Introduction

Tuberculosis (TB) is an infectious disease caused by the bacteria *Mycobacterium tuberculosis* (MTB)—when it affects the lungs—it is called PTB, and when the infection affects sites other than the lungs, it is called EPTB—the most common site of infection being lymph nodes followed by pleural effusion.1

Tuberculosis is responsible for nearly 8.6 million cases globally, according to the report World Health Organization (WHO) Global TB.1 India is an endemic country for TB, and only cases from India add 26% to this global load; among the five countries contributing the maximum to this global load, India occupies the first position.2,3

It is a great public health problem in India, leading to a large number of deaths, and hence is a great health hazard. Out of all the TB cases, 15–20% of cases are due to affection of extrapulmonary sites (EPTB), with nearly 50% of the cases being due to human immunodeficiency virus (HIV)–TB coinfection. In the worldwide scenario, of the 1,183,373 new TB cases annually, 234,029 (20%) are of EPTB origin.4

To reduce all the complications and mortality due to it, early diagnosis is very important. Conventional or fluorescent microscopy and culture are very important in the early diagnosis of this infection. Culture is the gold standard of diagnosis, being able to diagnose both viable and drug-sensitive/resistant bacteria, but it is expensive, time-consuming, and requires a long time (about 6–8 weeks) to detect the bacilli and has sensitivity through high specificity.5

In the same way, though conventional staining by Ziehl–Neelsen stain is simple, easy, cheap, and fast, it has low sensitivity and fails to identify a very low count of the bacteria (<10 bacilli/mL of the sample).6

On the contrary, diagnosis of the infection can be done using fluorescent dyes—auramine–rhodamine dyes and seeing the stained smear under [light emitting diode fluorescence microscopy (LED-FM)] light emitting diode fluorescence microscopy. The disadvantage of this method is that though it identifies about 5–10% more acid-fast bacilli (AFB), being more sensitive—the uptake of the dye is slow, less specific, expensive, and decreased quality control procedures.7

To overcome these limitations, CBNAAT or Xpert MTB/RIF assay, a fully automated real-time semi-nested polymerase chain reaction (PCR) system giving results within 2 hours, detecting RIF resistant gene was endorsed by WHO as the most rapid test for diagnosis of PTB in 2010 as a replacement for sputum smear microscopy. The assay has both high sensitivity and specificity but is expensive and can detect viable bacteria, including nontuberculous mycobacteria (NTM).8

The high performance of Xpert MTB/RIF in TB samples is well established, and the same principle was applied to extrapulmonary cases where the diagnosis is difficult because of the paucibacillary nature of the infection and the presence of a variety of clinical features.

Materials and Methods

Type of the study and design: It was a hospital-based prospective cross-sectional study.

- Place of study: Culture and drug sensitivity testing (C&DST) laboratory under the Department of Microbiology, Burdwan Medical College & Hospital, Bardhaman; Associate Professor and HOD, Department of Microbiology, Burdwan Medical College & Hospital, Bardhaman; Assistant Professor, Department of Microbiology, R.G. Kar Medical College and Hospital, Kolkata; Assistant Professor, Department of Microbiology; Associate Professor, Department of Community Medicine; Research Scientist-8, Department of Microbiology, Burdwan Medical College & Hospital, Bardhaman, West Bengal, India; *Corresponding Author


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Medical College & Hospital, Bardhaman, West Bengal, India.

- Period of study: July 2021–February 2022 following approval by the Ethics Committee.
- Study population: All nonrespiratory clinical samples from clinically suspected patients with symptoms of suspected EPTB attending the outpatient department or admitted in the chest ward or other departments—Medicine and Paediatrics Departments of Burdwan Medical & Hospital during this period were collected and sent to our C&DST laboratory for further processing.

**Inclusion Criteria**

All patients of both genders up to 80 years attending the hospital in this period with features of EPTB were included in the study.

**Exclusion Criteria**

- All sputum samples, blood, or urine.
- Patients not willing to give consent for the study.

**Sample Size**

A total of 593 extrapulmonary clinical samples collected from lymph nodes, pus, pleural fluid, CSF, ascitic fluid, and tissue aspirate from patients suspected of having EPTB were included in the study.

