apathy, papilledema, and cranial nerve palsies. A high degree of clinical suspicion is necessary especially in immunocompromised patients or the diagnosis may be missed. [49] Diagnosis can be confirmed through lumbar puncture and CSF analysis.

50 missing

1.6 Laboratory Diagnosis of Meningitis

1.6.1. Cerebrospinal fluid composition

The crucial step for the diagnosis of meningitis is CSF analysis including cell count, microscopic observation, identification and susceptibility testing of CSF. Tight junctions between the epithelial cells lining the choroid plexus have tight junctions and prevent cells and protein from flowing freely into the CSF. [50 Venkatesan] These tight junctions create what is known as the blood brain barrier. In healthy individuals the blood brain barrier (BBB) prevents the entry of cells and large amounts of protein into the CSF. Disease such as meningitis can result in protein and white blood cells, that are ordinarily prevented by the BBB, to enter the CSF. [51 Rumbaugh,]

The L4 to L5 location is used for lumbar puncture to obtain CSF for analysis due to lower risk of damaging the nerve roots. [51] Glucose, proteins and chloride enter the CSF by passive diffusion or pinocytosis. The normal protein concentration in the CSF is related to plasma concentration of the protein. When the BBB is damaged, however e.g., in bacterial meningitis, albumin, which is found

in higher concentration in the plasma, crosses the BBB resulting in an increased protein concentration in the CSF. [51]

Even though normal CSF is considered acellular, a very small number of cells can be found in healthy individuals. The number of cells found in the CSF changes according to the age of the individual. A cell count of 0 - 5 white blood cells and red blood cells in the CSF are considered normal in adults but in children may vary from 0-20 cells. In neonates, the premature BBB allows more cells to cross and the cell count may range from 0-32 cells. [51] A CSF sample with more than 2 polymorphonucleocytes observed, should always be considered abnormal. [52]

In bacterial meningitis the CSF colour may vary from clear to purulent. Turbidity of the CSF is a result of increased cells, protein concentration, or presence of bacteria. [51]

The typical CSF abnormalities in bacterial meningitis are the presence of elevated polymorphonuclear cells, hypoglycorrhachia and raised CSF protein level. [52] The leukocyte and protein count are the least influenced by antimicrobial therapy, while glucose starts increasing 24-48 hours after starting appropriate antibiotics. Protein and leukocyte may still be present until the end of treatment. [52]

1.6.2 Laboratory investigations

Routine investigations performed in the laboratory include Gram stain, cell count, chemistry and culture. When a CSF sample is received by the laboratory, a Gram stain should be performed as soon as possible. [17] Microscopy is a rapid laboratory test and a positive microscopy result will provide a presumptive diagnosis and direct treatment. *S. pneumoniae*, appears as Gram-positive cocci whereas *N. meningitidis* appears as Gram-negative diplococci. [17] The sensitivity of the Gram stain varies from 25% to 97% and is correlated to the bacterial load present in the CSF. [52] A negative Gram stain does not exclude meningitis. [52]

Laboratory examination of CSF using Gram stain permits quick identification of the aetiological microorganism in 60% to 90% of people that present with bacterial meningitis and the specificity is nearly 100%. [45] The greater the bacterial load, the more likely it is to obtain a positive

microscopy. With a CSF concentration of 10^5colony-forming units/ml 97% of cases will have a positive microscopy while this figure drops to 25% with concentrations of 10^3 or fewer colony forming units.

The frequency of seeing positive microscopy also varies depending on the bacterial pathogen concerned. Bacteria are seen on microscopy in 60 to 90% of cases of bacterial meningitis. [45] In cases of tuberculous meningitis, fewer than 15% to 25% of specimens have positive smears, and 20% of patients with tuberculous meningitis have negative CSF cultures.[45] CSF culture is the mainstay in the diagnosis of bacterial meningitis and is positive in 80% to 90% of patients with bacterial meningitis when CSF is obtained before starting antibiotic treatment. [45]

CSF leucocyte counts are also useful in distinguishing the different aetiologies but there are no absolute cut-off values. [17] A leucocyte count of > 1000 cells/ul with a predominance of neutrophils is highly suggestive of bacterial meningitis. [45] CSF Protein is usually elevated in meningitis of various aetiologies but is markedly increased in bacterial and TBM. [49] Hypoglycorrhachia or low glucose points towards bacterial meningitis but is also seen in tuberculous and cryptococcal meningitis. [17] No single CSF parameter can diagnose the cause of meningitis and even with clinical features, the diagnosis can be problematic. [17] Clinicians should rather err on the side of caution and treat for bacterial meningitis, if it is suspected, and wait for microbiological results. [53]

Additional investigations for the diagnosis of tuberculous meningitis include auramine, culture and polymerase chain reaction for *M. tuberculosis*.

Molecular GeneXpert MTB/RIF testing was officially started in South Africa in October 2011 and complete implementation was reported by 2013. [54] The testing was performed only on sputum samples and not on extra pulmonary specimens such as CSF. Rollout of the newer, more sensitive GeneXpert ultra was started nationally in October 2017, but was only introduced in the King Edward VIII Microbiology Laboratory in January 2018. At the same time extra pulmonary as well as CSF samples began to be processed using the GeneXpert ultra platform. During the earlier time period, in 2008, all CSF samples received for bacterial culture were also sent for M. tb culture but from July 2010, diagnostic stewardship measures were implemented and M. tb culture or GeneXpert ultra were only done on request from the clinician.

Establishing a gold standard for TBM diagnosis is difficult and Mitha *et al* looked at comparing laboratory tests to a uniform clinical case definition. [55] That study showed that 53.3% of patients with possible TBM had a positive TB culture. It also showed that the older GeneXpert test was positive in 53.5% of patients with possible TBM. The frequency of positive results decreased further in the probable TBM group with only 38.2 % of patients having positive cultures and 35.3 % of patients having positive GeneXpert results, highlighting the difficulties with TBM diagnosis both clinically and by laboratory methods. [55]

According to a 2021 Cochrane review the sensitivity of the newer GeneXpert ultra on CSF is 89% and specificity is 91% as compared to culture. [56] The GeneXpert ultra-assay has improved sensitivity in the diagnosis of TBM compared to the older GeneXpert test but there are few studies on its use in the diagnosis of TBM. [56]

Lateral flow assay for cryptococcal antigen on CSF in patients suspected of cryptococcal meningitis has a sensitivity of between 94-100% and is recommended by the WHO. [43] Initial cultures are positive in 75% of patients with cryptococcal meningitis and additional cultures increase the yield. [48]

1.7 Problem statement

No single study has investigated culture positive bacterial, cryptococcal and tuberculous causes of meningitis in both adults and children in Kwa-Zulu Natal (KZN) previously. The study will describe the laboratory characteristics of CSF samples from patients with the different types of meningitis with the aim of guiding clinicians in prompt treatment of meningitis. The study will look at patients with bacterial, tuberculous and cryptococcal meningitis and analyze the laboratory and demographic characteristics of these patients. Very little recent research has been done on drug resistant *Mycobacterium tuberculosis* causing meningitis in KZN and South Africa as a whole and this study will add important data.

