Original Article

String Test: A New Tool for Tuberculosis Diagnosis and Drug-Resistance Detection in Children

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Abstract

Background: There is a critical need to improve the diagnostic accuracy of tuberculosis (TB) in children. Several techniques have been developed to improve the quality of sputum samples; however, these procedures are very unpleasant and invasive and require hospitalization and trained personnel. This study aims to explore the potential use of a new and noninvasive tool, "string test," for TB diagnosis in children and in adults not able to render sputum samples and at risk of developing multidrug-resistant TB (MDR-TB). **Methods:** Children with clinical suspicion of TB attending the pediatric consultation at the Cetrangolo or Cordero Hospitals and adults suspected of MDR-TB and unable to produce sputum attending the Infectious Disease Unit of Cetrangolo Hospital were included in this study. **Subjects and Methods:** The "string test" is a string that is swallowed by the patients and exposed to gastrointestinal secretions that were late analyzed for TB diagnosis and drug-resistance detection by GenoType MTBDRplus. MedCalc software was used to perform statistical analysis. **Results:** This technique could be applied on 62.1% of selected children. About 11 (30.6%) children were diagnosed as TB cases, 8 (22.2%) from gastric aspirate and using the "string test." Six out of 19 adults were also diagnosed. Genotype directly on the string specimen detected two MDR-TB in adults and two isoniazid-resistant cases before obtaining the isolate. **Conclusion:** This test was safe, cheap, and easily implemented without requiring hospitalization. This research could represent a significant step forward to diagnose and rapidly detect drug-resistant TB in children.

Keywords: Children, diagnosis, drug-resistance, string-test, tuberculosis

Introduction

There is a critical need to improve the diagnostic accuracy of TB in children. According to the latest estimates, children account for a significant proportion of the global TB disease burden. At least 1 million children fall ill with TB each year, and they represent about 11% of all TB cases. In 2015, 210,000 children died of TB including 40,000 TB deaths among HIV-positive children.[1] Researchers have estimated that 67 million children are infected with TB (latent TB and LTBI) and are therefore at risk of developing disease in the near future. In addition, 25,000 children develop multidrug-resistant (MDR) TB every year.[2]

Certainty diagnostic of childhood TB continues being under investigation because still persist many difficulties in obtaining microbiologic confirmation of the disease, mainly in pulmonary cases. As a direct consequence of that, the situation is even worse in multidrug-resistant (MDR-TB) cases. Small children usually show the so called "closed forms of TB", cannot produce sputum and therefore, contribute little to disease transmission within the community.[3,4] Consequently, the diagnosis and treatment of TB in children have not been considered a priority by many TB control programs. Although acid-fast sputum smear microscopy and culture provide reliable diagnostic results in adults, diagnosis of TB in children is

Access this article online

Quick Response Code:

Website: www.ijmyco.org

DOI: 10.4103/ijmy.ijmy_54_18

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How to cite this article: Imperiale BR, Nieves C, Mancino B, Sanjurjo M, Tártara S, Di Giulio ÁB, *et al*. String test: A new tool for tuberculosis diagnosis and drug-resistance detection in children. Int J Mycobacteriol 2018;7:162-6.

usually not easily confirmed. The poor sensitivity of current approaches yields a microbiological diagnosis in only 40% of children with TB, but much lower confirmation is reached in young children.[5,6]

MDR-TB is a growing global health problem. MDR-TB is defined as strains of *Mycobacterium tuberculosis* resistant to at least rifampicin (RIF) and isoniazid (INH). It was also estimated for the year 2016 that there were >5 million people infected with drug-resistant forms of TB in the world, and in fact, the World Health Organization (WHO) has reported 600,000 people with MDR-TB or RIF-resistant TB in 2015.[1] Children represent a significant proportion of these cases. With the poor availability of new molecular tests and culture with drug susceptibility confirmation of MDR-TB in many settings, children suspected of being MDR-TB are very often unconfirmed and frequently put on long and toxic treatment regimens. A meta-analysis of MDR-TB treatment among children showed that >80% had positive outcomes when treated for MDR-TB and that pediatric patients tolerated second-line medications well.[7] Taking into account the increasing rates of MDR-TB, it is important to be able to confirm the bacteriological diagnosis of the disease and determine the drug susceptibility of the infecting TB strain to give the patient the best opportunity of being cured.

