Common Bacterial Isolates, Clinical Outcome and TB Meningitis in Children Admitted at Morogoro Regional Referral Hospital, Tanzania

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Abstract

Background: Bacterial meningitis is still one of the major causes of deaths, disabilities, and mental retardation in children in Morogoro region. To study the current meningitis burden, we evaluated the common bacterial isolates and clinical outcome of the disease in the region.

Methods: We conducted a hospital-based prospective study on 1352 children aged between 7 days and 12 years admitted in pediatric wards at Morogoro Regional Referral Hospital for 7 months. Cerebrospinal fluid (CSF) for laboratory microbiological examination was collected by lumbar puncture in 72 children with signs and symptoms of meningitis. Latex agglutination test was used to confirm the bacterial colonies in the culture. Chi-square test was used for relative risk with 95% confidence intervals; statistical analysis and tests were considered statistically significant when \( P < 0.05 \).

Results: Among 72 CSF samples, 23 (31.9%) were positive for Streptococcus pneumoniae, 6 (8.3%) for Haemophilus influenzae, 5 (6.9%) for Group B Streptococcus, 3 (4.2%) for Escherichia coli, and 1 (1.4%) was positive for Mycobacterium tuberculosis. Furthermore, 34 CSF samples showed no bacteria growth in the culture media. In addition, 39 children (54.2%) did not respond to the treatment, whereas 79.5% \((n = 39)\) of them died, while 20.5% \((n = 39)\) of them were referred to a tertiary hospital. Nevertheless, the incidence of meningitis infection was 5.3% \((n = 1352)\) among the admitted children.

Conclusions: S. pneumoniae was the major laboratory-confirmed bacterial isolate associated with meningitis in children. We report for the first time the presence of tuberculous meningitis in Morogoro region. Ziehl–Neelsen staining for acid-fast bacilli should be mandatory for any case clinically suspected for meningitis.

Keywords: Cerebrospinal fluid, childhood bacterial meningitis, culture, lumbar puncture, tuberculosis

INTRODUCTION

Bacterial meningitis remains a serious public health threat globally, accounting for an estimated annual 170,000 deaths worldwide. Every year, bacterial meningitis epidemics affect more than 400 million people living in 21 countries of the “African meningitis belt” (from Senegal to Ethiopia). An estimate of 2%–4% of all pediatric admissions with fever in African hospitals had meningitis, and a lumbar puncture was rarely performed. A survey of 13 hospitals in Tanzania showed that 38% of health workers failed to recommend a lumbar puncture without any specific reasons for children with fever, stiff neck, and positive malaria slide and 30% failed to recommend lumbar puncture to children with fever and atypical convulsions(3) although about 75% of bacterial meningitis can be detected only at the bedside by examining the cerebrospinal fluid (CSF) cloudiness or turbidity.

Pyogenic bacteria such as Streptococcus pneumoniae and Haemophilus influenzae type B are the leading cause of meningitis among children, while Neisseria meningitidis serogroup A, W135, C, and X are the main cause for large epidemics and are responsible for localized outbreaks. However, due to lack of data, Mycobacterium tuberculosis remains a rare but important agent in the causation of meningitis in children.

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Deaths and neurological complications in children due to meningitis are many; however, among them, only in a few cases, lumbar puncture was taken during admissions and the results were not readily out until their deaths.

Additional factors, such as advanced HIV infection, malnutrition, and antibiotic-resistant bacteria, complicate the management of the infected children. For these reasons, meningitis presents an exceptional challenge to physicians working in resource-poor settings such as Morogoro Regional Referral Hospital (MRRH).

In Tanzania, the last epidemic of meningococcal meningitis occurred in 2001 at the Goma Refugee Camp, which was spread from Burundi and Rwanda. Surveillance done by the Centers for Disease Control and Prevention in 2008 showed that Tanzania is one of the countries grouped as medium performers for the surveillance of pediatric bacterial meningitis as compared to other African countries.

Control of bacterial meningitis is very challenging, but recently, a vaccine against group A meningococcal meningitis has been successfully tested in clinical trials in India and African countries in the meningitis belt.

After the introduction of the conjugate H. influenza B (Hib) vaccines into national childhood immunization programs, the decrease in the incidence of Hib disease in children in several countries has been well documented worldwide and there are limited published data from Tanzania.

