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Original Article

Use of Xpert MTB/RIF for diagnosis of pediatric tuberculosis in Indonesia

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Abstract

Background The Xpert MTB/RIF assay demonstrated a better diagnostic value than sputum smear for TB in adults and children.

Objective To evaluate the use of Xpert MTB/RIF for TB diagnosis in children.

Methods We conducted a prospective study in Yogyakarta, Indonesia, involving 19 primary health centers (PHCs) and one provincial hospital. Children aged 0-14 years with suspected TB who visited the study sites were screened. Subjects underwent history-taking, physical examination, tuberculin skin test, chest X-ray, as well as sputum induction for Xpert MTB/RIF assay, sputum smear, and TB culture. The diagnosis of TB was made by doctors based on the results of investigations, as follows: certain TB (bacteriological confirmation), probable TB, and possible TB.

Results Of 80 subjects, 21 (26%) were diagnosed with TB disease (4 certain TB and 17 probable TB). Sputum induction was successfully performed in 79 children. None of the children had positive sputum smears. Mycobacterium tuberculosis was detected by Xpert MTB/RIF in 4 children, accounting for 5% of all children with suspected TB, or 19% among children with TB disease. The 4 Xpert MTB/RIF-positive subjects had severe TB disease and were rifampicin-sensitive.

Conclusion Xpert MTB/RIF may improve case finding among children with severe TB disease with negative sputum smear. **[Paediatr Indones. 2020;60:198-204; DOI: 10.14238/pi60.4.2020.198-204]**.

Keywords: *Xpert MTB/RIF; tuberculosis; children*

Identification of Mycobacterium tuberculosis in respiratory specimens is regarded as the gold standard of pulmonary TB diagnosis. While it is now the most important diagnostic approach in the era of increasing multidrug resistant TB, including in children, challenges remain due to difficulties in obtaining sputum specimens and the paucibacillary nature of TB in children.[1] The Xpert MTB/RIF assay, an automated molecular test using real-time polymerase chain reaction, provides several benefits such as simultaneously detecting the presence of Mycobacterium tuberculosis DNA and rifampicin sensitivity in less than 2 hours, and improved diagnostic value. The specificity of Xpert MTB/RIF compared to a clinical reference standard was at least 99%. In comparison to sputum smear, the Xpert MTB/RIF sensitivity was 37% higher on expectorated or induced sputum and 44% higher on gastric lavage

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specimens.[2,3] Despite low quality evidence, in 2014 the WHO recommended the use of Xpert MTB/RIF as the initial diagnostic test for pediatric TB.[3]

Indonesia ranked 3rd among the 22 countries with the highest TB burden, with an estimated 322,806 TB cases in 2014, which contributed 10% of total global cases.[4] Of these, only 7% of cases were under 15 years of age. The problem of underdiagnosis of pediatric TB has been acknowledged worldwide. Underdiagnosis is mainly observed at the primary health care level, which has limited resources for diagnosing TB. On the contrary, in urban areas of Indonesia pediatric TB tends to be overdiagnosed but underreported by doctors in private practice.[5,6] In line with the WHO recommendation and to improve case finding and diagnosis quality in children, the Indonesia National TB program recommended the use of Xpert MTB/RIF in children in 2015. We aimed to evaluate small-scale implementation of Xpert MTB/RIF for children in Yogyakarta, Indonesia, including the use of sputum induction method to collect sputum specimens in children.

Methods

We conducted a prospective study in Yogyakarta municipality from March to October 2015 in 19 primary health centers (PHCs). The PHC health workers were informed about a new recommendation to use Xpert MTB/RIF for pediatric TB. They were asked to screen children aged 0-14 years who visited the PHCs with TB symptoms or close contact to an infectious TB case, and to refer them to Dr. Sardjito Hospital, a provincial hospital, for further TB investigations. Tuberculosis symptoms included persistent cough (cough for 2 weeks or more that did not improve with antibiotics or anti-asthma treatment), fever (body temperature > 38°C for 2 weeks or more after common causes such as typhoid, urinary tract infection, or malaria had been excluded), and weight loss or failure to thrive. Screening of children with suspected TB was also done in the Department of Pediatrics Outpatient Clinic and Ward, Dr. Sarjito Hospital by a study pediatrician. Children suspected to have extrapulmonary TB (meningitis TB, miliary TB, etc.) were also screened by a study pediatrician and recruited into the study.

