Improving the diagnosis of pulmonary tuberculosis using line probe assay and determining the factors associated with the disease in children in Jos, Nigeria

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Abstract

Introduction **Diagnosing tuberculosis (TB), including pulmonary tuberculosis (PTB), in children remains a challenge, partly due to its paucibacillary nature in young children. Data on the use of line probe assay (LPA), on gastric and sputum samples, for diagnosing PTB in children are scarce. We determined the proportion of samples positive for *Mycobacterium tuberculosis* (MTB) by smear microscopy (SM) and LPA in presumptive PTB cases as well as the factors associated with PTB confirmed by LPA, in children in Jos, Nigeria.**

Methods **An observational study in children aged 6 months-16 years. Gastric and sputum samples were examined by SM and by LPA for MTB using GenoType MTBDR*plus* Ver 2.0 (Hain Lifescience). Multivariate logistic regression was performed to determine the factors associated with PTB.**

Results **Out of 103 children with presumptive PTB, 47 had confirmed PTB, 26 unconfirmed PTB and 30 unlikely PTB by LPA. In 67 gastric samples, MTB was identified by SM in 2 (3.0%) compared to 28 (41.8%) by LPA while in 31 sputum samples, MTB was identified by SM in 5 (16.1%) compared to 18 (58.1%) by LPA. The factors associated with pulmonary tuberculosis were an abnormal chest X-ray (adjusted odds ratio (AOR))=12.39 [3.75-40.90], p<0.001), sleeping in the same room with more than three persons (AOR=3.30 [1.23-8.85], p=0.018) and sleeping in a room with none or one window (AOR=2.86 [1.03-7.95], p=0.044).**

Conclusions **Line probe assay improves the diagnosis of pulmonary TB in children, especially with gastric samples, while an abnormal chest X-ray is a useful adjunct in PTB diagnosis. Avoiding overcrowding and having windows in sleeping rooms are a necessary part of TB prevention.**

Keywords **Line probe assay, smear microscopy, gastric sample, sputum sample, pulmonary tuberculosis, chest X-ray, children.**

Introduction

Nigeria has the highest tuberculosis (TB) burden in Africa as of 2018, ranking sixth among the 30 high burden TB countries in the world and it has a TB incidence rate of 219 per 100,000 population.[1] It is among the five countries that account for 80% of the global paediatric TB mortality.[2]

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Diagnosing tuberculosis (TB), including pulmonary tuberculosis (PTB) in children, remains a challenge, especially in developing countries.[3] This diagnostic difficulty is even more in younger children, who often are unable to expectorate sputum needed for smear microscopy (SM) and *Mycobacterium tuberculosis* (MTB) culture. Younger children will often swallow their sputum after coughing, thus necessitating the aspiration of their gastric content for identifying MTB in their gastric sample. The paucibacillary nature (fewer bacilli) of TB in children also adds to the diagnostic challenge. Identifying MTB in sputum in children with PTB is <10-15% by SM and 30-40% by culture,[4,5] while from gastric aspirate it is even lower – 4% for smear and 11% for culture.[6] Smear microscopy is cheap and easy to carry out, but its sensitivity is poor. And while culture is the gold standard, it is expensive and takes a longer time to get results. However, molecular line probe assay (LPA) although also expensive, provides a more accurate and very rapid result; hence an improved and faster PTB diagnosis.[7] LPA could have several advantages in resource-limited settings like ours. For instance, it could allow for prompt treatment of the disease and therefore indirectly reduce the duration and cost of hospitalization. The methods of nucleic acid amplification tests for the detection of mycobacterial DNA or RNA such as Xpert MTB/RIF and LPA (GenoType MTBDR*plus*) are only recently being used,[5] and these have good sensitivity and specificity for detecting MTB.[7] Nicol et al.[8] reported the first study in children with PTB that used Xpert MTB/RIF.[8] The HAIN GenoType MTBDR*plus* can process various clinical samples,[9] but its use in children with PTB using gastric and sputum samples is not well documented.

We therefore sought to determine the proportion of samples positive for MTB by SM and LPA in presumptive PTB cases as well as the factors associated with PTB confirmed by LPA, in children in Jos, Nigeria.

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Methods

Study design

This was an observational cross-sectional study carried out from October 2014 to May 2017 and from March 2018 to March 2019.