Although there are a number of reference log books and treatment documentation of suspected cases of meningitis at MRRH, the actual incidence of the disease and the outcome of the current management practices are not known which calls for a new evaluation and research on the disease in the region. In addition, the major causative agents associated with meningitis cases are not well documented.

The rationale of this study lies in the fact that the best way to evaluate the effect of the existing protocol is to perform this incidence study to assess changes in the causative organisms, management outcome, and mode of meningitis presentation.

**Methods**

A prospective study was conducted among admitted children in pediatric wards at the MRRH for 7 months with age ranging between 7 days (neonates) to 12 years. A convenient sample size of 72 children with clinical meningitis signs and symptoms was involved in the study. CSF culture and further analyses were performed at MRRH General Laboratory and the Department of Veterinary Medicine and Public Health Laboratory at Sokoine University in Morogoro, Tanzania.

**Inclusion criteria**

All children with fever and either of the following signs and symptoms such as convulsions, neck stiffness, impaired level of consciousness, severe headache, opisthotonos position, irritability, and petechial rashes were taken for lumbar puncture. Patients with signs of increased intracranial pressure such as bulging of fontanels lumbar puncture were not performed.

Other parameters such as body temperature, level of consciousness, nutritional status assessment, and bedside in vitro antigen-antibody HIV test were done using Tanzanian algorithm for HIV testing. The antibiotic usage information from parents/guardians who sought the care of children was asked regarding prior use of antibiotics or any other drugs for those who came without a referral letter.

Briefly, Gram stain was performed from centrifuged CSF; one to two drops each of CSF was streaked onto commercially prepared blood and chocolate agar media. Residual CSF was inoculated into 5% Fildes broth. The CSF broth was routinely subcultured after 24, 72, and 7 days of incubation. Positive growth cultures were tested for the presence of Hib, S. pneumoniae, E. coli, and Group B Streptococci by latex agglutination meningitis test system.

**Lumbar puncture procedure**

The kit for collection of CSF for children contained skin disinfectant, sterile gauze, lumbar puncture needle 23-gauge/2.5 inch, sterile screw-cap tubes, syringe and needle, transport container, and Trans-Isolate medium was used when CSF could not be analyzed in the microbiological laboratory immediately.

A child was lying on the side with his or her back arched forward so that the head almost touched the knees during the procedure. For conscious children, sedation was done to keep them calm. The skin around the needle insertion area was disinfected twice along the line drawn between the iliac crests with 70% alcohol, followed by povidone-iodine for further disinfection.

The needle was slowly introduced deeply between lumbar 3 and 4 or lumbar 4 and 5, and 1–2 ml of CSF depending on the opening pressure was collected into sterile, screw-cap tubes each. The specimens were then labeled according to the patient’s name and file number and were immediately sent to the laboratory.

**Ziehl–Neelsen staining procedure**

The CSF samples were kept on glass slides and slightly fixed and then the strong solution of carbol-fuchsin was flooded onto the slides. The heat was applied below the slide until the fumes arose and caution was taken not to overheat or boil the sample. After cooling for some few seconds, the heat was reapplied again until the fumes were seen. The slide was again allowed to cool and washed well with running water for 1–2 min. Then, the drops of decolorizer (20% sulfuric acid solution) were poured onto the smear and left to act for 1–2 min. The slide was washed in running tap water and then decolorized with 20%
sulfuric acid for a few minutes and washed thoroughly until the smear was colorless or faint. Few drops of the counterstaining Löffler’s methylene blue solution were poured on the smear and left to act for few minutes. After that, the slides were washed by the running tap water and blot dried ready to be examined under the microscope, and for AFB, if no AFB it appeared purple and the background was blue in all cases.

**Performing latex agglutination test**

The isolates to be tested were grown for 18–24 h on a BAP at 35°C–37°C in a candle jar. From overnight growth on a BAP, a sterile loop was used to prepare a light to moderate cell suspension (approximately equal to a 0.5 McFarland density standard) in 0.5 ml of 0.85% saline. On a glass slide or reaction card, 10 µl (1 droplet) of the latex reagent was added and then 10 µl of the cell suspension was added. The two suspensions were mixed together and the latex agglutination reaction was observed at an angle with oblique lighting.

A positive reaction was indicated by agglutination (cells clumping together) appearing within 2–10 min and a negative reaction was indicated by no agglutination and the suspension appearing homogenous milky, and if the reaction time exceeded 30 min, it was taken as false positive reaction.

