PEDIATRIC INFECTIOUS DISEASE SOCIETY OF THE PHILIPPINES

Vol.18 No.2
July - December 2017

PIDSP JOURNAL

Vol 18, No. 2
July - December 2017

ORIGINAL ARTICLE

A META-ANALYSIS ON GENEXPERT USING STOOL SAMPLES IN DIAGNOSING PEDIATRIC PULMONARY TUBERCULOSIS

Othella Mary Ann S. Cacayorin, MD*

*Philippine Children's Medical Center, Philippines

Correspondence:
Dr. Othella Mary Ann S. Cacayorin
Email: othella07@yahoo.com

The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

OBJECTIVES: To find an alternative specimen for GeneXpert assay in the diagnosis of pediatric pulmonary tuberculosis (PTB). To determine the sensitivity and specificity of using stool samples as an alternative to sputum for GeneXpert assay.

METHODS: A systematic search was done using an electronic database (e.g. Pubmed). Using the keywords "GeneXpert", "tuberculosis", "Stool samples". QUADAS-2 checklist was used in assessing the studies gathered. Revman 5.3 was used to determine the sensitivity and specificity of the study included in this meta-analysis.

RESULTS: A total of 185 stool samples were included in this review, which showed a median sensitivity of 77% (IQR 0.63-0.87) and median specificity of 98% (IQR 0.95-1.0).

CONCLUSION: Despite the heterogeneous sensitivity, GeneXpert has a high specificity, which enabled rapid diagnosis of pulmonary tuberculosis promoting timely initiation of appropriate therapy.

KEYWORDS:

gene xpert, stool, pulmonary tuberculosis

INTRODUCTION

Tuberculosis is one of the most prevalent causes of mortality and morbidity in developing countries affecting both the adult and pediatric population. The diagnosis of pulmonary tuberculosis (PTB) remains challenging in young children because they cannot expectorate spontaneously, making it difficult to obtain a representative specimen from the lower respiratory tract, and because PTB in children is typically paucibacillary. Thus, PTB in children is probably underdiagnosed, hence maybe left untreated due to the challenging diagnostic confirmation or late diagnosis is maybe the typical clinical scenario. (1). Most often, children are treated empirically based on clinical features, chest X-ray findings, tuberculin skin testing, and contact with an index patient.

Microbiological identification of *Mycobacterium tuberculosis* from cultures is the gold standard for diagnosing tuberculosis infection. However, culture of mycobacteria is not able to provide a rapid diagnosis for the clinical management of severe cases, and requires expensive and sophisticated laboratory facilities, which is not available in most resource-limited settings (2). GeneXpert was introduced in 2008 capable of identifying tuberculosis within a few hours and at the same time detect multidrug resistant Tuberculosis (MDRTB) with a high sensitivity and specificity. Extrapulmonary samples can also be tested using the GeneXpert to detect *Mycobacterium tuberculosis.*

A sample that is easy to obtain or obtained in a non-invasive means that will yield a high sensitivity and specificity using GeneXpert for the diagnosis of PTB in children would increase the likelihood of early and accurate diagnosis thus enable a clinician to institute early treatment for this vulnerable population.

Since sputum samples are difficult to obtain in pediatric patients, alternative specimen has been gastric aspirates. However, this specimen's diagnostic yield only ranges from 20-40% (3).

"Young children tend to swallow sputum when they cough or even when asleep. It is also known that *Mycobacterium tuberculosis* DNA can survive intestinal transit" (4). Thus, the rationale for getting gastric aspirate as an alternative to sputum for diagnosing pediatric PTB. Testing stool for *Mycobacterium tuberculosis* DNA from swallowed sputum with the bacteria surviving intestinal transit can possibly be a good alternative for diagnosing pulmonary tuberculosis. Besides, stool collection is easier and non-invasive.

