Performance of Xpert MTB/RIF Assay for Childhood Pulmonary Tuberculosis With Real World Evidence in China

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Abstract

BACKGROUND: Rapid and accurate notification of childhood pulmonary tuberculosis (PTB) is a worldwide challenge. Although the Xpert MTB/RIF assay (Xpert) has been endorsed as the initial test for suspected childhood pulmonary tuberculosis in many countries, limited studies have reported the real-world performance of Xpert for the detection of childhood PTB.

OBJECTIVE: To evaluate the real-world performance of Xpert for the detection of childhood pulmonary TB in China.

METHODS: We consecutively extracted the data of all patients ≤14 years with pulmonary disease through the electronic medical record (EMR) systems of Shanghai Public Health Clinical Center from January 2014 to December 2017. The clinical profile, the decision-making tests including AFB smear, solid/liquid culture, pathological examination and Xpert result were matched and assessed. The real diagnostic accuracy and the all-factors case notification rate for childhood PTB with the implementation of Xpert were evaluated.

RESULTS: 519 cases ≤14 years with pulmonary disease were extracted from the data base. Of these, 145 had matched results, there were 374 non-matched cases including 346 with incomplete or unavailable data and 28 with NTM, BCG or an unidentified strain. For matched data, the overall sensitivity and specificity of the Xpert assay were 66.7% (32/48, 95%CI 0.52-0.80) and 87.6% (85/97, 95%CI 0.87-0.98) respectively against the gold standard; 34.6% (44/127, 95%CI 26.6-43.7) and 100% against the composite clinical reference standard (CCRS). The all-factors case notification rate by Xpert was 29%.

CONCLUSIONS: Xpert/MTB RIF assay has acceptable sensitivity and excellent specificity for rapid diagnosis of children with pulmonary TB as well as for the detection of RIF resistance in China. However, implementation of Xpert for the initial diagnosis of childhood PTB is inadequate to meet the urgent requirement for rapid and accurate detection of childhood PTB.

Background

Tuberculosis (TB) is a vicious children-killer worldwide. According to the latest global report, about 1.04 million children had TB and 201,000 children died of TB in 2017. About 1/2 of the childhood tuberculosis cases were not microbiologically proven and for those under 5 years, this proportion rose to about 3/4 [1]. Lack of child-appropriate tools continues to make the diagnosis of childhood tuberculosis a big challenge. Traditional diagnostic tools are not sufficient to meet the requirement for a rapid and accurate diagnosis of childhood PTB. Clinical diagnosis of childhood PTB mainly relies on the clinical assessment combined with laboratory tests and the responses to anti-TB treatment (ATT). About 10 to 15% of childhood TB is smear-positive because samples are paucibacillary [2]. The sputum culture is only able to detect approximately 30–40% of the cases with probable tuberculosis in children [3] and the tuberculin skin test (TST) and interferon-gamma release assay (IGRA) cannot differentiate latent TB from active TB [4–5]. In recent years, molecular tests for TB were introduced into clinical practice and a rapid test

system, Xpert® MTB/RIF assay (Cepheid, USA) was endorsed by WHO for the detection of tuberculosis [6]. Xpert assays were reported to have better accuracy than sputum smear microscopy. For adults, this assay was at least as sensitive as culture methods [7]. For children, especially for those who experience difficulty in expectoration, the data for the accuracy of Xpert assay has been limited. Since 2018, Shanghai CDC has used the Xpert test as an initial method for early detection of PTB. We designed this retrospective study based on the electronic medical record (EMR) systems in order to evaluate the real diagnostic performance for childhood PTB among hospitalized patients in the context of an environment with a substantial TB burden.

Methods

Non-interventional cohort data traced from EMR system

We created a database from the EMR systems of Shanghai Public Health Clinical Center which is the exclusive tertiary hospital administered for the hospitalization of childhood tuberculosis in Shanghai, China. The EMR systems used in this study were the HIS (hospital information system, Kingstar Winning Software.co., Ltd, China) and the LIS (laboratory information system, Kingstar Winning Software.co., Ltd, China) systems which are widely used as the main systems in Shanghai. Case data were collected consecutively from January 2014 to December 2017. We searched all the cases with the following two criteria: (1) ≤14 years of age; (2) with pulmonary disease. The case information was analyzed for data integrity in the matching records from the EMR systems including the quality of samples, the results from Xpert MTB/RIF system, the sequencing for strain identification, the clinical profile and other laboratory examinations.

