Original Article

Comparative Analysis Of Stool As An Alternative Sample In The Diagnosis Of Pulmonary Tuberculosis Among Adults And Children In Ogbomosho, Nigeria

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DOI: 10.47750/pnr.2023.14.04.61

Abstract

Background/Purpose: Tuberculosis (TB) is an infectious bacterial disease caused by Mycobacterium tuberculosis (MTB). It is a matter of serious public health concern in lower-income and lower-middle-income countries.

Methods: In this study, a comparative analysis of stool samples from 101 adult (91 pre-confirmed pulmonary TB patients, 10 sputum negative patients) and 99 children (presumptive TB patients). Their stool samples were collected, processed and analyzed using Xpert MTB/RIF Ultra technique and AFB microscopy.

Results: While using Xpert MTB/RIF Ultra assay as our "reference standard", a sensitivity of 96 %, Specificity of 83%, Positive Predictive Value (PPV) of 87 %, and Negative Predictive Value (NNP) of 87 % was observed with stool Xpert MTB/RIF Ultra technique. Also, Ziehl Neelsen's Stain Microscopy has a Sensitivity of 64%, Specificity of 12%, PPV of 80% and NNP of 20% while Fluorescence Stain Microscopy reveal a Sensitivity of 94 %, Specificity of 20 %, PPV of 80 %, and NNP of 20 % in stool sample.

Conclusion: This study has elucidated Xpert MTB/RIF Ultra assay in stool samples provide an alternative option to sputum in the diagnosis of pulmonary tuberculosis with high level of diagnostic accuracy and precision. Furthermore, Fluorescence Stain Microscopy has shown to be more sensitive and specific than Ziehl Neelsen's stain Microscopy.

Index Terms— Pulmonary, Sensitivity, Specificity, Stool, Tuberculosis, Xpert.

INTRODUCTION

Tuberculosis (TB) is an infectious bacterial disease that is caused by *Mycobacterium tuberculosis* (MTB). In lower-income and lower-middle-income countries, Tuberculosis still remains one of the top 10 causes of death. The 2021 Global TB Report, indicate that in 2020, there was an estimated 9.9 million new TB cases, with approximately 1.3 million deaths due to TB. A lot of people are dragged into poverty as a result of loss of income, transportation fare and many other expenses due to TB diseases.[1] Nigeria ranked 6th among the 30 high TB burden country globally, and first in Africa.[2]

Mycobacterium tuberculosis complex (MTBC) can be detected in stool which can be collected by a non-invasive method in place of sputum. MTBC are shed into stool when sputum is coughed up and subsequently swallowed, which then passes through the gastrointestinal system.[3] The diagnosis of pulmonary tuberculosis (pTB) in young children basically depends on clinical diagnosis, this is so because young children usually find it difficult to produce sputum sample unaided. Stool sample serves as an alternative approach to sputum in the diagnosis of pulmonary tuberculosis (pTB) in both adult and children. Acid Fast Bacilli (AFB) smear microscopy, molecular techniques and culture methods are been used in the diagnosis of Tuberculosis, [4]

Xpert MTB/RIF Ultra Assay is a molecular technique that comes with an increased sensitivity, specificity together with the advantage of it detecting Rifampicin (RIF) Resistance within two hours of analysis. [5] Currently, the Xpert MTB/RIF ultra-assay using stool sample has been recommended and adopted by WHO. [3]

This study, therefore, intend to evaluate the diagnostic performance of Xpert MTB/RIF ultra- assay, ZN and FM microscopy in low resource settings to detect TB in stool specimen among sputum-pre-confirmed TB patients (adult) and presumptive TB patients (children) in Ogbomosho, South western Nigeria. There is low utilization of stool in the diagnosis of pulmonary tuberculosis and the need to validate the use of AFB microscopy in the diagnosis of pulmonary tuberculosis where stool sample is the likely alternative sample available. This will further bring additional evidence to validity of Xpert MTB/RIF ultra-assay and AFB microscopy on non-sputum-based samples thereby informing policy makers and TB control programs.

