COMPARISON OF BEDSIDE INOCULATION OF CULTURE MEDIA WITH TRADITIONAL CEREBROSPINAL FLUID CULTURE METHOD IN PATIENTS WITH SUSPECTED BACTERIAL MENINGITIS AT KENYATTA NATIONAL HOSPITAL

A Dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine in Internal Medicine by

ANTHONY OLIWA ETYANG

UNIVERSITY OF NAIROBI

2008

DECLARATION

I certify that this dissertation is my original work and that it has not been submitted for a degree to any other university.

Anthony Oliwa Etyang

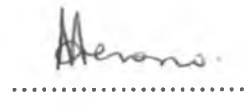
SUPERVISORS

Prof. E.O. Amayo,
Associate Professor,
Department of Clinical Medicine & Therapeutics,
University of Nairobi .

Prof. S.M. Bhatt,
Professor,
Department of Clinical Medicine & Therapeutics,
University of Nairobi.

Prof. I.A. Wamola,
Professor,
Department of Medical Microbiology,
University of Nairobi.

Dr. M.C. Maritim,
Tutorial Fellow,
Department of Clinical Medicine & Therapeutics,
University of Nairobi.



DEDICATION

To my father who has not only shown me the way, but has always been there to inspire me.

To my mother whose unending love and support has seen me endure the toughest ofchallenges.

To my brother,Philip and sisters,Linda,Caro and Maria who have always been there for me.

To God who has made it all possible.

Epolo Edeke.

ACKNOWLEDGEMENTS

I would like to thank my supervisors for their guidance,support and encouragement at all stages that have gone into preparing this dissertation.

I thank my sponsors, Katholischer Akademischer Auslander Dienst (KAAD) for providing me with a generous scholarship and research allowance to pursue this degree.

I thank the laboratory staff at the Department of Medicine, University of Nairobi for carrying out the bacterial cultures that formed the basis of this study.

I thank all the patients who agreed to participate in this study and all the clinicians who were ever so alert in notifying me about potential study patients.

I am grateful to all who in many different ways have contributed to the success of this study.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

List of Abbreviations

C B A .... Chocolate blood agar

C D C... Centers for disease control

C N S.... Central nervous system

C S F.... Cerebrospinal fluid

C T .... Computerized tomography

E L I S A...Enzyme linked immunosorbent assay

G C S... Glasgow coma score

HiB.... Haemophilus influenzae B

HIV.... Human Immunodeficiency virus

I C P.... Intracranial pressure

K N H.... Kenyatta National Hospital

L P .... Lumbar puncture

MGIT.... Mycobacterial Growth Indicator Tube

m l .... Milliliter

S B A .... Sheep blood agar

S O L .... Space occupying lesion

S P S S ....Statistical package for the social sciences

W H O .... World health organization

Z N..... Ziehl-Neelsen

ABSTRACT

Background: The yield of bacterial cultures from cerebral spinal fluid (CSF) at Kenyatta National Hospital (KNH) is very low. Bedside inoculation of culture media with CSF may improve yields.

Objective: To compare the culture yield of CSF inoculated onto culture medium at the bedside to CSF which is inoculated onto culture medium in the microbiology laboratory.

Hypothesis (Ha) : The yield of CSF inoculated onto culture medium at the bedside would be significantly higher than the yield of CSF inoculated onto culture medium in the microbiology laboratory .

Study design: Cross-sectional comparative study

Setting: Accident and emergency department and medical wards, Kenyatta National Hospital, Nairobi, Kenya.

Study population: CSF from patients admitted to the medical wards at KNH with a clinical diagnosis of meningitis .

Study method: Patients admitted with a clinical diagnosis of meningitis were subjected to lumbar puncture after excluding those in whom the procedure was contraindicated. CSF thus obtained was either inoculated onto culture medium at the bedside or put in a sterile specimen bottle. Both samples were then transported to the microbiology laboratory at the same time. CSF in the sterile bottle was inoculated onto culture medium at the laboratory and both samples

were then incubated for up to 72 hours under appropriate conditions. Isolated bacteria were identified using standard microbiologic techniques and sensitivity patterns to various antibiotics determined using disk diffusion method.

Data management and analysis: Data was entered into a computer database and analyzed using SPSS version 11.5. Comparison between the two culture methods was made using the z test.

Results : Two hundred and twenty CSF specimens were obtained during a four month period. *S.pneumoniae* was isolated from 24 CSF specimens and *H.influenzae* from 1. Bacterial cultures were positive in 25 (11.4% , 95% CI 7.0-15.6%) of samples inoculated at the bedside and 23 (10.5%,95% CI 6.5-14.5%) of samples inoculated at the laboratory. Bacteria were isolated 5 hours earlier in samples inoculated at the bedside(95% CI 4.34-6.86 hrs,$p<0.05$). Four percent of *S.pneumoniae* isolates were resistant to crystalline penicillin and chloramphenical.

Conclusion : There was no significant difference in culture yield after bedside inoculation of culture media with CSF compared to traditional CSF culture method. Bedside inoculation of culture media with CSF resulted in faster time to positive culture. The majority of bacterial isolates were susceptible to antibiotics currently used at KNH

INTRODUCTION

Meningitis is an inflammatory disease of the leptomeninges, the tissues surrounding the brain and spinal cord . It is associated with very high morbidity and mortality. Causative organisms include bacteria, viruses as well as fungi. Non infectious causes of meningitis for example tumors and irradiation also exist.

The diagnosis of bacterial meningitis requires the isolation of the causative agent from the cerebrospinal fluid (CSF).In the majority of patients treated for bacterial meningitis at Kenyatta National Hospital, a causative organism is not isolated.

The purpose of this cross-sectional comparative study was to determine whether bedside inoculation of culture media with CSF would improve the proportion of culture proven bacterial meningitis cases at KNH.

LITERATURE REVIEW

From its original recognition in 1805 by Vieusseux (1) until the early 1900s, bacterial meningitis was virtually 100 percent fatal. In 1913, Flexner's introduction of intrathecal meningococcalantiserum prevented some deaths, but the clinical outcome did not dramatically improve until the advent of systemic antimicrobial therapy more than 50 years ago [2].However despite advances in critical care and the introduction of newer antimicrobial agents morbidity and mortality from the disease remain high(3)

Worldwide, bacterial meningitis accounts for approximately 1.2million cases annually (4). Meningitis is among the ten leading infectious causes of death and is responsible for approximately 135,000 deaths throughout the world each year (4)

In a study performed at Kenyatta National Hospital in 1992 (5), bacterial meningitis was found to account for 23.1% of all neurological admissions.

The classic triad of acute bacterial meningitis consists of fever, nuchal rigidity, and a change in mental status, although an appreciable number of patients do not have all three features (one-third in one series) (6) . Most patients have high fevers often greater than 38°C (7) , but a small percentage have hypothermia; almost no patients have a normal temperature (8) .

Headache is common. The headache is typically described as severe and generalized. It is not easily confused with a normal headache.

The following findings at presentation and during hospitalization were noted in a review of 279 episodes of community-acquired meningitis (6) . The frequency of headache was not noted. Fever was present in 95 percent at presentation and developed in another four percent within the first 24 hours. Among survivors in whom such data were available, 19 percent had fever for ten or more consecutive days, but most had other possible causes of continued fever. Patients with no identifiable source of fever other than meningitis had an average of four

consecutive days of fever (range 0 to 14 days). Nuchal rigidity was present in 88 percent on initial examination, and persisted for more than seven days in some patients despite overall improvement. Mental status was altered in 78 percent. Most were confused or lethargic, but 22 percent were responsive only to pain and six percent were unresponsive to all stimuli.

Similar findings were noted in three other large series of adults with acute bacterial meningitis (7,9,10) . At presentation, fever was present in 77 to 85 percent, neck stiffness in 83 to 94 percent, headache in 79 to 94 percent, and altered mental status in 83 percent, including coma in 14 to 16 percent.

One or more of the classic findings on history (fever, neck stiffness, altered mental status) or physical examination (nuchal rigidity) are absent in many patients with bacterial meningitis (6,7,9,11) . In the 2004 review of 696 cases of community-acquired bacterial meningitis cited above, only 44 percent had the clinical triad of fever, neck stiffness, and, altered mental status (10) . This was much more likely to occur in patients with pneumococcal compared to meningococcal meningitis (58 versus 27 percent).

