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Xpert® MTB/RIF assay testing on stool for the diagnosis of paediatric pulmonary TB in Tanzania

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SETTING: Six health facilities in Dar es Salaam, Tanzania.
OBJECTIVE: To evaluate the use of stool specimens in the diagnostic workup of paediatric TB using the Xpert® MTB/RIF assay.
DESIGN: Between December 2018 and May 2019, we performed a cross-sectional diagnostic study of children aged between 1 month and 14 years with presumptive TB. A single stool specimen was tested using Xpert. The result was compared with the reference microbiological standard for respiratory or gastric specimens tested using Xpert and/or solid culture. The sensitivity, specificity and predictive values of stool Xpert assay were assessed.
RESULTS: A total of 225 children with a median age of 2.17 years (IQR 1.16–5.19) were enrolled; 165/225 (73.3%) were aged <5 years. Of 225 children, 8 (3.6%) were diagnosed with TB as they were culture- or Xpert-positive on sputum/gastric aspirate. The stool Xpert assay showed a sensitivity of 62.5% (95% CI 25–92) and specificity of 100% (95% CI 98–100) against the reference standard.
CONCLUSION: Use of the Xpert assay on stool specimens had a moderate sensitivity and high specificity in the diagnosis of pulmonary TB in children. Our data adds to the body of evidence for the use of Xpert assay on stool as a non-respiratory specimen to complement conventional methods used to diagnose the disease.

The WHO estimated that 10 million people developed TB in 2019, of which children (aged <15 years) accounted for approximately 12% of cases, with the Africa region having the second highest number of notifications after South-East Asia.[1] Public health programmes had not prioritised TB in children in the past, as more efforts were put into responding to the most infectious cases in adults.[2] However, recent efforts have been taken to prioritise the control and treatment of childhood TB in many national TB programmes.[3]

The diagnosis of pulmonary TB in children is challenging and relies mainly on a combination of factors, including prior TB exposure, clinical examination and relevant imaging and laboratory findings.[4–6] The WHO recommends bacteriological confirmation whenever possible; however, this is hardly accomplished in developing countries because of the limitation of available tests, as well as inadequate laboratory facilities.[6] In addition, obtaining specimens for the diagnosis of TB is difficult, especially in younger children who are not able to expectorate sputum. Gastric aspirate, which is an alternative method of obtaining a specimen, is invasive and has a poor yield, given the paucibacillary nature of this disease in children.[7,8] Other diagnostic tests for TB in children are non-specific, including the tuberculin skin test (TST) and chest radiograph (CXR). In many resource-limited settings, including Tanzania, a paediatric TB score chart is used to aid in the diagnosis of TB despite its widely varying sensitivity and specificity, especially in children with HIV co-infection.[9,10]

Studies have documented stool as a possible specimen for detecting TB using Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) among pulmonary TB patients.[11–21] Stool is a much easier sample to obtain, particularly in younger children, and could aid in the diagnosis of paediatric TB. We, therefore, conducted this study to evaluate the performance of stool Xpert assay in children suspected of having TB across six health facilities in Dar es Salaam, Tanzania, against microbiological confirmation as the reference standard.

STUDY POPULATION, DESIGN AND METHODS

Study design and setting of the study

This was a diagnostic cross-sectional study conducted in six health facilities in Dar es Salaam, Tanzania, between December 2018 and May 2019. These health facilities included three regional referral hospitals (Amana, Mwanayamala and Temeke), two district hospitals (Sinza and Mbagala Rangi Tatu) and one health centre at Buguruni. Regional referral hospitals operate at the regional level offering specialised tertiary services, whereas district hospitals serve at the district and council level, providing basic medical and surgical services. Health centres provide outpatient and some inpatient services at the lower ward level. At all levels, preventive and treatment services for TB is provided. Dar es Salaam is the most populous city in Tanzania and a major contributor to TB cases notified in the country, accounting for 20% of TB patients notified in 2018.[22]

Sample size

The sample size was estimated using Buderer's formula for sensitivity and specificity.[23] The study conducted by Seble et al. in a similar setting reported a sensitivity of 100% and a specificity of 89% in the performance of stool Xpert;[11] the latter was used in the calculation, as it yielded a larger sample size. A prevalence of

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17.6% was used based on a hospital study in South Africa looking into novel TB diagnostics.[24] With an absolute precision of 5%, at a 95% level of confidence, the final sample size calculated was 183. All participants meeting the criteria were enrolled consecutively on admission until the sample size was attained.