1.8 Research Question

This study will aim to describe the etiological profile of meningitis over two time periods, 2008 to 2010, and 2018 to 2019 at King Edward VIII Hospital which is a tertiary referral health care facility in KZN. This study will look at laboratory findings in cerebrospinal fluid and also analyze drug susceptibility patterns of *M. tb*. causing meningitis.

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CHAPTER 2

CAUSES OF MENINGITIS IN THE ERA OF HIV

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Causes of meningitis in the era of HIV

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2.1 Abstract

Background: Meningitis is a dangerous condition with high morbidity and mortality. Newer methods that can aid in the faster diagnosis of meningitis will improve the diagnosis and enable rapid initiation of the correct treatment.

Methods: The study was a retrospective laboratory-based analysis of cerebrospinal fluid (CSF) samples received over two time periods. Data was collected over a 30-month period from January 2008 to June 2010 and then a 24-month period from January 2018 to December 2019. By looking at two time periods the changes in aetiology can be evaluated. Only non-duplicate CSF samples that were culture positive or GeneXpert MTB/RIF ultra (GeneXpert ultra) positive were included in the analysis.

Results: A microbial aetiology was confirmed in a total 396 samples over the entire study period. Most patients were in the 21 to 40 age group. Of the 337 adult samples, *Cryptococcus neoformans* was cultured in 222 (65.9%) CSF samples, *Mycobacterium tuberculosis* was confirmed by culture or polymerase chain reaction in 86 (25.5%) samples and *Streptococcus pneumoniae* 16 (4.7%) of samples. More than a third (10/29) of the CSF samples positive using GeneXpert ultra were culture negative for *Mycobacterium tuberculosis* and would have been missed if only culture was performed. In children bacterial meningitis was more common than cryptococcal or laboratory confirmed tuberculous meningitis. 96% of CSF samples that cultured *C. neoformans* were antigen positive

Conclusion: *C. neoformans, M. tuberculosis* and *S. pneumoniae* are the leading causes of meningitis in this setting. The relative percentage of bacterial meningitis dropped from 23% in the 2008-2010 period to 14% in the 2018-2019 period. While there are rapid antigen tests for *C. neoformans,* the laboratory diagnosis of *M. tuberculosis* from CSF remains challenging.

Key words:

Meningitis, HIV, tuberculosis, *Cryptococcus neoformans*

2.2 Manuscript

2.2.1. Introduction

Meningitis is a dangerous infection of the central nervous system that has an associated high morbidity and mortality rate. [1] Empiric treatment is often started based on knowledge of common microbial causes. South Africa has a high burden of human immunodeficiency virus (HIV) disease. With HIV disease the immune system is impaired and the patient is at increased risk of infection especially with opportunistic pathogens such as *Cryptococcus neoformans*. *C neoformans* is an important cause of mortality in HIV infected patients, even in those on antiretroviral treatment.[2] In 2008 the number of HIV positive people in South Africa was 5.27 million and by 2018 this number had increased to 7,52 million. [3] To the authors knowledge there have been no published studies on the causes of meningitis at King Edward VIII Hospital or in Kwa-Zulu Natal, South Africa. A study performed in five Johannesburg hospitals from 1980 to 1982 also showed that the leading causes of meningitis in adults were *N. meningitidis, S. pneumoniae* and *H. influenzae.* [4] The same study also found that that the leading causes of neonatal meningitis were *E. coli* and *Group B Streptococcus.* The pneumococcal vaccine was introduced in South Africa in 2008 with vaccine coverage of 10% that year which increased to 72% in 2008 and this would have decreased the incidence of invasive pneumococcal disease including meningitis. [5] In a 4 year study at the Pretoria Academic Hospital from 1994 to 1998 Schutte C M *et al* concluded that "the HIV epidemic was responsible for a marked shift in the spectrum of meningitis towards chronic infections such as TB and cryptococcal meningitis." [6] During the period 2006 to 2008, Jarvis *et al* found that in a setting where 43 % of patients have clinically suspected HIV infection, the leading causes of meningitis was *Cryptococcal neoformans, followed by M. tuberculosis* and then other bacteria. *S pneumoniae* constituted 90% of the non-mycobacterial bacteria cultured followed by *N. meningitidis* which made up 3%. [7] A 10 year South African study from 2008 to 2017 showed that despite introduction of antiretroviral therapy(ART) and reflex cryptococcal antigen testing, there has been no decrease in cryptococcal meningitis and this was attributed to stopping antiretroviral therapy , poor adherence, or virological failure.[8] Another study had found that in hospital mortality of tuberculous meningitis (TBM) can be as high as 69.1% [9].

In the paediatric population, the common causes of meningitis as described in the Western Cape Province were *S. pneumoniae, Group B Streptococcus and N. meningitidis.*[10] The clinical signs and symptoms in children can be non-specific and lumbar puncture is recommended to make the diagnosis.[11]

The cell counts performed on CSF samples may be helpful in differentiating bacterial meningitis which has a polymorphonuclear predominance from tuberculous and cryptococcal meningitis which have a lymphocytic predominance, however in the early stages of tuberculous meningitis there may be a predominance of polymorphonuclear cells[11].