The real prevalence of PTB in the hospitalized children ≤14 years with pulmonary disease was estimated with an extended scale, based on comprehensive consideration of the EMR regarding clinical diagnosis, the follow-up records, the laboratory test results, the composite clinical reference standard (CCRS) and the experienced physicians' suggestions. With this estimation, we calculated the real notification rate of Xpert for suspected childhood PTB.

Case definition

The gold standard (microbiological reference standard) of PTB in this study was a composite of positive results for M. tuberculosis by culture method on a respiratory tract specimen (RTS) including induced or induced sputum (IS), nasopharyngeal aspiration (NA) or gastric aspiration (GA). In consideration of the low yield of MTB culture from childhood RTS, we also assessed the diagnostic performance against CCRS. In China CCRS involves the following 8 items [8]: (1) fever or cough for more than two weeks, weight loss or failure to gain weight in the previous 3 months, (2) a chest radiograph suggestive of TB, (3) household tuberculosis contact within the preceding 24 months, (4) a positive tuberculin skin test, (5) smear-positive RTS with/without a positive culture result, (6) effective anti-tuberculosis treatment, (7) other excluded pulmonary diseases like pneumonia, lung tumor, lung cyst, and interstitial lung diseases;

(8) pathology of lung tissue consistent with pulmonary TB. Besides meeting criteria 1 and 2, patients meeting any 2 criteria of the items 3, 4, 6, and 7 are defined as clinical diagnosed. Based on these criteria, all cases were categorized into three groups according to the Expert Panel [9] and the extensively-experienced childhood tuberculosis experts' decisions: (I) confirmed TB (culture-positive diagnosed TB); (II) probable TB (culture-negative clinically diagnosed TB); (III) non-TB (no TB evidence). According to the practice guideline, the sequencing method (Xpert was not included) and histopathological examination (HE) applied to lung biopsy specimens were used as definitive diagnostic methods. In the follow-up period, experts divided patients who had no definitely evidence into probable TB or non-TB by considering if they had effective response to anti-tuberculosis therapy (clinical features suggestive of tuberculosis disease that were present at baseline have improved, and there is no new clinical feature suggestive of tuberculosis) or no (clinical features suggestive of tuberculosis disease that were present at baseline have not improved).

Estimated prevalence at the site

We estimated the real prevalence with four scales: EMR recorded diagnosis, the follow-up record, the microbiological test, the experts' experience. The first index "EMR recorded diagnosis" was established on clinical judgment and suspicions, which often over-estimated the real prevalence, representing the maximum estimation. The second index "follow-up record" was created by a sampling method and was potentially more accurate. The third index "microbiological test" represented the minimum estimation of prevalence. The fourth index "experts' experience" was the most likely correct, being consistent with all the clinical criteria. Although this estimation was not very exact, it was considered important to calculate the real prevalence of the site. Some accuracy indices, such as positive and negative predictive values can only be comprehended in association with the prevalence. The case notification rate was also calculated from these estimates.

Sample processing and records

Direct smear microscopy and MTB culture of each RTS were performed following the WHO guideline [10]. Cultures positive for growth of acid-fast bacilli underwent confirmation of M tuberculosis complex by MPT64/MPB64 antigen detection [11]. Phenotypic drug-susceptibility testing was done on positive cultures with the BACTEC MGIT 960 SIRE kit (BD, Franklin Lakes, NJ, USA) in accordance with the manufacturer's instructions [12]. The Xpert MTB/RIF test was performed on the basis of the manufacturer's protocol, Results were reported as the following four results: "negative", "TB detected, rifampicin resistance detected", "TB detected, no rifampicin resistance detected" and "invalid result". The semi quantitative scale for Xpert results was divided into four grades which were measured by cycle-threshold (Ct): very low ($28<Ct \leq 38$), low ($22<Ct \leq 28$), medium ($16<Ct \leq 22$), or high ($Ct \leq 16$) [13].