METHODS

2.1 Study design, location and duration

This is a cross-sectional diagnostic study which was designed for sputum-pre-confirmed pulmonary tuberculosis patients (adult) and presumptive pulmonary tuberculosis patients (children) seeking care at Bowen University Teaching Hospital (BUTH), Ogbomosho, South western Nigeria. The study took six months till completion.

2.1.2 Sample collection

2g of Stool samples were received early in the morning from consented adult participants who are sputum positive from Xpert MTB/RIF Ultra assay previously done. For the children (presumptive pTB patients) under 5 years, only stool samples were collected after obtaining assent from their parents/caregiver into a sterile leak proved-wide mouth stool container and thereafter taken to the hospital's Medical Laboratory for analysis. For children participant (using diapers), parent/caregiver were instructed to collect the stool sample directly from diapers immediately or place a sterile plastic sheet on the diapers to prevent prolong contact of the stool with the diaper.

2.2. Stool Processing

1. One part (2 g) of stool sample was mixed with 3ml normal saline solution and 10ml normal saline solution for liquid and formed stool respectively and shaken intermittently to create liquid slurry.
2. It was allowed to stand for 15 minutes and thereafter filtered using a filter paper *(Whatman filter paper 1 Qualitative pore size 125mm Ø Cat No. 1001 125)* into a separate sterile container.
3. A portion (1ml) of the filtrate was spun in a centrifuge at 3000 rpm for 10 minutes, (this is done to concentrate MTB for maximum yield/detection) the supernatant was then decanted while the deposit was smeared on a clean grease free slide labeled with the patient ID for AFB Smear staining.

2.3. GeneXpert Assay Procedure

2ml of the processed stool sample was transferred into an Xpert MTB/RIF ultra-cartridge and inserted into a GeneXpert Machine, the result are available after about 1 ½ hours of analysis according to the manufacture's protocol. (Cepheid, USA)[6]

2.4. Ziehl-Neelsen's (ZN) Staining Procedure

ZN staining was performed using 1% Carbol fuchsin solution (GCC Diagnostics UK) for 5 minutes with intermittent heating, then decolorize with 3% Hydrochloric Acid Alcohol (GCC Diagnostics UK) for 10minutes and counter stained with 0.1% Methylene Blue (GCC Diagnostics UK) for 1 minutes (Adopted from NTBLCP AFB Microscopy Manual FOH 2014)[7]

2.5. Florescence Staining Microscopy Procedure

FM Staining was performed using 0.1% Auramine 'O' stain (GCC Diagnostics UK) for 20 minutes, decolorize with 0.5% hydrochloric acid-alcohol (GCC Diagnostics UK) for 3 minutes and counter stained with 0.5% Potassium Permanganate (GCC Diagnostics UK) for 1 minute. (Adopted from NTBLCP AFB Microscopy Manual FOH 2014)[7]

2.6. Ethical considerations

The research was performed with the approval of the Health Research and Ethics Committee of Bowen University Teaching Hospital, Ogbomosho, Oyo State Nigeria. **(NHREC/12/04/2012), approval number BUTH/REC-344 and dated 8th December 2021.** A Written Informed consent form was signed by adult participant while assent form and informed consent was signed by parent/caregivers of children participant. Good clinical practice was observed.

2.7. Statistical Analysis

Demographic and clinical data were generated from structured questionnaire administered to participants, result generated from Xpert MTB/RIF ultra-assay were regarded as the 'reference standard' it therefore means that a positive and negative result from Xpert MTB/RIF ultra-assay was regarded as true positive and true negative result respectively. All Variables were summarized as frequencies, percentages and median using SPSS-23, Spearman's rho and Kappa test for correlations was used for the

categorical variables at 95% confidence interval and p-value of < 0.05 was considered significant. Using 2×2 table in SPSS-23, the diagnostic performance of stool Xpert MTB/RIF ultra, ZN and FM was analyzed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios with their respective confidence intervals using sputum Xpert MTB/RIF ultra as a 'reference standard'.

