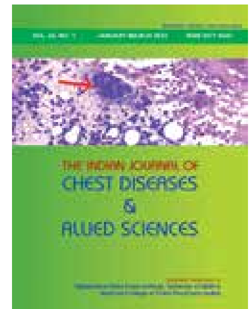
Original Article

Role of CBNAAT in the Diagnosis of Extra-Pulmonary Tuberculosis: Experience from a Tertiary Care Hospital in India



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ABBREVIATIONS USED IN THIS ARTICLE

MTB = *Mycobacterium tuberculosis*
RIF = Resistance of rifampin
CBNAAT = Cartridge-based nucleic acid amplification test
DMC = Designated Microscopy Centre
AFB = Acid-fast bacilli
TB = Tuberculosis
CRS = Composite reference standard
HIV = Human immunodeficiency virus
EPTB = Extra-pulmonary TB
CI = Confidence interval
LJ = Lowenstein-Jensen
ICMR = Indian Council of Medical Research
PPV = Positive predictive value
NPV = Negative predictive value
LNA = Lymph node aspirate
CSF = Cerebrospinal fluid
WHO = World Health Organization

Abstract

Background. Xpert-MTB/RIF assay or Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) helps in rapid diagnosis of tuberculosis (TB).

Methods. Specific samples were collected and carried to Regional Medical Research Centre where these were taken up for CBNAAT and culture in Lowenstein-Jensen media. Appropriate samples were sent to the Designated Microscopy Centre (DMC) of our institute for acid-fast bacilli (AFB) smear examination. Diagnostic measures, such as sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of Xpert-MTB/RIF were reported considering mycobacterial culture and a composite reference standard (CRS) as Gold standard.

Results. We studied 335 samples. Lymph node fine needle aspirate was the most common sample (32.5%) followed by pleural fluid (29.3%). The overall sensitivity and specificity of Xpert-MTB/RIF was determined to be 26.5% (95% CI [confidence interval] 20.8–32.8) and 100% (95% CI 96.8–100), respectively. The sensitivity and specificity of CBNAAT in relation to mycobacterial culture, however, was 78.8% (95% CI 61.1–91.0) and 89.1% (95% CI 85–92.4), respectively. Both were highest for pus, cerebrospinal fluid and lymph node fine needle aspirate samples.

Conclusions. Xpert-MTB/RIF may be useful for samples, like cold abscess and lymph node fine needle aspirate or biopsy specimens. However, its routine use in case of serosal fluids is not recommended because of its lower sensitivity.

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Introduction

Tuberculosis (TB) is a leading cause of mortality at present with an estimated 1.3 million deaths among human immunodeficiency virus (HIV)-negative people.[1] Extra-pulmonary TB (EPTB) has been defined as an infection by *Mycobacterium tuberculosis* which affects tissues and organs outside the pulmonary parenchyma.[2] The burden of EPTB ranges from 15% to 20% among HIV-negative patients. It rises to 40% to 50% of new TB cases in HIV-positive people.[3] EPTB usually causes symptoms specific to the organ involved; this along with the lower incidence of disease raises a diagnostic dilemma.

The diversity of sample types and difficulty in obtaining adequate tissue for analyses, along with unavailability of a rigorous Gold standard for comparison makes the diagnosis of EPTB challenging. The clinical samples obtained from relatively inaccessible sites, moreover, may be paucibacillary, decreasing the sensitivity of diagnostic tests. Such complications in obtaining a diagnosis increase the morbidity and mortality.[3]

The Gene-Xpert-MTB/RIF or cartridge-based nucleic acid amplification test (CBNAAT) is a semi-automated, cartridge-based nucleic acid amplification assay, that amplifies and detects mycobacterial DNA (deoxyribonucleic acid) using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). The results are available within 100 minutes. Although developed to test sputum samples in pulmonary tubercular infections, it is currently being recommended for the diagnosis of EPTB.

The evidence base for the role of CBNAAT in the diagnosis of EPTB is comparatively weak and many more studies assessing a variety of clinical samples other than sputum are, therefore, needed. Although the test has a positive profile in recent studies[4], the advantages of having a rapid test for EPTB must be weighed against the accuracy of the test and the possible harms from mis-diagnosis when considering the use of this test.

This study aimed at evaluating the role of CBNAAT in the diagnosis of EPTB and compared its efficacy with other diagnostic modalities, like mycobacterial culture and a composite reference standard (CRS).