Statistical analysis

The general sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated to evaluate the diagnostic accuracy of Xpert MTB/RIF for childhood PTB in this real-world setting. Stratification analysis was done for subgroup evaluation. Categorical variables were compared using the Fisher's exact test or Pearson Chi-square test. The P value of ≤0.05 was considered significant. 95% confidence intervals (95%CI) were estimated for data with binomial distributions. Kappa values were assessed to compare the agreement between categorical variables. The major statistical analysis was performed by IBM SPSS Statistics version 22, Microsoft Excel for Windows 10 and GraphPad Prism version 5.

Results

Database, case-flow and analytical procedures

35049 cases from database were screened and 519 cases ≤14 years of age with pulmonary disease were enrolled in this study. Of the 519 cases, 173 cases were matched with a clinical profile, AFB smear, solid/liquid culture result, Xpert and final diagnosis. 346 non-matched cases included 55 with incomplete clinical data (unable to define the case, such as dead or transferred to other hospitals), 87 with repeated hospitalization, 45 with no necessity for RTS test, 110 with no RTS test for other reasons (such as unable to acquire specimen), 49 with non-paired results for Xpert, AFB smear, and MTB culture test (only one or two results). In 173 matched cases, 28 cases were excluded for the following reason: 3 with NTM and 5 with BCG vaccine infection identified by sequencing, and 20 who were culture-positive but had no record of strain identification. The 145 matched cases were analyzed for accuracy, among them 33.1% (48/145) were culture-positive (30 smear-positive and 18 smear-negative), 66.9% (97/145) were culture-negative including 54.5% (79/145) probable TB and 12.4% (18/145) non-TB cases (*FIGURE 1.)*.. For the 145 matched cases, the mean age was 3.65 ± 3.62 years, male sex was 37.9% and 87.6% was clinically diagnosed TB. For the 346 non-matched cases, the mean age was 3.51 ± 3.50 years, male sex was 37.6%, and 91.0% was clinically diagnosed TB, no statistically difference between these two groups. *TABLE 1.* shows the clinical characters of the matched cases.

Estimated prevalence at the site and the notification rate from Xpert

The TB prevalence estimation via EMR record was approximately 82%, from the available follow-up records of this site it was about 70%, the total microbiologically positive rate of the cases was 45% and the eight experienced experts' estimation was 70%. The total prevalence was estimated to be 67% (*TABLE 2.)*.. From these estimates, the estimated number of TB cases was 259 after exclusion of repeated cases and those without a necessity of RTS testing. Of these cases, 75 had positive Xpert results. Thus, the all-factors case notification rate by Xpert was 29.0% (75/259) and was similar by culture (32.4%, 84/259, p = 0.391).

Diagnostic accuracy of the Xpert RIF assay

The diagnostic accuracy of the Xpert RIF assay was evaluated against different reference standards separately. Based on the gold standard, 48 cases were diagnosed as pulmonary tuberculosis. On this basis, the overall sensitivity and specificity of the Xpert assay were 66.7% (32/48, 95%CI 0.52–0.79) and 87.6% (85/97, 95%CI 0.87–0.98); the PPV and NPV were 72.7% (95%CI 0.57–0.85) and 84.2% (95%CI 0.74–0.90). The positive yield was 76.7% (23/30, 95%CI 0.57–0.89) in cases with both smear-positive and culture-positive results, and 50% (9/18, 95%CI 0.27–0.73) in the smear-negative culture-positive cases (*TABLE 3).*. The positive rates of the Xpert assay on different sample types (IS, NA and GA) were 29.6% (8/27), 30.0% (6/20), 30.6% (30/98), respectively and showed no statistically significant differences (P >0.05). The agreement between the Xpert and the culture results was 81% (kappa = 0.56) and the inconsistencies were 12 Xpert-positive culture-negative cases and 16 Xpert-negative culture-positive cases *(TABLE 4).*