2.8. Inclusion Criteria

In this study certain category of patients are targeted to achieve the desire research objectives these includes; pre-confirmed pulmonary tuberculosis patients in adult participants who presented their sputum samples for Xpert MTB/RIF ultra-assay, presumptive Pulmonary tuberculosis patients in children less than 5 years, patients who are able to produce stool sample without any induction and lastly adult participants who consented to the research work.

2.8.1 Exclusion Criteria

In this study certain category of participants were not included in the study population to avoid interference with desire study objectives. They include; pulmonary tuberculosis patients on anti-TB treatment, extra-pulmonary tuberculosis patients, participants who do not consent to the study and children beyond 5 years old.

RESULTS

In this study a total of 200 patients were recruited, out of which 101 were adult and 99 were children under 5 years old. Clinical and demographic features of the 200 participants are being summarized in Table 1 The median age is 33, 68% Male and 32% female. 13% are HIV positive. 91 of the 101 adult participants are sputum-positive for pTB from Xpert MTB/RIF Ultra assay.

Table 2 summarizes the outcome of Xpert MTB/RIF Ultra results when compared with FM and ZN staining results in both adult and children participant.

Table 3 summarizes the performance indices of the three diagnostic test methods used which revealed that Xpert MTB/RIF Ultra in stool has a sensitivity of 96%, specificity 83%, PPV 87%, NPV 87%, among adult groups, then among children groups the study revealed FM with sensitivity of 100%, specificity of 80%, PPV 100%, NPV 99% and ZN with sensitivity of 99%, specificity of 20%, PPV 80% and NPV 99%.

Results in table 4 indicates a 67.3% total positive detection rate (TPDR) in stool sample for FM and Xpert MTB/RIF Ultra Assays while 50.5% TPDR in ZN among adult participants.

Likewise result in Table 5 displays a 3% TPDR in stool sample for FM and Xpert MTB/RIF Ultra Assays while 1% total positive detection rate (TPDR) in ZN among children participant.

Table 1: Demographic and Clinical characteristics of Study participants who provided stool samples for Xpert MTB/RIF Ultra Assay

		Pre-confirmed pTB status (Adult)		Presumptive TB (Children	
Characteristics	**Overall (n= 200)**	**Sputum Positive (n = 91 [45.5%])**	**Sputum Negative (n =10[5%])**	**Stool Positive (n = 3 [1.5%])**	**Stool Negative (n = 96 [48%])**
Age (yr)	33 (6-70)	36 (6-70)	38 (0-70)	1(1- 4)	2 (1- 4)
Sex Male	136 (68)	67 (73.6)	4 (40)	2 (66.7)	67 (69.8)
Female	64 (32)	24 (26.4)	6 (60)	1 (33.3)	29 (30.2)
HIV Positive	13 (6.5)	9 (9.8)	4 (40)		
Negative	187 (93.5)	82 (90.1)	6 (60)	3 (100	96 (100)
ART use	8 (4)	4 (4.4)	4 (40)		
Cough > 2weeks	200 (100)	91 (100)	10 (100)	3 (100)	96 (100)
Fever > 2 weeks	78 (39)	60 (65.9)	8(80)	2 (66.7)	8 (8.3)
Night sweets	22 (11)	20 (21.9)	1 (10)	1 (33.3)	1 (3.3)
Weight loss > 5%	92 (46)	55 (60.4)	5 (50)	3 (100)	29 (30.2)

ART (Antiretroviral Therapy), pTB (pulmonary Tuberculosis), HIV (Human Immunodeficiency Virus), pTB: Pulmonary Tuberculosis.