Material and Methods

All individuals with presumptive EPTB who visited the out-patient department of our Institute and/or admitted during the period October 2017 to June 2019 were included in the study. *A presumptive case* was defined as "a patient with symptoms and signs of EPTB who needs to be investigated".[5] All patients with tuberculous pleural effusion, ascites, pericardial effusion, lymphadenopathy, cold abscess, meningitis and genito-urinary TB were included in the study after an alternative diagnosis had been ruled out.

All patients who were on anti-TB treatment for more than one month or those who did not give consent or were detected to be HIV positive were excluded from the study. The study was approved by the Institutional Ethics Committee. A written informed consent was obtained from all the patients at the time of enrolment and a patient information sheet was provided in Oriya and English.

All the patients were evaluated on the basis of detailed clinical history, physical examination and routine blood investigations, which were recorded in a standardised proforma. Specific samples from appropriate sites, viz, pleural fluid, peritoneal fluid, pericardial fluid, lymph node aspirate, pus, cerebrospinal fluid, endometrial curettage, bone tissue, synovial fluid, biopsy specimens were collected from the patients in two sterile containers, one of which was carried to the Regional Medical Research Centre (ICMR) through cold chain where it was processed and taken up for CBNAAT and culture in Lowenstein-Jensen (LJ) medium. One mL of the sample was put in the Xpert/RIF cartridge (Cepheid, USA); no decontamination or centrifugation was performed. The remaining sample was decontaminated using NALC-NaOH and centrifuged. No decontamination was, however, done for cerebrospinal fluid (CSF) samples. The precipitate was put for mycobacterial culture in LJ medium.

The other container was sent to the Designated Microscopy Centre (DMC) our institute for acid-fast bacilli (AFB) smear examination using Ziehl-Neelsen technique. The growth of mycobacteria on culture medium was noted at the end of two weeks and every week thereafter. The final results were reported at the end of eight weeks.

The results of CBNAAT were compared with the AFB smear, mycobacterial culture and CRS. The CRS was formulated including parameters, like clinical presentation, radiology, histology and cytology reports (for biopsy samples and aspirates, respectively), biochemical tests, such as adenosine deaminase levels (for pleural fluid, ascitic fluid, pericardial fluid and CSF).

Statistical Analysis

The data were entered in Microsoft Excel spread-sheet. Comparison of means was done using Fischer's exact 't' test (with sample size less than 40) or quantify agreement with Kappa value (sample size more than 40). The Kappa value <0.4 was considered as poor agreement

while values >0.7 were considered a strong agreement. Chi-square test was used to study association and a P value <0.05 was considered significant.The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were reported. Statistical analysis was done using Stata13.1.

Results

A total of 335 cases were included in the study; 182 (54.3%) were males (male:female = 1.19:1). Maximum number of patients [120 (35.8%)] were within the age group of <20 years (Figure 1). Male patients (mean age 38.2 ±22.2 years; 95% CI=34.9 to 41.4) were significantly older compared to female patients (mean age 32.5 ±18.4 years; 95% CI=29.6 to 35.5) (P=0.013).

Lymph node aspirates were the most commonly collected specimens [109 samples (32.5%)] followed by pleural fluid [98 samples (29.3%)] (Figure 2). The sensitivity of CBNAAT for lymph node fine needle aspiration samples in comparison with LJ culture was 75.0% and specificity was 78.5% (κ=0.38; poor agreement). The sensitivity of CBNAAT for cold abscess samples in comparison with LJ culture was 100% and specificity was 86.2% (κ=0.79; strong agreement) (Table).

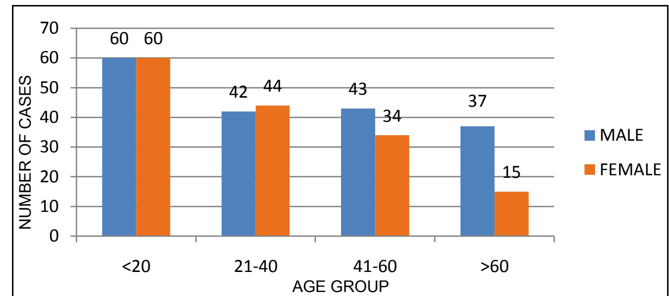

Figure 1. Age and gender distribution of the study patients (N=335).