Based on the CCRS, 127 cases were diagnosed as pulmonary tuberculosis, including 79 culture-negative clinically diagnosed TB. The sensitivities of AFB smear, MTB culture, and Xpert MTB/RIF assay were 23.6% (30/127, 95%CI 16.7–32.1), 37.8% (48/127, 95%CI 29.5–46.9) and 34.6% (44/127, 95%CI 26.6–43.7), respectively. The specificities and positive predictive values of the three assays were all 100%. The negative predictive values of AFB smear, MTB culture, and Xpert MTB/RIF assay were 15.7% (95%CI 9.8–23.9), 18.6% (95%CI 11.7–20.0), 17.8% (95%CI 11.2–27.0), respectively *(TABLE 5).* There were 12 children had Xpert(+)&smear(-)&culture(-) results, 2 children had Xpert(-)&smear(+)&culture(-) results. the combined sensitivity of Xpert plus culture, Xpert plus AFB smear, Xpert plus AFB smear and culture were 47.2% (60/127), 36.2% (46/127), 48.8% (62/127), respectively. They were all statistically higher than from a single test (P <0.05). Performance of Xpert MTB/RIF assay in different age groups were also analyzed. The sensitivities in 1–5 years group, 5–10 years group, 5–14 years group were 36.4%, 15.4%, 61.5%, respectively. *(TABLE 6)*

In routine clinical practice, non-tuberculous Mycobacterium (NTM) and BCG vaccine infection are not always pre-excluded. Therefore, we evaluated the situation when these cases arose in the analysis. In this study, NTM cases were consistently Xpert-negative and BCG vaccine infection cases were all Xpert-positive. The sensitivity, specificity, PPV and NPV of Xpert against the gold standard were 66.1% (95%CI 0.52–0.78), 87.6% (95%CI 0.79–0.93), 75.5% (95%CI 0.61–0.87) and 81.7% (95%CI 0.73–0.89), respectively. In comparisons against the CCRS, the sensitivity, specificity, PPV and NPV of Xpert were 34.7% (95%CI 0.26–0.44), 80.8% (95%CI 0.61–0.93), 80.8% (95%CI 0.78–0.97) and 20.2% (95%CI 0.13–0.29), respectively.

Detection of RIF resistance

A total of 11 cases had recorded drug-resistance via phenotype drug sensitivity test (DST) and had matched Xpert MTB/RIF test results. 5 were resistant to HRES (isoniazid, rifampin, ethambutol and streptomycin), 3 were resistant to HR, 1 was resistant to HE, and 2 were mono-resistant to H. The 8 cases

with phenotypic rifampin-resistance were also detected by Xpert assay. The consistency of culture-based DST and Xpert assay for rifampin resistance was 100%.

Discussion

In this study, the sensitivity of the Xpert test varied with the AFB smear status in culture-proven cases. Xpert showed an overall sensitivity of 66.7%, which was similar to AFB smear (30/48, 62.5%). The sensitivity was higher in cases where both culture and AFB smear were positive (76.7%) and lower in cases that were culture-positive but AFB smear negative (50%). This finding was consistent with other reports [14–15] and easy to understand: the trend for an Xpert-positive yield is associated with the bacterial load in specimens. In this study, we estimated the real prevalence of childhood TB to be 67%, but the real case notification rate was much lower. One reason of this situation was that the sensitivity of neither Xpert, culture nor smear was good enough for the notification of childhood PTB cases (34.6%, 37.8% and 23.6% against CCRS, respectively); another reason was that about 1/4 of the cases did not have valid specimen test results. However, the causes could not be fully assessed because this was a retrospective study. These results showed that the performance of Xpert remains suboptimum in a real-world setting.

For establishing a definitive diagnosis of childhood PTB, multiple or repeated decision-making tests (such as MTB culture, nucleic acid test or sequencing) on RTS are often required. The added diagnostic yield from combined tests seems much higher than repeated test [15–16], although this was not extensively assessed in our study. A study from South Africa suggested that the incremental increase in sensitivity from testing a second specimen was 27.8% for MTB/RIF in smear-negative cases [17]. But another study indicated that the collection of one sputum specimen should be adequate in establishing a diagnosis of TB; the sensitivity of MTB/RIF for MTB detection in suspected TB cases from whom only one specimen was analyzed did not differ significantly from the sensitivity achieved by testing two or three specimens [18]. In this study, we included data from only one specimen from each child, further studies would be needed to confirm that two specimens give a higher detection rate than one sample. Some studies indicated that sputum induction can be effectively performed and is well tolerated and safe even in infants and this induction was better than GA for the isolation of M. tuberculosis in both HIV-infected and uninfected infants and children [17,19]. In this study, children under five years old underwent GA, over five years old had IS or NA, Xpert performed equally well with the different respiratory specimens (IS, NA and GA). The sensitivity of Xpert varied significantly in different age groups, in 1–5 years group the sensitivity of Xpert was 36.4%, which was similar to the sensitivity of child-wide population against culture, it indicated GA was a desirable method to instead sputum in infant and young children. 5–10 years group had the lowest sensitivity (15.4%), we analyzed it was due to children among this group couldn’t exhaust sputum well. 10–14 years group had the highest sensitivity (61.5%), which was similar to adult [7], it indicated children of this age had the power to cough and could acquire high-quality specimens.

