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PATHOGENIC ISOLATES IN MENINGITIS PATIENTS IN DAR ES SALAAM, TANZANIA
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PATHOGENIC ISOLATES IN MENINGITIS PATIENTS IN DAR ES SALAAM, TANZANIA

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ABSTRACT

Objectives: **To determine, from laboratory records, the spectrum of bacterial and fungal pathogens isolated from cerebrospinal fluids (CSF) of in-patients with meningitis at Muhimbili Medical Centre (MMC) in Dar es Salaam and to ascertain the laboratory results (based on microscopy and culture) using the latex agglutination technique.**

Design: **A retrospective study based on laboratory records of CSF samples investigated between November 1999 and June 2000 and a cross-sectional study involving investigation of 60 freshly collected CSF samples by conventional (microscopy and culture) and antigen detection by latex agglutination technique (LAT).**

Setting: **Muhimbili Medical Centre in Dar es Salaam, Tanzania.**

Investigations: **Information from laboratory records, bacteriological examination of CSF by microscopy, culture and agglutination techniques.**

Results: **According to records, a total of 1144 CSF samples were investigated between November 1999 and June 2000, of which two hundred and twenty two specimens (19.4%) had a positive bacterial or fungal culture. Fifty five of the isolates were from children (aged less than 15 years) and were; 20 (36.4%) were *Streptococcus pneumoniae,* 12(21.8%) were *Cryptococcus neoformans,* and nine (16.4%) were *Haemophilus influenzae* type b. The remaining 14 (25%) isolates included three group B streptococci, three *Klebsiella* spp, two *E.coli,* two *Staphylococcus aureus,* two *Pseudomonas* spp, one Moraxella and one *Salmonella* group B. For adults a total of 167 positive cultures were reported and 163 (97.6%) of the isolates were *Cryptococcus neoformans,* two (1.2%) were *Pseudomonas* spp. and two were *S. aureus.* There was good agreement between conventional microscopy and culture with the latex agglutination technique in the identification of CSF pathogens.**

Conclusion: **In children, *S. pneumonia,* and bacteria in general constituted the majority of isolates. Adult cases of meningitis were almost exclusively due to *C. neoformans.* Overall, *C. neoformans* appears to be the most common isolate among meningitis cases. Based on LAT results, our routine diagnostic methods seem to be adequate in the identification of the common CSF pathogens.**

INTRODUCTION

Meningitis is common in Tanzania(1) and accounts for significant morbidity and mortality among infants, elderly and immunocompromised individuals especially those infected with HIV(2). Prompt laboratory diagnosis and therapy of meningitis, which requires knowledge of aetiological agents, is extremely crucial in improving prognosis. Studies done in various African countries seem to depict differences in the relative involvement of the various putative pathogens in the aetiology of meningitis(3-6). For example, a study of aetiological agents of meningitis in Kumasi, Ghana found no *C. neoformans* even in AIDS patients and recommended not to screen routinely for the fungus in meningitis(7). However, studies done in South Africa(4) and Rwanda(8) found the fungus to be the dorminant aetiological agent of meningitis. Similar differences are notable in bacteriological findings emanating from different studies on meningitis in Africa (3-7,9-11). These differences underline the need of having representative data, with well defined diagnoses, from all countries. This kind of information is highly desirable for planning both curative and preventive medical care for meningitis in a given catchment area, and is especially useful in providing treatment guidelines for doctors working in hospitals without the necessary laboratory facilities. In Tanzania there is no published data of the pathogens involved in meningitis. The objective of this study was to document the spectrum of bacterial and fungal pathogens isolated from the CSF of in-patients with

meningitis at MMC in Dar es Salaam. Due to the laboratory records showing a high number of culture negative results it became imperative to test a few CSF samples with both the routine (microscopy and culture) and with the more expensive and sensitive latex agglutination technique as a quality assurance measure.

MATERIALS AND METHODS

Information from laboratory records: Laboratory records of CSF specimens processed at the Department of Microbiology and Immunology of MMC in Dar es Salaam between November 1999 and June 2000 were retrieved. The information sought was age of the patient and the bacteria and fungi that were isolated.

Sample collection and processing: For ascertaining the laboratory records a few CSF samples (n = 60), collected from patients with clinical signs and symptoms of meningitis(12), were tested by routine microscopy and cultural methods and by latex agglutination. Specimens were collected aseptically by lumbar puncture in sterile screw-capped containers. Samples were immediately transported to the Department of Microbiology and Immunology for processing as described below.

Bacteriological examination of CSF: This consisted of detection of bacterial antigens by LAT, Gram and Ziehl-Neelsen stains and culture on different media.

Detection of bacterial antigens: CSF specimens were centrifuged and the supernatant was used for detection of *S. pneumoniae,* group B streptococci, *H. influenzae* type b, *N. meningitidis* A,B,C,Y and W 135 and *E.coli* Ki using the Wellcogen rapid antigen detection kit (Murex Wellcome Diagnostics, Temple Hill, Dartford, UK). The kit identifies the corresponding polysaccharide antigens in CSF using the provided instructions.

Identification by microscopy and cultural techniques: The deposit was inoculated on Sabouraud's dextrose agar (SDA), blood and chocolate agars. The SDA plates were incubated aerobically at 37°C for 36 hours. The blood and chocolate agar plates were incubated in gas jars provided with Gas Paks® (BBL, Cockeysville, MD) at 37°C for 24 hours. A portion of deposit was also used to make smears for Gram and Ziehl-Neelsen stains. Identification of *C. neoformans* was established by identification of the fungus in CSF by Indian ink preparation and positive fungal culture. Bacterial identification of the cultures was based on the combination of colony morphology and Gram stain characteristics.