Older laboratory methods for the diagnosis of tuberculous meningitis were culture based and took several weeks. Molecular testing has enabled the rapid detection of *M. tuberculosis*. One such molecular test is the GeneXpert MTB/RIF ultra (Cepheid, Sunnyvale, CA USA), which is an automated system that can both detect *M. tuberculosis* and rifampicin (RIF) resistance in the short time period of 2 hours[12]. The World Health Organization endorsed GeneXpert MTB/RIF in 2013, for the diagnosis of suspected tuberculous meningitis.[13] In 2018 the GeneXpert MTB/RIF ultra was introduced into the laboratory, which had improved sensitivity over the older GeneXpert MTB/RIF. Despite these advances, a 2018 study shows that meningitis is still one of the foremost causes of mortality in Africa and it is linked with the highest risk of inpatient death. [14]

This study described the commonest causes of laboratory confirmed meningitis, as well as key laboratory findings, in King Edward VIII Hospital, Kwa-Zulu Natal, a setting of high HIV prevalence where there has been no such study previously. It also looks at drug resistance in *M. tuberculosis* cultured from cerebrospinal fluid (CSF) samples which has not been studied extensively. In view of the high mortality rate of meningitis, information from this study may guide the clinician in initial management of patients in this setting. A 2021 Cochrane review that included 6 studies that looked at GeneXpert MTB/RIF ultra (GeneXpert ultra) for the diagnosis of TBM, showed that the sensitivity of GeneXpert ultra on CSF was 89% and specificity was 91% as compared to culture. [15] The study looked at the aetiological profile of laboratory confirmed meningitis over 2 time periods 2008 to 2010 and 2018 to 2019 as well as the impact of GeneXpert ultra on the diagnosis of TBM.

2.2.2 METHODS

This is a retrospective description of the causes of laboratory confirmed meningitis. The microbiology laboratory culture results of all patients seen at King Edward VIII Hospital from January 2008 to June 2010 and January 2018 to December 2019 were reviewed to identify patients from whom a cerebrospinal fluid sample was sent to the laboratory. Only culture positive and GeneXpert ultra-positive samples were included. In cases where there were multiple isolates per patient, only the first cultured isolate per patient was included in the analysis. Specimens for bacterial, mycobacterial as well as fungal culture were included. All specimens were processed in the laboratory according to standard operating procedure of the King Edward VIII Division of the National Health Laboratory Services. Gram positive and Gram negative bacteria were identified using the automated Vitek identification system (bioMerieux, Lyon, France). Culture and drug susceptibility testing of *M. tuberculosis* was performed at the Inkosi Albert Luthuli Central Hospital Academic Laboratory using standard laboratory procedures for culture and the 1% indirect agar proportion method on Middlebrook 7H10 agar for susceptibility testing. This is the reference laboratory for *M. tuberculosis* susceptibility testing for the province. Culture for TB was performed using BACTEC mycobacteria growth indication tubes (MGIT) 960 system [BACTEC MGIT Becton Dickinson, USA]. TB culture was performed on all CSF samples during the first time period and only on request by a clinician during the second time period due to diagnostic stewardship measures. Demographic data such as age and sex, where available, were noted. Susceptibility data, where available, were noted for *M. tuberculosis* and bacterial isolates. Susceptibility results to rifampicin, isoniazid, ceftriaxone and meropenem were recorded. Cell counts for polymorphonuclear cells and lymphocytes was recorded in cells per cubic millimetre. Chemistry results were obtained, where available, and included protein, glucose and chloride concentrations. Glucose and chloride concentrations were recorded in millimoles per litre and protein in grams per litre. Molecular testing was performed using the GeneXpert MTB/RIF ultra (Cepheid, Sunnyvale, CA USA) and the MTBDR plus line probe assay (Hain Lifesciences) according to manufacturer's instructions only during the second time period.[16]

SAMPLING

All cerebrospinal fluid (CSF) samples taken from patients at King Edward VIII from which a microorganism was cultured were included in the study. The first period under evaluation was a 30-month period from the 1 January 2008 to 30 June 2010. At the end of June 2010 routine culture of all CSF samples for *M. tuberculosis* was stopped and it was only performed if requested by the treating clinician. Where multiple specimens cultured the same organism from the same patient, only one isolate for the study period was counted to prevent overrepresentation. To examine the long-term trends in causative organism following pneumococcal vaccination as well as intensified tuberculosis screening, an analysis of the microbial causes and susceptibility of *M tuberculosis*, for a 24-month period from 1 January 2018 to 31 December 2019 was also undertaken. In 2018 the more sensitive GeneXpert MTB/RIF ultra became available, and so the positive results from this test were also collected for the second time period only. Line Probe assay was also introduced for molecular susceptibility testing of isolates to Rifampicin and Isoniazid.

Statistics

Categorical data were presented as percentages while continuous data were presented as means

ETHICAL CONSIDERATIONS:

The identity of the participants was not used and only the hospital numbers and specimen numbers were used to identify participants. There was no direct patient participation. Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of Kwa Zulu Natal (Ethical approval number BE 196/010)

2.2.3 RESULTS

A microbial aetiology was confirmed in a total 396 samples over the entire study period. Microorganisms were cultured from 258 non-duplicate CSF samples received by the laboratory during the period January 2008 to June 2010. In the 2018 to 2019-time period, microorganisms were cultured or diagnosed by GeneXpert ultra from 138 CSF samples.

Age analysis

The age of the patient was recorded for 203 out of 217 adults with culture positive meningitis (2008-2010). There were 4 patients from the age of 13 to 20, 67 patients aged 21 to 30, 89 patients aged 31 to 40, 14 patients aged 41 to 50 and 14 patients older than 50. Most patients were between the ages of 21 to 40 as shown in Figure 1

