Prevalence of Tuberculosis in Children Using Gene Xpert on Gastric Lavage Samples
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ABSTRACT
Background: Tuberculosis (TB), caused by Mycobacterium Tuberculosis (MTB), affects the lungs, kidneys, spine, and brain and is a significant global health challenge affecting 10% -20% of children. Zambia is ranked in thirty countries with a heavy burden. Early detection and intervention are crucial for managing the disease and preventing its progression to severe forms.

Objective: The aim of this study was to assess the prevalence of tuberculosis among children aged 0 to 10 years at the Arthur Davison Children's Hospital in Ndola, Zambia. The study specifically focused on children with coughs, and the preferred gastric lavage technique was used for sampling.

Methodology: A cross-sectional study was conducted, and we enrolled 138 children who were assessed using a structured questionnaire. To diagnose TB, we employed X-rays and GeneXpert analysis of gastric lavage samples collected from January 2022 to February 2022.

Results: The results revealed that out of the 138 participants, 119 (86.2%) tested negative for Mycobacterium tuberculosis, while 19 (13.8%) tested positive. GeneXpert detected 2 out of the 19 positives (1.4%), whereas chest X-ray identified all 19 positive cases (100%). Males showed a higher prevalence of MTB (52.6%) than females (47.4%), and the 0–4 age group had a higher MTB prevalence of 13 cases (68.4%) than 6 cases (31.6%) in the 5–10 age group.

Conclusion: In conclusion, the study showed a low prevalence of MTB (1.4%) in children under 10 years of age when using GeneXpert with gastric lavage samples. However, chest X-ray outperformed GeneXpert in detecting positive cases. The potential issues with the cumbersome process and the influence of gastric lavage sample acidity raise concerns about GeneXpert’s effectiveness as the preferred choice for TB diagnosis in this population.

This study evaluates methods for diagnosing tuberculosis in children, addressing challenges in routine sputum production for laboratory diagnosis due to their limited sputum production capacity.

Key Words: Tuberculosis; diagnosis; children; GeneXpert; chest X-ray; Zambia

Introduction
Mycobacterium Tuberculosis (MTB) is the bacterium that causes the disease known as tuberculosis. The kidney, spine, and brain are among the parts of the body that are typically attacked by bacteria. In Zambia, one of the leading causes of morbidity and mortality is tuberculosis [1,2]. According to the World Health Organization (WHO) 2013, Zambia is one of the thirty nations with the greatest TB burden in the world. According to the national TB prevalence study conducted in Zambia, the prevalence of TB was higher than what the WHO had previously predicted. The prevalence of confirmed TB was 638 per 100,000 persons, while the prevalence of all TB strains was 455 per 100,000 people [3]. Urban areas had a greater rate of TB prevalence than rural areas. With an estimated 77% of the nation’s TB burden, the Copperbelt and Lusaka Provinces had the greatest prevalence [4]. Additionally, the TB burden was higher among men, People Living with HIV (PLHIV), and persons between the ages of 25 and 44, a group that is economically productive, indicating that the TB burden could have a detrimental impact on the micro and macroeconomic status [2]. With 59% of all notified TB patients simultaneously having HIV infection, Zambia has a high rate of TB and HIV coinfection [4]. According to WHO 2013, there are 9 million new cases of TB worldwide each year, up to 500 000 of which are Multidrug Resistant (MDR-TB) cases. Although country-specific statistics are generally

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The accurate identification of pediatric TB still presents a number of diagnostic problems. Malnutrition, coinfection with HIV, and the general lack of specificity in most signs and symptoms have confounded clinical diagnostic algorithms. Children frequently acquire meningitis or extra pulmonary TB that is missed, such as lymph node disease. Recent transmission is represented by childhood TB. Sub-Saharan Africa (SSA) not only has the highest TB incidence rates but also has a high HIV incidence. The WHO reports that despite major technological advancements in detection procedures, TB reduction and eradication remain a long way off for medical professionals.

One of the leading causes of morbidity and mortality worldwide is tuberculosis. The WHO 2013 reports that although modern TB treatment and quick diagnosis methods have significantly decreased mortality by 47% since 1990, TB is still a leading cause of death, particularly in developing nations. Pneumonia and Extra Pulmonary Tuberculosis (EPTB) are the two kinds of tuberculosis that are caused by MTB. The most prevalent type of tuberculosis, which affects the lungs, is pulmonary tuberculosis.

Lung abnormalities can be detected using chest X-ray (CXR), a quick imaging approach. The thoracic cavity, which contains the airways, ribs, lungs, heart, and diaphragm, can be diagnosed with CXR. In the past, CXR has been one of the most important instruments for identifying tuberculosis. Especially when the X-ray is reviewed to look for any abnormality that is compatible with TB, CXR is a powerful tool to identify TB as a differential diagnosis for patients since it has high sensitivity for pulmonary TB. Although some CXR abnormalities (such cavities) are fairly specific for pulmonary TB, CXR has a low specificity. Numerous CXR abnormalities that are consistent with pulmonary TB are also present in a number of other lung illnesses, making them suggestive of both TB and other pathologies. Additionally, there is a sizable difference between individual observers when reading CXRs [7-24].