GeneXpert is a portable, highly sensitive, user-friendly and rapid molecular assay with a turnaround time of less than 2 hours (5). It is a nucleic acid amplification test which simulatenously detects DNA of *Mycobacterium tuberculosis* complex and resistance to Rifampicin in less than 2 hours. In comparison to standard cultures that can take 2-6 weeks for MTBC to grow and conventional drug resistance tests can add 3 more weeks. The primer in the XpertMTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes are able to diffentiate between the conserved wild-type sequence and mutation in the core region that are associated with rifampicin resistance (6).

The Centers for Disease Control and Prevention (CDC) recommends that testing be performed on at least one respiratory specimen from patients who have a moderate to high risk of having pulmonary TB (6).

"The GeneXpert MTB/RIF test offers a potential solution for improving

tuberculosis diagnosis. By 2013, the first year of full Xpert coverage, 30% more patients were diagnosed in the Xpert scenario than in the baseline scenario. The test appeared to be as sensitive as culture with smear-positive specimens, but less sensitive with smear-negative pulmonary and extrapulmonary specimens that include low numbers of bacilli."(7)

Stool sample can be a good alternative to sputum on detecting *Mycobacterium tuberculosis* using Gene Xpert MTB/RIF assay. This could be a solution to the current challenge of getting respiratory samples in children and become a routine means of diagnosing pulmonary tuberculosis in children.

OBJECTIVES OF THE STUDY

The general objective of the study is to determine the usefulness of geneXpert using stool samples in diagnosing pulmonary tuberculosis in children and to determine its sensitivity and specificity.

Ethical Considerations

The study will use secondary data from previously published studies. No actual patient- researcher interaction will be done. No ethical concerns were encountered in both the data gathering and methodology of this study.

MATERIALS AND METHODS

Systematic Search Strategy

We did a thorough literature search on available publicly accessible scientific journal databases such as MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, HERDIN, other non-English databases and unpublished trials. Using the keywords "GeneXpert", "tuberculosis", "Stool samples", wherein an exhaustive literature search was performed. A manual search in the references and citation lists of eligible studies was also done to further look into relevant studies. Authors of significant journals that were freely accessible were contacted via e-mail.

Inclusion Criteria

Inclusion criteria were used to select appropriate journals to be included in this meta-analysis, as outlined by the PRISMA guidelines. All studies included are randomized controlled trials with a target pediatric population between 1 to 15 years old that have been diagnosed to have pulmonary tuberculosis and PTB suspect. The eligibility, quality assessment and data extraction were done by the author.

Assessment of Study Quality

The study used the QUADAS- 2 checklist to assess the quality of the studies.

Statistical Analysis

A meta-analysis tool Review Manager version 5.3 downloaded from the Cochrane website was used to conduct the meta-analysis. It provided a forest plot of the variables in the studies being compared, as well as their homogeneity and sensitivity.

Studies included

Of a total of 13 citations, 4 potentially relevant citations were identified and 2 of these were further excluded after evaluation. Of these 4 studies, 2 used cross-sectional studies, 1 of which had limited information. Two remaining cohort studies were eligible for inclusion in the qualitative and quantitative synthesis, and reported 185 stool samples using cohort studies. The 2 studies were both done in South Africa. "South Africa is one of the countries with the highest burden of TB, with the World Health Organization (WHO) statistics giving an estimated incidence of 450,000 cases of active TB in 2013. About 1% of the population of about 50 million develop active TB disease each year." (8)

Figure 1. Selection of studies reporting on the use of GeneXpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis using stool samples.

