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**Original article**

# Rifampicin resistance among individuals with extra pulmonary tuberculosis at tertiary care hospital, Ahmedabad

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## ABSTRACT

**Introduction:** Tuberculosis is an infectious disease caused by the Mycobacterium tuberculosis which typically involve lung but can affect other sites called extra pulmonary tuberculosis (EPTB). Common disease manifestations of EPTB include meningitis, lymphadenitis, ocular, oral, pleuritis, pericarditis, peritonitis, cutaneous, musculoskeletal, abdominal, genitourinary, and miliary forms of tuberculosis. EPTB can be either primary (at the site of initial infection) or secondary (disseminated), which usually occurs due to haematogenous or lymphatic spread of bacteria from the primary organ, reactivation of Latent TB Infection (LTBI), ingestion of infected sputum, or spread locally from adjacent organs. EPTB cases accounted for 16% of the 7.5 million incident cases worldwide in 2019. Cartridge based nucleic acid amplification test (CBNAAT) have high sensitivity in both respiratory and non-respiratory specimens.

**Materials and Methods:** A laboratory based retrospective observational study. The study was conducted in the department of Microbiology, Narendra Modi medical college, Ahmedabad, Gujarat over a period of one year i.e. from January 2022 to December 2022. The main Aim of study to determine the rate of rifampicin resistant M. tuberculosis among EPTB and to determine the age and gender dependent preponderance of EPTB. A total of 1523 EPTB specimens were received from various clinical sites suspected of EPTB infections. All received EPTB specimens were processed in CBNAAT laboratory with aseptic precautions and tested in Gene Xpert MTB / RIF, according to NTEP guidelines.

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**Results:** Overall isolation rate of Mycobacterium tuberculosis causing EPTB infection was 208 (13.7 %). Among that rifampicin resistant were 23 (11%). EPTB infections were found in various specimens like pleural fluids 57(15%), FNACs 52 (29 %), pus 50 (54%), gastric aspirates 34 (5%), CSF 7 (8 %), ascitic fluids 5 (7%), other body fluids 2 (10%) and synovial fluid 1 (25%). In present study rifampicin resistant were seen in females 19 (83%) as compared to male 4 (17%). Age group of 11-20 years - 11 (24%) showed highest rifampicin resistance followed by 21-30 years - 08 (16 %). Rifampicin resistance was highest in "medium" level EPTB load - 10 (21%) specimens of EPTB.

**Conclusions:** The study shows a high proportion of rifampicin resistance at tertiary care hospital, Ahmedabad. The review showed that the incidence of extra pulmonary Rifampicin Resistance Tuberculosis has continued to become a serious public health problem. Hope thus, this finding could help the programmatic management of the disease within the context of the National TB Control program (NTEP).

**Keywords:** EPTB, Gene Xpert MTB, Rifampicin resistant

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## Introduction

Tuberculosis (TB) is one of the leading causes of death from a single infectious agent [1]. TB mainly affects the lungs (pulmonary TB), but it can also affect other body sites, which refer, to extra pulmonary TB (EPTB) [1]. EPTB is defined as any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs. The body organs other than the lungs that are mainly affected by Mycobacterium tuberculosis include the pleura, lymph nodes, abdomen, genitourinary tract, skin, joints, bones, and meninges [2–4]. Worldwide extra pulmonary tuberculosis accounts for 25% of all TB cases and even higher percentage in HIV infected individual and children [5]. EPTB cases accounted for 16% of the 7.5 million incident cases worldwide in 2019 [6.] Drug-resistant (DR) TB is the main challenge of the global TB control program due to its high risk of relapse, treatment failure, prolonged transmission of the bacilli, and death[7]. The treatment of DR-TB is more difficult than the treatment of drug-susceptible TB because it requires the use of second-line drugs that are of a longer duration, costlier, more toxic, and less effective. Patients who are infected with strains resistant to isoniazid and rifampicin are practically incurable by standard first-line TB drugs [8,9]. The conventional Mycobacterium tuberculosis detection technique based on microscopic examination of acid-fast bacilli is relatively insensitive[10]. Xpert MTB/RIF (Cepheid) is an automated, cartridge-based assay designed to simultaneously detect Mycobacterium tuberculosis (MTB) and resistance to rifampicin (RIF) directly in clinical specimens using hemi-nested real-time PCR. The single use cartridge contains reagents for DNA extraction, PCR amplification, internal controls and five partially overlapping fluorescent probes A, B, C, D and E, targeting the 81 bp Rifampicin Resistance Determining Region (RRDR) of MTB rpoB gene. The test provides semi-quantitative MTB detection based on the probes Cycle Threshold (Ct) – number of PCR cycles required to amplify MTB DNA to a detectable level. MTB detection result is reported as “High”, “Medium”, “Low” and “Very low”[10]. CBNAAT/Gene Expert MTB/RIF have been the game changer as they have far high sensitivity than smear in both respiratory and non-respiratory specimens[10]. CBNAAT is real time PCR technique for diagnosis of TB and detection of rifampicin resistance conferring result within 2 hours [10].