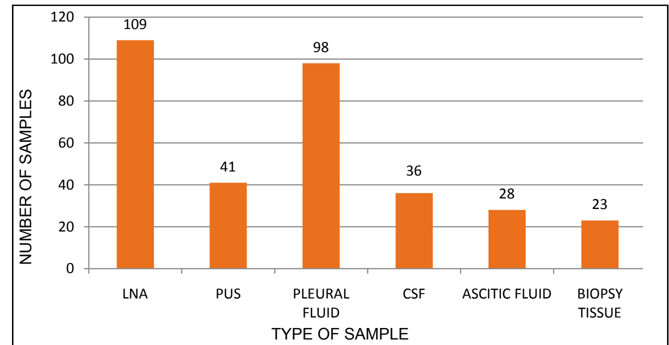

Figure 2. Sample distribution of the study patients (N=335).

Table Performance of Xpert MTB/RIF with culture and composite reference standard

Sample	LNA (N=109)	Pus (N=41)	Pleural Fluid (N=98)	CSF (N=36)	Ascitic Fluid (N=28)	Tissue Biopsy (N=23)	Overall (N=335)
Sensitivity							
LJ culture	75 (47.6–92.7)	100 (73.5–100)	0 (0–84.2)	100 (2.5–100)		50 (1.3–98.7)	78.8 (61.1–91.0)
CRS	33.0 (23.8–43.3)	55.2 (35.7–73.6)	5.9 (1.6–14.4)	22.2 (2.8–60.0)	11.1 (0.3–48.3)	36.4 (10.9–69.2)	26.5 (20.8–32.8)
Specificity							
LJ culture	78.5 (68.8–86.3)	86.2 (68.3–96.1)	95.8 (89.7–98.9)	97.1 (85.1–99.9)	96.4 (81.7–99.9)	85.7 (63.7–97.0)	89.1 (85–92.4)
CRS	100 (73.5–100)	100 (73.5–100)	100 (88.4–100)	100 (87.2–100)	100 (82.4–100)	100 (73.5–100)	100 (96.8–100)
PPV							
LJ culture	37.5 (27.1–49.2)	75 (54.7–88.2)	0	50 (12.7–87.3)	0	25 (5.5–65.5)	44.1 (35.3–53.2)
CRS	100	100	100	100	100	100	100
NPV							
LJ culture	94.8 (88.8–97.7)	100	97.9 (97.9–98.0)	100	100	94.7 (81.7–98.6)	97.5 (95.2–98.7)
CRS	15.6 (13.8–17.5)	48 (38.1–58.0)	31.9 (30.6–33.2)	79.4 (73.1–84.5)	70.4 (65.3–75.0)	63.2 (52.3–72.8)	40.6 (38.7–42.5)
Diagnostic accuracy							
LJ culture	78.0 (69.0–85.4)	90.2 (76.9–97.3)	93.9 (87.2–97.7)	97.2 (85.5–99.9)	96.4 (81.7–99.9)	82.6 (61.2–95.1)	88.1 (84.1–91.3)
CRS	40.4 (31.1–50.2)	68.3 (51.9–81.9)	34.7 (25.4–45.0)	80.6 (64.0–91.8)	71.4 (51.3–86.8)	69.6 (47.1–86.8)	51.0 (45.6–56.5)

**Data presented as percentage (95% CI) unless otherwise stated*

The CBNAAT could not detect mycobacterial DNA in any of the pleural fluid and ascitic fluid samples, and thus, the sensitivity was nil in comparison to culture; the specificity in both the cases, however, was high (κ=0.02; poor agreement) and 96.4% (P=1.00) respectively. The sensitivity of CBNAAT for CSF samples in comparison with LJ culture was 100% and specificity was 97.1% (P=0.05).

The sensitivity of CBNAAT for biopsy tissue samples in comparison with LJ culture was 50% and specificity was 85.7% (P=0.32). Pooling all the results of CBNAAT together, the test was positive in 33 (9.9%) cases and negative in 7 (2.1%) cases. The overall sensitivity of CBNAAT in comparison with LJ culture was 78.8% and specificity was 89.1% (χ^2=89.796; P<0.0001) (Table).

The sensitivity of CBNAAT for lymph node samples in comparison with the CRS was 33.0% and specificity was 100% (κ=0.09; poor agreement). The sensitivity of CBNAAT for cold abscess samples was 55.2% (95% CI=35.7–73.6%) and specificity was 100% (κ=0.42; fair agreement) (Table). The sensitivity of CBNAAT for pleural fluid samples in comparison to CRS was 5.9% and specificity was 100% (κ=0.04; poor agreement). The results were similar in case of ascitic fluid (P=0.32) (Table).