The samples were divided into two parts—one part was stained by Ziehl–Neelsen stain and auramine–rhodamine stain and examined under the conventional microscope under oil immersion (100 ×) magnification for 300 fields. Smears were similarly stained by auramine dye which enters the cell wall of the bacteria, making it glow golden-yellow when examined by fluorescence microscopy under ultraviolet (UV) light for AFB.

Samples from slides showing AFB were cultured on Lowenstein–Jensen media. Scraping from positive culture was again stained by Ziehl–Neelsen stain and confirmed by finding AFB.

**Sample**

Two parts—first part stained by

- Ziehl–Neelsen stain—seen in conventional microscope under oil immersion (100 ×) magnification.
- Auramine–rhodamine stain—seen in a fluorescence microscope.

Positive slides—samples cultured in Lowenstein–Jensen media—stained by Ziehl–Neelsen stain and confirmed.

Second part—sample tested by CBNAAT—in universal falcon tubes (30 mL capacity) + sampling reagent (NaOH and isopropanol) at 2:1 ratio—kept for 15 minutes at room temperature with intermittent shaking—3 mL of this mixture was added to the CBNAAT cartridge—result read within 2 hours.

**Result**

A total of 593 samples of suspected EPTB were received in the study period from different extrapulmonary sites—with samples from lymph nodes being the highest (Fig. 1).

Of the lymph nodes, the cervical lymph node was the most affected site (13/22), followed by the axillary (06/22) and inguinal (03/22).

Of the 593 extrapulmonary samples processed, MTB was detected in 52 cases (8.77%) by CBNAAT, while 21 (3.5%) samples showed AFB by Ziehl–Neelsen stain, 33 samples (5.56%) were detected positive by fluorescent stain and the culture on Lowenstein–Jensen media detected AFB in 41 (6.9%) cases only (Table 1).

Smear microscopy by Ziehl–Neelsen stain could not detect 31 and fluorescent microscope 19 cases and falsely declared them to be negative, but CBNAAT being a very accurate test, detected the deoxyribonucleic acid (DNA) of TB bacilli in the samples and declared them to be positive (Tables 2 and 3).

Culture in Lowenstein–Jensen media yielded positive results in 41 CBNAAT-positive cases but could not detect 11 CBNAAT-positive cases (Table 4).

The sensitivity and specificity of CBNAAT in comparison with Ziehl–Neelsen smear is (Table 5):

- Sensitivity = 21/21 + 0 × 100 = 100%
- Specificity = 541/572 × 100 = 94.58%

The sensitivity and specificity of CBNAAT on comparison with FM smear is:

- Sensitivity = 33/33 + 0 × 100 = 100%
- Specificity = 541/560 × 100 = 96.6%

The sensitivity and specificity of CBNAAT on comparison with culture results on Lowenstein–Jensen is:

![Fig. 1: Distribution of the CBNAAT-positive EPTB samples from different sites](image)

<table>
<thead>
<tr>
<th>Total EPTB sample</th>
<th>CBNAAT positive</th>
<th>CBNAAT negative</th>
<th>Microscopy ZN positive</th>
<th>Microscopy FM positive</th>
<th>Culture in LJ media positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>593</td>
<td>52</td>
<td>541</td>
<td>21</td>
<td>33</td>
<td>41</td>
</tr>
<tr>
<td>%</td>
<td>8.77</td>
<td>91.2</td>
<td>3.5</td>
<td>5.5</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Table 1: Distribution of total extrapulmonary samples according to CBNAAT and microscopic findings.
• Sensitivity = 41/41 + 0 × 100 = 100%
• Specificity = 541/552 × 100 = 98%

Smear microscopy by auramine–rhodamine stain and examining them under a fluorescence microscope for AFB is a simple, rapid, cheap, but very specific method with low sensitivity. Sputum culture on Lowenstein–Jensen media is a very sensitive and specific method, but it takes 6–8 weeks to give a positive result—so not very useful in a very infectious disease like TB where there is a great need for early diagnosis and treatment.