Participants, specimen and data collection

Children aged 1 month to 14 years were consecutively recruited from the wards or clinics of the six health facilities. TB screening was done per standard of care; presentations of cough for ⩾2 weeks (or cough of any duration in HIV-positive children), unexplained fever ⩾2 weeks, poor growth or weight loss over the preceding 3 months. Furthermore, children were included if they had a history of TB contact regardless of symptoms or children presenting with a CXR suggestive of TB. Children were considered irrespective of whether they could produce sputum; gastric aspiration was conducted on patients who were unable to produce sputum. Patients who were on treatment for any form of TB were excluded from the study. HIV status, history of previous TB treatment or presumptive extrapulmonary TB were not considered as exclusion criteria.

Children who were able to expectorate provided two sputum specimens – one for Xpert testing and a second specimen for culture. Trained health workers collected early morning gastric aspirates using nasogastric tubes from children who were unable to produce sputum. All children were asked to provide one stool specimen for Xpert testing. Specimens were collected in wide-mouthed and screw-capped containers and stored at the health facility. Sputum Xpert assay was performed as per routine operating procedures. One respiratory specimen and a stool specimen were transported to Central Tuberculosis Reference Laboratory (CTRL; Dar es Salaam, Tanzania) for culture and stool Xpert testing. A structured questionnaire was used to collect demographic and clinical information, which included age, sex, details of the clinical presentation and physical examination. Information was obtained from the parents/guardian, patient and clinical notes at the health facility

Laboratory procedures

At CTRL, specimens were processed within 7 days of collection; in case the specimens were not processed on the same day of collection, they were stored at 2–8°C. Laboratory personnel were blinded to the patients' clinical status.

Stool Xpert MTB/RIF assay

Stool specimens were processed by adding approximately 10 mL distilled water to 2 cm^3 of faecal specimen, and then homogenised by vortex mixing. This mixture was then incubated for 15 min at room temperature to allow the particulate matter to settle. The supernatants from each processed specimen were then collected in another container and mixed with sample reagent according to the manufacturer's instructions (2:1 ratio of Xpert reagent to sample). The mixture from each specimen was then vortexed and incubated for a further 15 min at room temperature; 2 mL was then transferred to a GeneXpert cartridge and analysed using Xpert. Stool Xpert results were interpretated the same way as sputum Xpert results. In case of invalid results, the specimens were reanalysed using Xpert, and the final reports drawn up based on the obtained results.

Culture

Culture was performed using Löwenstein-Jensen (LJ) media slants in accordance with standard operating procedure in a contained Biosafety Level 3 laboratory at CTRL. Briefly, respiratory specimens were homogenised using *N*-acetyl-cysteine to free the bacilli from the mucus, cells or tissue. Before culture, the specimens were subjected to a harsh decontamination procedure (using 4% sodium hydroxide) that liquefied the organic debris and eliminated the unwanted normal flora. Processed sediments were used to inoculate LJ media slants and incubated at 35–37°C until growth was observed or discarded as negative after 8 weeks. A culture was considered contaminated following observation of overgrowth of microorganisms that were lacking characteristics of mycobacteria.

Data analysis

Data were coded and entered in the Statistical Package for Social Sciences v20 (IBM Corp, Armonk, NY, USA). The data were then transferred to Stata v13 (Stata Corp, College Station, TX, USA) for analysis. Baseline demographic characteristics were analysed using percentages for categorical variables, and medians and interquartile ranges (IQRs) for continuous variables. Microbiologically confirmed TB was defined as a positive result on a respiratory specimen using Xpert or culture. A negative microbiological reference was defined as a respiratory specimen that was neither positive using Xpert nor culture. The results of the two respiratory samples (if both were available) was used to define the negative microbiological reference.

Sensitivity, specificity and predictive values for the stool Xpert were calculated using respiratory samples with, including sputum and gastric aspirate tested using Xpert and/or solid culture considered the reference standard. The Stata command '*diagt*' was used to estimate the sensitivity, specificity and predictive values of the tests.