Study site

The study site was the Jos University Teaching Hospital (JUTH). JUTH being a referral hospital, sometimes receives referrals from other health facilities in Jos city, including Faith Alive Hospital and Vom Christian Hospital. It is the largest hospital in Jos. Jos city with a population of about 900,000 is the capital of Plateau State which has a population of approximately 3,206,531.[10] The state has over forty indigenous tribes and several other ethnic groups from various parts of Nigeria reside in Jos, making Jos a cosmopolitan Nigerian city.

Study population

The children studied were in the age group 6 months to 16 years. Those with a presumptive PTB diagnosis and who were consented were consecutively enrolled in the study. Presumptive PTB (previously called suspected PTB) in a child is as defined by the Nigeria National Tuberculosis and Leprosy Control Programme (NTBLCP) guidelines. This definition includes cough of 2 weeks or more duration with any of the following symptoms: low-grade fever not responding to malaria treatment, night sweats, loss of appetite, loss of weight or failure to thrive.[11] Children already receiving antituberculosis treatment (ATT) were excluded from the study.

The calculated minimum sample size was 60. This was calculated using OpenEpi,[12] where p=4%, is the detection rate for MTB in the sputum of children by SM.[6]

Operational definitions

Presumptive PTB was defined as above. A child with presumptive TB who has clinical features (history and physical examination) with an abnormal chest X-ray (CXR) suggestive of PTB was regarded as having clinically diagnosed PTB. A child with presumptive PTB or clinically diagnosed PTB, with or without a smear-positive sample, who is positive by LPA was classified as a

case of confirmed PTB. A child with clinically diagnosed PTB who is smear-negative and is LPA negative was classified as a case of unconfirmed PTB. A child with presumptive PTB who ended up neither having clinically diagnosed PTB nor confirmed or unconfirmed PTB was classified as a case of unlikely PTB (not TB).[13] Cases of unlikely PTB are of other diagnoses such as pneumonia or heart diseases complicated by pneumonia. A child with PTB could also have in addition, other forms of tuberculosis such as TB meningitis, pleural effusion and abdominal TB. TB treatment outcomes (whether cured, completed treatment or died) was also as defined by the national guidelines.[11] The World Health Organization (WHO) AnthroPlus software (WHO, Geneva, Switzerland)[14] was used to determine body mass index (BMI) of the children and a BMI Z-score of ≤–3 was considered as severe malnutrition.

Abnormal features in a CXR included any one or more of the following: hilar/paratracheal adenopathy, segmental/lobar consolidation, bronchopneumonic changes, pleural effusion, reticulonodular infiltration, segmental/lobar lung collapse.

Recruitment of study participants

During weekdays at the various paediatric units of the study site, children who met the inclusion criteria were consecutively enrolled in the study. Every enrolled child received a detailed history and physical examination. Relevant data – socio-demographic, clinical (incorporating TB screening questions) and laboratory, were collected in a case report form. Patients were recruited by Consultant Paediatricians, and their CXRs were read by both the paediatricians and a Consultant Radiologist, before obtaining their LPA results. Patient recruitment was on an outpatient basis, and only very sick children were admitted into the paediatric wards.

Specimen collection and processing

For older children who were able to expectorate sputum, a spontaneous sputum sample was collected. For younger children, especially those five years and below, and any child who was unable to expectorate, gastric aspirate samples were collected. To do this, the child fasted overnight for 4-6 hours and the next day in the morning around 6-8 AM, an appropriate size nasogastric tube was passed into the child's stomach. Once the tube is in-situ, a 10 mL syringe was used to aspirate 5-10 mL of the gastric content into a specimen container. Where the aspirate was less than 5 mL, 20 mL of sterile water was instilled into the stomach and immediately aspirated to obtain a sample. Pleural fluid samples were collected via a thoracotomy tube. Children with a significant amount of pleural effusion usually have a chest tube inserted. Only one specimen sample was collected for each child, either sputum or gastric and pleural where indicated. Every collected specimen was immediately processed. A loop of the specimen was taken and placed on a slide to make a smear of about 1 cm x 2 cm, which was dried in air and fixed with gentle heat. Three slides were prepared for each specimen. Ziehl-Neelsen (ZN) staining was then carried out and slides read, according to the national guidelines.[11]

