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COMPARISON OF BEDSIDE INOCULATION OF CULTURE MEDIA WITH CONVENTIONAL CEREBROSPINAL FLUID CULTURE METHOD IN PATIENTS WITH BACTERIAL MENINGITIS
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ABSTRACT

Background: **The yield of bacterial cultures from cerebrospinal 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 that of CSF inoculated onto culture medium in the microbiology laboratory.**
Design: **Cross-sectional comparative study.**
Setting: **Accident and Emergency Department and medical wards at Kenyatta National Hospital.**
Subjects: **Cerebrospinal fluid from patients at KNH with a clinical diagnosis of acute meningitis.**
Results: **Two hundred and twenty CSF specimens were obtained during a four month period. *S. pneumaniae* was isolated from 24 CSF samples and *H. influenzae* from one. Bacterial cultures were positive in 25 (11.4%, 95% CI 7.0-15.6%) samples inoculated at the bedside and 23 (10.5%, 95% CI 6.5- 14.5%) 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 per cent of *S. pneumaniae* isolates were resistant to crystalline penicillin.**
***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.**

INTRODUCTION

Acute bacterial meningitis is a neurological emergency that accounts for about 23% of all neurological admissions at Kenyatta National Hospital (1). Mortality from bacterial meningitis remains high despite advances in critical care and the introduction of newer antimicrobial agents (2).

A definitive diagnosis of bacterial meningitis requires the performance of cultures on standard media which have a turn-around time of approximately 48-72 hours and have varying sensitivities (3-7). The time taken to obtain a bacterial diagnosis can be shortened by use of serological methods which are also more sensitive than cultures but these do not give antimicrobial sensitivities of the bacterial isolate (8). As a result clinicians are often compelled to treat patients with suspected bacterial meningitis using empirical drugs.

The World Health Organisation (WHO) recommends that cerebral-spinal fluid (CSF) obtained from lumbar punctures be transported to the microbiology laboratory within one hour so as to optimise yields from cultures that are performed (9). However in many instances this does not occur, leading to poor yields obtained from cultures. Other contributing factors to poor culture yields are prior antibiotic use and use of inappropriate culture media.

Bedside inoculation of culture media has been shown in several studies to increase the sensitivity of bacterial cultures obtained from ascitic fluid of patients with suspected spontaneous bacterial peritonitis (10-12). To test whether this strategy

could be employed in culturing CSF from patients with suspected bacterial meningitis, we conducted a cross-sectional study comparing inoculation of CSF onto culture media at the bedside with the traditional method of laboratory inoculation at the Kenyatta National Hospital.

MATERIALS AND METHODS

The study was conducted between June and October 2007. This was a cross-sectional comparative study carried out on CSF from patients presenting to the KNH accident and emergency department with suspected bacterial meningitis. Patients were suspected to have bacterial meningitis if they had any three of the following five signs and symptoms for duration of one week or less: headache, fever, neck stiffness, photophobia and altered mental state.

Cerebrospinal fluid was tapped using aseptic techniques from the subarachnoid space of consenting patients using a sterile wide bore needle inserted between the fourth and fifth lumbar vertebrae. Patients who had contra- indications to lumbar puncture were excluded. Approximately 1ml of CSF thus obtained was then allowed to drop directly onto chocolate and sheep blood agar plates at the bedside which were then sealed. Another 4 ml of CSF was collected at the same time and placed in sterile bijou bottles to await transport to the microbiology laboratory located within the department of clinical medicine and therapeutics at KNH. Both the CSF inoculated culture plates and CSF containing bijou bottles were transported at the same time to the microbiology laboratory, and in all cases this was done within one hour of the lumbar puncture in keeping with WHO guidelines for the isolation of bacteria from CSF (9).

Cerebrospinal fluid contained in the sterile bijou bottle was centrifuged at 3000 rpm for 5 minutes. The sediment was then gram stained. A drop from the sediment was collected using a sterile wire loop and streaked onto chocolate and sheep blood agar plates which were then incubated in a carbon dioxide (CO_2) rich environment at 37°C for a minimum of 72 hours. The culture plates inoculated at the bedside were incubated at the same time and under the same conditions as those inoculated at the laboratory. Standard microbiological techniques were used to identify bacteria that were cultured. Antibiotic sensitivity tests were carried out on the bacteria isolated using the disk diffusion method.

Data were collected using a questionnaire and analysed using SPSS version 11.5. The Z test for the difference between independent proportions was used to compare the results of each of the diagnostic methods using 95% confidence intervals.

Approval to carry out the study was obtained from the Ethics and Research Committee of the Kenyatta National Hospital.

RESULTS

Two hundred and thirty eight patients met the inclusion criteria for the study. After excluding eighteen patients, cerebrospinal fluid was obtained from two hundred and twenty patients. Reasons for exclusion were papilloedema in five, focal neurological deficits in twelve and lack of consent in one. Table 1 outlines the baseline characteristics of the 220 patients who were recruited into the study.