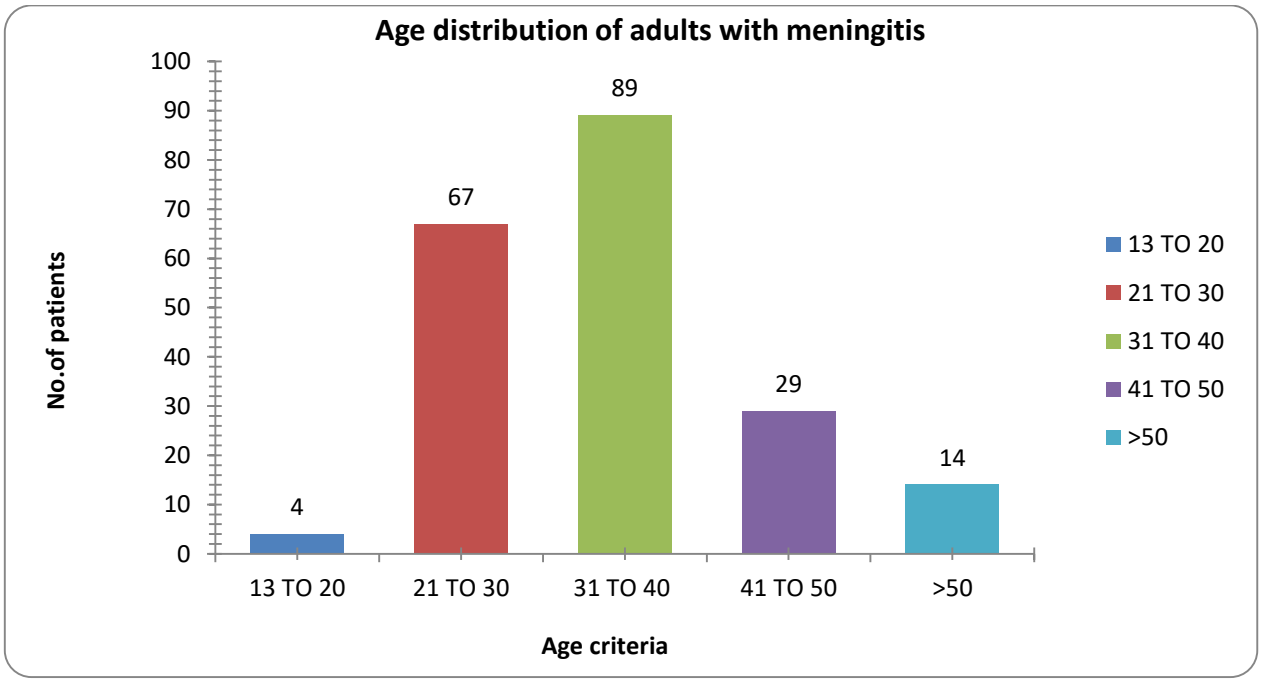

Figure 1: Age distribution of adults with meningitis

The age of the paediatric patients was recorded for 38 out of 43 children with culture positive meningitis (2008-2010). There were 17 patients less than a year of age, 12 patients aged 1 to 3 years, 7 patients aged 4 to 6 years, and 2 patients older than 6 years. Most patients were younger than 1 year as shown in Figure 2

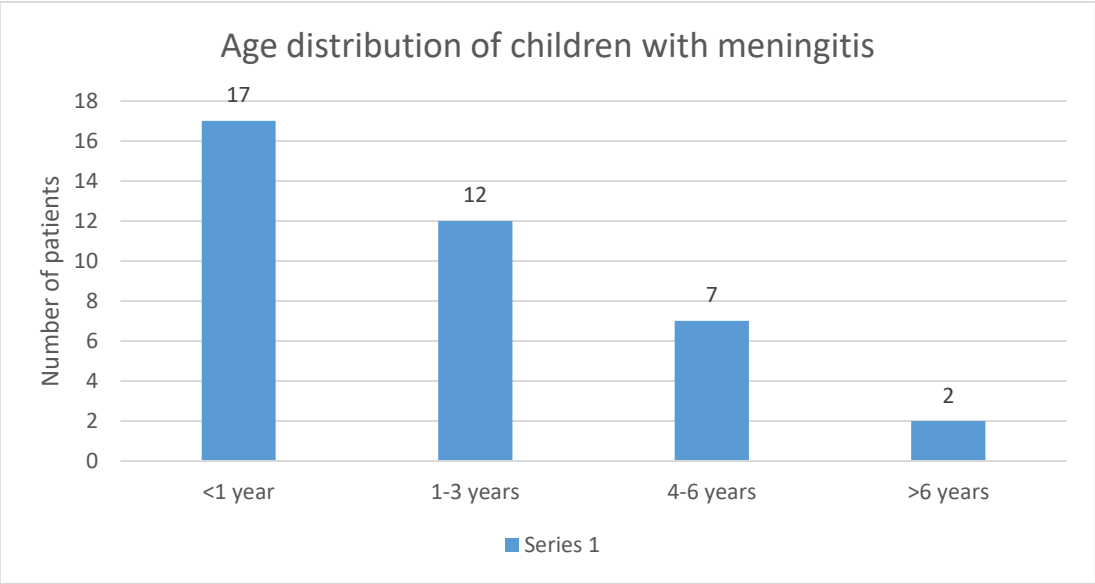

Figure 2: Age distribution of children with Meningitis

Microbial causes

Out of the 258 isolates (adult and paediatric cases) in the 2008-2010 period, 135/258(52%) were *C. neoformans*, 64/258(25%) were *M. tuberculosis* and 59/258 (23%) were other bacteria. In the 2008 -2019 period, 90/138 (65 %) were *C. neoformans*, 29/138(21%) were *M. tuberculosis* and 19/138 (14%) were other bacteria. From the adult only population of 215 patients in the 2008-2010 period, *C. neoformans* and *M. tuberculosis* made up 195/215 (91%) of isolates cultured with the remaining patients having *S. pneumoniae* 11(5%) and other bacteria 9(4%). From the adult only population of 122 patients in the 2018-2019 period, *C. neoformans* and *M. tuberculosis* contribute 113/122 (93%) of isolates cultured with the remaining 4 (4%) patients having *S. pneumoniae* and 9(4%) having other bacteria. The number of adult patients with laboratory confirmed meningitis dropped from an average of 86 cases per 12-month period in the 2008-2010 period to 61 cases per 12-month period in the 2018-2019 period. Figure 3 and Figure 4 show the microbial aetiologies in the two time periods.

There was a decrease in pneumococcal meningitis in both children and adults between the earlier and later time periods from 14 to 0 cases in children and 11 to 5 cases in adults respectively. There was also a decrease in tuberculous meningitis from the 2008-time period to the 2018-time period in adults from 62 cases to 24 cases. The relative decrease in tuberculous and bacterial meningitis resulted in a proportionate increase of the fraction that *C. neoformans* contributed to the aetiology of meningitis.