However, virtually all patients have at least one of the findings of the classic triad of fever, neck stiffness, and altered mental status with a sensitivity of 99 to 100 percent for the presence of at least one finding in a 1999 critical appraisal of ten studies of 845 episodes of meningitis (12) . Similarly, 99 percent had at least one classic feature in the 2004 series of 696 cases (10) . Thus, the absence of all of these findings essentially excludes the presence of bacterial meningitis.

In addition to the classic findings, a number of other manifestations, both neurologic and non-neurologic, can occur in patients with bacterial meningitis:

a) Significant photophobia.
b) Neurologic complications such as seizures, focal neurologic deficits (including cranial nerve palsies), and papilledema may be present early or occur later in the course (6,7,10,12) . Seizures have been described in 15

to 30 percent of patients and focal neurologic deficits in 20 to 33 percent (6,9,10,12) .

c) Hearing loss is a late complication. Dexamethasone therapy may reduce the rate of neurologic sequelae, particularly in selected patients with pneumococcal meningitis of intermediate severity (9) .

d) Certain bacteria, particularly *N. meningitidis*, can cause characteristic skin manifestations, such as petechiae and palpable purpura. In two large series of patients with community-acquired bacterial meningitis, rash was present in 11 and 26 percent; among those with rash, 75 and 92 percent were associated with meningococcal meningitis (6,10) . A petechial rash is not specific for meningococcal infection and some patients with meningococcal meningitis have a maculopapular rash (6) .

e) Arthritis occurs in a significant minority of patients with bacterial meningitis. In a case series of 696 episodes of community-acquired bacterial meningitis, arthritis was diagnosed in 48 (seven percent) of the episodes with *N. meningitidis* the etiologic agent in two-thirds of these joint infections (13) . Joint fluid aspiration was performed in 23 patients and was positive by culture in six (26 percent). Recognition of the concurrent arthritis is important as prolonged antibiotic therapy is necessary. It may be helpful in establishing the total duration of therapy.

Bacterial meningitis tends to spare other organs unless severe sepsis ensues. However, if meningitis is the sequela of an infection elsewhere in the body, there may be features of that infection still present at the time of diagnosis of meningitis (eg, otitis or sinusitis).

The causative agents for bacterial meningitis vary with age. In adults the most frequently isolated organisms are *Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae* .

In a review of 248 patients with community-acquired acute bacterial meningitis seen in 1995 in acute care hospitals in four states in the United States, the following frequencies of the major pathogens were noted in adults. (14):

Up to age 60, *S. pneumoniae* was responsible for 60 percent of cases, followed by *N. meningitidis* (20 percent), *H. influenzae* (10 percent), *L. monocytogenes* (six percent), and group B streptococcus (four percent). Age 60 and above, almost 70 percent of cases were due to *S. pneumoniae*, approximately 20 percent to *L. monocytogenes*, and three to four percent each to *N. meningitidis*, group B streptococcus, and *H. influenzae*. An increased prevalence of *L. monocytogenes* in the elderly has been noted in other reports (15).

A similar distribution of causes was noted in a later series of 696 episodes of community-acquired bacterial meningitis in adults (mean age 50±20 years) in the Netherlands from 1998 to 2002 (10). *S. pneumoniae* was responsible for 51 percent of cases, followed by *N. meningitidis* (37 percent) and *L. monocytogenes* (four percent). The remaining 8 percent were due to *H. influenzae* (two percent), *S. aureus*, streptococci, gram-negative bacilli, *Haemophilus parainfluenzae*, and *Staphylococcus epidermidis*.

A definitive diagnosis of meningitis is made via CSF culture and only culture proven cases of bacterial meningitis were included in the above (10,14,15) mentioned studies. In a randomized controlled trial of dexamethasone in acute bacterial meningitis, a total of 301 adults were recruited. The Gram's stain was positive in 74 percent and CSF culture was positive in 78 percent [9]. Comparable values (64 percent positive Gram's stain, 77 percent positive culture) were noted in another report (7). The Gram's stain is positive in 10 to 15 percent of patients with negative CSF cultures (6).

Studies in Africa on bacterial meningitis, while reporting essentially similar patterns of organisms, have had different rates of culture yields. In addition the majority of studies have been on the pediatric age group with few studies being performed in adults.

Nesbitt *et al* (16) in a study on children with pyogenic meningitis at KNH found positive cultures in 46% of the cases. Studies perfomed in Malawi (17) and Zambia (18) yielded positive culture results in about 50% of samples.

Wanyoike *et al* (19) studied 92 patients at KNH, 52 of them being adults. Culture reports were positive in 75% of patients. Bacteria isolated in adults in this study were *S.pneumoniae* (48%), *N.meningitidis* (15%) .

In a prospective study by Honnas et al.(20) to investigate mortality and antibiotic resistance in meningitis patients (children and adults), thirty-two meningitis cases were seen over a three month period in a rural health facility in Mumias. Mean age was 11.3 years (range one month-60 years). Cerebrospinal fluid cultures were positive in 26 patients (81.3%). *S.pneumoniae* was responsible for 15 cases (46.9%), followed by *H.influenzae* in seven (21.9%). Salmonella infection was seen in two patients, and *E.coli* and *N.meningitidis* in one each.

A retrospective review of data from children admitted with acute bacterial meningitis to Kilifi district hospital from 1994 through 2000 was performed by Mwangi et al(21). Three hundred ninety cases were identified; 224 (57%) had culture –confirmed bacterial meningitis. Among the children with available CSF results the frequency of bacterial causes was as follows: *S.pneumoniae* 92(41%), *H.influenzae* 77 (34%), non-typhi salmonella 19(9%), other enterobacteriaceae 13(6%), Streptococcus Group A or B 17 (8%), other organisms 5 (2%).

A cross sectional survey was conducted by Youssef et al. (22) in 12 Egyptian hospitals from May 1998 to December 2000 for children <6 years of age with meningitis in order to determine the etiology of disease and design prevention strategies. In 2047 patients with suspected meningitis, culture results were positive in 228 (11 %) of CSF samples. Organisms isolated included: 89 (39%) patients with *H. influenzae* , 68 (30%) with *Streptococcus pneumoniae* , 30 (13%) with *N. meningitidis* , 18 (8%) with *Mycobacterium tuberculosis* and 23 (10%) with other bacteria [*E.coli* (6 isolates), *Klebsiella sp* (4), *S.typhi* (5), *P. mirabilis* (1)].

A study on neonatal meningitis at KNH in 2000 performed by Laving (23) reported four (4.8%) positive bacterial cultures in a study population of 84 patients.

Unpublished data from KNH microbiology laboratory indicates that in the three months August to November 2006, 330 CSF samples from adults were processed. In none of the samples was a bacterium cultured .

Possible explanations for the poor yield of bacteria from CSF at KNH include prior antibiotic use , delay in transport of CSF to the laboratory , inappropriate handling of CSF and use of inappropriate culture media . The contribution of each of these factors to the poor bacterial yields at KNH has not been studied.

To what extent does prior antibiotic therapy alter CSF findings? In most instances, antibiotics have little effect on the CSF white cell count, protein or glucose concentrations during the first 2 to 3 days of therapy (24). In some patients, however, antibiotic therapy has been associated with conversion of the CSF to a predominant lymphocytic pleocytosis (24,25). Prior antibiotic therapy reduces the yield from Gram stain by 20% and culture by 30% (24, 25,26), a finding supported by Feldman *et al* who also demonstrated decreased bacterial load in partially treated patients, particularly in respect of Hib and meningococcal meningitis (27). In contrast, Davis et al reported no difference in respect to CSF findings (white cell count, glucose,Gram stain) in patients with Hib meningitis (28).

World health organization (WHO) guidelines (29) recommend that CSF reaches the microbiology laboratory within one hour of performing lumbar puncture and that chocolate and sheep blood agar be used for isolating the three most common causes of bacterial meningitis , namely *S.pneumoniae* , *N.meningitidis* and *H.influenzae* .