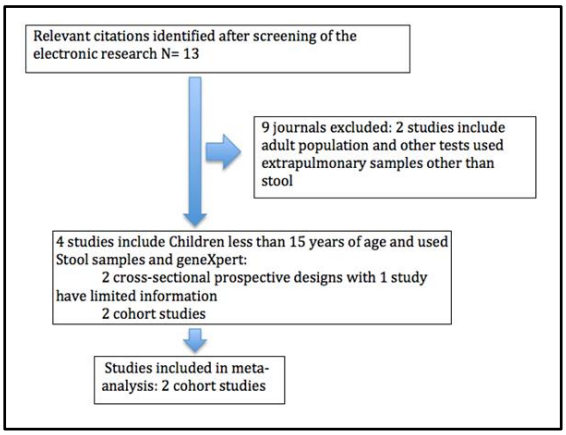


Figure 2. Inclusion criteria used in the study

	Nicol 2013 (published article) N= 115	Banada 2016 (published article) N= 71
Study design: RCT	Cohort	Cohort
Population	< 15 years	< 15 years
Diagnosed with PTB or PTB suspects	Suspected PTB	TB positive
Sample	Stool	Stool
Diagnostic test	GeneXpert	GeneXpert
Gold standard	Induced sputum	Induced sputum
Analysis	Liquid culture	Liquid culture (MGIT)

Diagnostic accuracy of GeneXpert in stool samples for PTB

In the 2 studies included, the study of Nicol et al. showed a sensitivity of 54% (CI 95% 0.25-0.81) and specificity 98% (CI 95% 0.93 -1.0) while the study of Banada et al. showed sensitivity of 85% (CI 95% 0.69-0.81) and specificity of 100% (CI 95% 0.93 – 1.0).

In the study of Nicol, the 0.15 g of thawed stool (confirmed by weighing) was retrieved using pediatric FLOQSwabs (Copan Italia, Brescia, Italy). Swabs were then placed in 2.4 mL PBS and vortexed briefly before being removed. The sample was left undisturbed for 20 minutes at room temperature to allow large particles to settle before 2 aliquots of 1-mL supernatant were removed. One aliquot was tested immediately with Xpert and the other was stored at 4°C for later duplicate testing (within 1 week). Prior to Xpert testing, the sample was centrifuged at 3200 x g for 15 minutes. The supernatant was discarded and pellet was resuspended in 1 mL PBS. Xpert testing was then performed per the manufacturer's instructions using a 2:1 ratio of Xpert reagent to sample (1).

Figure 3. Summary of Sensitivity and Specificity with its PPV and NPV studies

Study	True Positive	False Positive	False Negative	True Negative	Sensitivity	Specificity	PPV	NPV
Banada 2016	33	0	6	32	85%	100%	100%	84.2%
Nicol 2013	7	2	6	99	54%	98%	77.8%	94.3%

In the study of Banada, between 0.2g to 1.2g of stool were processed. Two mls of a stool processing buffer (SPB), containing AL buffer (Qiagen, Valencia, CA) and 10% Polyvinylpyrrolidone (Sigma Aldrich, St. Louis, MO), two ml of Xpert MTB/RIF sample reagent (SR) (Cepheid), and 3 mm glass beads (Fisher Scientific, Pittsburgh, PA) were added to the mixture. The final stool and buffer combination were mixed by snap vortexing (for <10 seconds), incubated for 30 min at room temperature and then passed through a syringe filter (fitted with glass wool to capture the stool debris) into a clean collection vial. Two ml of this filtrate was then loaded into the sample loading chamber of an Xpert assay cartridge. Subsequent sample processing and PCR were performed in accordance with the manufacturer's recommendations using G3 cartridges in GeneXpert instrument (11)

RESULTS

In this meta-analysis, 2 studies were evaluated on the diagnostic accuracy of the GeneXpert MTB/RIF when used to test non-respiratory samples, specifically

stool samples. The specificity of the 2 studies are very high, highlighting its utility as a rule-in test for tuberculosis diagnosis that can be used to reliably prescribe the start of TB treatment when positive. In contrast, sensitivity of the 2 studies was heterogeneous. This heterogeneity could be attributed to how the stool samples have been prepared.