Several techniques have been developed to improve obtaining good quality sputum samples. These include sputum induction and gastric aspiration (GA). However, these procedures are very unpleasant and invasive for children and require hospitalization and trained personnel.[8] Due to their invasiveness, none of these methods have attained widespread acceptance. On the other hand, some adults are also not able to produce sputum and must be referred to bronchoscopy for bronchoalveolar lavage (BAL), which is a very invasive procedure to obtain respiratory clinical specimens.[9] In addition, it is well known that the burden of MDR-TB in adults, with or without HIV coinfection, poses a problem to the TB control programs and the efforts necessary to improve TB diagnosis in the near future.[1,10]

Recently, a noninvasive method for improving sputum collection called the "string test," formerly designed to obtain enteric pathogens such as *Giardia lamblia*, was preliminarily evaluated for TB diagnosis in Peru with interesting results.[11-13]

This preliminary study was aimed to explore the potential use of this new tool, the string test (TBST) for TB diagnosis in children and in adults not able to render sputum samples (ANSP) and at risk of developing MDR-TB. The specific objectives of the study were as follows: (a) to evaluate the recovery rate of *M. tuberculosis* from the gastric content by TBST, as compared to GA in children suspected of pulmonary TB, and in BAL in ANSP; (b) to investigate the feasibility and accuracy of the new TBST; and (c) to explore combining TBST with the GenoType MTBDRplus™ (GTMTBDR) for MDR-TB detection.

Methods

Recruitment of patients and samples

All eligible children between 3 and 15 years old with clinical suspicion of TB attending the pediatric consultation at the Dr. Cetrángolo or Petrona V. De Cordero Hospitals were invited to participate in the study.

Adults attending the Infectious Disease Unit of Dr. Cetrángolo Hospital, highly suspected of MDR-TB and unable to produce spontaneous sputum (ANSP), were also asked for participation.

All participants or the relatives responsible for the children were properly informed about the study and read and signed the informed consent form, (previously approved by the teaching and Research Councils of the involved hospitals) indicating their willingness the children or themselves to participate in the study.

Clinical procedure

TBST consists of 80 cm length coiled nylon string inside a weighted gelatin capsule. Once swallowed by the patient, the string unravels in the stomach and become coated with gastrointestinal secretions containing whatever pathogens are present. An hour later, the string is retrieved through the mouth, set on a tube containing 2.0 ml of 10% $NaHCO_3$ and analyzed by usual TB laboratory methods.

For optimal performance, samples were taken on three consecutive days. The 1st day children underwent one TBST. For this study, a homemade version of the commercial pediatric Entero-test string test™ was prepared with a gelatin capsule which has been previously filled with the suture Leinen-Operationszwirn Et.Nr. 100 (Leinen-ET Heike Dietrich, Mülheim-Saarn, Germany).

The procedure was performed early in the morning after an overnight fasting. Once swallowed the TBST, the trailing string was taped to the subject's cheek, remaining *in situ* for 1 h before controlled removal by the study physician.

After 1 h, the patient was asked to wash the mouth with water, and the string was retrieved. The first 50 cm portion of the retrieved string, approximating the section between the taped end of the string and the part sitting in the pharynx, was cut and discarded. The remaining portion of the string of 30 cm in length was cut in two gastric sections of 15 cm each. Each section was placed in its own 15 ml Falcon tube containing 2 ml of 10% $NaHCO_3$, transported on the day of collection to the laboratory, processed for culture or refrigerated overnight.

On the second and 3rd day, children were subjected to GA or induced sputum procedures. ANSP went to bronchoscopy for BAL specimens.[14]

Laboratory procedure

The removed strings were transported to the laboratory within a maximum of 3 h of obtaining the samples in sterile 15 ml Falcon tubes containing 2 ml 10% $NaHCO_3$. Secretions adsorbed to the string were eluted and the

Table 1: Number of suspected patients and the global diagnosis of tuberculosis regarding the total tuberculosis string test, gastric aspirate, and bronchoalveolar lavage specimens

Included patients	Test								
	GA, *n* (%)	BAL, *n* (%)	TBST total, *n* (%)	TBST+/ GA+, *n* (%)	GA+/ TBST− (*n*)	GA−/ TBST+ (*n*)	BAL+/ TBST+ (*n*)	BAL+/ TBST− (*n*)	BAL−/ TBST+ (*n*)
Children	58 (44.6)	-	36 (62.1)	8	3	0	ND	ND	ND
Adults	-	72 (55.4)	19 (34.5)	ND	ND	ND	5	0	1
Total	130 (100.0)		55 (42.3)	8	3	0	5	0	1