While no published literature could be found comparing culture rates when CSF is inoculated onto culture medium at the bedside as opposed to the conventional method in the laboratory , several authors (30,31,32) have

demonstrated that the yield of bacteria cultured from ascitic fluid in patients with spontaneous bacterial peritonitis is significantly increased by inoculating blood culture bottles with ascitic fluid at the bedside . Runyon *et al* (30) demonstrated this in a study in which ascitic fluids from patients suspected of having spontaneous bacterial peritonitis were inoculated into blood culture bottles (i) at the bedside and (ii) in the laboratory after a delay . In 29 episodes in which the bedside bottles were culture positive , only 22 of the laboratory-inoculated sets demonstrated growth; this 31 % difference was statistically significant ($P < 0.02$).

Similar results were obtained by Bobadilla *et al* (31) and Such *et al* (32) . Based on these results it is likely that CSF culture yields can be increased by bedside inoculation of culture medium but the validity of this assumption and the extent to which the yield would be increased had not been tested .

STUDY JUSTIFICATION

Acute bacterial meningitis constitutes a significant public health problem worldwide(3). It is a medical emergency requiring prompt diagnosis and the administration of appropriate antibiotics as soon as is possible. In most instances at KNH a definitive diagnosis i.e isolation of bacteria from CSF is not made. As a result, no recent data regarding causative agents and their sensitivity patterns exist. Bedside inoculation of culture medium with ascitic fluid has been shown to increase the rates of positive culture in patients with spontaneous bacterial peritonitis (30,31,32).Thus it would be useful to determine whether bedside inoculation of culture media with CSF would result in an increase in the proportion of positive CSF bacterial cultures at KNH.

OBJECTIVES

Broad Objectives:

To compare the yield between immediate inoculation of culture medium with CSF and conventional culture method .

Specific Objectives:

1.To determine culture yield of bedside inoculation of culture medium with CSF .

2.To determine the culture yield of CSF inoculated onto culture medium after transport to the microbiology laboratory .

3. To compare the culture yield between (i) bedside inoculation of culture medium with CSF and (ii) laboratory inoculation of culture medium with CSF .

MATERIALS AND METHODS

Study design: Cross-sectional comparative study

Study location: Kenyatta National Hospital Accident and Emergency Department

Study population: CSF from patients undergoing lumbar puncture for suspected bacterial meningitis

Sample size

$$n=\left[\frac{z_{\alpha}\sqrt{2\pi_1(1-\pi_1)} - z_{\beta}\sqrt{\pi_1(1-\pi_1) + \pi_2(1-\pi_2)}}{\pi_1 - \pi_2}\right]^2$$

$\alpha = 0.05$

$\beta = 0.20$

$\pi_1 = 0.85$

$\pi_2 = 0.75$

The minimum sample size (n) was thus determined to be 216.

The study was designed to have 80 % power of detecting a 10 % difference based on previous studies on ascitic fluid (23) if it existed between the two methods with a p value set at 0.05.

Sampling technique: CSF from consecutive patients with clinical diagnoses of meningitis undergoing lumbar puncture were recruited until the desired sample size was achieved.

INCLUSION CRITERIA

1. CSF from patients admitted to the medical wards at K N H with a clinical diagnosis of meningitis undergoing lumbar puncture for diagnostic purposes.

2. **Case definition:** Patients with any 3 of the following five clinical features present for less than one week's duration:

Headache

Fever

Neck stiffness

Photophobia

Altered mental state

3.Patients who gave consent to participate in the study.

EXCLUSION CRITERIA

1.Patients in whom lumbar puncture was contraindicated:

a)patients with papilloedema on fundoscopy

b) patients with focal neurologic deficits

c) patients with space occupying lesions as revealed by cranial CT

2. Patients who had received antibiotics within the preceding 48 hours.

METHODOLOGY

Clinical methods:

Patients presenting to KNH in whom after evaluation at the accident and emergency department, bacterial meningitis was suspected as the cause of illness were re-evaluated by the investigator. A complete medical history was taken and a physical examination including fundoscopy performed. Consenting patients in whom lumbar puncture was not contraindicated were then subjected to the procedure.

CSF Collection

CSF was tapped from the subarachnoid space maintaining aseptic techniques using a sterile wide bore needle inserted between the fourth and fifth lumbar vertebrae. Approximately 1 ml of CSF was inoculated onto chocolate and sheep blood agar plates. Another 4 mls of CSF was collected and placed in two separate specimen bottles, one for biochemical tests and one sterile bottle for microscopy

culture and sensitivity.

The CSF inoculated chocolate agar plate as well as the CSF contained in one of the sterile bottles was then transported to the microbiology laboratory at the same time (within 1 hour of the LP as per WHO guidelines).

Laboratory methods

The culture plates inoculated at the bedside with CSF were incubated in the laboratory and bacterial culture carried out according to WHO guidelines for the isolation of bacteria from CSF (27). Bacteria isolated were then identified and antibiotic sensitivity determined using appropriate antibiotic disks.

CSF contained in the sterile bottle was centrifuged after which a drop from the sediment was collected using a sterile wire loop and streaked onto chocolate and sheep blood agar plates which were then handled in the same manner as the plate inoculated at the bedside.Gram staining was performed on the remaining sediment.

DATA ANALYSIS

Data was recorded on a proforma , entered into a database and analyzed using SPSS version 11.5.

The data was cleaned by running frequencies and all missing entries were corrected .

The main outcome variable was the proportions of CSF samples yielding positive bacterial cultures using the two different methods .

The z test for the difference between independent proportions was used to compare the results of each of the diagnostic methods with a confidence interval set at 95 % . The α level was 0.05.

ETHICAL CONSIDERATIONS

1.The study was carried out after obtaining approval from the ethical research committees of the department of medicine ,University of Nairobi and the Kenyatta National Hospital .

2. Informed consent for participation in the study was obtained from patients or guardians if too ill to give consent .

3. Patients' identities were kept confidential .

4. The entire procedure was carried out while maintaining strict aseptic techniques .

5. CSF of all patients including those excluded from the study was sent for other standard investigations as required or requested by clinicians in the ward .

6 . The need to perform a lumbar puncture or CT scan was not used as a reason to deny patients from receiving antibiotics and other treatment as deemed appropriate by the attending physician .

7.Results from the study were relayed to the patients and attending clinicians for use in patient management.

DEMOGRAPHIC DATA

The mean age of the entire group of 220 patients was 32.5 ± 7.14 years with a range of 18 – 78 years. The peak age group was 26-35 years. Figure 2 depicts the age distribution of the 220 patients whose CSF was studied.

The majority (62%) of the 220 patients were female. The difference between the proportions of male and female patients was statistically significant ,$p<0.001$. Figure 3 illustrates the gender distribution of the patients studied.

Results of testing (ELISA) for HIV were available for 215 (97%) of the patients from whom CSF was obtained. One hundred eighty (83.7%) of these patients were positive for HIV. Ten patients knew of their HIV status prior to admission and two were on HAART.

Most of the patients lived in Nairobi or on the outskirts of Nairobi (86%).The majority of patients were unemployed (62 %).

Figure 2: Age distribution of patients with suspected bacterial meningitis (n=220)