Ethics approval and consent to participate

Ethical clearance to conduct this study was obtained from the Muhimbili University of Health (Dar es Salaam) and Allied Sciences Ethical Committee (Dar es Salaam, Tanzania). Written informed consent was provided by parents or guardians and participant assent from older children.

RESULTS

Patient demographics and clinical information

Of 258 children invited to participate in the study, 17 did not produce stool specimens, 10 did not produce either gastric aspirate or sputum specimens, and five did not provide consent, and were therefore excluded

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Conflict of interests: none declared.

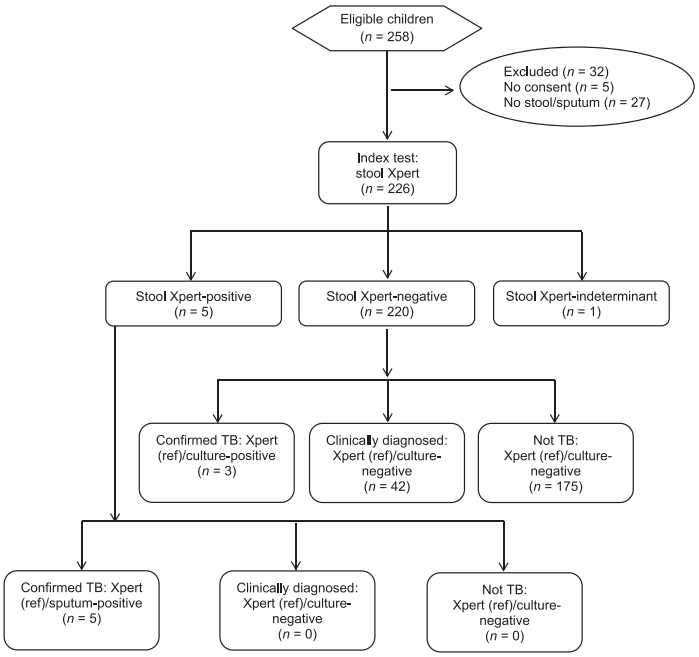

FIGURE Flow diagram showing patient enrolment and laboratory results.

from our analysis. One participant index test was indeterminant and excluded; 225 patients were therefore included in the final analysis (Figure).

The participants' median age of was 2.17 years (IQR 1.16–5.19), and about half (47.1%) of the study population were female. The majority of participants (n = 165, 73.3%) were aged <5 years. Of 212 patients whose HIV status was known, 14 (6.8%) were HIV-positive, and 92.9% were on antiretroviral therapy at the time of enrolment. Evidence of bacille Calmette-Guérin immunisation was present in 195 (86.7%) children. History of TB contact was reported in 88 (39.1%) participants, of which 77% were smear-positive. Baseline characteristics between microbiologically confirmed TB and non-confirmed TB participants are given in Table 1.

Proportion of study participants with microbiologically confirmed TB

Among the 225 participants, 8 (3.6%) were microbiologically confirmed to be positive using culture or Xpert on sputum/gastric aspirate. Forty-two participants (18.7%) were clinically diagnosed to have TB and started on anti-TB medication, and a total of 175 (77.7%) were not diagnosed with TB.

Sensitivity, specificity, positive predictive value, negative predictive value

Stool test for Xpert assay showed a sensitivity of 62.5% (95% CI 25–92) and specificity of 100% (95% CI 98–100) when compared with sputum or gastric aspirate specimens using Xpert or/and culture, which were considered the reference standard (Table 2). Positive and negative predictive values were respectively 100% (95% CI 47–100) and 98.6% (95% CI 96–100). When compared by age, the sensitivity was 83.3% in the above-five age group compared to those aged <5 years, where none of the participants confirmed by the reference standard were detected using the stool Xpert assay (Table 3). The specificity was 100% in both age groups.