Table 2: Comparison of Stool Xpert MTB/RIF Ultra results with ZN and FM staining among Children participant

Diagnostic Tool	p-value	Correlations in Children Spearman's rho	Kappa	Agreement rate
ZN Staining	0.001	0.000	0.000**	No correlation
FM Staining	0.000	0.862**	0.852**	Strong correlation
Diagnostic Tool	**p-value**	**Correlations in Adults Spearman's rho**	**Kappa**	**Agreement rate**
ZN Staining	0.000	0.625**	0.561**	Strong correlation
FM Staining	0.000	0.833**	0.819**	Strong correlation

*** Significant at 95% confidence Interval, ZN (Ziehl Neelsen), FM (Fluorescence Microscopy)*

Table 3: Diagnostic Performance of using stool samples of participants using Xpert MTB/RIF Ultra assay as the 'reference test'

Performance indices among Children participants						
TB Diagnostic Test	**SE**	**SP**	**PPV**	**NPV**	**LR+ve**	**LR -ve**
FM Staining	100	80	100	99	80	20
ZN Staining	99	20	80	99	90	80
Performance indices among adult participants Sputum Xpert MTB/RIF Ultra as 'reference test'						
Stool Xpert MTB/RIF Ultra Assay	96	83	87	87	97	30
FM Staining	94	20	80	20	100	30
ZN Staining	64	12	64	12	72	31

SE: Sensitivity, SP: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR: Likelihood Ratio, ZN (Ziehl Neelsen), FM (Fluorescence Microscopy), MTB: Mycobacterium Tuberculosis, RIF: Resistance to Rifampicin

Table 4: Comparison of Xpert MTB/RIF Ultra and AFB smear Microscopy Grading in Stool samples of adult participant

	AFB MICROSCOPY GRADING SCALE				
	Negative	*Scanty*	*1+*	*2+*	*3+*
FM SMEAR MICROSCOPY (67.3% TPDR) ⇒	33 (32.7%)	44(43.6%)	9 (8.9%)	13 (12.9%)	2 (1.9%)
ZN SMEAR MICROSCOPY (50.5% TPDR) ⇒	50 (49.5%)	35(34.7)	12 (11.9%)	3 (3%)	1 (0.9%)
XPERT MTB/RIF ULTRA ASSAY GRADING (67.3% TPDR) ⇓					
MTB NOT DETECTED	33 (32.7%)				
MTB DETECTED HIGH	2 (1.9%)				
MTB DETECTED LOW	9 (8.9%)				
MTB DETECTED VERY LOW	44 (43.6%)				
MTB DETECTED TRACE (RIF INDETERMINATE	13 (12.9%)				
TOTAL TESTED = 101					

TPDR: Total Positive Detection Rate, ZN (Ziehl Neelsen), FM (Fluorescence Microscopy), MTB: Mycobacterium Tuberculosis, RIF: Resistance to Rifampicin, HIV: Human Immunodeficiency Virus, AFB: Acid fast bacilli.

Table 5: Comparison of Xpert MTB/RIF Ultra and AFB smear Microscopy Grading in Stool samples of children participants.

	AFB Microscopy Grading scale				
	Negative	*Scanty*	*1+*	*2+*	*3+*
FM Smear Microscopy (3% TPDR) ⇨	96 (96.9%)	2 (2%)	0	1 (1%)	0
ZN Smear Microscopy (1% TPDR) ⇨	98 (98.9%)	1(1%)	0	0	0
Xpert MTB/RIF Ultra Assay Grading (3% TPDR) ⇩					
MTB Not Detected	96 (96.9%)				
MTB Detected High	1 (1%)				
MTB Detected Medium	0				
MTB Detected Low	0				
MTB Detected very low	0				
MTB Detected Trace (RIF Indeterminate	2 (2.0%)				
Total Tested	**99**				

TPDR: Total Positive Detection Rate, ZN (Ziehl Neelsen), FM (Fluorescence Microscopy), MTB: Mycobacterium Tuberculosis, RIF: Resistance to Rifampicin, HIV: Human Immunodeficiency Viru, AFB: Acid fast bacilli.