Using CXR alone for TB diagnosis increases the risk of both over diagnosis and underdiagnoses. Always put forth great effort to confirm a TB diagnosis using bacteriological means, such as sputum smear microscopy, culture, or a molecular test. If the diagnosis of TB is based on bacteriological confirmation, it is classified by the WHO as either clinically diagnosed TB or bacteriologically confirmed TB.

A principle tool for quick identification of MTB and medication resistance is the GeneXpert assay. A two-hour turnaround time and excellent sensitivity and specificity make it an appealing point-of-care test that makes it easier to diagnose and treat TB pleuritis and pericarditis on time. The Xpert MTB/RIF assay has the major benefits of speedy results and little technical expertise needed to perform the test. The Xpert MTB/RIF assay can also promptly detect potential MDR TB.

This study aimed to determine the prevalence of tuberculosis in children at Arthur Davison Children’s General Hospital, determining the detection rate, gastric lavage as the best sample, and TB as the primary cause of cough.

Methodology

Ethical considerations
Ethical approval was sought from the University of Zambia, School of Medicine Ethics Committee (UNZASOMUREC) assurance number FWA00000338/reference number 27-07-2022 and National Health Research Authority reference number Ref No: NHRA0018/05/06/2023 before the commencement of the research. Informed assent and consent were sought, and clarification that participation will be voluntary was made available to the study participants. The results were kept confidential, and access to the information was restricted to the supervisors, researchers and attending clinicians only. Permission to conduct the study and sample analysis was sought from the Laboratory Director, Arthur Davison Children’s Hospital.

Study design and site
This was a cross-sectional study performed from October 2021 to February 2022 at Arthur...
Davison Children’s Hospital (ADCH) in Ndola, Zambia. The ADCH is a specialized children’s hospital covering all pediatric patients in the northern part of Zambia.

**Study population and eligibility criteria**

Children below the age of 10 who consented to be part of the research and were also presenting with mycobacterium TB symptoms were enrolled in this study. A sample size of 138 participants was determined and attained.

**Data collection**

Data were collected from a Microsoft Excel spreadsheet collecting information on age, gender, and results for both X-ray and GeneXpert. A structured questionnaire was given to enrolled patients whose parents and/or guardians agreed to be part of this study. The questionnaire validity was verified before the commencement of the study. Gastric lavage samples were collected from pediatric patients by inserting a 5 ml syringe nasopharyngeal tube with litmus paper to check the acidity of the sample. Specimens were then placed in a clean container and sent to the laboratory for diagnosis.

**Laboratory analysis**

The Gene X-pert Mycobacterium method of detecting gastric lavage was based on the Centre for Disease Control and Prevention. The X-pert MTB/RIF assay is a Nucleic Acid Amplification (NAA) test that uses a disposable cartridge with the GeneXpert Dx 4.7b Software System. GeneXpert is a product of Cepheid, a molecular diagnostics company.

The company’s headquarters are located in Sunnyvale, California, United States. The GeneXpert system is widely used worldwide for the diagnosis of various infectious diseases, including TB [25, 26].

In the laboratory, each gastric lavage sample received a unique identifier for tracking purposes. The samples were prepared following the manufacturer’s instructions using the reagent provided with the assay as a routine specimen. Subsequently, a cartridge containing this prepared mixture was loaded into the GeneXpert machine. The machine then processed the specimens, and the results obtained were recorded and stored for further analysis.

**Data analysis**

Data were analyzed using SPSS statistical package version 21, and descriptive statistics were used to summarize the data. Proportions for gender were described using percentages, while age was described into groups of 0 years - 4 years and 5 years - 10 years. The chi-square test was used once assumptions were met to analyze variables.

**Results**

Due to its serious physical, social, and economic repercussions, tuberculosis is one of the most pervasive public health issues, especially in poor nations [26]. This study included a total of

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**Figure 1:** Demonstrates that, for the two months under study, the overall prevalence of tuberculosis (TB) among children under the age of ten was 13.8% and that, of the 138 children who were tested, 19 received TB treatment.
138 children under the age of 10 years. Overall prevalence of TB (Figure 1)

Figure 2 depicts the hospital’s TB prevalence when gastric lavage samples were submitted for testing using Gene X-pert. Out of the 138 samples examined, only two were positive, translating to a 1.4% prevalence.

Using the age ranges of 0-4 and 5-10, Figure 3 demonstrates the prevalence of TB by age. Thirteen out of 19 children with TB were between the ages of 0 and 4, which resulted in a prevalence of 68.4%, while 6 were between the ages of 5-10, which resulted in a prevalence of 31.6%.

Regarding sex, males were more affected by TB (52.6%), while females were affected by TB (47.4%), which was slightly lower (Figure 4).

Figure 5 indicates Comparison of clinical diagnosis using X-ray to TB diagnosis using GeneXpert while processing gastric lavage samples. The findings showed that just 2 of the 138 children tested positive by Gene X-pert when the same patients were subjected to both methods of diagnosis, whereas 19 of the same patients who were screened using X-rays were confirmed to have TB. Gene X-Pert had a detection rate of 1.4%, while X-ray had a detection rate of 13.8%.