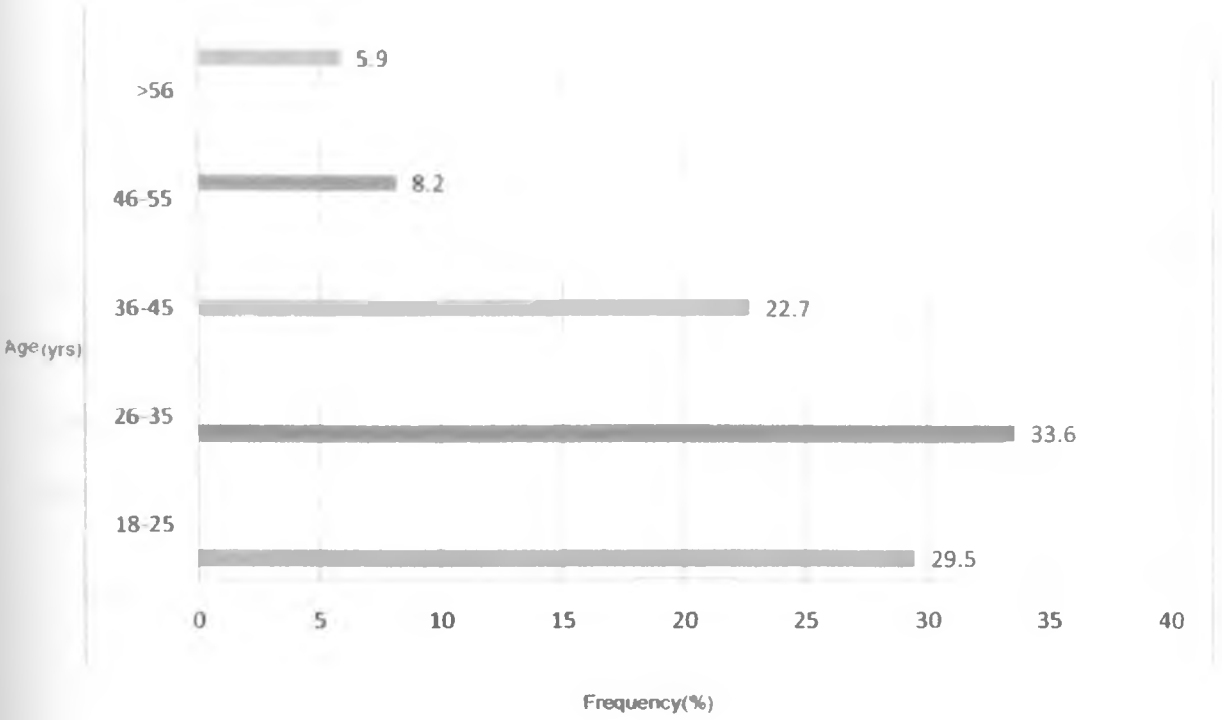
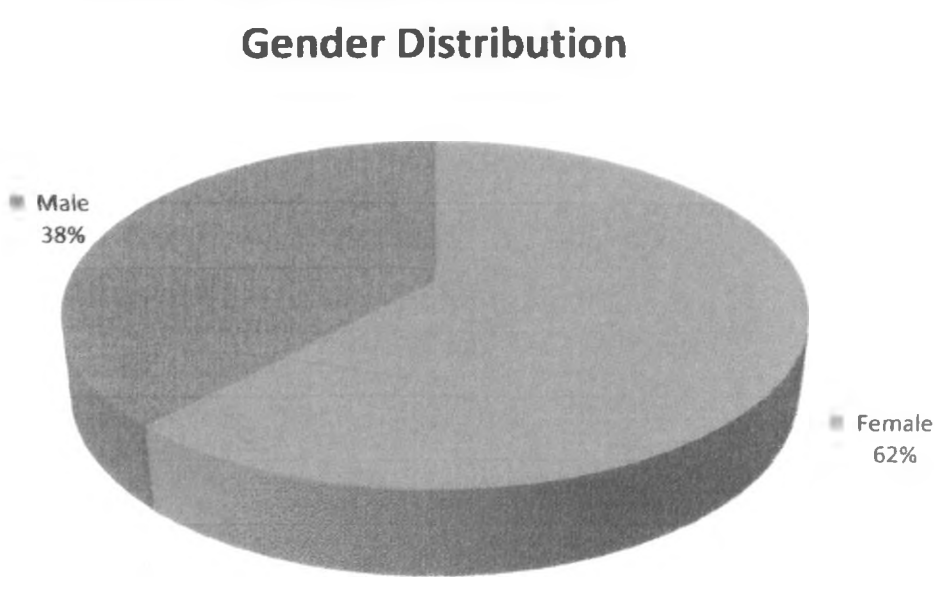


Figure 3 : Gender distribution of patients with suspected bacterial meningitis (n=220)

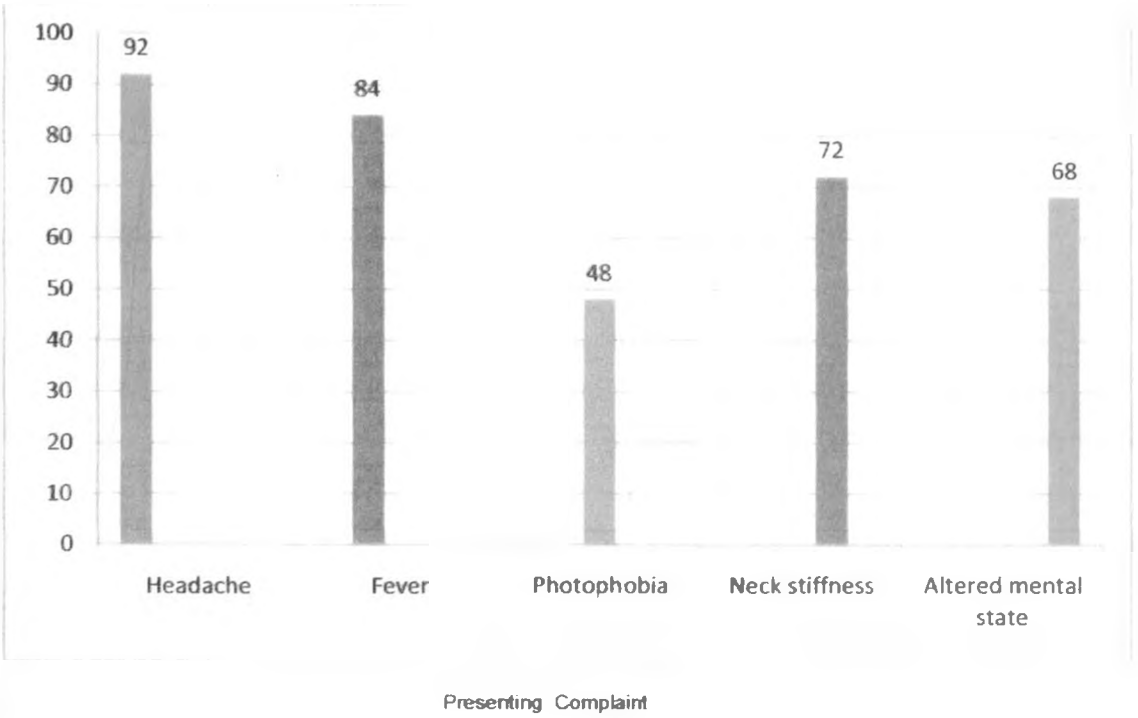


Twenty five patients had positive CSF cultures. Their mean age was 32.7 ± 7.2 years. Thirteen (56%) of these patients were female. Ninety percent of the patients hailed from Nairobi and its outskirts and 60% were unemployed. HIV test results were available for all 25 patients and 13 (52%) tested positive. When compared to the entire group of 220 patients from whom CSF was obtained, the 25 with positive bacterial cultures had a significantly ($p=0.03$) smaller proportion of patients positive for HIV and a significantly ($p=0.04$) smaller proportion of female patients. There was however no statistically significant difference between these two groups in terms of age ($p=0.45$) and other demographic characteristics.

CLINICAL PRESENTATION

The most common presenting complaint was headache which was present in 203 (92.3%) of patients with suspected meningitis and 23 (92%) of patients in who had positive CSF bacterial cultures. The average duration of all symptoms was 3.8 ± 2.2 days. Figure 2 depicts the prevalence of presenting complaints among patients who had positive CSF bacterial cultures.

Figure 4 : Prevalence of presenting complaints in patients with bacterial meningitis



The most common presenting complaint was headache in 92% of patients. The most common combination of symptoms was the triad of headache , fever and altered mental state present in 65 % of patients. The combination of headache , fever , and neck stiffness was present in 42 % of patients.Table 2 illustrates the initial Glasgow coma score of these patients at admission.

Table 1 : GCS score at admission of patients with acute bacterial meningitis

GCS	Frequency (%)
3-8 (Severe)	3 (12)
9-11 (Moderate)	13 (52)
12-15 (Mild)	9 (36)
Total	**25 (100)**

COMORBID ILLNESS

Table 2 illustrates the comorbid illnesses present in patients with proven bacterial meningitis .Positive bacterial cultures were more likely to be obtained from HIV negative patients (13/35 vs 12/180 *OR* 5.53 , 95% CI 3.20-9.52)

Two patients who had positive CSF cultures for bacteria had diabetes and both were admitted in diabetic ketoacidosis. The organism isolated in both of these patients was *S.pneuomoniae*.