In a real-world setting, NTM and BCG vaccine infection cannot be pre-excluded before laboratory test. We noticed that if NTM and BCG vaccine infection were included in the analysis, the specificity of Xpert

against CCRS would decrease from 100% to 80.8%. The added false positive was from BCG vaccine infection. BCG was derived from *Mycobacterium bovis* and the sensitivity to anti-TB drugs is different. The BCG strain has low to medium level resistance to isoniazid and is intrinsically resistant to pyrazinamide which is much different from the routine MTB strains [20]. For clinicians, the possibility of a BCG infection should be carefully assessed if an Xpert-positive result is obtained from a young infant sample. The major symptoms of BCG infection for infants are abscess and lymphadenitis at the site of injection. If fever and hepatosplenomegaly presented, disseminated BCG infection should be considered. Confirmation of BCG infection should rely on the identification of *M. bovis* by PCR or sequencing methods.

Even though the sensitivity of smear microscopy for the diagnosis of childhood TB has remained less than 15% [3], advances have been made in the performance of the test for the rapid detection of MTB. There were two children with positive-smear, negative-culture & Xpert results, they all had clinical manifestations of probable active tuberculosis, and had good clinical response to ATT at follow-up visits, this result showed AFP smear was still irreplaceable. There were 48 children had positive culture results, accounting for 33.1%. Inclusion of the three tests improved the rate of definite tuberculosis detection to 48.8%, Xpert plus culture give a higher positive yield (47.2%) and Xpert plus AFB smear provided a better solution for the rapid detection of PTB with a sensitivity of 36.2%. Our study confirmed that combined use of smear, culture and Xpert was superior to the use of any of the three methods separately. When all three methods yielded negative results, we could improve the diagnosis by including the child's clinical and radiological findings. The development of better diagnostic methods for childhood tuberculosis clearly remains a major priority.

The Xpert MTB/RIF assay detects rifampicin-resistance mutations in the rpoB gene, based on the finding that 95% of all rifampicin-resistant M tuberculosis strains contain mutations localized within the 81bp core region of the bacterial RNA polymerase β subunit (rpoB) gene, which encodes the active site of the enzyme [21]. Rifampicin resistance is frequently associated with concomitant isoniazid (INH) resistance [22], so rifampicin resistance is strongly indicative of MDR tuberculosis and Xpert was used as the initial screen for MDR tuberculosis in this study. There were 11 cases of drug-resistant tuberculosis, 8 of whom were of MDR-TB, 3 were resistance to INH but sensitivity to RFP. The MTB/RIF assay was positive to all MDR-TB and negative to all rifampin-sensitive, isoniazid-resistant tuberculosis patients. It indicated Xpert can find out MDR-TB with high sensitivity and specificity.

Our study has some limitations. Firstly, it was a retrospective study and a single-center study an endpoint that we were unable to analyze due to our retrospective design. Secondly, Incorporation bias can arise when the unknown strain identification results influences the diagnosis of active tuberculosis.

Conclusion

Compared with the poor sensitivity of AFB smear and the long turnover time of culture, Xpert is a point-of-care diagnostic tool for rapid detection of childhood PTB with high sensitive and excellent accuracy for

RIF resistance. However, using Xpert or culture alone can lead to the missed diagnosis of childhood tuberculosis. The combined use of smear, culture and Xpert is superior to the use of any of the three methods alone.

Declarations

Abbreviations:

TB: Tuberculosis; PTB: pulmonary tuberculosis; ATT: anti-TB treatment; TST: tuberculin skin test; IGRA: interferon-gamma release assay; Xpert: Xpert® MTB/RIF assay (Cepheid, USA); DST: drug sensitivity test; EMR: electronic medical record; RTS: respiratory tract specimen; HIV: Human Immunodeficiency Virus; IS: induced sputum; NA: nasopharyngeal aspiration; GA: gastric aspiration; CCRS: composite clinical reference standard; PPV: positive predict value; NPV: negative predict value; CI: confidence interval.