Patient care and treatment

Children diagnosed with TB including drug-sensitive (DS) and drug-resistant (DR) TB by LPA, received ATT based on national guidelines[11] and those with other diagnoses (unlikely TB cases) such as pneumonia and heart diseases/failure received the appropriate treatment including antibiotics. Children diagnosed with HIV at the time of PTB diagnosis were first started on ATT, then later commenced on antiretroviral therapy based on national guidelines.[15]

Laboratory procedure for the LPA

Following the manufacturer's (Hain Lifescience, Nehren, Germany) instruction for the GenoType MTBDR*plus* Ver 2.0,[9] the LPA was performed in three stages involving extraction of DNA from decontaminated samples, amplification by polymerase chain reaction (PCR) and reverse hybridization. The DNA strips were evaluated using the provided manufacturer's template by aligning strips against each locus and locus control bands. For a valid result, the locus band for Conjugate

Control, Amplification Control and *M. tuberculosis* Complex bands must be present in addition to other corresponding bands of *rpoB*, *katG*, *inhA*, wild type probes and mutation probes. The result was then interpreted according to the manufacturer's instruction as mono-resistance or multi-resistance.

Smear microscopy for AFB was done following ZN staining of samples. MTB culture for further drug sensitivity testing (DST) was not done on samples that showed mono-drug resistance (DR) TB by LPA. No TB culture was done on any of the specimens. All laboratory procedures were carried out at the SANAS accredited APIN Laboratory, JUTH.

Statistical analysis

Stata software version 10.0 (Stata Corporation, College Station, Texas, USA) was used for the data analysis. Confirmed PTB (a positive LPA test) was the outcome variable, and the other variables were independent variables. For an initial examination of the association between the outcome (dependent) and independent variables, Chi-square or Fisher's exact tests were performed for categorical variables while for non-normally distributed variables Wilcoxon-Mann-Whitney test was performed. Data were also presented by means of descriptive statistics. Bivariate logistic regression was first performed. Then variables that were significantly associated with the outcome in the bivariate analysis were fitted into a multivariate logistic regression modelling to determine the factors associated with PTB. Forward step-wise modelling was performed, in which variables with $p<0.05$ remained in the model and those with $p>0.10$ exited the modelling at each step. Results were then expressed as odds ratio (OR) for the bivariate analysis and adjusted OR (AOR) for the multivariate analysis, with their 95% confidence intervals (CI). The AOR adjusts for potential confounding variables in the multivariate analysis. All tests were two-sided with p-values of <0.05 as statistically significant.

Ethical approval

Ethical approval (reference number-JUTH/DCS/ADM/127/XIX/5943) for the study was granted by the Ethics committee of the Jos University Teaching Hospital. Written informed consent was obtained from parents/guardians for the recruitment of their children/ward into the study.

Results

Characteristics of the study population

The total number of children with presumptive PTB enrolled in the study was 103. Their median (IQR) age was 3.3 years (1.2-9.0) and 56 (54.4%) were females (Table 1). Majority of the children studied, slept in the same room with three or more persons (62.1%) while 68 (66.7%) slept in rooms with either one or no windows at all, and 68 (66%) lived in a compound type of housing (Table 1). Also, the majority of the children studied had cough (95.1%), fever (77.7%), weight loss (83.3%), BCG vaccination (85.7%) and BMI Z-score $\leq$-3.0 (68.7%). Children who had night sweats were 47.1%, and those who had a history of contact with a TB case were 35.9%. And there were 69 (67%) children with an abnormal CXR (Table 2). Other characteristics of the study population are shown in Tables 1 and 2.

In categorizing PTB diagnosis based on the results of a positive LPA, of the 103 children studied: 47 (45.7%) had confirmed PTB, 26 (25.2%) unconfirmed PTB and 30 (29.1%) unlikely PTB. Out of the 69 that were clinically diagnosed with PTB, 43 (62.3%) were positive by LPA.

There were 8 (20%) HIV positive children among the 40 cases of confirmed PTB who were screened for HIV and of the 69 clinically diagnosed PTB cases, 13 (18.8%) were HIV positive.

In 67 gastric samples, MTB was identified by SM in only 2 (3.0%) compared to 28 (41.8%) by LPA. For 31 sputum samples, MTB was identified by microscopy in 5 (16.1%) compared to 18 (58.1%) by LPA. However, MTB was not identified by SM in any of the 5 pleural fluid samples, whereas it was identified by LPA in 1 (20%) of these samples (Table 3).