All the EPTB samples were subjected to Ziehl–Neelsen stain, FM stain culture in Lowenstein–Jensen media, and CBNAAT assay. The sensitivity and specificity of CBNAAT on comparison with culture results in FM smear-negative cases are:

- Sensitivity = 08/08 + 11 × 100 = 42.1%
- Specificity = 541/541 × 100 = 100%

Extrapulmonary samples were received from different sites like a lymph node, pus (22), pleural fluid (11), CSF (10), tissue aspirate (04), pus (03), ascitic fluid (01), and synovial fluid (01) but the maximum samples were from the lymph node followed by the pleural fluid.

The CBNAAT-positive samples were found more in females (27) than males (25) at a 1:08 ratio. The maximum number of cases was found in the age-group 21–30 years (32.70%), followed by the age-group 31–40 years of age (17.31%) (Table 6).

Among the 52 samples declared positive by CBNAAT, in six samples, RIF resistance was detected, and in the rest 46 samples, no RIF resistance was identified (Table 7).

### Discussion

Extra pulmonary TB accounts for approximately 25% of TB cases caused by Mycobacterium complex worldwide, thus being responsible to a great extent for the morbidity and mortality due to the bacteria. Because EPTB infection is usually deep-seated, biopsy by surgery is required to collect a sample for testing, making the diagnosis further difficult.

Culture by liquid media by mycobacteria growth indicator tube (MGIT) system is costly and needs a lot of expertise to do it by trained laboratory technicians. Cartridge-based nucleic acid amplification test is a simple, rapid, closed system working on the principle of nested semi-quantitative nucleic acid amplification method, which can be done quite easily, giving early accurate results within 2 hours.

Cartridge-based nucleic acid amplification test is also useful in the diagnosis of EPTB, but its use in this aspect has not been used much, probably because of a lack of knowledge, and our study aims to highlight this fact.

A study by Denkinger et al. reported that samples from the lymph node were more sensitive (83%) than that from the pleural fluid (46%).

The same was also corroborated by Rai et al. and Penz et al., who also found that the sensitivity of the lymph node is much higher (87%) than that of pleural fluid (37%).

The same finding was also reported by us—MTB was isolated from the lymph node in 22 (42.31%) cases while that from the pleural fluid in 11 (21.16%) cases.

Tubercular pleural effusion was the most common form of EPTB in the study conducted by Mukherjee et al., being found in 58.17% of cases, followed by lymphadenopathy (22.71%).

Met al. study on EPTB and found that MTB was isolated more in the younger age-group. Similar results were also found in the study conducted by Singh et al., who reported that the maximum cases were in the age-group 21–30 years, with MTB detected in 23 (34.3%) cases.

### Table 2: Comparison of results from CBNAAT and ZN smear

<table>
<thead>
<tr>
<th>CBNAAT status</th>
<th>ZN smear</th>
<th>Grand total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smear positive</td>
<td>Smear negative</td>
</tr>
<tr>
<td>CBNAAT positive</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>CBNAAT negative</td>
<td>0</td>
<td>541</td>
</tr>
<tr>
<td>Grand total</td>
<td>21</td>
<td>572</td>
</tr>
</tbody>
</table>

### Table 3: Comparison of results from CBNAAT and FM smear

<table>
<thead>
<tr>
<th>CBNAAT status</th>
<th>FM smear</th>
<th>Grand total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smear positive</td>
<td>Smear negative</td>
</tr>
<tr>
<td>CBNAAT positive</td>
<td>33</td>
<td>19</td>
</tr>
<tr>
<td>CBNAAT negative</td>
<td>0</td>
<td>541</td>
</tr>
<tr>
<td>Grand total</td>
<td>33</td>
<td>560</td>
</tr>
</tbody>
</table>

### Table 4: Comparison of results from CBNAAT and culture on LJ media

<table>
<thead>
<tr>
<th>CBNAAT status</th>
<th>Culture on LJ media</th>
<th>Grand total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture positive</td>
<td>Culture negative</td>
</tr>
<tr>
<td>CBNAAT positive</td>
<td>41</td>
<td>11</td>
</tr>
<tr>
<td>CBNAAT negative</td>
<td>0</td>
<td>541</td>
</tr>
<tr>
<td>Grand total</td>
<td>41</td>
<td>552</td>
</tr>
</tbody>
</table>