Adult Meningitis

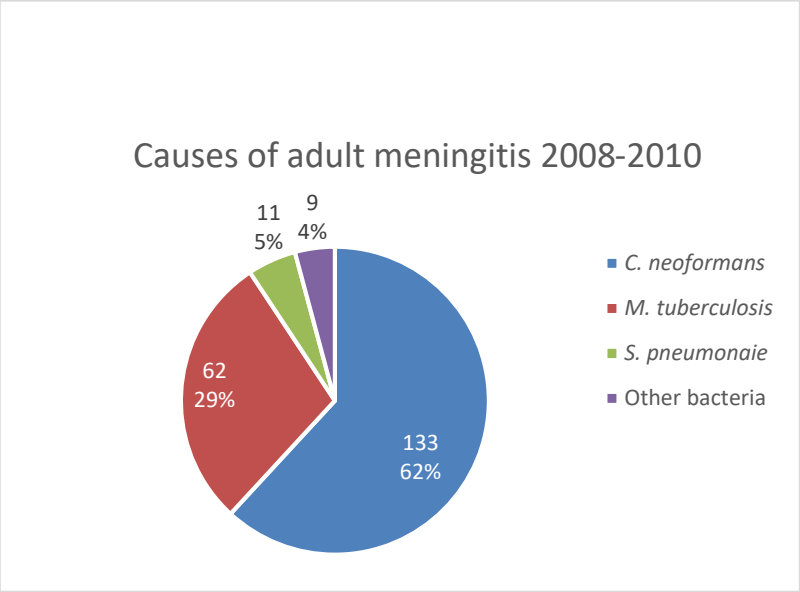

Figure 3. Causes of adult meningitis 2008-2010 from a total of 215 patients

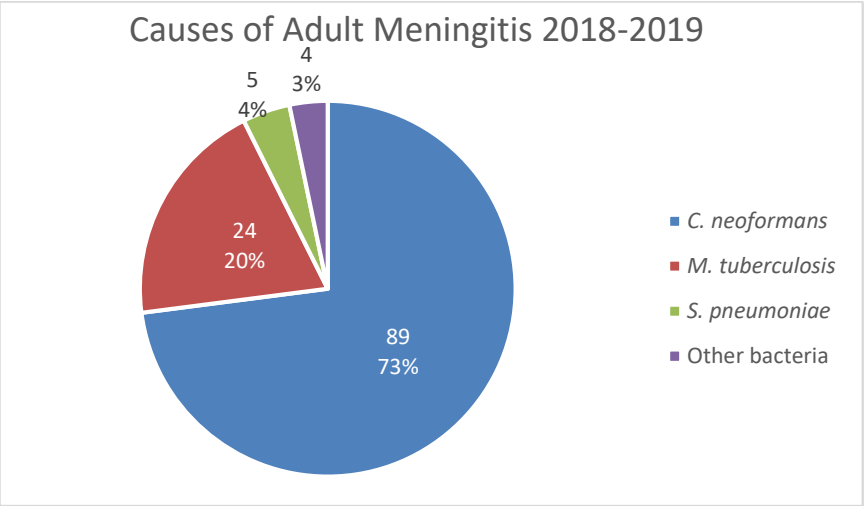

Figure 4. Causes of adult meningitis 2018-2019 from a total of 122 patients

Table 1. Common Causes of bacterial meningitis in children compared to adults 2008-2010 and 2018-2019

	Children				**Adult patients**			
	2008-2010		**2018-2019**		**2008-2010**		**2018-2019**	
Organism	**No.**	**%**	No.	**%**	**No.**	**%**	**No.**	**%**
Streptococcus pneumoniae	14	35,9	0		11	55	5	55,6
Group B streptococcus	5	12,8	0		0		0	
Neisseria meningitidis	3	7,7	0					
Haemophilus influenzae	2	5.1	2	20				
Escherichia coli	2	5,1	0		3	15	0	
Staphylococcus aureus	2	5,1	2	20	2	10	0	
Klebsiella pneumoniae	2	5,1	2	20	0		1	11
Salmonella species	0		1	10	2	10	0	
Enterobacter cloacae	2	5,1						
Acinetobacter lwofii	0		1	10				
Serratia liquefascians	0		0		1	5	0	

Coagulase negative staphylococci	6	15.4	1	10	1	5	1	11
Enterococcus faecium	0		1	10				
Viridans streptococcus	1	2,6			0		1	11
Listeria monocytogenes	0				0		1	11
total	39		10		20		9	

All *S. pneumoniae* isolates in both time periods were susceptible to ceftriaxone. All isolates of *Group B Streptococcus, N. meningitidis, Haemophilus influenzae, Salmonella species* were also susceptible to ceftriaxone. One isolate out of the six isolates of *S. aureus* was resistant to cloxacillin and susceptible to vancomycin. There were 2 isolates of *Enterobacter cloacae* in the first period that was resistant to ceftriaxone and susceptible to meropenem. In the second time period there was 1 isolate of *Serratia liquefascians* that was resistant to ceftriaxone and susceptible to meropenem.

There was a large decrease in the number of *S. pneumoniae* between the two time periods from 25 to 5 cases.

Paediatric meningitis

Children were classified from 0 to 12 years of age. The predominant cause of meningitis in children was bacteria rather than *C. neoformans* or *M. tuberculosis*. The leading cause of meningitis in children in the 2008-2010 period was *S. pneumoniae* 14/43 (34, 1%) followed by *Group B streptococcus* 5/43(12.2%) and other microbes shown in Table 1. The 3 paediatric patients that came from nursery cultured *E. coli, K. pneumoniae* and *S. pneumoniae*.

In children, in contrast to adults, bacterial meningitis was more frequent with only a small percentage of cryptococcal and tuberculous meningitis being present.

In the 2018 to 2019-time period there were only 10 cases of bacterial meningitis, 5 cases of tuberculous meningitis and 1 case of cryptococcal meningitis. The bacteria isolated from 10 CSF samples in children during the 2018-2019 period were *Haemophilus influenzae* (n=2), *Klebsiella pneumoniae* (n=2), *Staphylococcus aureus* (n=2), *Staphylococcus epidermidis* (n=1), *Enterococcus faecium* (n=1), *Acinetobacter lwoffii* (n=1), *Salmonella species* (n=1). Of note there were no cases

of pneumococcal meningitis, Group B streptococcal meningitis or meningococcal meningitis in children for the second time period

Of the 5 paediatric GeneXpert ultra positive cases in the 2018-2019 period, only 1 was culture positive which indicates that GeneXpert ultra detected an additional 4 TBM cases.