Subjects underwent history-taking, physical examination, nutritional assessment, tuberculin skin test (TST), chest X-ray (CXR), and sputum induction in the hospital. The sputum was collected for Xpert/MTB RIF assay, smear, and solid culture for Mycobacterium tuberculosis. A pediatrician collected cerebrospinal fluid from a child who was suspected to have TB meningitis. A trained study nurse performed TST by intradermally injecting 0.1 mL of 2 TU tuberculin purified protein derivate RT 23 in the volar aspect of the forearm. The TST results were assessed by measuring the transverse induration diameter at 72 hours, and were considered positive if the diameter was > 10 mm, regardless of BCG vaccination status. The CXR was performed in both antero-posterior and lateral views, and was interpreted by a radiologist and a pediatrician who were blinded to subjects' clinical information. In case of disagreement, a consensus was made by both radiologist and pediatrician.

A trained nurse attempted to collect sputum at least once using the sputum induction method. A second sputum specimen was obtained on the same day, a minimum of 4 hours after the first specimen was obtained. The following procedure was done according to standard protocol after 2-3 hours of fasting: the child received 200 ug salbutamol via jet nebulizer for 20 minutes to prevent bronchoconstriction, followed by 5 mL of 3% sterile saline via jet nebulizer for 20 minutes. The nurse then performed chest percussion over the anterior and posterior chest wall. If the child could not expectorate sputum unassisted, sputum was obtained by suctioning through the nasopharynx with a sterile mucus extractor of catheter size 6 or 7 as the child was coughing. The procedure was performed with continuous monitoring of pulse and oxygen saturation. If oxygen saturation fell below 92% then the procedure was halted. The sputum trap was sealed and the specimen was transported directly to the Microbiology Laboratory. A laboratory technician assessed the sputum macroscopically for quality. Good quality sputum was considered to have adequate volume (2 mL), the presence of mucoid or mucopurulent material, and no obvious food particles or other solid particulate in the sputum. Poor quality specimens were thin and watery (saliva). Another specimen (cerebrospinal fluid) was collected from a child with suspected TB meningitis.

The specimens were tested for acid fast bacilli (AFB), cultured for mycobacteria on Lowenstein-Jensen media, and used for Xpert MTB/RIF assay (*Cepheid,* Sunnyvale, California). The specimens were processed for Xpert MTB/RIF as follows: 1 mL of uncentrifuged specimen was mixed with the sample reagent (*Cepheid*) in a 1:2 ratio. The mixture was shaken and allowed to stand for 10 minutes, then shaken again and allowed to stand for another 5 minutes. Two mL of the mixture was put into an Xpert cartridge and inserted into the Xpert MTB/RIF machine (*Cepheid Inc.*). The result was obtained within 2 hours.

A study pediatrician established the diagnosis of TB disease based on the following criteria: certain TB (bacteriologic confirmation for Mycobacterium tuberculosis, either from the positive result of sputum smear, Xpert MTB/RIF, of sputum culture); probable TB (had at least one TB symptom, and CXR was consistent with intrathoracic TB or there was supportive evidence of extrapulmonary TB; and there was a positive clinical response to anti TB treatment); possible TB (had at least one TB symptom and either of the following: a positive clinical response to anti-TB treatment or CXR was consistent with intrathoracic TB). Latent TB infection was diagnosed in children with positive TST result in the absence of TB disease. Ethical approval of the study was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Indonesia. Informed consent was obtained from parents/guardians prior to the procedures. The data were analyzed descriptively as proportion or frequency for categorical variables and as mean or median for continuous variables. Statistical analysis was performed using *STATA software version 12* (*StataCorp*, College Station, Texas).