Table 1. Socio-demographic characteristics of children diagnosed with pulmonary tuberculosis using line probe assay

Characteristics	Total N (%)	Tuberculosis (LPA)		P value
		Present N (%)	Absent N (%)	
Age (years)				0.167
0-5	58 (56.3)	23 (48.9)	35 (62.5)	
6-15	45 (43.7)	24 (51.1)	21 (37.5)	
Median (IQR)	3.3 (1.2-9.0)	5.1 (1.5-12.0)	3.0 (1.1-8.3)	0.089
Sex				0.077
Male	47 (45.6)	17 (36.2)	30 (53.6)	
Female	56 (54.4)	30 (63.8)	26 (46.4)	
Religion				0.442
Christianity	59 (57.3)	25 (53.2)	34 (60.7)	
Islam	44 (42.7)	22 (46.8)	22 (39.3)	
Education level of child				0.636
Pre-school	58 (56.3)	24 (51.1)	34 (60.7)	
Primary	22 (21.4)	12 (25.5)	10 (17.9)	
Secondary	9 (8.7)	4 (8.5)	5 (8.9)	
Tertiary	4 (3.9)	3 (6.4)	1 (1.8)	
No formal education	10 (9.7)	4 (8.5)	6 (10.7)	
Type of housing				0.667
Flat	35 (34.0)	17 (36.2)	18 (32.1)	
Compound type	68 (66.0)	30 (63.8)	38 (67.9)	
Family size				
Median (IQR)	6 (4-9)	6 (5-10)	6 (4-8)	0.145
Number of persons sleeping in the same room with the child				0.018
1-2	39 (37.9)	12 (25.5)	27 (48.2)	
3-7	64 (62.1)	35 (74.5)	29 (51.8)	
Median (IQR)	3 (2-4)	3 (2-4)	3 (2-3)	0.004
Number of windows in room where child sleeps				0.021
0-1	68 (66.7)	37 (78.7)	31 (56.4)	
2-3	34 (33.3)	10 (21.3)	24 (43.6)	
Median (IQR)	1 (1-2)	1 (1-1)	1 (1-2)	0.027

LPA – line probe assay; **IQR** – interquartile range.

There were 12 cases of DR TB, of which 8 were RIF mono-resistant and 4 INH mono-resistant. None of these cases had their samples cultured for DST.

Outcomes

Of the 103 children studied, 73 were TB cases (47 confirmed and 26 unconfirmed), and 30 were unlikely TB cases. Out of the 73 TB cases, 70 completed TB treatment while 3 left the hospital against medical advice before commencement of treatment. There were a total of 7 deaths of which 6 were TB cases (5 confirmed TB, 1 unconfirmed TB) and 1 unlikely TB and all deaths occurred within 48 hours for those who were hospitalized. Out of the 6 TB cases that died, 2 died before starting ATT and 4 died while on ATT and of these 4 who died while on ATT, 2 had both HIV with TB meningitis, 1 had severe malnutrition and 1 had pleural effusion.

Table 2. Clinical and laboratory characteristics of children diagnosed with pulmonary tuberculosis using line probe assay

Characteristics	Total N (%)	Tuberculosis (LPA)		P value
		Present N (%)	Absent N (%)	
BCG vaccination				0.250
Yes	84 (85.7)	40 (90.9)	44 (81.5)	
No	14 (14.3)	4 (9.1)	10 (18.5)	
History of TB contact				1.000
Yes	37 (35.9)	17 (36.2)	20 (35.8)	
No	59 (57.3)	27 (57.4)	32 (57.1)	
Don't know	7 (6.8)	3 (6.4)	4 (7.1)	
Cough				1.000
Present	98 (95.1)	45 (95.7)	53 (94.6)	
Absent	5 (4.9)	2 (4.3)	3 (5.4)	
Fever				0.097
Present	80 (77.7)	40 (85.1)	40 (71.4)	
Absent	23 (22.3)	7 (14.9)	16 (28.6)	
Weight loss				0.769
Present	91 (88.3)	42 (89.4)	49 (87.5)	
Absent	12 (11.7)	5 (10.6)	7 (12.5)	
Night sweats				0.796
Present	48 (47.1)	21 (45.6)	27 (48.2)	
Absent	54 (52.9)	25 (54.4)	29 (51.8)	
TB-HIV co-infection				0.650
Present	20 (22.2)	8 (20.0)	12 (24.0)	
Absent	70 (77.8)	32 (80.0)	38 (76.0)	
BMI Z-score				0.205
≤-3.0	68 (68.7)	28 (62.2)	40 (74.1)	
>-3.0	31 (31.3)	17 (37.8)	14 (25.9)	
Median (IQR)	-2.1 (-3.3–0.5)	-2.3 (-3.5–1.0)	-1.8 (-3.1–0.4)	0.178
MUAC (N=97)				
Median (IQR) in cm	13.2 (11.5-15.2)	13 (11.3-15.2)	13.5 (11.5-15.5)	0.364
Abnormal CXR				<0.001
Yes	69 (67.0)	43 (91.5)	26 (46.4)	
No	34 (33.0)	4 (8.5)	30 (53.6)	
Mantoux (N=44)				0.228
Median (IQR) in mm	0 (0-7.5)	0 (0-6)	3 (0-10)	
ESR (N=50)				0.443
Median (IQR) in mm/hr	55.5 (25-91)	56 (32-119)	55 (16-80)	