Each lot of latex suspension was tested for positive agglutination reactions using specific organism’s reference strains with known capsular serotypes. For the biosafety reasons, a solution of 5% formalized physiological saline was used to kill the bacterial isolates after the identification. Antiserum were stored in the refrigerator at 4°C and warmed to room temperature (25°C) before use. It was kept back in the refrigerator as soon as the testing was finished to prevent the loss of binding activity of the antibody.

**Statistical methods**

Laboratory record audits were conducted by comparing logbooks (CSF specimen records) information with study laboratory reports to identify missed meningitis cases. Data obtained were coded and analyzed using SPSS computer program version 22 (SPSS Inc., Chicago, IL, USA). Categorical comparisons were performed with the Chi-square test. Test statistics were evaluated with a significance level of \( P < 0.05 \). The strength of association was expressed using odds ratios with 95% confidence intervals (CIs).

**Ethical considerations**

Ethical clearance was sought from the Ethical Clearance Committee of Sokoine University, and permission to conduct

| Bacteria                                | Number | Percentage |
|-----------------------------------------|--------|------------|
| **Gram stain**                          |        |            |
| Gram positive                          | 30     | 41.7       |
| Gram negative                          | 7      | 9.7        |
| No stain                               | 35     | 48.6       |
| Total                                   | 72     | 100        |
| **Culture**                             |        |            |
| *S. pneumoniae*                         | 23     | 31.9       |
| *H. influenzae*                         | 6      | 8.3        |
| Group B streptococci                    | 5      | 6.9        |
| *E. coli*                               | 3      | 4.2        |
| No growth                               | 35     | 48.6       |
| Total                                   | 72     | 100        |
| **ZN-Stain**                            |        |            |
| Positive for AFB                        | 1      | 1.4        |
| Negative for AFB                        | 71     | 98.6       |
| Total                                   | 72     | 100        |

**Table 1: Bacteria isolates from cerebrospinal fluid culture, Gram stain, and ZN stain results**

![Figure 1](chart.png)

*Figure 1: Flowchart summary that shows the trend of the study from 1352 admitted children at Morogoro Regional Referral Hospital for 7 months*
The incidence of meningitis among children aged 7 days to 12 years was 5.3% [Figure 1].

Only one CSF specimen was Ziehl–Neelsen (ZN) stain positive for acid-fast bacilli (AFB) [Table 1]. Bacterial culture revealed *S. pneumoniae* in 23 (31.9%). CSF samples, *H. influenzae* in 6 (8.3%) samples, Group B *Streptococcus* in 5 (6.9%) samples, and *E. coli* in 3 (4.2%) samples; however, 35 (48.6%) samples showed no bacterial growth.

A total of 32 (44.4%) children responded well to the treatment, while 31 (43.1%) children died either before or during treatment, whereas 19 (26.4%) children recovered fully and 14 (19.4%) children recovered with signs of neurological deficit. The remaining 8 (11.1%) children were referred to a tertiary hospital. Furthermore, optical observation of the 72 CSF samples revealed turbidity in 33 (45.8%) specimens, whereas 39 (54.2%) samples were clear [Table 2].

Of 72 children with meningitis symptoms, the CSF culture samples yielded 23 (31.9%) *S. pneumoniae*, 6 (8.3%) *H. influenzae*, 5 (6.9%) Group B *Streptococcus*, 3 (4.2%) *Escherichia coli*, and 35 (48.6%) had no bacterial growth.

Furthermore, among children with meningitis symptoms, the CSF Gram staining showed 30 (41.7%) Gram-positive, 7 (9.7%) Gram-negative, and 35 (48.6%) were no staining at all.

Among 72 children with meningitis symptoms treated, 34 (47.2%) responded to treatment, whereas 38 (52.8%) did not respond to treatment. Of the children who responded treatment, 17 (50%) had CSF bacterial culture positive and 17 (50%) had CSF bacteria culture negative. On the other hand, 17 (48.6%) children with meningitis symptoms and CSF culture negative responded to the treatment regimen.

Moreover, 68 children with known HIV status, 31 (45.6%) responded to the meningitis treatment, while 37 (54.4%) did not respond to treatment. Of the children who responded to treatment, 10 (32.3%) were HIV positive and 21 (67.7%) were HIV negative. However, of 37 (54.4%) of the children who did not respond to the treatment, 13 (35.1%) were HIV positive and 24 (64.9%) were HIV negative.

