**Mycobacterium tuberculosis** and **Mycobacterium africanum** in stools from children attending an immunization clinic in Ibadan, Nigeria

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Tuberculosis; Stool; Diagnosis; Pediatric

**Summary**

**Background:** Tuberculosis is a major cause of childhood morbidity and mortality in Nigeria. Diagnosis of childhood tuberculosis is a global challenge making early treatment a mirage. In this study we investigated the stools of children for the presence of mycobacteria.

**Methods:** Stool samples from children aged 3 days to 3 years who presented for postnatal immunization at a large university-based clinic in Nigeria, were subjected to Ziehl–Neelsen staining. Samples with acid-fast bacilli were further processed using mycobacterial culture, spoligotyping, and deletion typing.

**Results:** One hundred and ninety-two stool samples from different children were collected and processed. Thirty (15.6%) had acid-fast bacilli. Of these, eight had *Mycobacterium tuberculosis* and one had *Mycobacterium africanum*.

**Conclusions:** Approximately 5% (9/192) of apparently well children had evidence of potentially serious tuberculosis infection. The usefulness of stool specimens for diagnosing pediatric tuberculosis warrants further investigation.

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**Introduction**

More than 2 billion people, approximately one-third of the global population, are infected with **Mycobacterium tuberculosis**, the major causative organism of tuberculosis (TB).¹
Mycobacterium africanum and Mycobacterium bovis, also members of the Mycobacterium tuberculosis complex, are much less frequent causes of TB in humans. The incidence and prevalence of pediatric TB varies significantly across the globe, driven largely by the burden of the disease in different countries. About 1 million children under 15 years of age develop TB worldwide annually, representing 11% of all TB cases. The majority of these cases occur in low-income countries where the prevalence of HIV/AIDS is high. Nigeria currently ranks fourth on the list of TB burdened nations globally, and pediatric TB accounts for a substantial proportion of these cases. Almost 2 million people per year die as a result of TB, mostly in developing countries like Nigeria, but the mortality in children is often underreported. Despite this, TB is one of the ten leading causes of childhood mortality.

Young children and especially newborns are at a high risk when exposed to a contagious source. A comprehensive review of the natural history of childhood TB showed that primary infection before 2 years of age frequently progressed to active disease within 12 months. The majority of these cases occur in low-income countries where the prevalence of HIV/AIDS is high. Nigeria currently ranks fourth on the list of TB burdened nations globally, and pediatric TB accounts for a substantial proportion of these cases. Almost 2 million people per year die as a result of TB, mostly in developing countries like Nigeria, but the mortality in children is often underreported. Despite this, TB is one of the ten leading causes of childhood mortality.

The diagnosis of pulmonary TB (PTB) in children is challenging. Children rarely expectorate adequate amounts of sputum, and the limitations of using other specimens or techniques, such as first morning gastric aspirates (considered the best clinical specimens for young children with suspected PTB), nasopharyngeal swabs, sputum induction, and laryngeal swabs, are well known. Accordingly, there is a strong imperative to evaluate the diagnostic utility of clinical specimens that are more readily collectable. Some investigators have suggested that stool microscopy and culture for Mycobacterium tuberculosis may be diagnostic in some children with tuberculosis, but other investigators have described stool evaluation as ‘worthless’ since non-pathogenic acid-fast bacilli (AFB) may be found in the normal intestinal contents of adults.

Following the identification of AFB in the stools of apparently well children who were being screened for cryptosporidiosis in our immunization clinic, we designed this study to characterize AFB in the stools of children attending the clinic.

Methods

Subjects

Subjects were consecutive children who presented for immunization at the University of Ibadan Health Services Clinic, Ibadan, Nigeria. Stool samples from the children were evaluated using AFB staining. All the AFB-positive stool specimens were evaluated further for the presence of mycobacteria.

Ethical approval

The Institutional Review Committee of the University of Ibadan and the University College Hospital, Ibadan, Nigeria approved the study. Oral informed consent was obtained from the parents of the children.

Clinical specimens

Stool samples were collected from each child into a sterile plastic container and kept in the refrigerator at 4 °C prior to processing using Ziehl–Neelsen (ZN) staining. The ZN stain was carried out as described by Shrestha et al. The use of deletion analysis for the typing of Mycobacterium tuberculosis complex strains has been previously described. For this work, the deletion typing method described by Warren et al. was used. In our analysis, we used primers directed against the RD4 and RD9 loci to generate a deletion profile.
that would allow speciation of the isolate. The multiplex master mix system from Qiagen was used for the PCRs, with primers previously described by Warren and colleagues. 19

The PCR mixture was a multiplex reaction, with each PCR reaction containing 1 µl of DNA template, 5 µl Q-buffer, 12.5 µl multiplex master mix (Qiagen), and 0.5 µl of each primer (50 pmol/µl). The total volume of the reaction was made up to 25 µl with water. The reaction was allowed to run for 15 min at 95 °C, followed by 45 cycles at 94 °C for 1 min, 62 °C for 1 min, and 72 °C for 1 min. After the last cycle, the samples were incubated at 72 °C for 10 min. Products were visualized by electrophoresis on 3% agarose gels.

The positive controls included a known M. bovis isolate (AHS) and a known M. tuberculosis isolate provided by the Medical Research Council, Center for Molecular and Cellular Biology, Stellenbosch University, Cape Town, South Africa, whilst the negative control was water. The resulting gel images were analyzed on the basis of their alignment on the gel (i.e., same band size with either of the controls). The RD9 deletion analysis was done to discriminate M. tuberculosis from other Mycobacterium tuberculosis complex (MTC). Those with a deletion at this region were further investigated with primers targeting the RD4 region and this discriminated deletion analysis confirmed these isolates as M. tuberculosis.