### Table 5: Comparison of CBNAAT results with culture on LJ media and FM smear-negative cases

<table>
<thead>
<tr>
<th>CBNAAT status</th>
<th>FM smear negative</th>
<th>Grand total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture positive</td>
<td>Culture negative</td>
</tr>
<tr>
<td>CBNAAT positive</td>
<td>08</td>
<td>11</td>
</tr>
<tr>
<td>CBNAAT negative</td>
<td>0</td>
<td>541</td>
</tr>
<tr>
<td>Grand total</td>
<td>08</td>
<td>552</td>
</tr>
</tbody>
</table>

### Table 6: Age-sex distribution of the CBNAAT-positive samples

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 years</td>
<td>02</td>
<td>01</td>
<td>03</td>
<td>(5.77%)</td>
</tr>
<tr>
<td>11–20 years</td>
<td>06</td>
<td>05</td>
<td>11</td>
<td>(17.31%)</td>
</tr>
<tr>
<td>21–30 years</td>
<td>07</td>
<td>10</td>
<td>17</td>
<td>(25.69%)</td>
</tr>
<tr>
<td>31–40 years</td>
<td>05</td>
<td>08</td>
<td>13</td>
<td>(21.25%)</td>
</tr>
<tr>
<td>41–50 years</td>
<td>06</td>
<td>00</td>
<td>06</td>
<td>(11.54%)</td>
</tr>
<tr>
<td>51–60 years</td>
<td>01</td>
<td>01</td>
<td>02</td>
<td>(3.68%)</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>00</td>
<td>02</td>
<td>02</td>
<td>(3.68%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>27</td>
<td>52</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

*Among the total 52 extrapulmonary samples, the number of female patients outnumbered the male one*
Diagnosis of Extrapulmonary Tuberculosis by CBNAAT

Table 7: RIF resistance in CBNAAT-positive cases

<table>
<thead>
<tr>
<th>Presumptive MTB samples</th>
<th>EPTB</th>
<th>CBNAAT positive</th>
<th>CBNAAT negative</th>
<th>RIF resistance detected</th>
<th>RIF resistance not detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1513</td>
<td>593</td>
<td>52 (8.77%)</td>
<td>541 (91.23%)</td>
<td>6 (11.54%)</td>
<td>46 (88.46%)</td>
</tr>
</tbody>
</table>

In our study, we also found the maximum prevalence of MTB was in the age-group 21–30 years; 17 (32.70%) cases were closely followed by the age-group 31–40 years (13–25%). Singh et al., in their study, found that MTB was found more in the cases of EPTB in males at the ratio of 3:2 male:female ratio. We in our study, however, found that more EPTB cases were found in females than males (ratio 27/25—1.08).

We in our study, however, found that more EPTB cases were found in females than males (ratio 27/25—1.08).

In the study done by Singh et al. in 2020 detected RIF resistance by CBNAAT in 52 (6.8%) samples. They also found that out of 46 samples stained by Ziehl–Neelsen, which were negative, CBNAAT found MTB in 27 samples—thus proving the fact CBNAAT is a very sensitive test that could identify correctly false negative results given by Ziehl–Neelsen stain.

In our study, CBNAAT gave RIF-resistant reports in six cases out of 52 positive samples (11.54%).

**Conclusion**

Cartridge-based nucleic acid amplification test is a very useful test able to diagnose a highly infectious disease like MTB rapidly within 2 hours, and hence treatment can be initiated very fast, thus preventing the development of resistant cases. India is a TB-endemic country, and increased use of CBNAAT for diagnosis of it—both pulmonary and extrapulmonary cases may act as a boon in disguise.

**Acknowledgment**

We are grateful to the Principal, Dean, and Medical Superintendent of Burdwan Medical College for allowing us to conduct the study. We are also thankful to all the staff of the Microbiology laboratory for cooperating with us in carrying out the study. Lastly, we are very much grateful to all the participants of this study for their full-hearted support and cooperation.

**References**