In addition, of the clear CSF samples for culture, 7 (17.9%) showed positive for *S. pneumoniae*, 2 (5.1%) for *H. influenzae*, 3 (7.7%) for Group B *Streptococcus*, 1 (2.6%) for *E. coli*, while 26 (66.7%) had no bacterial growth. On the other hand, of the 33 (45.8%) turbid CSF samples, 24 (72.7%) were bacterial culture positive, 16 (48.5%) showed *S. pneumoniae*, 4 (12.1%) had *H. influenzae*, 2 (6.1%) were Group B *Streptococcus*, 2 (6.1%) *E. coli*, and 9 (27.3%) did not show any bacterial growth.
which reported that *S. pneumoniae* was the most prevalent isolate in the CSF of children with clinical signs and symptoms of meningitis.[16]

The current management of meningitis at MRRH is not optimal since 20 (54.1%) children with CSF bacterial culture positive [Table 2] did not respond to the treatment regimen; however, the *P* value was not significant for a positive management response (*P* = 0.8235). This might suggest either presence of resistant bacteria or other causes of meningitis in children such as fungal or virus infection.[17]

Moreover, optical turbidity were only significant for *S. pneumoniae* CSF culture [Table 2] and the relative risk (RR) for positive bacterial culture growth was almost 3 times more than in clear CSF culture (RR 2.7, 95% CI 1.45-5.05, *P*<0.001). This might be due to the effect of antibiotic used prior admission in peripheral hospitals, or the sensitivity and specificity of the method used to culture the CSF samples were not good enough.[9] Therefore, these findings suggest that proper culture and sensitivity of the CSF sample are mandatory regardless of the optical observation and high sterility should be considered during a lumbar puncture procedure to avoid contamination and interference with the culture growth results.

This study also showed that meningitis can affect any child, regardless of the HIV status, however, the treatment response was good for HIV-negative children, although the *P* value was not significant (*P* = 0.8027) may be due to a small sample size [Table 2]. On the other hand, the effect of HIV being tested once *in vitro* by rapid antigen-antibody test reading without considering the window period and maternal antibody calls for further studies with the use of DNA-polymerase chain reaction methods, which is the conclusive diagnosis of HIV present in the body.

In some optically clear CSF, the culture became positive; hence, this strongly suggests for the initiation and continuation of antibiotic irrespective of the turbidity of the CSF. For all of the children who failed to recover, the possibility of comorbidity with other terrible diseases such as protein-energy malnutrition, pneumonia, severe malaria, and diarrhea could be a strong reason, which is not an unusual finding in children in resource-limited areas such as Morogoro region.

To the best of our knowledge, this study showed for the first time in Morogoro region the presence of (TB) meningitis by identifying one child with TB meningitis after a positive ZN stain results without prior clinical features, suggestive of any form of TB. This shows that ZN staining is very important, useful and should be recommended to all other children suspected of meningitis so as to start anti-TB treatment early, prevent the spread of the disease, and save the life of children.

Delayed reporting for medical services was the main obstacle as many children died before lumbar puncture performed and thus possibly interfered with the exact number of clinical cases of meningitis recorded at the hospital. Second, blood culture was also very important in diagnosis and confirmation of bacterial meningitis, and to rule out other systemic causes of the disease, however, it was not done due to limited agar plates and incubators. Third, many of the examined children were diagnosed with meningitis or upper respiratory infection earlier in other hospitals and probably had received antibiotics before admission, hence interfered with the clinical presentation of the disease on admission.

**Conclusion**

The present study has demonstrated with laboratory confirmation for the first time the presence of TB meningitis among children admitted at MRRH by ZN stain positive for AFB. This is very crucial information that calls for an alert to health-care workers and the public health sector and that TB meningitis may be present without typical TB features in children. According to this study, in the future, suspected cases of meningitis should be handled as emergency cases; lumbar puncture and initiation of antibiotics treatment should not be delayed in anyhow. Furthermore, laboratory results should be out within 24 h after hospital admission to conclude on the causative organisms for the disease. It is recommended that all samples from meningitis suspected cases, regardless of the turbidity of the CSF, should be subjected to bacterial culture and sensitivity testing.

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**Conflicts of interest**

There are no conflicts of interest.

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