The sensitivity of CBNAAT for CSF samples in comparison with clinical diagnosis was 22.2% (95% CI=2.8–60.0) and specificity was 100% (95% CI=87.2–100) (P=0.04). The sensitivity of CBNAAT for biopsy tissue samples in comparison with clinical diagnosis was 36.4% (95% CI=10.9–69.2) and specificity was 100% (95% CI=73.5–100) (P=0.04).

Discussion

In our study male to female ratio was 1.19:1. Although the WHO reported the global male: female (M:F) ratio for notifications to be 1.8[2], a recent study in Karnataka[6] reported a male to female ratio of 1.06:1 among EPTB cases in the study mapping the patterns and trends of the disease. Another study[7] reported a male to female ratio of 1.17:1. Similar results have also been obtained from Ghana in a retrospective study.[8] In most low-income countries, twice as many men are notified with TB as women.[9] Socio-economic and cultural factors leading to barriers in accessing health-care facilities may cause under-notification in women. The increasing awareness of the disease and attempts at reducing the stigma associated with it has definitely improved the reporting of the disease among women in recent times. Studies[10,11] have reported higher reporting and incidence of EPTB among females.

EPTB is primarily a disease of the young. Approximately one-third of the world's population is infected with TB, and the greatest burden of disease occurs in 15–49 years old.[9] In the present study, majority of the patients belonged to the age group of ≤20 years. Prevalence of TB is similar in males and females until adolescence, after which it increases in males. In high prevalence countries, however, women of reproductive age have higher rates of progression to disease than men in this age group. This along with aggressive case reporting could explain the significantly lower age of disease detection among females (P=0.013). Our results could also be due to the fact that most of the patients aged ≤20 years had evidence of malnutrition (BMI [Body Mass Index] 17.6±2.8Kg/m^2) that could predispose to the development of the disease in previously exposed cases (75% of the cases had a history of contact).

The study population mostly included cases of lymphadenopathy (32.5%) followed by pleural effusion (29.3%). This is in accordance with the national data which states that peripheral lymph node involvement is the commonest form of EPTB and the cervical region is the most frequently affected site.[10,11] Similar observations have been reported in other studies.[6,7]

All the samples in the present study were subjected to CBNAAT and mycobacterial culture simultaneously. The sensitivity was detected highest for pus samples and CSF and the specificity was high for CSF, ascitic fluid, pleural fluid and pus. A study[12] examined multiple databases to determine the accuracy of Xpert compared with culture and CRS and identified 18 studies with 4461 samples. In lymph node tissues or aspirates, Xpert pooled sensitivity was 83.1% (95% CI 71.4–90.7) and specificity was 93.6% (95% CI 87.9–96.8) *versus* culture. A systematic literature search of seven electronic databases[13] reported the pooled sensitivity and specificity of CBNAAT in lymph node samples to be 1.0 (0.7–1.0) and 0.9 (0.7–1.0), respectively. Another study[14] reported a sensitivity of 88% (95% CI 82–92.5) for lymph node aspirates. In a study by Lawn *et al*[15] sensitivity exceeded 75% for tissue biopsies and fine-needle aspirates (88.3%; 95% CI: 82–95). The lower sensitivity and specificity reported in the present study could be attributed to the lower sample size; also all previous studies included lymph node fine needle aspiration and biopsy samples as one entity while we considered biopsy samples separately.

A study[12] reported the pooled sensitivity for CSF samples to be 80.5% (95% CI 59.0–92.2) and pooled specificity 97.8% (95% CI 95.2–99.0) against culture. Tortolli *et al*[16] reported a pooled sensitivity of 84.6% (95% CI: 65–104) and specificity of 98.3%. The sensitivity for CSF sample in our study varied widely between 2.5% to 100%, while the specificity was 97.1%.

In case of pleural fluid most studies have reported a low sensitivity of CBNAAT. Studies[13,16] have reported a sensitivity of less than 40%. One study[15] reported a sensitivity of 44.4% while another study[14] reported the sensitivity to be 40% (95% CI: 31.1–50.9). All these studies have reported a very high specificity, similar to our study. The sensitivity, in our study, however, was nil. This could be attributed to the paucibacillary nature of the disease and the fact that pleural biopsy is actually the sample of choice for the diagnosis of pleural TB. Due to similar reasons, sensitivity in cases of ascitic fluid was undetermined, whereas specificity was high.