RESULTS

A total of 1144 CSF samples were investigated during the study period from November 1999 to June 2000. Two hundred and twenty two specimens (19.4%) of the CSF samples collected had a positive culture (Table 1). Of the 55 isolates from children, 20 (36.4%) were *S. pneumonia,* 12 (21.8%) were *C. neoformans,* and nine (16.4%) were *H. influenzae* type b (Table 1). All the other isolates were 14 (25%) and the results are shown in Table 1. For adults a total of 167 isolates were isolated and identified (Table 1). One hundred and sixty three (97.6%) of the isolates were *C. neoformans.* Two isolates were *Pseudomonas spp.* and two were *S. aureus* (Table 1).

Table 1

Bacteria and fungi isolated from CSF samples (n = 1144) of patients with clinical signs of meningitis

Isolate	Patients aged less 15 years	Patients aged 15 years	Total
Crypococcus neoformans	12 (21.8%)*	163 (97.6%)	175 (78.8%)
Streptococcus pneumoniae	20 (36.4%)	-	20 (9.0%)
Haemophilus influenzae type b	9 (16.4%)	-	9 (4.0%)
Neisseria meningitidis	-	-	-
Group B streptococcus	3 (5.5%)	-	3 (1.4%)
E. coli	2 (3.6%)	-	2 (0.9%)
Salmonella group B	1 (1.8%)	-	1 (0.45%)
Moraxella spp	1 (1.8%)	-	1 (0.45%)
Staphylococcus aureus	2 (3.6%)	2 (1.2%)	4 (1.8%)
Klebsiella spp	3 (3.6%)	-	3 (1.4%)
Pseudomonas spp	2 (3.6%)	2 (1.2%)	4 (1.8%)
No growth	577 (91.3%)**	345 (48.1%)**	922 (19.4%)**

*Percentage of positive cultures
**Percentage of all specimens
- No isolate

Table 2

CSF samples (n = 60) from adult meningitis cases as identified by both conventional (microscopy and culture results) and the antigen detection by agglutination test

	Microscopy and culture results	Antigen detection results*
C. neoformans	44	Not determined**
S. pneumoniae	3	2
N. menigitidis	-	-
H. influenzae type b	1	1
E.coli	1	–
Klebsiella spp	1	–
No detection	10	57

*The antigen detection kits were for detection of *S. pneumonia,* Streptococcus group B, *Haemophillus influenzae* type b, *Neisseria meningitidis* A,B,C, Y and *W135* and *E.coli* K1
** Cryptococcal antigens (CryAg) were not determined

Neither *N. meningitidis* nor *M. tuberculosis* was reported. Comparisons of results obtained from a combination of microscopy and culture results with those of antigen detection is shown in Table 2. The latex agglutination technique, like the conventional diagnostic technique, showed most of the CSF samples had no bacteria. A few differences were noted. An additional *S. pneumoniae* isolate and *E. coli* isolate were identified by the combination of the microscopy and culture techniques compared with the latex agglutination technique. The other isolates found by routine methods, namely *C. neoformans* and *Klebsiella* spp could not be ascertained since they were not within the range of detection by the agglutination kits.

DISCUSSION

Our findings show a clear difference in the aetiological agents of meningitis between children and adults. For children, bacteria were more dominant than *C. neoformans* accounting for 70.2% of all culture positives, while the fungus constituted 21.8%. This finding is in keeping with those of other African studies(5,7,9,11). In these studies, *S. pneumoniae, H. influenzae, S. agalactiae, E.coli* and *Klebsiella* spp accounted for more than 80% of all bacterial isolates, although differing in the isolation frequencies.

For adults *C. neoformans* was by far the major cause of meningitis accounting for over 97% of all positive cultures, while bacteria accounted for less than three per cent. These findings seem to represent a shift from bacteria-associated meningitis in pre-AIDS period (unpublished observations). It is important to note that *C. neoformans* was the single most frequent cause of meningitis, being the most common isolate in adults and the second most common in children (Table 1). This predominance of *C. neoformans* is most probably due to the high prevalence of HIV among paediatric(13) and adult(2,14) patients in this hospital, given the strong association between cryptococcal meningitis and HIV seropositivity(4,6,8,10). This pattern is likely to apply in many hospitals in Tanzania given the reported high HIV seroprevalence among patients in various parts of the country(13,14). In this study, however, records regarding the HIV serostatus of the patients could not be obtained.

The large number of culture negative CSF samples prompted the need to ascertain the laboratory records. The results obtained by LAT showed a good agreement with the conventional methods (microscopy and culture), both techniques showing bacteria in only a few samples. Deductively, the culture negative CSF in our records could suggest; aseptic meningitis, non-traditional bacterial pathogens or viral meningitis, emphasising the need for further investigation. It may be particularly interesting to investigate for the viral causes of meningitis given the reported magnitude in Ethiopia(5).

In order to improve the current routine diagnostic methods we propose an improvement in microscopy with emphasis on examining and documenting the various cellular components in the background of the smear(15) and to search for 'non-traditional' bacterial agents of meningitis(16-18). There is also a need for more regular antimicrobial susceptibility testing of the culture isolates. The large isolation frequency of *C. neoformans* does advocate routine mycological surveillance of CSF and immediate institution of appropriate chemotherapy for cryptococcal meningitis according to the current treatment guidelines (19-21).

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