One patient whose CSF was positive for acid alcohol fast bacilli on ZN staining was found to have multidrug resistant tuberculosis on sputum culture

Table 2:Comorbid conditions in patients with bacterial meningitis

Comorbidity	Frequency	Percentage
HIV	12	48%
Diabetes mellitus	2	8%
Tuberculosis(disseminated)	1	4%
No comorbid illness	10	40%
Total	25	100%

LABORATORY CHARACTERISTICS

1. TIME TO LABORATORY

The average transport time for CSF samples to the microbiology laboratory was 42.5 ± 12.83 minutes. The time for CSF samples that eventually yielded bacteria on culture (n=25) was 41.4 ± 11.67 while CSF samples that did not yield bacteria on culture (n=195) took 43.8 ± 14.26 minutes. There was no statistically significant difference in the time(mean difference -1.9 minutes ,CI -4.4 to 0.6 minutes,p=0.23).

2. GROSS APPEARANCE OF CSF

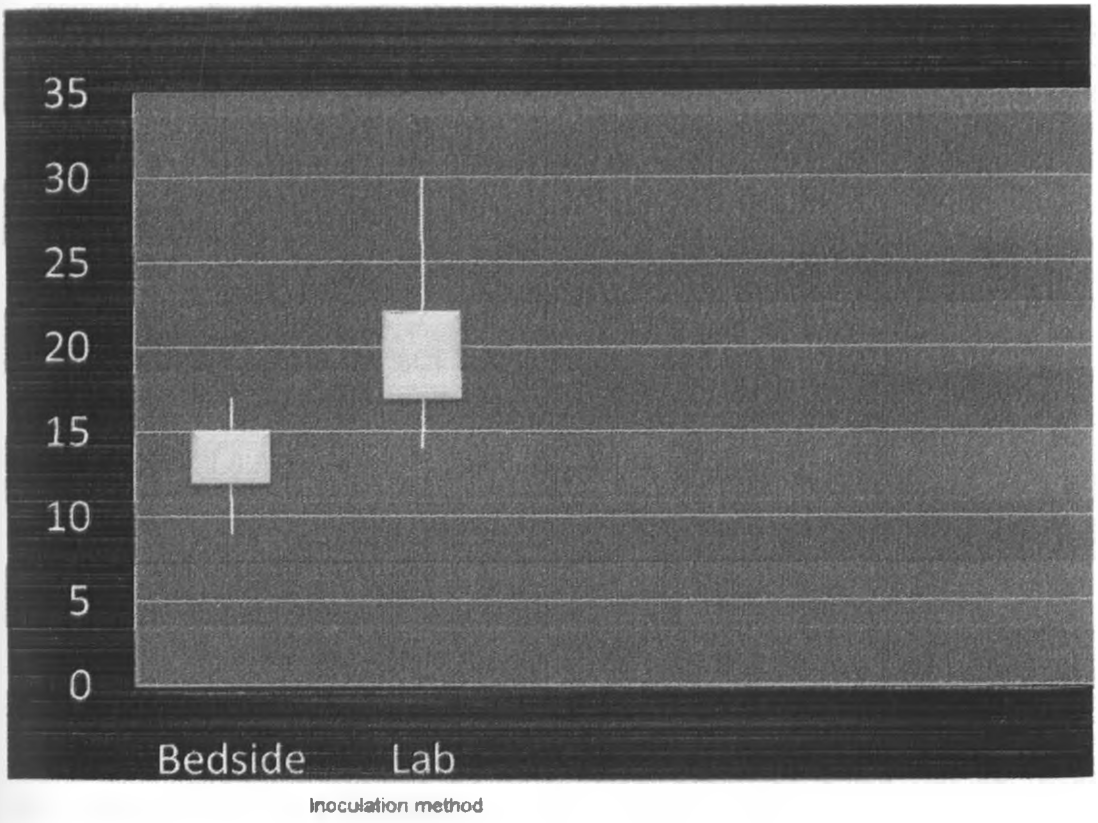
Seventy eight percent of the 25 positive CSF samples were turbid . Among the 195 CSF samples that failed to yield bacteria on culture,23(11.8%) were turbid .

3. TIME TO POSITIVE CULTURE

The mean time it took for samples inoculated on to culture medium at the bedside to be positive (n=25) was 14.7 ± 1.87 hours while those inoculated at the laboratory turned positive (n=23) after a mean of 20.3 ± 2.54 hours. The mean

difference in time to positive results between bedside and laboratory inoculated culture media was -5.6 hours,95% *CI* -6.86 to -4.34 hours, $p<0.05$.

Figure 4: Time to positive culture



4. CULTURE OUTCOME

Twenty five (11.4%,95% CI 7.0-15.6%) of the 220 CSF samples yielded bacteria on culture. Twenty four (96%) of the 25 positive samples grew *S.pneumoniae* while 1 (4%) sample yielded *H.influenzae*.

5. OTHER MICROORGANISMS ISOLATED

C.neoformans was isolated using india ink stain from 15 (7%) CSF specimens while *M.tuberculosis* was isolated from 1(0.5%) specimen on Ziehl-Neelsen staining. Contaminants consisted of aerial fungi and *Micrococcus spp.* More contaminants were isolated from bedside inoculated culture media (6%) than laboratory inoculated media (2%),p=0.012.

6. BEDSIDE CULTURE OUTCOMES COMPARED TO LABORATORY CULTURE

Twenty five (11.4%,95%CI 7.0-15.6%) of 220 CSF samples that were inoculated on to culture media at the bedside yielded bacteria while 23 (10.5%,95%CI 6.5-14.5%) of 220 samples that were inoculated at the laboratory yielded bacteria on culture.These two proportions were compared using the z test.

The z statistic was found to be 0.099 which is within the critical values of I1.96I. The 95% confidence interval for this value is -0.167-0.185. Therefore the null hypothesis that the two proportions are the same could not be rejected and the conclusion was that there was no significant difference between the proportion of CSF samples that yielded bacteria after bedside inoculation of culture media and the proportion of CSF samples that yielded bacteria after laboratory inoculation of culture media.

6. SENSITIVITY PATTERNS

Antibiotic sensitivity of bacterial isolates were performed using the disk diffusion method. Table 4 summarizes the results of antibiotic sensitivity testing for the 24 isolates of *S.pneumoniae* and 1 isolate of *H.influenzae*.

Table 3: Antibiotic sensitivity of CSF bacterial isolates

Drug / Bacteria	Sensitive	Resistant
S.pneuomoniae (n=24)		
Penicillin	23	1
Chloramphenical	23	1
Ceftriaxone	24	0
Ceftazidime	24	0
Cefuroxime	24	0
Vancomycin	24	0
H.influenzae (n=1)		
Penicillin	1	0
Chloramphenical	1	0
Ceftriaxone	1	0
Ceftazidime	1	0
Cefuroxime	1	0
Vancomycin	1	0

DISCUSSION

Bacterial meningitis constitutes a neurological emergency and prompt diagnosis is essential in ensuring that appropriate therapy is administered. The yield from bacterial cultures of CSF at KNH is very low and thus clinicians frequently have to give empirical therapy for bacterial meningitis without knowledge of the causative organisms and their antimicrobial susceptibility patterns. While there are no studies that have been carried out to investigate the cause of poor yields at KNH, several reasons have been postulated. These include prior use of antibiotics by patients before presentation to hospital, a problem not unique to KNH (33),delayed performance of lumbar punctures, delays in transporting CSF to the laboratory, inappropriate handling and storage of CSF and use of inappropriate culture media.

This is the first study in Africa which has addressed the issue of bedside inoculation of culture media with CSF as compared to traditional methods. Due to ethical reasons specimens were expedited to the laboratory, reaching there well within the WHO recommended time limit of one hour. If this study was done in the real world situation at KNH where specimens generally take more than one hour to reach the laboratory, the evidence in favor of bedside inoculation may have been overwhelming.

The study patients had a mean age of 32.5 years. Previous studies of a similar nature performed at KNH (34,35) found a similar age, further exposing the young age of patients seen at KNH.

The male:female ratio of 1:1.6 was not unexpected since most of the patients seen at KNH are female. There exists no published data showing that females are more susceptible to contracting meningitis. Most of the patients recruited into the study (86%) lived in or around Nairobi and 62% of them were unemployed. These findings were similar to those of other investigators at KNH (34,35).