Table 2. Causes of meningitis in children 2008-2010

Organism	**No.**	**Percentage (%)**
Streptococcus pneumoniae	14	32.6
Group B streptococcus	5	11.6
Neisseria meningitidis	3	7.0
Escherichia coli	2	4.7
Haemophilus influenzae	2	4.7
Klebsiella pneumoniae	2	4.7
Staphylococcus aureus	2	4.7
Enterobacter cloacae	2	4.7
Streptococcus viridans	1	2.3
Coagulase negative staphylococci	6	14
Cryptococcus neoformans	2	4.7
Mycobacterium tuberculosis	2	4.7
total	43	

Table 3. Causes of meningitis in children 2018-2019

Organism	**No.**	**Percentage (%)**
Haemophilus influenzae	2	13,3
Klebsiella pneumoniae	2	13,3
Staphylococcus aureus	2	13,3
Enterococcus faecium	1	6.7
Acinetobacter lwoffii	1	6.7
Salmonella species	1	6.7
Coagulase negative staphylococci	1	6.7
Cryptococcus neoformans	1	6,7

Mycobacterium tuberculosis	5	33,3
total	16	

Tuberculous meningitis

In the 2008-2010 period, samples in which *M. tuberculosis* was cultured, the mean CSF protein was 3 g/l, the mean glucose was 1,8 millimoles per litre and the mean chloride was 110,6 millimoles per litre. The average lymphocyte count was 93.5 cells and the average polymorph nuclear cell was 63.7 cells per cubic milliliter. There were no acid fast bacilli seen on microscopy and 0 lymphocytes on cell count in 5 % of CSFs. . A study done by Kaerstaed *et al* that showed that up to 24 % of CSF samples from patients with tuberculous meningitis can have acellular fluid. [9] During the 2008-2010 period, 54/64 (84.4%) isolates were susceptible to rifampicin and isoniazid, 8/64 (12.5%) isolates were multidrug resistant (MDR) i.e., resistant to both rifampicin and isoniazid and 2/64 (3.1%) were isoniazid monoresistant.

In the 2018 – 2019 period, GeneXpert was performed as the first test on CSF for the diagnosis of TB. Of the 29 GeneXpert positive CSF samples, only 14/29 (48%) were culture positive. Acid fast bacilli were seen in 4/29(13.8%) CSF samples on microscopy. 10/29 were culture negative, 2 were insufficient as sample was used up in GeneXpert and 3 samples werecontaminated with other bacteria. 13.8% (4/29) of the GeneXpert positive specimens also tested positive for rifampicin resistance. 3 of the 4 rifampicin resistant samples had line probe assays that confirmed the rifampicin resistance while the remaining one was culture negative. 2 of the 4 Rifampicin resistant samples were MDR. Over the entire study period there was 10.8% (10/93) of MDR TB.

Cryptococcal meningitis

For the 2008-2010-time period, the mean CSF protein was 1.1 g/l, mean glucose was 2,1 millimoles per liter and mean chloride was 120.9 millimoles per liter. Cryptococcus was seen on microscopy in 112 out of 135 (83%) of culture positive CSF samples. Cryptococcal antigen test was positive in 96% of patients with cryptococcal meningitis. The mean lymphocyte count was 43.2 cells and the mean polymorph nuclear cell was 21.7 cells per cubic milliliter.

Bacterial meningitis

In the 2008-2010 period, the mean CSF protein was 3.1g/l, the mean glucose was 1.5 millimoles/litre and mean chloride was 120 millimoles /litre. The mean polymorph count was 468.1 cells per cubic milliliter and the mean lymphocyte count was 63 cells per cubic milliliter.

Table 2. Laboratory findings in bacterial, tuberculous and cryptococcal meningitis in all patients for 2008-2010 period

Mean Laboratory indices	Bacterial meningitis	Tuberculous meningitis	Cryptococcal meningitis
Polymorph count (cells per cubic milliliter)	468.1	63.7	21.7
Lymphocyte count (cells per cubic milliliter)	63	93.5	43.2
Protein (g/l)	3.1	3.0	1.1
Glucose (mmol/l)	1.5	1.8	2.1
Chloride (mmol/l)	120	110	120.9

2.2.4 DISCUSSION

This study described the commonest etiologies of culture positive meningitis in King Edward VIII Hospital, Kwa-Zulu Natal, a setting of high HIV prevalence. The findings of this study are also in keeping with a large study by Jarvis *et al* who also found that in 2010, C. *neoformans* and *M. tuberculosis* were the commonest causes of meningitis.[7] The etiological profile of meningitis in adults has changed from bacteria such as *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* that were the predominant pathogens in the 1980s to *C. neoformans* and *M. tuberculosis* and this is due to HIV infection. In a 4-year South African study from 2009 to 2012 Britz *et al* found that the relative proportions of different aetiologies were, cryptococcal meningitis 62.3%, TBM 24.6 % and pneumococcal meningitis 10.1%. [17] This study found similar figures for cryptococcal meningitis 62%, TBM 29% and pneumococcal meningitis 5%.

The main causes of paediatric meningitis in the 2008-2010 period, *S. pneumoniae, Group B Streptococcus* and *N. meningitidis* are similar to findings in the Western Cape by Jans *et al* who found that between 2010 and 2012, 31 bacteria cultured from children were, *Streptococcus pneumoniae* (*n*=9; 29.0%), Group B *Streptococcus* (*n*=6; 19.4%), *Neisseria meningitidis* (*n*=5; 16.1%), and other bacteria (n=11; 35.4%). The relative percentage of bacterial meningitis decreased from 23% of causes in 2018-2010 period to 14 % in the 2018-2019 period and there

was a corresponding increase in the proportion of cryptococcal meningitis from 52% in 2008-2010 to 65% in 2018-2019 period. The large decrease in the number of S. pneumoniae between the two time periods from 25 to 5 cases is due to the national Expanded Programme for Immunization as shown previously. [17] The expansion of ART coverage from 2008 to 2012 along with earlier detection of tuberculosis through intensified case-finding and improved molecular diagnostics, has been suggested as having been responsible for the decrease of microbiologically confirmed tuberculosis. [17] Previous studies have shown that, similar to this study, that the incidence of tuberculous and pneumococcal meningitis decreased more significantly among adult populations over the reported period, with the proportion of cases of cryptococcal meningitis increased when compared to those of pneumococcal and tuberculous meningitis.

The larger declines in bacterial and tuberculous meningitis as compared to cryptococcal meningitis are possibly due to the effects of vaccination and the introduction of the enhanced tuberculosis control programme in 2011. A 2017 review article showed that in most parts of Sub-Saharan Africa, the incidence of cryptococcal meningitis is not declining in spite of the availability of antiretroviral therapy, due to lack of adherence and retention in HIV care. Over the past 5 years, advances have been made in reflex screening of cryptococcal antigen in blood sent for CD4 count testing and early screening for meningitis which may have resulted in earlier detection in the CSF. [18]

This study showed that bacterial meningitis had a predominance of polymorphonuclear cells while the cryptococcal and tuberculous meningitis had a predominance of lymphocytes which is in keeping with the literature. [11] Previous studies, however, have shown that there is no agreement in the literature regarding precise cut-off points in CSF values for various meningitis aetiologies. [10] The cell count alone cannot differentiate between bacterial, tuberculous and fungal meningitis. Increased lymphocyte count in the CSF is be seen in viral, fungal, and tuberculous infections of the CNS, although a predominance of PMNs may occur in the early stages of these infections.