TABLE 1 Baseline characteristics of presumptive TB cases in children who visited select health facilities in Tanzania, 2018–2019

Characteristic	All children (n = 225) n (%)	Confirmed* (n = 8) n (%)	Non-confirmed† (n = 217) n (%)
Sex			
Male	119 (52.9)	5 (62.5)	114 (52.5)
Female	106 (47.1)	3 (37.5)	103(47.5)
Age group, years			
<5	165 (73.3)	2 (25)	163 (75.1)
⩾5	60 (26.7)	6 (75)	54 (24.9)
Age, years, median [IQR]	2.17 [1.16–5.19]	9.8 [5.9–13.1]	2.09 [1.16–5]
HIV status			
Positive	14 (6.5)	1 (12.5)	13 (6.3)
Negative	200 (93.5)	7 (87.5)	193 (93.7)
On ART	13 (92.9)	1 (100)	12(92.3)
Presence of BCG scar	195 (86.7)	8 (100)	187 (86.2)
History of TB contact	88 (39.1)	4 (50)	84(38.7)
Clinical findings			
Cough >2 weeks	78 (34.7)	5 (62.5)	56 (25.8)
Fever >2 weeks	61 (27.1)	6 (75)	72 (33.2)
Night sweats	102 (45.3)	7 (87.5)	94 (43.3)
Weight loss	54 (24)	4 (50)	50 (23)

* Microbiologically positive TB on Xpert/culture.
† Microbiologically negative TB on Xpert/culture.
ART = antiretroviral therapy; BCG = bacille Calmette-Guérin.

DISCUSSION

In this study, the use of stool for Xpert had a 62.5% sensitivity and a 100% specificity when compared to the reference standard. The sensitivity and specificity from our results is consistent with findings of a recent systematic review and meta-analysis on the diagnostic accuracy of stool Xpert in children which reported a sensitivity of 67% and specificity of 99%.[20] The results are also comparable to a study conducted among children suspected to have TB in Zimbabwe in which sensitivity was reported at 68% and specificity at 98%.[12] However, the sensitivity in our study was lower than that reported by Moussa et al. from Egypt,[21] which showed a sensitivity of 83% with almost similar specificity. The increased sensitivity in Moussa et al.'s study could be accounted for by testing two samples per patient, which may have resulted in an incremental diagnostic yield, while in our study participants were only required to provide one sample.

The diagnostic accuracy for stool Xpert assay was higher in the above-five age group compared to the under-five group, diagnosing 83% of the children above the age of five who were microbiologically confirmed TB and none of the children under 5 years. The test did not perform as well in children under five, who would largely benefit from the non-invasive diagnostic test; however, the small number of confirmed cases may have affected the accuracy of the results of this subgroup analysis. At this age, children are usually unable to expectorate sputum compared to their older counterparts, and may therefore need to undergo further procedures such as gastric aspiration and sputum induction.[8] Likewise, due to the low number of confirmed cases, comparing stool Xpert assay sensitivity among HIV-positive and HIV-negative children was not done.

There is currently no standard stool processing method. Studies employ different methods when collecting, storing and pro-

TABLE 2 Diagnostic performance of stool Xpert assay compared to culture/Xpert assay of respiratory samples

	Sputum/gastric aspirate culture/Xpert assay			
Stool Xpert assay	Sensitivity *n/N* (%) (95% CI)	Specificity *n/N* (%) (95% CI)	PPV *n/N* (%) (95% CI)	NPV *n/N* (%) (95% CI)
	5/8 (62.5) (25–92)	217/217 (100) (98–100)	5/5 (100) (47–100)	217/220 (98.6) (96–100)

Xpert = Xpert® MTB/RIF; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

TABLE 3 Diagnostic performance of stool Xpert assay compared to culture/Xpert assay of respiratory samples by age

	Sputum/gastric aspirate culture/Xpert					
	Positive (sensitivity)			Negative (specificity)		
Stool Xpert	All *n/N* (%)	Age <5 years *n/N* (%)	Age ⩾5 years *n/N* (%)	All *n/N* (%)	Age <5 years *n/N* (%)	Age ⩾5 years *n/N* (%)
Positive	5/8 (62.5)	0/2 (0)	5/6 (83.3)	0/217 (0)	0/163 (0)	0/54 (0)
Negative	3/8 (37.5)	2/8 (100)	1/6 (16.6)	217/217 (100)	163/163 (100)	54/54 (100)

Xpert = Xpert® MTB/RIF.

cessing of stool specimens, which could lead to differences in results. The processing method used in our study was fairly simple, employing readily available distilled water with no filtering or centrifugation step. The advantage of this method is that it can be used in lower-level facilities with minimal training and supervision. In our study, samples were collected, either processed on the same day or stored at 2–8°C and processed within 7 days, mainly due to logistical reasons and finite resources.