The overall seroprevalence for HIV was 83.7%. This is compared to an estimated background HIV prevalence of 39 % in inpatient care (36). Previous studies in Kenya (16,19,20,21,23) did not report the proportion of HIV positive patients studied although Wanyoike (19) reported that the 4 patients in his study with cryptococcal meningitis were all HIV positive. Immunosuppression due to HIV infection is known to predispose individuals to frequent infections including those affecting the CNS and bacterial meningitis is listed by the WHO (37) and CDC (38)as an opportunistic infection in patients with HIV. The role of HIV infection on the prevalence of meningitis although not a primary domain of the study became obvious. Hence one can conclude a positive association between the two.

Other diseases may also predispose individuals to bacterial meningitis. Two patients from whom *S.pneumoniae* was isolated had diabetes mellitus and were in diabetic ketoacidosis at admission. Patients with diabetes may be predisposed to bacterial infections due to defects in neutrophil function (39).Impairment of innate immunity may also predispose diabetics to more severe infections (39). Furthermore , severe infections such as bacterial meningitis in the two patients in this study are known to precipitate diabetic ketoacidosis (40).

The overall yield of bacteria from cultures carried out in this study was 11.4%. The yield could have varied between 7.0 – 15.6 %. The yield would appear to be low as compared to that found in the study by Wanyoike et al (19). In this study the patients included both adults and children. Previous studies have noted higher yields among children (7,20). In addition Wanyoike et al used both clinical and laboratory criteria (presence of neutrophils) in recruiting CSF specimens that would be cultured while this study relied only on clinical criteria for meningoencephalitis. Cloudy CSF specimens (an indicator of increased neutrophils) are more likely to yield bacteria on culture. as demonstrated by Scarborough et al (33) in Malawi and Mirza et al (41) in Nairobi. Restricting cultures to cloudy CSF specimens would have resulted in a yield of 60% in this study.

The high prevalence of HIV infection among study patients (83.7%) could also have contributed to the low yield. It is possible for HIV meningoencephalitis to present with symptoms similar to those of meningitis thus accounting for the low yield. Studies done on patients with HIV have found reduced sensitivity for detecting bacteria in CSF (42). Indeed on sub analysis of study data, excluding HIV infected patients would have resulted in a yield of 37%,more than thrice what was obtained. Bacteria were also five times more likely to be isolated from the CSF of HIV negative patients.

The average time it took to get CSF samples in this study to the laboratory was 42.5 ± 12.83 minutes after performance of lumbar puncture.This was well within the WHO recommended 1 hour limit (29) . Although samples that eventually grew bacteria happened to have arrived in the laboratory earlier than samples that did not grow bacteria , the difference was not statistically significant (mean difference -1.9 minutes , CI -4.4-0.6 min , p=0.23). Wanyoike et al (19) in their study ensured that CSF specimens reached the laboratory within 30 minutes which could account for the higher yield of bacteria obtained in that study.

It was possible that recruited patients had previously taken antibiotics but in the absence of assays of blood and urine levels of antibiotics this could not be proven and the patients were recruited on the basis of what they told the principal investigator.This could have further reduced the yield from cultures as shown in previous studies (26,27,28).

It was anticipated based on findings from previous studies on ascitic fluid that the yield from bedside cultures would be significantly higher. In the real world situation at KNH where specimens are left on the bench for hours a difference would most likely have been noted. It has already been mentioned above that in the period spanning August to November 2006 not a single bacterium was isolated from the CSF of 330 patients who underwent lumbar puncture for suspected meningitis giving a yield of 0 %. This study demonstrated that if CSF

is handled according to WHO recommendations, then the expected yield should be about 10 %.

The absence of a significant difference in yield could also have been due to several factors: Ascitic fluid has different characteristics from CSF. The bacteria isolated from studies on ascitic fluid was mainly *E.Coli* while *S.pneumoniae* was the predominant organism isolated from CSF in this study. *N.meningitidis* and *H.influenzae* are known to be much more fastidious organisms regarding their isolation from CSF (29). In this study only one CSF sample grew *H.influenzae* and no isolates of *N.meningitidis* were made. It is possible that bedside inoculation would have resulted in a better yield if these organisms had been studied. The low yield of cultures (11.4%) also reduced the power of the study to detect a significant difference between the two methods and it is likely that a larger study with more positive cultures will detect a significant difference.

A significant finding in this study was that cultures from samples inoculated at the bedside grew approximately 5 hours earlier than samples inoculated at the laboratory and this difference was statistically significant. This being the first study of its kind, there are no previous studies to compare this finding with. Runyon et al (30) Bobadilla et al (31) and Such (32) in studies on ascitic fluid from patients with spontaneous bacterial peritonitis did not report a difference in time to culture positivity between ascitic fluid specimens inoculated onto culture medium at the bedside compared to those inoculated after transport to the laboratory. Kaplan et al (43) in a study of febrile infants reported that CSF cultures done via conventional methods turned positive after a median time of 18 hours which is comparable to the 20 hours found in the present study for cultures inoculated at the laboratory. Other studies (5,10,16-23)did not report how long it took for cultures to turn positive. Further studies are needed to confirm this finding as bedside inoculation would then result in earlier identification of meningopathogens with attendant earlier determination of drug susceptibility patterns which would be useful in guiding clinicians caring for patients with bacterial meningitis.

Patients who were recruited into the study had to have any three of five presenting complaints namely headache , altered mental state , fever , photophobia or neck stiffness present for not more than one week. The most common presenting complaint was headache which was present in 92% of patients recruited. Meningeal inflammation resulting in increased CSF levels of cytokines such as TNF and IL-1 leads to increased permeability of the blood brain barrier , resulting in vasogenic edema which results in increased intracranial pressure clinically manifested as headache (44,45). Headache also results from stimulation of nociceptors located in the meninges by inflammatory mediators in addition to other mechanisms (44). Wanyoike (19) did not mention the frequency of headache or other symptoms and signs of meningeal inflammation in his study carried out at KNH. Hussein (34) who performed 307 lumbar punctures in a study on cryptococcal meningitis at KNH , reported headache in 82% of his study patients which was slightly lower than the frequency found in the present study. It is however important to note that the study by Hussein was in a slightly different population of patients who tended to have chronic meningitis. Given the longer duration of symptoms in patients recruited in his study , recall bias may have led to fewer of his patients reporting headache compared to the present study. The most common combination of symptoms and signs in the present study was headache, fever and altered mental state which was found in 65 % of study patients. The classic triad of fever , neck stiffness and change in mental status was present in 42 % of patients which was similar to a study by Van de Beek et al (10) in which 44 % of patients in the Netherlands had this classical triad.

The average duration of symptoms reported by patients in this study was 3.8 days which while similar to that reported by Scarborough et al (33) in Malawi was significantly longer than that reported by de Gans et al (9) in a study performed in Europe. It is possible that some patients had sought treatment in other health facilities before coming to KNH but this was not documented. Delays in reporting to hospital have been proposed as a factor contributing to increased mortality of patients with acute bacterial meningitis in Africa when compared to patients in the developed world (33).

S.pneumoniae was the main bacteria isolated in this study accounting for 24 of 25 isolates (96%). *H.influenzae* was isolated from the CSF of one patient who also happened to have *C.neoformans* .Previous studies (6,10,14,15,17,) had shown that *S.pneumoniae* is the main cause of bacterial meningitis in adults. Wanyoike et al (19) found *S.pneumoniae* in 48% and *N.meningitidis* in 25% of patients with bacterial meningitis and no *H.influenzae*. Honnas et al (20) working in Mumias,Kenya found a relatively higher proportion of *H.influenzae* (21.9%) although this study was done in both adults and children and the mean age of study participants was 11.3 years. There was no epidemic of *N.meningitidis* reported during the present study as well as that by Wanyoike et al and it is possible that the differing proportions of the bacterial species isolated in these two studies could point to a changing epidemiology of bacterial meningitis at KNH with a relative increase in cases attributable to *S.pneumoniae*. The availability and widespread use of amoxicillin for a variety of ailments by the general public could explain the fall in the prevalence of meningitis caused by the bacterium as it is very sensitive to the drug.