Of importance, Jarvis *et al* found that 16% (81) patients with confirmed Cryptococcus, 5% (12) with TB and 4% (3) with bacterial meningitis had normal CSF cell-counts and biochemistry [(7)]. In this study, 6.7 % (14patients), with confirmed Cryptococcus, 3.8 % (4) with TBM and 4.4% (1) with bacterial meningitis had normal CSF cell counts and biochemistry.

No acid fast bacilli and lymphocytes were seen on microscopy in 5 % of CSFs that cultured *M. tuberculosis*. A study done by Kaerstaed *et al* showed that up to 24 % of CSF samples from patients with tuberculous meningitis can have acellular fluid. [9]
Markedly increased protein with a low glucose is found in bacterial meningitis and TBM and was seen in this study also. [19] This study found that CSF samples from patients in 2008-2010 period with TBM had lower CSF chloride than both bacterial and cryptococcal meningitis. It

has been found however that CSF chloride reflects whole body chloride and is not useful for the diagnosis of meningitis. [20] CSF chloride was discontinued as a laboratory test and was not available for the more recent study period.

Previous studies performed in KwaZulu Natal between 1999 and 2002 have shown that 30/350 (8.6 %) patients with tuberculous meningitis had MDR TB [21]. 73% of patients in that study had a previous history of receiving anti-TB treatment. This study however showed a higher rate of drug resistance of 10.8%. This may be due to increasing resistance over time. Unfortunately, clinical history was not available for this study. The presence of drug resistant *M. tuberculosis* causing meningitis has serious implications for management of these patients as the drug susceptibility pattern is not known initially and 1st line therapy is inadequate. [21]
Microscopy for *Mycobacterium tuberculosis* has a low yield, with this study showing acid fast bacilli in only 13.8% of CSF samples, in the second time period, and culture can take up to 6 weeks. This study found that in CSF samples with a high lymphocyte count and high protein count, together with a negative cryptococcal antigen, TBM should be a clinical consideration. Of the 29 GeneXpert ultra positive specimens, only 14 were culture positive. GeneXpert ultra contributed to the diagnosis of an additional 15 patients that may have been missed by culture alone.

The laboratory diagnosis of tuberculosis is still problematic and in a recent study by Mitha *et al*, the sensitivity of the older GeneXpert MTB/RIF for the diagnosis of tuberculous meningitis was 63.2% and that of culture was 65, 7%, when compared to five other diagnostic tests. [21] A recent Cochrane review found that the sensitivity of the GeneXpert ultra was 89% and the specificity was 91 % in comparison to culture. This is a significant improvement in sensitivity compared to the older GeneXpert MTB/RIF which had sensitivity of 71.1% and specificity of 98%. [15] The imperfect specificity, however, could mean that some of the GeneXpert ultra positive, culture negative specimens could be false positive results.

The authors added, however, that in patients with suspected TB meningitis, treatment should be based on clinical judgement, and not withheld based on an GeneXpert result. [15] A 2020 meta-analysis of deaths from HIV-associated meningitis in Africa showed that the short-term pooled mortality from cryptococcal meningitis, TBM and pneumococcal meningitis are 44%, 46% and 54% respectively, and that strategies are needed for decreasing mortality. Earlier diagnosis through optimal laboratory testing is one strategy to decrease mortality. [23]

There are several limitations to this study. As it was a retrospective study, clinical information was not available as all clinical chart records were not archived. HIV testing was commonly done using rapid tests that were not captured on the laboratory system but in clinical chart records and this information was not available for most patients.

Conclusion

C. neoformans and *M. tuberculosis* remain the leading causes of meningitis in adult patients with *S. pneumoniae* being the commonest cause of adult bacterial meningitis. There has been a decrease in pneumococcal meningitis especially in children due to the Expanded Programme for Immunization; and also, in adults due to the effects of herd immunity on adults. The introduction of reflex cryptococcal antigen screening on blood sent for CD4 count allows for earlier screening and detection of meningitis but better virological suppression is needed to prevent cryptococcal meningitis. While there are sensitive, rapid antigen tests for the diagnosis of cryptococcal meningitis, tuberculous meningitis continues to present a diagnostic dilemma to the clinician. Meningitis can present with acellular CSF and treatment should be initiated if the clinical suspicion is high. The increased rate of drug resistance in M. tuberculosis isolated from CSF needs continued attention and has serious management implication for patients with this condition. GeneXpert ultra has aided significantly in the diagnosis of tuberculous meningitis, however clinical correlation is needed as the specificity is not 100%. Better diagnostic tests for the diagnosis of TBM are needed as well comprehensive ART programmes.

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3.1 APPENDICES

APPENDIX 3.1.1

PROTOCOL SUBMITTED TO BREC

The Protocol

RESEARCHER: Dr Praksha Ramjathan

CO-INVESTIGATORS: SUPERVISOR: Dr Khine Swe Swe Han

DEPARTMENT: Dept. of Medical Microbiology – Academic Complex (King Edward VIII Hospital / Inkosi Albert Luthuli Central Hospital (IALCH))

Title:
Causes of Meningitis in the Era of HIV infection

Type of Study:

Laboratory Based – retrospective review of results

The Project

1. Aim:
   The aim of the study is to review the laboratory records of all the patients who had a cerebrospinal fluid specimen sent to the microbiology laboratory and to determine the organisms cultured from that specimen:

   Objectives:

   Primary -
   - To determine the causative organisms in patients presenting with suspected meningitis in King Edward VIII Hospital.

   Secondary -
   - The correlation between microorganism cultured and the demographic data from cerebrospinal fluid specimen.

2. Hypothesis:

- The primary objective is a descriptive retrospective analysis of the laboratory records of patients with suspected meningitis to determine the culture positive aetiology. No specific hypothesis will be tested for this objective.

Background and Literature

Meningitis is a life-threatening infection of the central nervous system that has a high morbidity and mortality rate. Empiric treatment is often started based on knowledge of common microbial causes.