Figure 4. Sensitivity and specificity of Xpert MTB/RIF on stool samples in the 2 studies

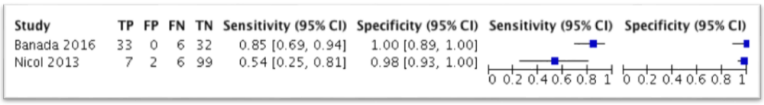
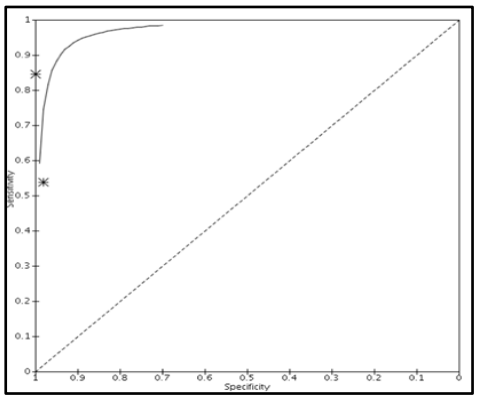


Figure 5. Summary of results of sensitivity and specificity of GeneXpert using stool samples with median sensitivity of 77% (IQR 0.63-0.87) and median specificity of 98% (IQR 0.95-1.0)

BANADA 2016			
	Disease	No Disease	Total
Positive	33	0	33
Negative	6	32	38
Total	39	32	71
NICOL 2013			
	Disease	No Disease	Total
Positive	7	2	9
Negative	6	99	105
Total	13	101	114

Figure 6. Comparison of the Positive Predictive Value and the Negative Predictive Value of both studies



DISCUSSION

In a study done by Hillemann et al., "3 strains were recovered from urine specimens from 1 patient (all of them were culture positive and Xpert assay positive), 2 stool specimens from 1 patient were positive for MTBC (both of them were culture positive and Xpert assay positive)" and in a study done by Taylor et al., 2 studies that evaluated the sensitivity of the GeneXpert system with actual patient stool samples showed a sensitivity of 100% compared to culture, although only 3 samples were tested."(8) These demonstrate that stool samples can be a good alternative to gastric aspiration, using GeneXpert to help in the diagnosis of tuberculosis in children.

In a study done by Walters et al, they found that stool sampling is "child-friendly" and has minimal infectious risk, and can overcome some barriers to bacteriologic investigation of tuberculosis in children. Rapid confirmation using noninvasive sampling may be particularly useful in young children with extensive tuberculosis or suspected drug resistance, and its potential clinical impact should be systematically investigated." (9)

In a cross-sectional prospective study done by Welday et al., "stool Xpert showed 100% sensitivity and 89.36% specificity without missing any positive from sputum ZN smear microscopy. Thus, using stool as a sample is a good alternative to the invasive procedure for collecting respiratory samples or sputum from children. (12)

In a study done by Kokuto et al in which the subjects were adults with difficulty in obtaining sputum samples, "The sensitivity of testing stool samples using the GeneXpert MTB/RIF was 100% (81.7%–100%) for detection of MTB in specimens from sputum smear-positive (1+ to 3+) patients, 81.0% (58.1%–94.6%)

in specimens from sputum smear scanty positive patients, and 50.0% (15.7%–84.3%) in specimens from sputum smear-negative patients. Meanwhile, each of the fecal specimens from the non-TB group was negative for MTB (specificity 100%; 95% confidence interval, 86.2–100)" (5)

In a study by Marcy et al, which uses extrapulmonary samples for the geneXpert in HIV infected children, GeneXpert performed on 1 stool sample had intention-to-diagnose and per-protocol sensitivities of 62.1% and 68.8%, respectively (13).

In some studies using extrapulmonary samples and a small number for stool samples, both the sensitivity and specificity are high as mentioned in the study of Hillemann et al, (8) Welday et al., (12), and Kokuto et al., (5). Meanwhile, in this analysis, the fecal GeneXpert MTB/RIF method is associated with lower levels of sensitivity, and this could be due to the paucibacillary nature of the pediatric TB. Another factor that could have also affected the result of this study is the age of the population. Older children in this study probably already know how to expectorate sputum, hence, spitting the phlegm out instead of swallowing it compared to the younger population of the study.