One patient in this study had a positive ZN smear for acid alcohol fast bacilli in CSF. She later turned out to be having multidrug resistant *M.tuberculosis* infection on sputum culture. Wagana (35) using BACTEC® MGIT cultures had found *M.tuberculosis* in the CSF of 15.7% of HIV positive patients presenting with meningoencephalitis at KNH.

The fungus *C.neoformans* was identified using india ink from 15 (7%) patients all of whom were HIV positive. This was similar to Wanyoike et al's findings (19) where 8% of samples were positive for the organism.

Contaminants were isolated in 6 % of bedside cultures compared to 2 % of laboratory inoculated cultures (p=0.012). The main organisms were aerial fungi and *Micrococcus spp*. Maintenance of strict aseptic technique during lumbar puncture should reduce the incidence of contamination of culture medium.

The treatment of bacterial meningitis is an evolving field. Selected third-generation cephalosporins, such as cefotaxime and ceftriaxone, have emerged as the beta-lactams of choice in the empiric treatment of meningitis (47). These drugs have potent activity against the major pathogens of bacterial meningitis with the notable exception of *Listeria monocytogenes*. With the worldwide increase in the prevalence of penicillin-resistant pneumococci, most infectious disease specialists would add vancomycin to cefotaxime or ceftriaxone as empiric treatment until culture and susceptibility results are available (47). However in this study, ninety six percent of *S.pneumoniae* isolates were found to be sensitive to crystalline penicillin and chloramphenical which are the two drugs used empirically at KNH for the treatment of acute meningitis. Previous studies at KNH by Wamola et al (46),Nesbitt et al (16) and Wanyoike et al (19) had found a higher incidence of penicillin resistant pneumococci ranging from 7-10% of isolates. The findings of this study would therefore suggest that the incidence of resistance to penicillin is reducing. However in vitro sensitivity to drugs does not necessarily translate into better outcomes for patients. Experimental studies have shown that in vitro susceptibility tests (including tests of bacterial killing) can overestimate the efficacy of drugs in the treatment of infection. In a mouse model of pneumococcal meningitis, for example, a CSF drug concentration that was 30 times greater than the in vitro minimum bactericidal concentration was the optimal concentration of a beta-lactam (48). Adjunctive dexamethasone has been shown to improve outcomes in some studies (3,9) while other studies have shown no clinical benefit (33). Recommendations regarding choice of antibiotics as well as adjunctive therapy for use in acute meningitis in the KNH should ideally be based on clinical trial evidence.

CONCLUSIONS

Bedside inoculation of culture media with csf did not result in a significantly higher yield of bacteria when compared to laboratory inoculation of the culture media.

This study demonstrated that CSF samples inoculated onto culture media at the bedside grew bacteria approximately 5 hours earlier than samples that were inoculated onto culture media at the microbiology laboratory.

Bacteria were more likely to be isolated from the CSF of HIV negative than HIV positive patients regardless of the inoculation method used.

The most common cause of bacterial meningitis was *S.pneumoniae.*

Most of the bacteria isolated were sensitive to antibiotics currently used at KNH.

LIMITATIONS

1.The overall bacterial isolation rate was low (11.4%).

2. Prior antibiotic use could not be objectively assessed among patients.

3. A number of patients with lateralizing signs and papilledema were excluded.

RECOMMENDATIONS

1. A larger study with a higher proportion of positive bacterial cultures with more power to detect a significant difference between the two methods should be performed.
2. CSF should be transported to the laboratory and handled according to WHO guidelines.
3. The two methods of inoculation of culture media should be studied under normal KNH conditions in the medical wards.
4. Further studies are required to correlate in-vitro drug susceptibility of *S.pneumoniae* to clinical outcomes.

REFERENCES

1.Viesseux M : Memoire sur le maladie qui a regne a Geneve au printemps de 1805. J.*Med Chirug Pharm* 1806 ;**11 :** 163 – 82

2. Flexner S : The results of serum treatment of thirteen hundred cases of epidemic meningitis . *J Exp Med* 1913 ; **17** : 553 .

3.Van de Beek D , de Gans J , Mc Intyre P , Prasad K . Corticosteroids in acute bacterial meningitis . *The Cochrane Database of Systematic Reviews* 2003 , **2** Art No : CD004405 . DOI : 10. 1002/14651858 CD 004405 .

4.World Health Organization . Epidemic meningococcal disease . *WHO Fact Sheet* 105 . Geneva:WHO ; 1998 .

5.Kwasa T O. The pattern of neurological disease at Kenyatta National Hospital. *East Afr Med J* 1992 ; 69 : 236-239

6. Durand M L , Calderwood S B , Weber D J , *et al* . Acute bacterial meningitis in adults . A review of 493 episodes . *N Engl J Med* 1993 ; **328** : 21 – 8 .

7. Aronin S I , Peduzzi P , Quagliarello V J . Community acquired bacterial meningitis : Risk stratification for adverse clinical outcome and effect of antibiotic timing . *Ann Intern Med* 1998 ; **129** : 862 – 9 .

8. Domingo, P, Mancebo, J, Blanch, L, et al. Fever in adult patients with acute bacterial meningitis. *J Infect Dis* 1988; **158**:496.

9. de Gans J , van de Beek D . Dexamethasone in adults with bacterial meningitis . *N Engl J Med* 2002 ; **347** : 1549 – 56 .

10. Van de Beek D , de Gans J , Spanjaard L , *et al* . Clinical features and prognostic factors in adults with bacterial meningitis . *N Engl J Med* 2004 ; **351** : 1849 – 59 .

11. Thomas, KE, Hasbun, R, Jekel, J, Quagliarello, VJ. The diagnostic accuracy of Kernig's sign, Brudzinski's sign, and nuchal rigidity in adults with suspected meningitis. *Clin Infect Dis* 2002; **35**:46.

12. Attia, J, Hatala, R, Cook, DJ, Wong, JG. The rational clinical examination. Does this adult patient have acute meningitis?. *JAMA* 1999; **282**:175.

13. Weisfelt, M, van de, Beek D, Spanjaard, L, de Gans, J. Arthritis in adults with community-acquired bacterial meningitis: a prospective cohort study. *BMC Infect Dis* 2006; **6**:64.

14. Schuchat A , Robinson K , Wenger J D *et al* . Bacterial meningitis in the United States in 1995 . Active Surveillance Team . *N Engl J Med* 1997 ; **337** : 970 – 6 .

15.Hussein A S , Shafran S D . Acute bacterial meningitis in adults . A 12 year review . *Medicine (Baltimore)* 2000 ; **79** : 360 – 8 .

16. 12. Nesbitt A , Jitta J S , Ndinya – Achola J O . Pyogenic meningitis in Nairobi children . *E Afr Med J* . 1988 ; **65** : 189 .

17. Brown K G E . Meningitis in Queen Elizabeth Center Hospital Blantyre , Malawi . *E Afr Med J* . 1975 ; **52** : 376 .

18. Bhushan V , Chintu C . Changing pattern of pyogenic meningitis in Lusaka . *E Afr Med J* .1979 ; **56** : 548 – 556 .

19. Wanyoike M , Waiyaki P , McLigeyo S , Wafula M .Bacteriology and sensitivity patterns of pyogenic meningitis at KNH. *E Afr Med J* .1995 ; **72** : 658-660

20. Honnas A , Petersen L . Bacterial meningitis in a rural Kenyan hospital . *E Afr Med J* . 1998 ; **5** : 396 – 401 .

21. Mwangi I , Berkley J , Lowe B , *et al* . Acute bacterial meningitis in children admitted to a rural Kenyan hospital : increasing antibiotic resistance and outcome . *Pediatr Infect Dis J* . 2002 ; **21** : 1042 – 8 .

22. Youssef F G , El – Sakka H , Azab A , *et al* . Etiology , Antimicrobial susceptibility profiles and mortality associated with bacterial meningitis among children in Egypt . *Ann Epidemiol* 2004 ; **14** : 44 – 48 .

23. Laving A . Neonatal Bacterial meningitis at Kenyatta National Hospital . M .Med Dissertation
University of Nairobi 2000.

24. Greenlee J E . Approach to diagnosis of meningitis : CSF evaluation . *Infect Dis Clin North Am* 1990 ; **4** : 583 – 98 .