The diagnosis of TB in pediatric patients is often based on case definitions that incorporate signs and symptoms of TB, suggestive chest radiograph, positive tuberculin skin test, and contact with an active TB patient. It is less frequently based on laboratory isolation of M. tuberculosis. Existing algorithms, however, have serious shortcomings, and the development of reliable and widely applicable algorithms is a high research priority.

In this study, we diagnosed M. tuberculosis in 27% (8/30) and M. africanum in 3% (1/30) of children who had AFB-positive stool specimens. Other investigators have diagnosed tuberculosis based on isolation of M. tuberculosis from stool specimens, but in different patient populations. Mwachari et al., 20 cultured M. tuberculosis from the stools of 10 (13%) HIV-infected adults with chronic diarrhea in Kenya. Manatsathit et al., 21 also found M. tuberculosis in the stools of eight (18%) adult AIDS patients in Thailand. In South Africa, 8% and 5% of 66 children with suspected PTB had stool specimens that were AFB-positive and M. tuberculosis culture-positive, respectively. 9 In that study, AFB were identified only in the stools of children who had PTB that was confirmed with positive gastric aspirates, but stool testing was less sensitive than gastric aspirates overall. Our study is unique because the study population included apparently well children who were brought to the clinic for routine immunization. None of the children yielded a growth in nine (30%). Spoligotyping and deletion analysis confirmed these isolates as M. tuberculosis complex (eight M. tuberculosis and one M. africanum; Table 1 and Figure 1). The patients with the positive stool mycobacterial cultures included three males and six females, with ages ranging from 1 week to 15 months (Table 1).

**Results**

One hundred and ninety-two children were recruited into the study, comprising 95 males and 97 females, aged 3 days to 3 years. Thirty children had AFB present in their stool specimens. Mycobacterial culture of stool samples from the 30

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age</th>
<th>SpolDB4 type</th>
<th>Family in SpolDB4 type</th>
<th>Species</th>
</tr>
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<tbody>
<tr>
<td>JC1</td>
<td>F</td>
<td>2 months</td>
<td>52</td>
<td>T2</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>JC2</td>
<td>F</td>
<td>1 week</td>
<td>53</td>
<td>T1</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>JC3</td>
<td>M</td>
<td>6 weeks</td>
<td></td>
<td></td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>JC4</td>
<td>F</td>
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<td>61</td>
<td>LAM10_CAM</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>JC5</td>
<td>M</td>
<td>3 months</td>
<td>774</td>
<td>T1</td>
<td>M. tuberculosis</td>
</tr>
<tr>
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<td>1 year, 3 months</td>
<td>61</td>
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<tr>
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<td>JC8</td>
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<td>3 months</td>
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<td>New strain (probably</td>
<td>M. africanum (deletion analysis)</td>
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<tr>
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<td>F</td>
<td>6 months</td>
<td>358</td>
<td>T1</td>
<td>M. tuberculosis</td>
</tr>
</tbody>
</table>

Table 1 Profile of pediatric patients with a positive culture result for Mycobacterium species

**Figure 1** Spoligotype profile of pediatric patients with a positive culture result for Mycobacterium tuberculosis complex.
the children had diarrhea, but four had cough, although clinical data were not collected prospectively in all children. The yield might be higher in children clinically suspected of having TB.

Some findings of this study provide potentially important epidemiological information. Firstly, *M. tuberculosis* has been implicated in most cases of TB in children; however, we found a case of *M. africanum*. This is, to our knowledge, the first published isolation of *M. africanum* in the stools of a Nigerian child. Cases of TB caused by *M. africanum* have been previously reported in adults from Nigeria and other African countries. In Cameroon, there has been a decline in STB strains around the world (Figure 1). This implies the possible isolation of *M. africanum* has not been previously reported in the SpolDB4 database, which contains a comprehensive listing of the *M. tuberculosis* strains around the world (Figure 1). This implies the possible circulation of poorly characterized or emerging *M. tuberculosis* strains in Nigeria.

This study should be interpreted in the context of its limitations. Since we did not have records of HIV testing in the mothers or children, we were unable to correlate our findings with the HIV infection status of the subjects. Also, full contact tracing was possible only in some of the children with positive stool tests, seriously constricting our ability to comment on the public health significance of the results. Our data cannot be extrapolated to older children because the oldest subject in this study was 3 years old. Finally, we do not have complete data on the subsequent clinical course of the patients. Despite these limitations, our findings add to the data cannot be extrapolated to older children because the oldest subject in this study was 3 years old. Finally, we do not have complete data on the subsequent clinical course of the patients. Despite these limitations, our findings add to the evidence that directed stool studies may be useful in pediatric TB diagnosis.

In conclusion, we have described the isolation of *M. tuberculosis* from the stools of a significant proportion of apparently well children attending the University of Ibadan Health Services Clinic. We have also described the first isolation of *M. africanum* from the stools of a Nigerian child. The problematic nature of diagnosing TB disease in this age group justifies further investigation of the diagnostic potential of stool specimens and other readily obtainable specimens, perhaps using more sensitive techniques. The limitations of such testing and the population for which it would be applicable are also fertile areas for clinical and laboratory studies.

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Conflict of interest: No conflict of interest to declare.

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