In case of biopsy specimens, however, the sensitivity varied between studies. One study[17] reported a sensitivity of 69% for CBNAAT. However, other studies reported sensitivities of 86.6% (95% CI: 79–94)[16] and 0.88% (95% CI: 77–95).[13] The specificity reported by these two tests were, however, high (95.5% and 0.98, respectively). Our study reported a sensitivity of 50.0% (95% CI: 1.3–98.7) and a specificity of 85.7% (95% CI: 63.7–97).

The sensitivity and specificity for pus samples from the analysis of 195 samples was 85.1% (95% CI: 75–95) and 94.6%, respectively.[16] In a study[14] that included 153 pus samples reported a sensitivity of 95% (87.4–98.4). We had included 41 pus samples and the sensitivity against culture gold standard was determined to be 100% (95% CI: 73.5–100) while the specificity was 86.2% (95% CI: 68.3–96.1).

The pooled sensitivity and specificity of CBNAAT against mycobacterial culture was 78.8% (95% CI: 61.1–91.0) and 89.1% (95% CI: 85–92.4). A study[17] reported an overall sensitivity 77.3%, and specificity of 98.2% which was similar to our study. Another study[18] reported a sensitivity of 73% (95% CI: 68–77) for CBNAAT against culture gold standard.

Comparing the results of CBNAAT with CRS, the sensitivity was least for pleural fluid and ascitic fluid. The sensitivity was similar for lymph node fine needle aspiration and biopsy specimens. A meta-analysis[12] included five studies to assess Xpert in lymph node samples against CRS showed a pooled sensitivity of 81.2% (95% CI 72.4–87.7). The specificity improved to 99.1% (95% CI: 94.5–99.9), in comparison to the culture reference standard. Results of our study of 110 lymph node fine needle aspiration samples showed the pooled sensitivity and specificity to be 33.0% (95% CI: 23.8–43.3) and 100% (95% CI: 73.5–100), respectively. The lower sensitivity could be attributed to a smaller sample size and exclusion of immunocompromised cases.

A meta-analysis[12] of six studies with 598 samples evaluated Xpert in pleural fluid *versus* CRS. In case of pleural effusions, compared with the pooled estimate with culture as the reference standard, the CRS subgroup yielded a lower sensitivity (21.4%, 95 CI 8.8–33.9) with a slightly higher specificity of 100% (95% CI 99.4–100). A low sensitivity was observed by us as well in 98 study samples.

In case of CSF, we have included 35 samples and the sensitivity and specificity was found to be 22.2% (95% CI: 2.8–60) and 100% (95% CI: 87.2–100), respectively. A meta-analysis[12] included five studies (711 samples) assessing Xpert in CSF samples *versus* a CRS found variable sensitivity (20%–86%) with a pooled sensitivity of 62.8% (95% CI 47.7–75.8) and specificity of 98.8% (95% CI 95.7–100). Another study[18] also reported a sensitivity of only 29% (95% CI: 8–65) in case of CSF. None of the CSF samples were detected positive by CBNAAT in a study.[17] A study from India[14] reported a sensitivity of 33% to 100% for CSF samples against CRS.

In case of 41 pus samples we observed a sensitivity and specificity of CBNAATof 55.2% (95% CI: 35.7–73.6) and 100% (95% CI: 73.5–100), respectively. Another study[18] reported a sensitivity of 64% for CBNAAT in case of pus samples. Higher sensitivities have been reported in other studies (85.1%; 95% CI: 75–95)[16]; (86-94).[14] However, both these studies[14,18], had a large sample size and included samples from immunocompromised cases.

Similar results were also reported for biopsy samples. However, in 23 biopsy samples from various sites in the present study reported a sensitivity and specificity of 36.4% (95% CI: 10.9–69.2) and 100% (95% CI: 73.54–100), respectively. The pooled sensitivity and specificity of CBNAAT against the CRS in our study was calculated to be 26.5% (95% CI: 20.8–32.8) and 100% (95% CI: 96.8–100), respectively. The low sensitivity in our study could be attributed to the higher number of pleural fluid and ascitic fluid samples. The sensitivity of the Xpert test in the study by Vadwai *et al*[18] against the CRS was found to be 81% (228/283) specimens. Their study, however, had a higher proportion of biopsy and cold abscess samples than various body fluids, which might account for the higher sensitivity. Similarly, a meta-analysis reported the overall sensitivity and specificity of Xpert to be 81.3% and 99.8% against clinical diagnosis.