Results

A total of 106 children were screened and suspected as having TB disease in the PHCs (78 children) and the hospital (28 children) during the study period. Twenty children screened in the PHCs and referred did not visit the hospital due to parent refusal (12 children), having no time to visit the hospital during working hours (7 children), or further TB investigation being done in a private hospital (1 child). Six children from the PHCs were excluded because their symptoms were not consistent with TB (**Figure 1**). Hence, 80 children were included; their characteristics are presented in **Table 1**. Sixty (75%) children had TB exposure with 29 reporting contacts, 5 children had positive TST, and 26 had both contact and positive TST. Of those

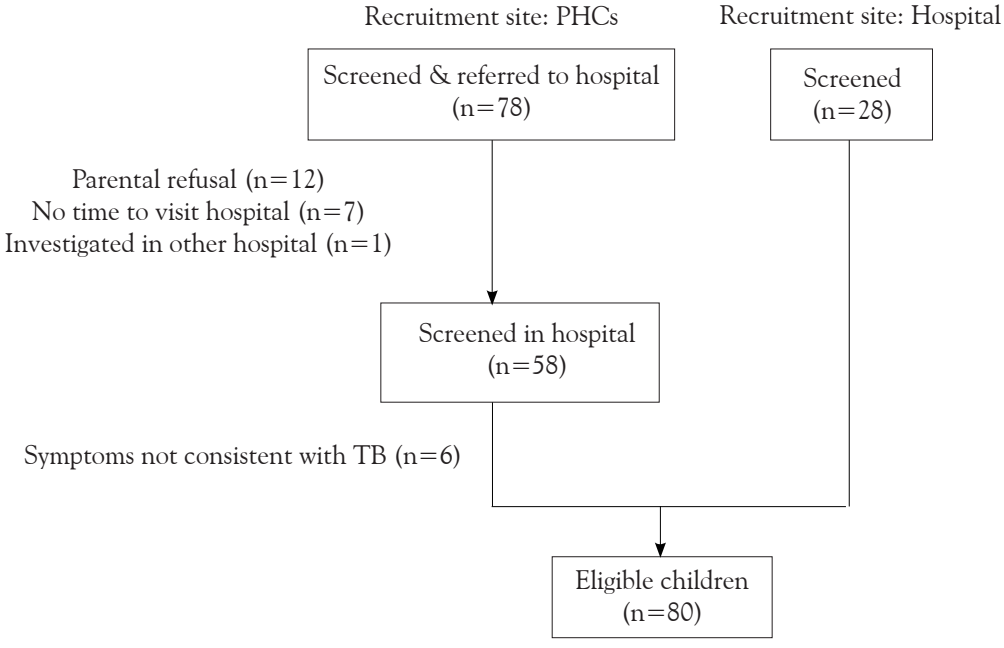

Figure 1. Screening of children with suspected TB

Table 1. Characteristics of subjects

Characteristics	(N = 80)
Male sex, n (%)	50 (62.5)
Median age, years (IQR)	4.4 (1.9; 8.5)
Age < 5 years, n (%)	43 (53.8)
Nutritional status, n (%)	
Well-nourished	43 (53.7)
Malnourished	30 (37.5)
Severely malnourished	7 (8.8)
Referral from PHC, n (%)	52 (65)
BCG vaccination, n (%)	79 (99)
Close contact with a TB case, n (%)	55 (68.8)
HIV (+), n (%)	2 (2.5)

with contact, 9 were multidrugs resistant (MDR) TB contacts. Two children had been diagnosed with HIV infection before screening for TB. HIV status of the other subjects was unknown, as HIV test was not performed in this study. Almost all children had received BCG vaccination, but BCG status was unknown for one child (no BCG scar, no immunization record, and the parent did not remember).

Of 80 subjects, 67 (83.3%) had TB symptoms. The 13 children without TB symptoms were included due to their past close contact with a TB case. Most children presented with symptoms of fever, cough, and weight loss. Other symptoms documented were seizure (1 child), stridor (1 child), and dyspnea (5 children). Tuberculin skin test was positive in 31 (38.8%) children. Miliary pattern on CXR was identified in 2 children, whereas pleural effusion and consolidation were identified in one child each.