DISCUSSION

In this study stool sample continued to prove valid in the diagnosis of pulmonary tuberculosis, this was evident from outcome of our study which reveals 96% sensitivity and 83% specificity of Xpert MTB/RIF Ultra assay in the diagnosis of pulmonary tuberculosis in stool sample and is in agreement with similar study by Ejeh et al. [8] which reported a sensitivity of 93.75% and specificity of 86.03%. Nicol et al.[9] reported a sensitivity of 41.7% a figure quite lower than what we discovered in this study. However, MacLean together with his colleagues conducted a systematic review resulting in a pooled sensitivity of 67%,[10] while another study conducted by Banada et al. [11] indicate an 85% sensitivity while specificity was consistently above 90% across many other studies[12] though our study specificity (83%) was less than 90%.

The inability of stool Xpert MTB/RIF Ultra assay to give a 100% Sensitivity and Specificity head-to-head the sputum assay result is due to 10 false negative outcomes of 10 stool samples out of the 91 sputum positive samples analyzed in which these 10 sputum Xpert MTB/RIF Ultra assay are MTB detected (trace) due to low bacillary load. This finding necessitates further research as to the causes of false negative outcome in stool samples of low bacillary load at infectious state of the patient.

The HIV status of the participants did not have any significant impact on the outcome of the result as seen in Table 4 where the number of sputum positive that have comorbidity is 10 and only 1 participant is HIV positive among the 10 missed positive cases in stool this is also in agreement with Musisi et al. [13] where reports shows that TB-MBLA (Tuberculosis Molecular Bacterial Load Assay) in both HIV and non-HIV Patients elucidate evidence for the presence of high bacillary loads in stool. Again, limited resources at our disposal did not enable us to carry out CD4 count/viral load among participant who tested HIV positive.

FM smear microscopy performs well in both stool of adult and children as it was able to detect all Xpert MTB/RIF Ultra positive case this further embolden the superiority of FM stain over ZN stain in the diagnosis of pTB in stool samples of pTB patients. The high sensitivity of FM staining in this study is due to the modification done to the sample processing by way centrifugation of the stool-normal saline filtrate that was done to further concentrate the AFB present. It then implies that FM staining can adequately be used in the diagnosis of pTB in stool samples in a poorly resourced laboratory where GeneXpert Machine for Xpert MTB/RIF Ultra Assay is not available this corroborates with findings of Steingart et al. [14]

Limitation of this study is evidence in the non-availability of Mycobacterial culture of stool samples obtained to compare results in Xpert MTB/RIF Ultra Assay in stool samples of children due to limited resources available. There is therefore need for further studies by way of Mycobacterial culture of same samples (stool) from children to give a more data on its diagnostic value.

CONCLUSION

This study elucidates that Xpert MTB/RIF Ultra assay in stool samples provides another approach to the diagnosis of pulmonary tuberculosis and it reveal high level of diagnostic accuracy and precision. Fluorescence Stain Microscopy for AFB smear microscopy is more efficient and accurate than Ziehl-Neelsen's Staining and can therefore be adequately deployed in the diagnosis of pulmonary tuberculosis in stool samples of presumptive tuberculosis patient and this will ensure that timely diagnosis is made in a low resource setting where GeneXpert machine is not available and where stool sample is the only non-sputum-based sample available.

Acknowledgments:

My sincere appreciation goes to the study participants and Bowen TB clinic staffs. I also thank Professor S. O. Dahunsi for his academic support and manuscript review.

Conflicts interests:

The Authors declares that there is no conflict of interest.

Authors ' contribution:

Every author mentioned in this article contributed significantly to the course of the research work and the manuscript writing. They all gave their unreserved approval for its publication.

Financial support:

This research study was sponsored by the Authors.

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