Discussion

The findings indicate that the prevalence of TB among children in this study, using both CXR and GeneXpert, was 13.8%, which falls within the range identified in previous research projecting a global burden of 10-20% TB cases in children under 15 years old [6, 5]. This suggests that the prevalence of TB Arthur Davison Children’s Hospital is consistent with the global trend. This prevalence is slightly higher than the rate reported by Nishtar et al. in 2022, which was 9.7%.

The study also revealed that GeneXpert identified 8.6% of the 1825 patients with positive CXR results. This rate is significantly higher than the 1.4% positive rate reported in the current study. The substantial difference in detection rates...
between GeneXpert and CXR indicates that GeneXpert may be more effective in identifying TB cases in this context, supporting its potential as a valuable diagnostic tool for tuberculosis in children.

Comparing these results with the study by Wekesa et al. in 2014, it is evident that the GeneXpert positivity rates also reported a lower positivity rate of GeneXpert to CXR, which was 17% and 42.5%, respectively.

Children have traditionally been considered a lower public health risk for TB because they rarely develop lung cavities, and most of them do not produce sputum [13-15]. As a result, diagnosing TB in children can be challenging. The accurate identification of pediatric TB still presents several diagnostic problems [20]. This poses a significant obstacle to effectively managing and treating TB in children.

This study reveals that children in the age range of 0-4 years were slightly more affected, with a prevalence rate of 68.4%, compared to those between 5-10 years, who had a prevalence rate of 31.6%. This disparity may be due to various factors, such as differences in exposure or immune responses among different age groups.

Interestingly, this study found that out of the 19 patients who tested positive for TB, only 2 (11%) were positive on Gene X-pert and X-ray examinations. In contrast, all 17 patients (89%) were only identified as positive through X-ray imaging and not detected by Gene X-pert. This
Research Article  Chanda P, et al.

confirms the hypothesis that when gastric lavage samples are used on Gene X-pert, there is a lower TB positivity rate in children under 10 years of age at this hospital compared to when X-ray imaging is employed.

Studies conducted by Wekesa et al. in 2014 and Nishtar et al. in 2022 have reported higher rates of false positives on CXR in the context of TB diagnosis. False positives occur when the CXR results suggest the presence of TB, but upon further evaluation, the individuals do not actually have active TB infection.

In the study by Wekesa et al. (2014), they found a higher rate of false positives with CXR, where 42.5% of patients had CXR results suggestive of TB, but only 17% of them tested positive using GeneXpert, which served as the gold standard. This discrepancy in the results indicates that CXR may lead to an overestimation of TB cases, leading to unnecessary treatment or additional investigations for those who do not have active TB.

Similarly, in the study by Nishtar et al. (2022), the sensitivity of CAD4TB, a diagnostic tool based on CXR, was reported to be 83.2%, while its specificity was only 12.7%, using GeneXpert as the reference standard. The low specificity indicates that CAD4TB had a high rate of false positives, where many individuals who did not have TB were identified as potentially having the disease based on CXR results.

Overall, the discussion highlights the importance of considering different diagnostic methods’ performance in diagnosing TB among children. While CXR remains a widely used tool, GeneXpert appears to demonstrate higher specificity and may be more effective in detecting TB cases as evidenced by the significantly higher positivity rate in the larger patient sample analyzed in this study. However, further research and larger-scale studies are required to validate these findings and assess the diagnostic accuracy of GeneXpert and CXR in various populations. Understanding the strengths and limitations of each diagnostic tool will aid in developing targeted and efficient TB detection strategies for children, particularly in high-burden regions such as Zambia [27-30].

Limitations
The study was conducted at a single hospital, Arthur Davison Children’s Hospital, for only a two-month period. This limited scope may not capture the full range of TB prevalence and diagnostic challenges in pediatric populations in Zambia. Additionally, the study may have been subject to selection bias, as it focused only on patients within a specific age range who sought treatment at a single hospital. This could introduce a bias toward more severe cases or exclude certain subpopulations, potentially impacting the accuracy and generalizability of the findings.

Conclusion
Diagnosing TB in children remains challenging due to their inability to produce spuutm. Instead, gastric lavage is often used as a diagnostic method, but it frequently yields a low TB detection rate. In this study, the prevalence of TB in children at Arthur Davison Children’s Hospital was found to be 1.4% when utilizing Gene X-pert alone and 13.8% when using X-ray imaging. This highlights the importance of employing multiple diagnostic modalities and considering the limitations of each method when diagnosing TB in children.

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Competing interests
The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors’ contributions
P. conceived the study, conducted the formal analysis and wrote the first draft. D. conducted the formal analysis and review and editing of the final manuscript. R. M, M. C and C. performed data curation and reviewed the manuscript. Moses Chakopo supervised the conduct of the study.
Prevalence of Tuberculosis in Children Using Gene Xpert on Gastric Lavage Samples

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