BCG – bacillus Calmette-Guerin; **BMI** – body mass index; **CXR** – chest x-ray; **ESR** – erythrocyte sedimentation rate; **HIV** – human immunodeficiency virus.; **LPA** – line probe assay; **MUAC** – mid-upper arm circumference; **TB** – tuberculosis; **IQR** – interquartile range

Factors associated with PTB

An initial analysis showed that the variables that were significantly associated with confirmed PTB (positive LPA) were: three or more persons sleeping in the same room with a child, child sleeping in a room with one or no windows at all and an abnormal CXR (Tables 1 and 2).

In the bivariate logistic regression, the same three variables above were significantly associated with confirmed PTB (positive (LPA). Thus, the odds of having PTB was twelve times higher in

Table 3. Identification of MTB from various samples by smear microscopy and LPA

Sample type	Smear microscopy		LPA	
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
Gastric (n=67)	2 (3.0)	65 (97.0)	28 (41.8)	39 (58.2)
Sputum (n=31)	5 (16.1)	26 (83.9)	18 (58.1)	13 (41.9)
Pleural (n=5	0 (0.0)	5 (100)	1 (20.0)	4 (80.0)
Total (n=103)	7 (6.8)	96 (93.2)	47 (45.6)	56 (54.4)

LPA – line probe assay; **MTB** – *Mycobacterium tuberculosis*.

Table 4. Factors associated with pulmonary tuberculosis in children

Characteristics	Bivariate analysis		Multivariate analysis	
	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Abnormal chest x-ray		<0.001		<0.001
No	1.00 (Ref)		1.00 (Ref)	
Yes	12.40 (3.92-39.22)		12.39 (3.75-40.90)	
Number of persons sleeping in the same room with the child		0.006		0.018
≤ 3	1.00 (Ref)		1.00 (Ref)	
> 3	1.54 (1.13-2.11)		3.30 (1.23-8.85)	
Number of windows in room where child sleeps		0.019		0.044
2-3	1.00 (Ref)		1.00 (Ref)	
0-1	2.90 (1.19-6.90)		2.86 (1.03-7.95)	

children with an abnormal CXR compared to those with a normal CXR (p<0.001), while the odds were one and a half times higher in children sleeping in the same room with three or more persons compared to those sleeping in a room with only one or two persons (p=0.006). And the odds were about three times higher in children sleeping in a room with one or no window at all compared to those sleeping in a room with at least two windows, p=0.019 (Table 4).

The multivariate regression showed that the odds of having PTB were three times higher in children sleeping in the same room with three or more persons compared to those sleeping in a room with only one or two persons (p=0.018). And the odds of having PTB was about two and a half times higher in children sleeping in a room with one or no windows at all compared to those sleeping in a room with at least two windows, p=0.044. The odds of having PTB was again twelve times higher in children with abnormal compared to a normal CXR (Table 4).

Discussion

In 67 gastric samples, MTB was identified by SM in 3.0% compared to 41.8% by LPA while in 31 sputum samples, MTB was identified by SM in 16.1% compared to 58.1% by LPA. The associated factors for pulmonary tuberculosis were an abnormal CXR, sleeping in the same room with more than three persons and sleeping in a room with one or no windows.