One hundred thirty-two sputum specimens were collected from 79 children and one cerebrospinal specimen was collected from one child. Fifty-three children provided two sputum specimens each, whereas 26 children only provided one specimen each. The most common reason for not collecting the second sputum was parent refusal. Adverse event of epistaxis occurred in 2 children. Macroscopic assessment of sputum specimens revealed that only 45 (34.1%) specimens were good quality. None of the specimens showed positive smear results.

Mycobacterium tuberculosis was detected by Xpert MTB/RIF assay in 4 (5%) subjects (in three sputum specimens and one cerebrospinal fluid specimen). This accounted for 6% of the 67 children with TB symptoms or 19% of 21 children with TB disease. Among good quality sputum specimens, the proportion of positive Xpert MTB/RIF was 6.7% (3/45). None was resistant to rifampicin. All of the positive Xpert/MTB RIF subjects had negative sputum smears. Solid culture of *Mycobacterium tuberculosis* was grown in one subject. None of the asymptomatic subjects had positive results of microbiological investigations.

Based on the investigation results, 21 (26%) children were diagnosed with TB disease (4 certain TB and 17 probable TB) and 14 (17%) children with latent TB infection. Of 21 children with TB disease, 5 had severe TB, 2 had miliary TB, 1 had TB meningitis, 1 had TB HIV, and 1 had pleural effusion. The characteristics and investigation results of the 4 children with positive Xpert MTB/RIF are shown in **Table 2**. None of the children recruited from the PHCs had positive Xpert MTB/RIF results. The 4 children with positive Xpert MTB/RIF results had severe TB and the following conditions: TB-HIV (1), TB meningitis (1), miliary TB (1), and laryngeal and miliary TB (1).

Discussion

Our study shows that Xpert MTB/RIF assay identified more TB cases than sputum smears. None of the

Table 2. Characteristics of the 4 subjects with positive Xpert MTB/RIF results

No	Gender	Age	Site	Symptoms	Close contact	CXR	TST, mm	Culture	HIV
1	M	9 yrs	Hospital	Cough, fever, dyspnea	Yes	Consolidation	0	No growth	Positive
2	F	7 mo	Hospital	Fever, cough, stridor, weight loss	Yes	Miliary pattern	7	No growth	Not tested
3	M	14	Hospital	Fever, seizures	Yes	Hilar	0	Growth	Not tested
4	M	7 mo	Hospital	Fever, cough	Yes	Miliary pattern	0	No growth	Not tested

smear specimens was positive for TB, whereas Xpert MTB/RIF was positive in 5% of children who were suspected to have TB. Among symptomatic children, the proportion was 6%, and increased to 19% in children with TB disease. A meta-analysis of 15 studies on the use of Xpert MTB/RIF in children reported an average positive result of 11%, which ranged between 1% in Malawi to 45% in Vietnam.[2] Xpert MTB/RIF has 35-44% higher sensitivity compared to sputum smears. Compared to culture, the sensitivity of Xpert MTB/RIF ranged from 25% to 100%, with a pooled sensitivity of 62% and specificity of 93%-100%, with a pooled sensitivity of 98%.[2] Our study was not designed to determine sensitivity and specificity of Xpert MTB/RIF, but if it is compared to culture as the gold standard, the sensitivity of Xpert MTB/RIF was 100% with a specificity of 98%.

The majority of studies on Xpert MTB/RIF in children were hospital- based studies that involved inpatient children.[7-11] One study from South Africa included outpatients from a primary care setting and documented positive Xpert MTB/RIF results in 7% of 384 children.[12] Our study recruited children from both community (PHCs) and hospital (both inpatient and outpatient) settings, but the positive Xpert MTB/RIF results were only identified in children who were hospitalized with severe TB. Higher proportions of positive Xpert MTB/RIF among hospitalized children compared to outpatients were shown by a stratified analysis of a meta-analysis.[2] Hospitalized children often have severe TB with higher bacterial loads which are more likely to have positive smears and cultures.[13] The performance of Xpert MTB/RIF was associated with smear status, in which sensitivity of Xpert MTB/RIF was greater in individuals who had higher mycobacterial load.[3,14] Xpert MTB/RIF detected 96% of smear-positive children from samples of expectorated or induced sputum, compared to 55% of smear-negative culture positive children with the same specimen.[3] The role of Xpert MTB/RIF for TB diagnosis among child contacts has also been evaluated.[15] The sensitivity of Xpert MTB/RIF was higher than sputum smear. Nevertheless, compared to hospital-based studies the sensitivity of Xpert MTB/RIF compared to culture in the context of contact investigation was lower.