25. Dalton H P , Allison M J . Modification of laboratory results by partial treatment of bacterial meningitis . *Am J Clin Pathol* 1968 ; **49** : 410 – 413 .

26. Blazer S , Berant M , Alon U . Bacterial meningitis . Effect of antibiotic treatment on CSF . *Am J Clin Pathol* 1983 ; **80** : 386 – 7 .

27. Feldman W E , Craft J C , Nelson J D . Clinicopharmacological evaluation of amoxicillin and probenecid against bacterial meningitis . *Antimicrob agents chemother* 1979 ; **16** : 346 – 52 .

28. Davis A T , Yogev R . Oral chloramphenical therapy in hemophilus influenza meningitis . *Infection* 1981 ; **9** : 42 – 44

29. Caugant D , Nicolas P , Perkins B , *et al* .Laboratory methods for the diagnosis of meningitis caused by *Neisseria meningitidis , Streptococcus pneumoniae , and Haemophilus influenzae .* World Health Organization 1999

30. Runyon B A , Antillon M R , Akriviadis E A . Bedside inoculation of blood cultures with ascitic fluid is superior to delayed inoculation in the detection of spontaneous bacterial peritonitis . *J Clin Microbiol* 1990 ; **12** : 2811 – 2 .

31.Bobadilla M , Sifuentes J , Garcia – Tsao G . Improved method for bacteriological diagnosis of spontaneous bacterial peritonitis . *J Clin Microbiol* 1989 ; **27** : 2145 – 47 .

32. Such J , Runyon B A . Spontaneous bacterial peritonitis . *Clin Infect Dis* 1998 ; **27** : 697 – 704 .

33. Scarborough M., Gordon S, Whitty C. Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. *N Engl J Med* 2007;357: 2441-50

34.Hussein ,M. Sensitivity patterns, serotypes of Cryptococcus neoformans and diagnostic value of India ink in patients with cryptococcal meningitis at Kenyatta National Hospital. M.Med dissertation.University of Nairobi 2007

35. Wagana, L.N. Clinical utility and cost effectiveness of CSF TB BACTEC MGIT cultures in the evaluation of HIV patients with meningoencephalitis at the Kenyatta National Hospital. M.med dissertation. University of Nairobi 2007.

36. Arthur,G, Bhatt,SM, Gilks,CF,et al.Trends in Bloodstream Infections among Human Immunodeficiency Virus-Infected Adults Admitted to a Hospital in Nairobi, Kenya, during the Last Decade.*Clin Infect Dis* 2001;**33**:248-256.

37. World health organization.WHO clinical staging of HIV/AIDS. WHO 2005. www.who.org/hivaids/staging (Accessed Jan 3 2007)

38. Centers for disease control. CDC Classification system for HIV infected adults and adolescents.MMWR 42 (No RR-17) December 18,2006

39. Lorente L,De La Fuerte H, Patin Y,et al.Innate immune response mechanisms in non insulindependent diabetes mellitus patients assessed by flow cytoenzymology. *Immunol Lett* 2000 ;74:239-44

40. Kitabchi A E, Umpierez GE,Murphy MB,et al.Hyperglycemic crises in patients with diabetes mellitus. *Diabetes Care* 2003 ; 26 Suppl S109

41. Mirza N B, Estambale B B, Wamola I A,et al.Bacterial meningitis in children admitted in hospitals within Nairobi. *East Afr Med J* 1998 ; 75 : 73-77

42. Jowi J O, Mativo P.M,Musoke S.S. Clinical and laboratory characteristics of hospitalized patients with neurological manifestations of HIV/AIDS at the Nairobi Hospital. *East Afr Med J* 2007; **84**:67-43.

43. Kaplan R L,Harper MB,Baskin MN,et al.Time to detection of positive cultures in 28-90 day old febrile infants. *Pediatrics* 2000;106:74-78

44. Koedel U, Scheld WM, Pfister H. Pathogenesis and pathophysiology of pneumococcal meningitis.*Lancet Infect Dis* 2002;2: 721-36

45. Roos K L. Acute bacterial meningitis. *Semin Neurol* 2000 ; 20 :293

46. Wamola I A,Mirza NB,Nzanzumuhire H.Penicillin resistant pneumococcal meningitis in Kenyatta National Hospital,Nairobi. *East Afr Med J* 1981 ; **58** :12-17

47. van de, Beek D, de Gans, J, Tunkel, AR, Wijdicks, EF. Community-acquired bacterial meningitis in adults. *N Engl J Med* 2006; **354**:44.

48. Tauber, MG, Doroshow, CA, Hackbarth, CJ, et al. Antibacterial activity of beta-lactam antibiotics in experimental meningitis due to Streptococcus pneumoniae. *J Infect Dis* 1984; **149**:568.

APPENDIX I : CONSENT FORM

Purpose of the study : To determine whether a modification of the traditional method of collecting cerebrospinal fluid (CSF) will increase the rate of detection of bacteria.

Procedure : Details of your medical history will be entered into a questionnaire . You will then undergo a physical examination and a lumbar puncture that involves obtaining fluid from your lumbar spine performed . The fluid obtained will then be placed in an appropriate medium and transported to the laboratory for analysis . The results of the analysis will be communicated to you and the doctor treating you as soon as possible.

Benefits : This study will help in effective diagnosis and management of your condition .

Risks : There will be no additional risks involved as you will not be actively participating in the study .

Participation : Participation is voluntary

Costs : There will be no additional costs for the additional laboratory tests that will be carried out for the purposes of the study.

Confidentiality : Your identity and the results of the laboratory tests shall be kept confidential .

CONSENT FORM

I ..... hereby consent to participate in the proposed research on bacterial meningitis.I am aware that the study involves a physical examination and laboratory studies on the cerebrospinal fluid obtained from my lumbar spine.

I understand that my identity and the results of the laboratory tests shall be kept strictly confidential .

I have been explained to the risks and the benefits of the study to myself and others with the same condition .

Signature of the participant/next of kin

.....

Signature of investigator

.....

Telephone Contact of investigator

0722 417507

SOURCE OF FUNDING : Katholischer Akademischer Auslander Dienst

(KAAD) ,a Non governmental organization affiliated with the Catholic church

in Germany provided full funding for the study . The address is :

KAAD,

151,Hausdorffstr,

D-53129 Bonn,

Germany

www.kaad.de

The sponsors of the study did not play any role in the design and conduct of the study or in the reporting of the results of the study.

APPENDIX III: TIME FRAMEWORK

Proposal development	Nov 2006 to Jan 2007
Ethical approval	Feb 2007 to March 2007
Data collection	April 2007 to Jun 2007
Data analysis	July 2007 to Aug 2007
Thesis write-up	Sep 2007 to Nov 2007

APPENDIX IV STUDY PROFORMA

Investigator : **Date:**

Personal History

Patient's name

IP No

Study No

Ward

Sex M=1 F=2 ( )

Age (yrs) ( )

Residence

Occupation

Medical History

Fever Y=1 N=2 ( )

Duration (no of days) ( )

Headache Y=1 N=2 ( )

Duration (no of days) ( )

Altered mental state Y=1 N=2 ( )

Duration (no of days) ( )

Neck stiffness Y=1 N=2 ( )

Photophobia Y=1 N=2 ( )

Duration(no of days) ( )

Antibiotic use Y=1 N=2 ( )

OTHER UNDERLYING DISEASE

Y=1 N=2 **( )**

HIV (Y=1 N=2) ( ) TB (Y=1 N=2) ( ) Diabetes (Y=1 N=2) ( )

PHYSICAL EXAMINATION

Absent=1 Present=2

Neck stiffness ( )

Fever ( )

Kernigs sign ( )

G C S

3 – 8 Severe coma (1) ( )

9 – 11 Moderate Coma (2) ( )

12 – 15 Mild coma (3) ( )

C S F Culture Results

Turbid? N=1 Y=2 ( )

Time to lab (minutes) ( )

Time to culture (hours/minutes)

S.pneumoniae (1) ( )

N.meningitidis (2) ( )

H.influenzae (3) ( )

Other organism (pls specify) (4) ( )

No growth obtained (5) ( )

Antibiotic sensitivity

Resistant=1 sensitive=2

Ceftazidime ( )

Ceftriaxone ( )

Chloramphenical (

Crystalline penicillin ( )