In this meta-analysis, only 2 studies were included due to the limited numbers of studies done on stool samples using geneXpert, probably due to the fact that this machine is relatively new and has only been introduced a few years ago. Other than that, an optimized protocol on testing stool sample needs to be developed to permit proper testing of stool likely to enhance the test's sensitivity. On the other hand, with the high specificity, GeneXpert enabled the rapid diagnosis of pulmonary tuberculosis enabling timely initiation of the appropriate therapy. The findings in this analysis confirm that the geneXpert MTB/RIF assay is an important advancement in the diagnosis of tuberculosis. More so, in the pediatric population, using a specimen that can be obtained in a non-invasive means using a new technology like geneXpert with a high specificity will lessen overtreatment of children who doesn't have TB. It will also lessen delays in the treatment of children that does not fulfill the current 3 out of 5 criteria of diagnosing pulmonary tuberculosis and cannot produce an adequate sputum specimen for further diagnosis.

RECOMMENDATIONS

Due to the limited number of journals that were accessed, it can be recommended that a larger scale clinical randomized controlled trial be done in testing stool samples using GeneXpert. The promise shown by this test using an accessible specimen cannot be overemphasized. Once an acceptable specificity and sensitivity is shown, it can lead to a change in the current diagnostic protocol of pulmonary tuberculosis in children.

REFERENCES

1. Nicol MP, Workman L, Isaacs W, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. Lancet Infect Dis 2011; 11:819–24.
2. Alvarez-Uria et. Al(2012) Rapid Diagnosis of Pulmonary and Extrapulmonary Tuberculosis in HIV-Infected Patients. Comparison of LED Fluorescent Microscopy and the GeneXpert MTB/RIF Assay in a District Hospital in India, hindawin Publishing Corporation Tuberculosis Research and Treatment Volume 2012 Article ID 932862 page 4
3. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of

pulmonary tuberculosis in infants and young children: a prospective study. Lancet. 2005; 365(9454):130–4. PMID: 15639294

4. Cordova, J., Shilho, R., Gilman, R.H., *et al*. (2010) Evaluation of Molecular Tools for Detection and Drug Susceptibility Testing of Mycobacterium Tuberculosis in Stool Specimens from Patients with Pulmonary Tuberculosis. *Journal of Clinical Microbiology*, **48**, 1820-1826. http://dx.doi.org/10.1128/JCM.01161-09
5. Kokuto et. al, Detection of Mycobacterium tuberculosis (MTB) in Fecal Specimens From Adults Diagnosed With Pulmonary Tuberculosis Using the Xpert MTB/ Rifampicin Test (2015), OFID, page 1
6. http://www.cdc.gov/tb/publications/factsheets/testing/xpert_mtb-rif.htm?ez_cid=CLIENT_ID(AMP_ECID_EZOIC)
7. Meyer-Rath,Schnippel, Long et. al (2012) The Impact and Cost of Scaling up GeneXpert MTB/RIF in South Africa;PLoS ONE; www.plosone.org; Vol 7,Issue 5, page 1,6
8. Doris Hellimann et. al.,(2011); Rapid molecular detection of Extrapulmonary tuberculosis by the Automated GeneXpert MTB/RIF system; http://jcm.asm.org/content/49/4/1202 ; 10.1128/JCM.02268-10(4)
9. Walters E, et. al., (2012) Rapid diagnosis of pediatric intrathoracic tuberculosis from stool samples using the Xpert MTB/RIF Assay: A pilot study. Pediatric Infectious Diseases Journal 2012;31:1316.
10. http://www.tbfacts.org/tb-statistics-south-africa/
11. Banada et.al, (2016), A Novel Sample Processing Method for Rapid Detection of Tuberculosis in the Stool of Pediatric Patients Using the Xpert MTB/RIF Assay, PLOS one, Page 3
12. Welday SH. Gene Xpert Testing Of Stool Samples for the Diagnosis of Pulmonary Tuberculosis in Children Less an 15 Years in Hospitals In and Around Nairobi 2015.
13. Marcy et. al, Performance of Xpert MTB/RIF and Alternative Specimen Collection Methods for the Diagnosis of Tuberculosis in HIV-Infected Children (2016), Clinical Infectious Diseases, Major Article, Page 1