In this study, we were able to microbiologically diagnose 8/225 (3.6%) children with TB using both respiratory and stool specimens, although a higher number (n = 42, 18.7%) was diagnosed clinically. This finding is consistent with the known paucibacillary nature of the disease in children.[7] The low prevalence of confirmed TB noted in this study is comparable to a study conducted among children with presumptive TB in Mwanza, a similar urban setting in Tanzania, confirming TB in 5.2% of children.[25] We performed diagnostic testing on patients with one or more of the signs or symptoms of TB, and as expected, the results differed from other studies that used more signs and symptoms or diagnostic algorithms. Hasan et al. reported microbiologically confirmed diagnosis in 18.7% of participants, which could be attributed to the narrower inclusion criteria in their study, which enrolled patients with Kenneth Jones score >5 only.[16,26.] Stool Xpert assay is a simpler and feasible alternative in situations where infants and children are unable to provide a respiratory sample; it should be noted that the WHO has recently recommended the use of non-pulmonary specimens such as stool.[27] Using a simple stool testing method, we were able to identify TB in 62% of children and was negative in all children not confirmed with TB. While results with stool testing on Xpert assay, and more recently, with Xpert® MTB/RIF Ultra (Cepheid) assay[28] in children have been promising, not enough evidence exists to replace testing of respiratory samples rather the need to offer complementary testing with stool.

There were some limitations to our study. First, the small number of confirmed cases limited the power of the study, and further subgroup analyses such as the performance of the test among HIV-positive children were not done. Second, CXR and culture were not performed on all patients, although these could have further assisted in the diagnosis of TB and assessment of the performance of the stool Xpert assay. Finally, the results of fresh and stored stool specimens were not reported separately, although this could have provided further insight into stool processing methods.

In conclusion, the stool for Xpert assay performed moderately well in confirmed TB cases with a moderate sensitivity and high specificity. Our results support the increasing body of evidence in favour of using stool Xpert assay to complement conventional methods used to diagnose TB in children. Further studies are recommended to operationalise the use of the stool Xpert assay in diagnosing pulmonary TB in our context.

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CONTEXTE : Six structures de santé à Dar es Salaam, Tanzanie.
OBJECTIF : Evaluer l'utilisation d'échantillons de selles dans le bilan diagnostique de la TB pédiatrique en utilisant le test Xpert® MTB/RIF.
SCHÉMA : Entre décembre 2018 et mai 2019, nous avons réalisé une étude transversale de bilans d'enfants âgés d'un mois à 14 ans avec la TB présumée. Un échantillon unique de selles a été testé par l'Xpert. Le résultat a été comparé avec comme référence le standard microbiologique d'échantillons respiratoires ou gastriques testés par test Xpert et/ou culture solide. La sensibilité, la spécificité et les valeurs prédictives de l'Xpert sur les selles ont été évaluées.
RÉSULTATS : Ont été enrôlés 225 enfants d'âge médiane 2,17 ans (IQR 1,16–5,19) dont 165 (73,3%) avaient moins de cinq ans. Huit (3,6%) enfants ont eu un diagnostic de TB par culture ou test Xpert positif sur aspiration de crachats/gastrique. Le test Xpert sur les selles a montré une sensibilité de 62,5% (IQR 25–92) et une spécificité de 100% (IQR 98–100) vis-à-vis du standard de référence.
CONCLUSION : Le recours au test Xpert sur des échantillons de selles a montré une sensibilité modérée et une spécificité élevée dans le diagnostic de la TB pulmonaire des enfants. Nous données confirment l'intérêt de l'utilisation du test Xpert sur les selles comme échantillon non respiratoire pour compléter les méthodes conventionnelles de diagnostic de la maladie.

Public Health Action (PHA) welcomes the submission of articles on all aspects of operational research, including quality improvements, cost-benefit analysis, ethics, equity, access to services and capacity building, with a focus on relevant areas of public health (e.g. infection control, nutrition, TB, HIV, vaccines, smoking, COVID-19, microbial resistance, outbreaks etc).