Sputum induction was initially used in the late 1980s to collect sputum specimen in HIV-infected patients to identify *Pneumocystis jirroveci*.[16] In the last decade, this method was recommended to collect sputum in children for TB diagnosis and has been shown to be safe, feasible, and improve case finding.[17-19] This method is simpler than gastric lavage and can be performed in an outpatient clinic. Furthermore, a previous study reported that one sputum specimen from sputum induction was as effective as two sputum specimens from gastric lavage.[20] In Indonesia this method has not been routinely performed. Sputum induction in our study was conducted in the hospital by trained nurses. In general, it was feasible for sputum induction to be performed in our study, with only 2 children experiencing epistaxis. Nevertheless, only 34.1% of sputum specimens were good quality. This could have been due to an improper sputum collection technique, an uncooperative child, or the child not able to produce sputum. Most children in this study were aged less than 5 years, and 50% of them were less than 2 years of age. In addition, most children in this study had mild symptoms, in whom sputum production was minimal or absent.

Bacteriological confirmation for the diagnosis of TB in children is now recommended by the WHO.[21] Not only does it improve the quality of diagnosis, but also is important in the era of increasing cases of multidrug resistant TB. The majority of previous studies, including our study, processed the specimens for bacteriological test at university hospital laboratories. Nevertheless, sputum collection should be able to be done at the primary health care level, making hospital referral unnecessary. Our study documented that a quarter of children from PHCs did not come to the hospital for further investigations, which may have contributed to underdiagnosis. Despite ease and feasibility, operational and logistic issues should be considered for implementation of sputum induction in PHCs.[22] These include lack of skill administering the procedure, lack of facilities (hypertonic saline, suction machine, mucous extractor), specimen transfer to a laboratory, and time constraints of busy health workers. In addition, a cost-effectiveness study of sputum induction as well as Xpert MTB/RIF for TB diagnosis in children is required.

In conclusion, Xpert MTB/RIF improves case finding and quality of pediatric TB diagnosis. Sputum induction is a feasible method to collect sputum in children, but more training is required to obtain good quality specimens in PHCs and hospitals alike.

Conflict of Interest

None declared.