South Africa has a high burden of human immunodeficiency virus (HIV) disease With HIV disease the immune system is impaired and the patient is at increased risk of infection especially with opportunistic pathogens such as Cryptococcus neoformans. In 2007 Kwa-Zulu Natal had an HIV prevalence of 37.4% in antenatal clinics. [1]

To the authors knowledge there have been no published studies on the causes of meningitis at King Edward VIII Hospital or in Kwa-Zulu Natal. In 1967, Jones et al [2] showed that the leading causes of meningitis at the Johannesburg Fever Hospital were *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae*. Another study performed in five Johannesburg hospitals from 1980 to 1982 also showed that the leading causes of meningitis in adults were *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae.* [3] *The* same study also found that that the leading causes of neonatal meningitis were *E. coli* and *Group B Streptococcus.*

In a 4 year study at the Pretoria Academic hospital from 1994 to 1998 Schutte C M et al concluded that "the HIV epidemic was responsible for a marked shift in the spectrum of meningitis towards chronic infections such as TB and cryptococcal meningitis." [4]

During the period 2006 to 2008, Jarvis et al found that in a setting where 43 % of patients have clinically suspected HIV infection, the leading causes of meningitis was *Cryptococcal neoformans, followed by Mycobacterium tuberculosis* and then other bacteria. *Streptococcus pneumoniae* constituted 90% of the non-mycobacterial bacteria cultured followed by *Neisseria meningitidis* which made up 3%. [5]

Cryptococcal meningitis was also the leading cause of meningitis in a study involving a group of patients where 77.9% were HIV positive. [6] This study will describe the commonest causes of culture positive meningitis in King Edward VIII Hospital, Kwa-Zulu Natal, a setting of high HIV prevalence where there has been no such study previously.

Summary of proposed research:

STUDY DESIGN.

This is a simple retrospective description of the causes of culture positive meningitis.
The microbiology laboratory culture results of all patients seen at King Edward VIII Hospital from January 2008 to June 2010 will be reviewed to identify patients from whom a cerebrospinal fluid specimen was sent to the laboratory. Only culture positive specimens will be included. Only one isolate per patient will be included in the analysis. Specimens for bacterial, mycobacterial as well as fungal culture will be included. All specimens are processed in the laboratory according to standard operating procedure which is standardised in the laboratory. Demographic data such as age and sex, where available, will be noted. Susceptibility data, where available will also be noted for *Mycobacterium tuberculosis* and bacterial isolates. Susceptibility to rifampicin, isoniazid, ethambutol, streptomycin, ofloxacin and kanamycin will be recorded. Cell counts for red blood cells, polymorphonucleo and lymphocytes will be recorded in cells per cubic millimetre. Chemistry results will be obtained, were available, and include protein, glucose and chloride concentrations. Glucose and chloride concentrations will be recorded in millimoles per litre and protein in grams per litre.

SAMPLING
All cerebrospinal fluids taken from patients at King Edward VIII from which a microorganism was cultured or detected by GeneXpert ultra were included in the study. The first period under evaluation will be 1 January 2008 to 30 June 2010 and the second will be 1 January 2008 to 31 Decmber 2019. Where multiple specimens cultured the same organism from the same patient, only one isolate for that entire time period will be counted to prevent overrepresentation.

SAMPLE SIZE

The sample size will depend on the number of cerebrospinal fluid specimens sent to the laboratory for culture. Since the objective of this study is purely descriptive, no sample size calculations were performed.

DATA COLLECTION

Data will be obtained from the computerised patient database at IALCH and entered into a data collection sheet for analysis.

DATA ANALYSIS TECHNIQUES
Data will be entered into Microsoft Excel spreadsheets.

All categorical variables will be summarised as counts and percentages. Age categories will be determined as following:

Group 1 – neonates - birth to 28 days
Group 2 – children - 29 days to 12 years
Group 3 – young adults – 13 to 20 years
Group 4 adults - 21 to 40 years
Group 5 older adults - 41 to 60 years
Group 6 elderly - greater than 60 years of age

STUDY LOCATION

National Health Laboratory Services, Microbiology Laboratory IALCH in Durban, Kwa Zulu-Natal, South Africa

STUDY PERIOD

The study period will be from January 2008 to June 2010

LIMITATIONS:

HIV results not available.
Clinical information not available.
Some data may be unavailable

ETHICAL CONSIDERATIONS:

The identity of the participants will be withheld and only the hospital numbers and specimen numbers will be used to identify participants.

There will be no direct patient participation.

Keywords
Meningitis Causes HIV

Key References

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APPENDIX 3.1.2

DATA EXTRACTION FORM

DATA EXTRACTION FORM

Abbreviations

Poly. – polymorphonuclear cell

Concen. – concentration

g/l – grams per litre

Mmol/l – millimoles per litre

Study number	Specimen number	Organism cultured	age	sex	Red cell count	Lymphocyte count	Poly. Count	Protein concen. g/l	Glucose concen. Mmol/l	Chloride concen. Mmol/l

APPENDIX 3.1.3
ETHICS APPROVAL

UNIVERSITY OF
KWAZULU-NATAL

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
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Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

23 November 2010

Dr P Ramjathan
Department of Medical Microbiology
Inkosi Albert Luthuli Central Hospital (IALCH)
Level 4
800 bellair Road
Cator Manor
4058

Dear Dr Ramjathan

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application dated 05 October 2010.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 19 November 2010 to queries raised on 01 November 2010 has been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 23 November 2010.

This approval is valid for one year from **23 November 2010.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.

APPENDIX 3.1.4
ETHICS AMMENDMENT TO INCLUDE RECENT DATA

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

12 August 2020

Dr. P Ramjathan
Department of Medical Microbiology
Inkosi Albert Luthuli Central Hospital (IALCH)

Dear Dr Ramjathan

PROTOCOL: Causes of Meningitis in the area of HIV Infection. REF: BE196/010

We wish to advise you that your correspondence received on 04 August 2020 submitting an application for amendments (inclusion of more recent data to look at trends in the causes of meningitis over a longer period) for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee.

The committee will be notified of the above at its next meeting taking place on 08 September 2020.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

APPENDIX 3.1.5

TURNITIN REPORT

Dissertation May

ORIGINALITY REPORT

7%	%	7%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Mark W Tenforde, Alida M Gertz, David S Lawrence, Nicola K Wills, Brandon L Guthrie, Carey Farquhar, Joseph N Jarvis. "Mortality from HIV - associated meningitis in sub - Saharan Africa: a systematic review and meta - analysis", Journal of the International AIDS Society, 2020 Publication	7%

Exclude quotes	On	Exclude matches	< 6%
Exclude bibliography	On		