Comparing to clinical diagnosis of the disease, CBNAAT was positive in 59 (17.6%) cases and negative in 164 (49%) cases. The overall sensitivity of CBNAAT in comparison to CRS was 26.5% (95% CI=20.8–32.8) and specificity was 100% (95% CI=96.8–100) (κ= 0.193; poor agreement).

Conclusions

Cartridge-based nucleic acid amplification test has already been established for the diagnosis of pulmonary TB by WHO. The role of CBNAAT in case of EPTB is being studied extensively and has been recommended

for the samples, like cold abscess and lymph node fine needle aspirate or biopsy based on previous studies and is also evident from the resultsof the present study. The easy availability of results also makes it ideal for the Indian health-care settings. However, its routine use in case of serosal fluids is not recommended because of its lower sensitivity. Immunocompromised cases were not included in the present study.

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References

1. India M. TB India Report 2018. Ministry of Health and Family Welfare [Internet]. Tbcindia.gov.in. 2020 [cited 21 December 2020]. *Available from URL:* https://tbcindia.gov.in/showfile.php?lid=3314. Accessed on July 20, 2019.
2. Global Tuberculosis Control 2009 - Epidemiology Strategy Financing - World [Internet]. ReliefWeb. 2020 [cited 21 December 2020]. *Available from URL:* https://reliefweb.int/report/world/global-tuberculosis-control-2009-epidemiology-strategy-financing. Accessed on July 21, 2019.
3. Gallant V, Ogunnaike-Cooke S, McGuire M. Tuberculosis in Canada: 1924-2012. *Can Commun Dis Rep* 2014;20;40:99–107. doi: 10.14745/ccdr.v40i06a02.
4. India M. Index-TB guidelines:: Ministry of Health and Family Welfare [Internet]. Tbcindia.gov.in. 2020 [cited 21 December 2020]. *Available from URL:* https://tbcindia.gov.in/showfile.php?lid=3245. Accessed on July 22, 2019.
5. Organization W. Global tuberculosis report 2018 [Internet]. Apps.who.int. 2020 [cited 21 December 2020]. *Available from URL:* https://apps.who.int/iris/handle/10665/274453. Accessed on July 25, 2019.
6. Prakasha S, Suresh G, Shetty S, D'sa I, Kumar S. Mapping the pattern and trends of extrapulmonary tuberculosis. *J Global Infect Dis* 2013;5:54.
7. Shrivastava D, Brahmachari D, Pathak D, Kumar D, Sainia D, Patel D, *et al*. Clinico-epidemiological profile of extra-pulmonary tuberculosis in Central India. *Int J Med Res Rev* 2015;3:223–30.
8. Ohene S, Bakker M, Ojo J, Toonstra A, Awudi D, Klatser P. Extra-pulmonary tuberculosis: a retrospective study of patients in Accra, Ghana. *PLoS One* 2019;14:e0209650.
9. Connolly M, Nunn P. Women and tuberculosis. *World Health Stat* Q1996;49:115–9.
10. Velingker A, Lawande D, Dcosta L. Clinico-epidemiological profile of extrapulmonary tuberculosis in Western India. *Int J Contemp Med Res* 2018;5:B1–B4.
11. Gaur P, Suryakant S, Bhaskar R, Singh S, Saxena P, Agnihotri S. Incidence and clinical profiles of pulmonary and extra-pulmonary tuberculosis patients in North Indian population: a hospital based retrospective study. *Int J Res Develop Pharm Life Sci* 2017;6:2773–8.
12. Denkinger C, Schumacher S, Boehme C, Dendukuri N, Pai M, Steingart K. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2014;44:435–46.
13. Maynard-Smith L, Larke N, Peters J, Lawn S. Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. *BMC Infect Dis* 2014;14:709. doi: 10.1186/s12879-014-0709-7.
14. Sharma SK, Kohli M, Chaubey J, Yadav RN, Sharma R, Singh BK, *et al*. Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India. *Eur Respir J* 2014;44:1090–3.
15. Lawn S, Zumla A. Diagnosis of extrapulmonary tuberculosis using the Xpert®MTB/RIF assay. *Expert Rev Anti-Infect Therapy* 2012;10:631–5.
16. Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, *et al*. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *Eur Respir J* 2012;40:442–7.
17. Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *J Clin Microbiol* 2011;49:1202–5.
18. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extra pulmonary tuberculosis? *J Clin Microbiol* 2011;49:2540–5.