Table 1
Baseline characteristics of study patients (n=220)

Characteristic	
Median age (range)	32.5 years (18-78)
Female sex (%)	136 (62%)
Education > 8 years	118 (54%)
Employed	135 (62%)
HIV positive (ELISA)*	180 (83.7%)
Presenting symptoms	
Headache	203 (92%)
Fever	184 (84%)
Photophobia	105 (48%)
Neck stiffness	158 (72%)
Altered mental status	149 (68%)

*Results of HIV testing (ELISA) were available for 215 (97%) of the 220 study patients. Two patients were on trimethoprim-sulfa prophylaxis

The median time it took to transport CSF specimens to the laboratory from the time of lumbar puncture was 42 minutes (range 31-54 minutes). Positive bacterial cultures were obtained in 25 of the 220 CSF samples processed (11.4%). The organ isms isolated and their frequencies are detailed in Table 2.

Table 2
Microorganisms isolated from CSF of patients with suspected bacterial meningitis (n=220)

Microorganism	Frequency No.	(%)
S. pneumoniae	24	11
H. influenzae	1	0.5
*C. neoformans**	7	15
*M. tuberculosis***	1	0.5
Micrococcus spp (contaminants)	13	6

*Identified using india ink stain
**Identified using ZN staining

Forty eight per cent of patients who had positive bacterial cultures were found to be HIV positive by ELISA. Positive bacterial cultures were more likely to be obtained from HIV negative (13/35) compared to HIV positive (12/180) patients (OR 5.53,95% CI 3.20-9.52).

Twenty five (11.4%, 95% CI 7.0-15.6%) of two hundred and twenty CSF samples that were inoculated at the bedside yielded bacteria. This was in comparison to twenty three (l0.5%, 95% CI 6.5-14.5%) of two hundred and twenty samples inoculated at the laboratory. There was no significant difference between the two yields (z=0.09, 95% CI-0.17-0.19).

Table 3
Antibiotic sensitivity patterns

Antibiotic	Number sensitive	Number resistant
S. pneumoniae (n=24)		
Penicillin	23	1
Chloramphenicol	23	0
Ceftazidime	24	0
Cefuroxime	24	0
Vancomycin	24	0
H. influenza (n=l)		
Penicillin	1	0
Chloramphenicol	1	0
Ceftazidime	1	0
Cefuroxime	1	0
Vancomycin	1	0

The median time it took for samples inoculated onto culture media at the bedside to turn positive (n=25) was 14.7 ± 1.9 hours while those inoculated at the laboratory turned positive (n=23) after a median of 20.3±2.5 hours. The mean difference in time to positive results between bedside and laboratory inoculated culture media was -5.6 hours, 95% Cl-6.86 to -4.34 hours, $p< 0.05$.

Antibiotic sensitivity results of the cultured bacteria performed using disk diffusion are shown in Table 3.

DISCUSSION

Prompt diagnosis of bacterial meningitis is critical in ensuring that appropriate therapy is given. The yield from bacterial cultures of CSF at the Kenyatta National Hospital is very low and clinicians frequently have to administer empirical therapy without getting to know the causative organisms and their antimicrobial sensitivity. While there have been no studies to investigate the cause of poor yields, several reasons have been postulated as follows: prior use of antibiotics, a problem not unique to KNH (13), 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 had been done in the real world situation in which specimens generally delay in reaching the laboratory the evidence in favor of bedside inoculation may have been overwhelming.

The absence of a significant difference in yield between the two methods could have been due to several factors: Ascitic fluid on which studies that formed the basis of the current study were conducted, has different characteristics from CSF. The bacteria isolated from studies on ascitic fluid were mainly *E. coli* while *S. pneumoniae* was the main bacterium isolated from CSF in this study. *N. meningitides* and *H. influenza* are known to be much more fastidious organisms regarding their isolation from CSF (9). It is possible that bedside inoculation would have resulted in a better yield if more of these bacterial species had been studied. The low overall yield of cultures (11.4%) also reduced the power of the study to detect a significant difference between the two methods. A possible explanation for the low yield could have been prior use of antibiotics by patients, which could only be ruled out by performing blood and urine assays which were not available during the course of the study. Several studies (14-16) have demonstrated reduced yield from CSF cultures of patients who had previously taken antibiotics. The high prevalence of HIV infection among study patients (83.7%) could also have contributed to the low yield of bacteria as shown in a study done in Nairobi (17).

A significant finding in this study was that cultures from samples inoculated at the bedside grew approximately five 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* (10), Bobadilla *et al* (11) and Such *et al* (12) in studies on ascitic fluid from patients with spontaneous bacterial peritonitis did not report a difference in time to positive culture between the two methods. Kaplan *et al* (18) 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 culture inoculated at the laboratory. Other studies (4,6,13, 19) did not report how long it took for cultures to turn positive. Further

studies are needed to confirm this finding as earlier detection of meningopathogens would lead to faster reporting of antimicrobial sensitivity patterns which would be useful in guiding clinical decisions.

Ninety six per cent of *S. pneumoniae* isolates in this study were sensitive to penicillin. This is in contrast to previous studies that found higher levels of penicillin-resistant pneumococci at KNH (3, 6, 20). However *in-vitro* sensitivity to drugs does not necessarily translate into better outcomes for patients as the tests 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 (21). Recommendations regarding choice of antibiotics as well as adjunctive therapy for use in acute meningitis should ideally be based on clinical trial evidence.

In conclusion, while no difference in yield was found between bedside and laboratory inoculation of culture media with CSF in this study, a significant difference in time to positive culture was detected. This may have potentially useful clinical implications and further studies are warranted to address this issue.

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