TBST: Tuberculosis string test, BAL: Bronchoalveolar lavage, GA: Gastric aspirate, + and −: Positive and negative culture results, ND: Not done

sample decontaminated with 2% NaOH-NALC. After the decontamination, Ziehl–Neelsen (ZN) staining and microscopy examination were performed. All samples were cultured in Löwenstein–Jensen and Stonebrink solid media and BACTEC MGIT960™ vials and analyzed by GTMTBDR™ test.[15-17] All procedures were performed in accordance with standard protocols. Briefly, ZN smear: two drops of processed sample were placed on a slide and stained by ZN according to the WHO standard protocol.[18] For liquid cultures, 200 µL of decontaminated sample was inoculated into an MGIT tube following the manufacturer's protocol.[15] The tubes were incubated into the BACTEC MGIT960 system at 37°C, and the results automatically reported.

The GTMTBDR was conducted on AFB smear-positive decontaminated samples following the manufacturer's standard operating procedures.[17] Negative smear samples with a positive culture were tested by the GTMTBDR and the MGIT960 system using the SIRE Drug susceptibility test kit (MGIT SIRE DST, Becton Dickinson, USA) following the manufacturer's protocol.[19]

Statistical analysis

MedCalc (Applied Math, Mariakerke, Belgium, version 17.9.7) was used to perform statistical analysis of data and results.[20] Accuracy measures (sensitivity, specificity [SP], positive and negative predictive values (NPVs) and area under the curve [AUC]) of the evaluated tests were calculated and analyzed. Due to the low number of clinical specimens analyzed, calculation of accuracy measures was performed by a 2 × 2 table.

Results

During the period comprised between January 2014 and December 2015, a total of 261 respiratory specimens (mean: 2.5; range: 2–3) obtained by GA or sputum from 103 suspected TB children were processed. Nearly 58 (56.3%) of the children complied with the inclusion criteria for the TBST (mean age: 5.3 years old; range: 3–8). The main exclusion criteria were young age (mean; 14.2 months) and less than the three required samples as specified in the clinical procedures section.

A total of 19 ANSP with medical decision to be submitted for bronchoscopy agreed to participate in the study. Fifteen cases were HIV co-infected and six were women. One of each TBST and BAL specimens were obtained from the above-mentioned patients.

Table 2: Drug-resistant detection reached by the tuberculosis string test combined with direct and indirect GenoTypeMTBDRplus

Patients	Combined tests					
	TBST plus D-GTMTBDR, (*n*)			TBST plus I-D-GTMTBDR (*n*)		Total (*n*)
	FDS	DR-TB	MDR-TB	FDS	MDR-TB	
Children	3	1	-	4	0	8
ANPS	0	1	2	2	1	6
Total	3	2	2	6	1	14

TBST: Tuberculosis string test, D-GTMTBDR and I-D-GTMTBDR: GenoType MTBDRplus, applied directly on samples or on the isolate, respectively, FDS: Mycobacterium tuberculosis susceptible to first line drugs, DR-TB: Mycobcterium tuberculosis resistant to isoniazid, MDR-TB: Mycobacterium tuberculosis simultaneously resistant to isoniazid and rifampicin, ANPS: adults not able to produce sputum samples

The TBST technique could be applied in 36 out of the 58 (62.1%) selected children. About 11 (30.6%) children were diagnosed as TB cases, 8 (22.2%) by the classical GA and the TBST, and three only by GA. A total of 6 out of 19 ANPS included were also diagnosed. One of these cases was only diagnosed by TBST with negative BAL culture.

Table 1 shows the results obtained from both children and adults relating the total number of suspected and included patients with the TBST reached by BAL, GA, and/or TBST samples.

The usefulness of combining the TBST with GTMTBDR for drug-resistant detection was assessed by applying this technique directly on AFB positive specimens (D-GTMTBDR) or indirectly on the isolates obtained by culture of AFB-negative specimens (I-D-GTMTBDR). Results are shown in Table 2.