Ethics approval and consent to participate:

This study is a retrospective study and the informed consents are not possible to acquire from participants' parental guardians. The tests performed in this study were routine clinical practice according to domestic practice guidelines and benefited the participants. The study procedure was approved by the Ethics committee of Shanghai Public Health Clinical Center and the informed consent is not required in this study (2019S02401).

Consent for publication:

The authors and their affiliations agreed to publish this study in *BMC Infectious Diseases.*

Availability of data and material:

Data and material information can be acquired via the corresponding author's email (tubercle@shphc.org.cn) according to domestic laws.

Competing interests:

The authors declare that there are no competing financial or none-financial interests with the funder of this study and other institutions.

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Author Contributions statement:

Lu Xia and *Xuhui Liu* contributed equally in conducting study and writing the original draft; *Shuihua Lu* and *Ping Liu* contributed in conceptualization, methodology and reviewing the original draft; *Xueqin Qian, Aimei Zhang, Tao Li, Xiuhong Xi,* and *Xiaoyong Fan* contributed in data curation, investigation and validation; *Yao Zhang* and *Yuhong Liu* provided the editing or analysis to the work.

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Tables

Due to technical limitations, Tables 1 - 6 are only available for download from the Supplementary Files section.

Figures

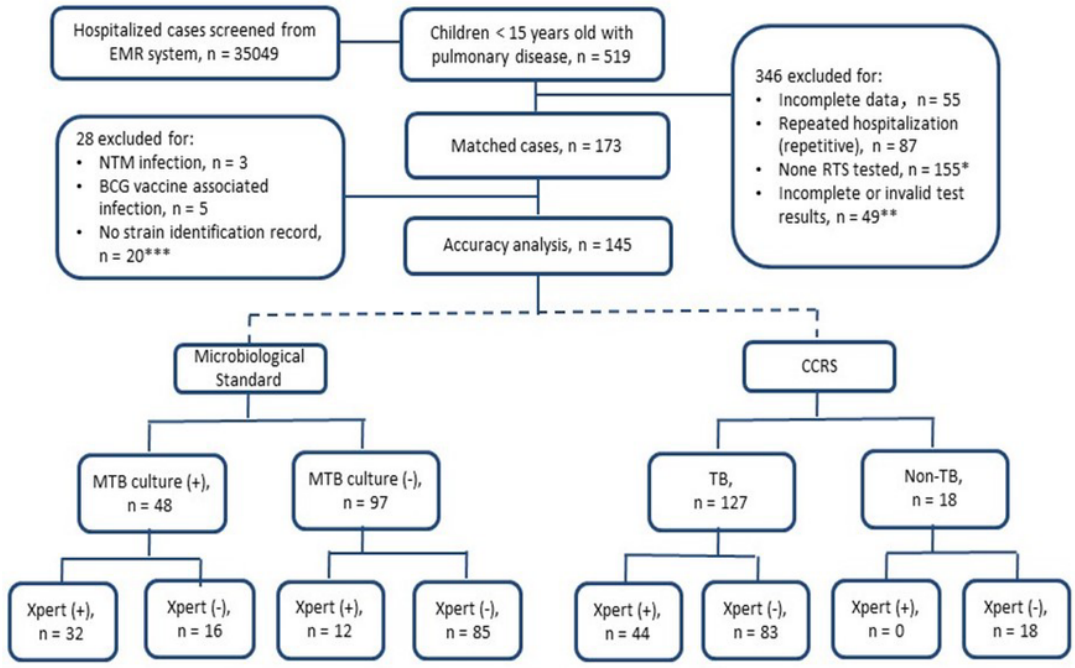

Figure 1

Flowchart of the participants. Legends: a) Of these cases, 45 were confirmed with TB before visiting our site therefore repetitive tests were not performed; 110 didn't accept invasive procedure or failed to obtain specimens. b) These include those with only one test done (Xpert, smear or culture), and those with invalid test results (Xpert error or culture contamination) thus test results were not matched. c) These cases were MPB 64 (-). NTM and BCG may result in false culture positive. The rest 20 cases were culture positive but MPB 64 (-), suspected with NTM but the stain identification was not performed.

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