In the present study, the proportion (3.0%) of children with smear-positive gastric samples was comparable to the 4% obtained in a study among children with pulmonary TB from a children's hospital in Texas, USA.[6] These similar low figures are not unexpected because TB in children is paucibacillary (fewer bacilli), especially with gastric samples. However, the slightly higher USA figure may be due to the examination of 3

gastric samples per child in that study compared to 1 sample in our study. Examination of more samples per patient is usually expected to increase the yield of MTB. We observed in the present study that 16.1% of children had smear-positive sputum sample, which was only slightly higher than the range of 2.2%-12.2% obtained in one systematic review for sputum smear positivity in children.[16] And in another study in children from Yemen, sputum smear positivity was even lower, only 7%.[17] Our higher sputum smear positivity could be attributed to a higher TB burden in Nigeria, where our study setting is.

In our study, MTB was detected by LPA in 58.1% of sputum samples (83.9% were smear-negative, Table 3) while in a study from India among adults, MTB was detected in 38.2% of sputum samples all (100%) of which were smear-negative.[18] Even though the latter study was in adults, both studies are still comparable in terms of their very high smear negativity, and in the ability of LPA to detect MTB in smear-negative samples. In contrast to these two studies, another study in India among adults showed a lower sputum LPA positivity (26.2%).[19] A study from Delhi among children with a presumptive diagnosis of multidrug-resistant (MDR) TB, 61.8% of the sputum samples were smear-positive by ZN staining and 92.8% were positive by LPA.[20] The higher sputum smear and LPA positive results of this study, when compared to ours, was not surprising because their study population was already those with a presumptive MDR-TB. All the above three studies[18-20] used LPA to identify MTB drug resistance, whereas, in the present study, we used LPA as a tool for detecting MTB to improve the diagnosis of PTB in children. Also, these three studies used only sputum samples, unlike our study that also used gastric samples, with the LPA detecting MTB in 41.8% of the gastric samples. Thus, our study was valuable in the context of diagnosing PTB in children by LPA using gastric samples.

The present study showed that a high proportion, 43/69 (62.3%), of children with clinically diagnosed PTB ended up having a positive LPA result. This may be attributed to the fact that these children were recruited by Consultant Paediatricians and also had their CXRs read by a Consultant Radiologist. Such specialists are needed for a better interpretation of CXRs in making a clinical diagnosis of PTB. Our study showed that an abnormal CXR was strongly associated with a PTB diagnosis. Hence, in settings where the expertise of specialists is available, even in the absence of facilities for doing LPA or even if the LPA is negative, PTB could still be clinically diagnosed with little risk of overdiagnosis and overtreatment.

We observed that sleeping in the same room with more than 3 persons was associated with pulmonary TB. Having more than 2 persons per bedroom is considered as crowding.[17]

A WHO document[22] on housing and health cited several studies showing the association between household crowding and risk of TB. Some of these cited studies and others, showed household crowding, including the number of persons per bedroom, is associated with an increased risk/incidence of TB.[21,23,24] One of these studies from Zimbabwe found crowding with more than 2 to 4 persons per room to be a major risk factor for TB.[24]

We found that sleeping in a room with one or no windows at all was associated with PTB. It is well established that having a window(s) in a room is essential for cross-ventilation. Studies have shown that rooms with windows enhance cross-ventilation between doors, which help to reduce transmission of airborne infections, including TB.[25,26]

The limitation of this study was that we were unable to do an MTB culture. MTB culture was also needed for further drug sensitivity testing (DST) on those samples that initially showed mono DR TB by LPA. Also, since we were unable to do MTB culture (the diagnostic gold standard), we could not in the present study compare the performance of the LPA and SM with culture in PTB diagnosis. Such a comparison in terms of sensitivity and specificity could have further added value to our study, in addition to enhancing its comparability with several other studies where TB cultures were done.

Conclusions

Line probe assay improves the diagnosis of PTB in children, especially with gastric samples,

while an abnormal CXR is a useful adjunct in PTB diagnosis. Avoiding overcrowding and having windows in sleeping rooms are a necessary part of TB prevention.

Authors' contributions statement: AOE was responsible for the concept and study design, statistical analysis and drafting of the manuscript. AOE, SO, IIA, YOI, CCA, HOA, MMI, ESY, MOO, ASS participated in data acquisition and interpretation and critical revision for intellectual content. All authors reviewed and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

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