The use of the D-GTMTBDR on the TBST specimen allowed the diagnostic of 2 MDR-TB in ANSP and 2 INH-resistant (R) cases in children and ANSP before having the isolate by conventional culture. These MDR-TB and INH-R cases were later confirmed by I-D-GTMTBDR and SIRE MGIT 960 performed on the isolates. One more MDR-TB ANPS case was detected by I-D-GTMTBDR. To estimate the shorter time in which drug-resistant results could be obtained, a 2 × 2 table

Table 3: Statistic parameters found for tuberculosis diagnosis and drug-resistance detection by applying the combined tuberculosis string test plus direct GenoTypeMTBDRplus

Test	Parameter (%)				
	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	AUC (95% CI)
TBST	82.4 (56.6-96.2)	100 (90.7-100.0)	100.0	92.7 (81.9-97.3)	91.2 (80.4-97.1)
TBST + D-GTMTBDR	80 (28.4-89.5)	100 (66.4-100.0)	100.0	90.0 (60.9-98.1)	90.0 (62.4-99.4)

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, TBST: Tuberculosis string test, D-GTMTBDR: GenoTypeMTBDRplus directly applied on acid-fast bacilli-positive clinical samples, CI: Confidence interval

was constructed considering only the drug-resistance detected by combining the TBST plus the D-GTMTBDR techniques.

Table 3 shows the statistic parameters found for the TBST contribution to TB and drug-resistance diagnosis. For TBST results, the sensitivity (S), NPV, and the AUC showed the wide dispersion of their respective 95% confidence interval (95% CI) calculated. The same happened when assessed the performance of TBST plus D-GTMTBDR for direct detection of drug resistance but with the addition of a wide 95% CI dispersion for SP.

Discussion

This study was mainly attempted to explore the possible usefulness of the TBST to improve the diagnosis of TB in children and eventually in ANPS and to potentially advance the detection of drug resistance from one simplified clinical sampling. This research could represent a significant step forward to diagnose TB and simultaneous detection of drug resistance in children.

The TBST proposes another method to obtain a respiratory sample in children suspected of suffering from TB. On the other hand, adults with difficulties to produce spontaneous sputum but under strong suspicion of TB might be investigated by TBST instead of undergoing bronchoscopy procedures. Both classical GA and BAL specimens are invasively obtained by also unpleasant procedures. Besides, the general procedures specify that a serial of 3 GAs must be collected from consecutive days requiring, moreover, a child partial hospitalization. This experience revealed TBST like a very safe and cheap technique, easily implemented without requiring any hospitalization, and well accepted by pediatricians and by ANSP's physicians.

This was only a preliminary study in which one of the major limitations was the low number of included children. According with the analysis, in the sites of recruitment, most of the children suspected of suffering from TB were very young (<3 years old) or with an age in which spontaneous sputum samples could be produced. Nevertheless, the SP and PPV were 100% for TBST and the combined TBST plus GTMTBDR. The low number of drug-resistant cases – both children and ANSP – within the whole patients' sample could be the reason explaining a wide dispersion in the 95% CI of S, SP, NPV, and AUC for TBST and TBST plus GTMTBDR. It could be expected an improvement of the statistical parameters, mainly the general performance of these tests measured by the AUC value, by increasing the final number of patients to be included in future studies. Anyway, the fact of high S and SP for TBST and the combined TBST plus GTMTBDR would allow giving the physician a true result for both TBST and drug-resistance detection.

As suggested by these preliminary results, if the TBST could be combined with a rapid molecular test, such as the line probe assay GenoType MTBDRplus or Xpert MTB/RIF™ assay for the simultaneous species identification and drug-resistance detection, it could represent a significant step forward to save time in the diagnosis of TB and the resistant forms of the disease in children and eventually in ANSP at risk of developing MDR-TB. Due to the encouraging results obtained in this study, a prospective evaluation is ongoing in several countries.

Conclusion

Tuberculosis (TB) caused by Mycobacterium tuberculosis remains one of the most serious infectious disesases afecting human beings. In 2015 more than 10.4 million people fall ill and 1.4 million people died about TB.

Acknowledgment

The authors would like to thank Miss. Bibiana Zingoni for her technical work and Mr. Guillermo Alonso also for the preparation of the TBST kit.

Financial support and sponsorship

This study was partially supported by the Damien Foundation, Brussels, Belgium.

Conflicts of interest

There are no conflicts of interest.

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