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References

1. Marais BJ, Pai M. New approaches and emerging technologies in the diagnosis of childhood tuberculosis. Paediatr Respir Rev. 2007;8:124-33. DOI: 10.1016/j.prrv.2007.04.002.
2. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, *et al.* Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med. 2015;3:451-61. DOI: 10.1016/S2213-2600(15)00095-8.
3. WHO. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update. Geneva, Switzerland: WHO; 2013.
4. WHO. Global tuberculosis report. Geneva: WHO; 2015.
5. Coghlan R, Gardiner E, Amanullah F, Ihekweazu C, Triasih R, Grzemska M, *et al.* Understanding market size and reporting gaps for paediatric TB in Indonesia, Nigeria and Pakistan: supporting improved treatment of childhood TB in the advent of new medicines. PLoS One. 2015;10:e0138323. DOI: 10.1371/journal.pone.0138323.
6. Lestari T, Probandari A, Hurtig AK, Utarini A. High caseload of childhood tuberculosis in hospitals on Java Island, Indonesia: a cross sectional study. BMC Public Health. 2011;11:784. DOI: 10.1186/1471-2458-11-784.
7. Bates M, O'Grady J, Maeurer M, Tembo J, Chilukutu L, Chabala C, *et al.* Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: a prospective descriptive study. Lancet Infect Dis. 2013;13:36-42. DOI: 10.1016/S1473-3099(12)70245-1.
8. Chisti MJ, Graham SM, Duke T, Ahmed T, Ashraf H, Faruque AS, *et al.* A prospective study of the prevalence of tuberculosis and bacteraemia in Bangladeshi children with severe malnutrition and pneumonia including an evaluation of Xpert MTB/RIF assay. PLoS One. 2014;9:e93776.
9. LaCourse SM, Chester FM, Preidis G, McCrary LM, Arscott-Mills T, Maliwichi M, *et al.* Use of Xpert for the diagnosis of pulmonary tuberculosis in severely malnourished hospitalized Malawian children. Pediatr Infect Dis J. 2014;33:1200-2. DOI: 10.1371/journal.pone.0093776.
10. Nicol MP, Workman L, Isaacs W, Munro J, Black F, Eley B, *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. DOI: 10.1016/S1473-3099(11)70167-0.
11. Pang Y, Wang Y, Zhao S, Liu J, Zhao Y, Li H. Evaluation of the Xpert MTB/RIF assay in gastric lavage aspirates for diagnosis of smear-negative childhood pulmonary tuberculosis. Pediatr Infect Dis J. 2014;33:1047-51.
12. Zar HJ, Workman L, Isaacs W, Dheda K, Zemanay W, Nicol MP. Rapid diagnosis of pulmonary tuberculosis in African children in a primary care setting by use of Xpert MTB/RIF on respiratory specimens: a prospective study. Lancet Glob Health. 2013;1:e97-104. DOI: 10.1097/INF.0000000000000403.
13. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The bacteriologic yield in children with intrathoracic tuberculosis. Clin Infect Dis. 2006;42:e69-71. DOI: 10.1086/502652.
14. Geleta DA, Megerssa YC, Gudeta AN, Akalu GT, Debele MT, Tulu KD. Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in sputum specimens in remote health care facility. BMC Microbiol. 2015;15:220. DOI: 10.1186/s12866-015-0566-6.
15. Togun TO, Egere U, Sillah AK, Ayorinde A, Mendy F, Tientcheu L, *et al.* Contribution of Xpert® MTB/RIF to the diagnosis of pulmonary tuberculosis among TB-exposed children in The Gambia. Int J Tuberc Lung Dis. 2015;19:1091-7. DOI: 10.5588/ijtld.15.0228.
16. Pitchenik AE, Ganjei P, Torres A, Evans DA, Rubin E, Baier H. Sputum examination for the diagnosis of Pneumocystis carinii pneumonia in the acquired immunodeficiency syndrome. Am Rev Respir Dis. 1986;133:226-9.
17. Joel DR, Steenhoff AP, Mullan PC, Phelps BR, Tolle MA, Ho-Foster A, *et al.* Diagnosis of paediatric tuberculosis using sputum induction in Botswana: programme description and findings. Int J Tuberc Lung Dis. 2014;18:328-34. DOI: 10.5588/ijtld.13.0243.
18. Planting NS, Visser GL, Nicol MP, Workman L, Isaacs W, Zar HJ. Safety and efficacy of induced sputum in young children hospitalised with suspected pulmonary tuberculosis. Int J

Tuberc Lung Dis. 2014;18:8-12. DOI: 10.5588/ijtld.13.0132.

19. Geldenhuys HD, Kleynhans W, Buckerfield N, Tameris M, Gonzalez Y, Mahomed H, *et al.* Safety and tolerability of sputum induction in adolescents and adults with suspected pulmonary tuberculosis. Eur J Clin Microbiol Infect Dis. 2012;31:529-37. DOI: 10.1007/s10096-011-1344-5.
20. 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 p.rospective study. Lancet. 2005;365:130-4. DOI: 10.1016/S0140-6736(05)17702-2.
21. WHO. Guidance for national tuberculosis programmes on the management of tuberculosis in children. 2nd ed. Geneva: WHO; 2014.
22. Moore HA, Apolles P, de Villiers PJ, Zar HJ. Sputum induction for microbiological diagnosis of childhood pulmonary tuberculosis in a community setting. Int J Tuberc Lung Dis. 2011;15:1185-90. DOI: 10.5588/ijtld.10.0681.