**Aims and objectives:**

- To determine the rate of Rifampicin Resistant M. tuberculosis among EPTB.
- To determine the age and gender dependent preponderance of EPTB.

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## Materials and Methods

The study was a retrospective record based. Total 1523 extra-pulmonary specimens were received from Sheth L.G. General Hospital, different UHCs and private hospital at CBNAAT laboratory of Microbiology department at Narendra Modi Medical College, Ahmedabad, during a period of one year i.e. January 2022 to December 2022. All received specimens were processed as per the standard protocol of Gene-Xpert instrument System.

**Inclusion criteria:**

All suspected patients of extra pulmonary tuberculosis as per NTEP guidelines.

**Exclusion criteria:**

Extra pulmonary specimens received for follow-up.

**Basic Procedure:**

1. Mix specimen (sputum) and reagent in equal volume.
2. Wait for 15 minutes.
3. Transfer reagent treated specimen into cartridge sample well by using a dropper. (Volume up to mark in dropper)
4. Go to the desktop and click create test.
5. Scan cartridge bar code by the scanner and fill the form mainly patient name and specimen type.
6. Click the Start test button and finally load the cartridge on the holder as shown by default (A1/A2/A3/A4) also notice clicking orange signal.
7. After that push the door and wait for the result (1 hour 51 minutes)


**GeneXpert Cartridge**

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**Interpretation of GeneXpert results:**

- MTB not detected
- MTB detected with grading either
    1. Very low
    2. Low
    3. Medium
    4. High
- With Rif Resistance NOT DETECTED or
- With Rif Resistance DETECTED

Results from the Xpert MTB/RIF assay indicate whether or not MTBC was detected in the specimen. In some instances, the result is "invalid," whereby the test should be repeated [11,12].

**If MTBC was detected, the results will also state whether resistance to RIF was**

- **Detected**: Mycobacteria have a high probability of resistance to RIF; should be confirmed by additional testing. If RIF resistance is confirmed, rapid molecular testing for drug resistance to both first-line and second-line drugs should be performed so that an effective treatment regimen can be selected.
- **Not detected**: Mycobacteria are probably susceptible to RIF; All tests that are positive for MTBC should have growth-based susceptibility testing to first-line TB drugs.
- **Indeterminate:** the test could not accurately determine if the bacteria are resistant to RIF. Growth-based susceptibility testing to first-line TB drugs should be performed.

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## Results

Out of 1523 various clinical EPTB specimens, Mycobacterium tuberculosis complex causing EPTB infection were detected in 208 specimens. Among them 23 (11%) were rifampicin resistant while 185 (89%) were rifampicin sensitive. In the rifampicin resistance, 83% were females and 17 % were males whereas among rifampicin sensitive 48% were females and 52% were males, respectively **(Table-1)**.

Rifampicin resistant were higher in the age group of 11-20 years 11 (24%) followed by 21-30 years 08 (16%) as compared to other age group. **(Table-2)**.

In our study, it was found that maximum number of rifampicin resistant MycobacteriumTB Complex (n= 208) were recovered from pleural fluids 57(15%) followed by FNACs 52 (29 %), pus 50(54%), gastric aspirates 34 (5%), CSF 7 (8 %), ascitic fluids 5 (7%), other fluids 2 (10%) and synovial fluid 1 (25%). **(Table-3)**.

Among the 208 positive EPTB specimens, 88 (42%) specimens show “Low” level detection, followed by “Very Low” in 59 (29%) samples, “Medium” level in 48 (23%) specimens and “High” level in 13(6%). (**Table-4)**

**Table 1: Overall distribution of EPTB Rifampicin resistant and sensitive Mycobacterium tuberculosis complex**

| Total specimen | No. (%) | Sex | |
|---|---|---|---|
| | | Male | Female |
| | | No. (%) | No. (%) |
| Rifampicin resistant | 23 (11%) | 4 (17%) | 19 (83%) |
| Rifampicin sensitive | 185 (89%) | 97 (52%) | 88 (48%) |
| Total | 208 | 101 | 107 |

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**Table 2: Age wise distribution of EPTB Rifampicin sensitive and resistant Mycobacterium tuberculosis complex**

| Age in years | Total (n=208) | Rifampicin sensitive | Rifampicin resistant |
|---|---|---|---|
| 0 -10 | 18 | 16 (87%) | 02 (13%) |
| 11-20 | 57 | 46 (76%) | 11 (24%) |
| 21-30 | 57 | 49 (84%) | 08 (16%) |
| 31-40 | 33 | 32 (97%) | 01 (3%) |
| 41-50 | 20 | 19 (95%) | 01 (5%) |
| 51-60 | 11 | 11 (100%) | 00 |
| 61-70 | 09 | 09 (100%) | 00 |
| 71-80 | 03 | 03 (100%) | 00 |

**Table 3: Rate of positive EPTB in different extra-pulmonary specimens**

| Specimens | Total | Positive (n=208) |
|---|---|---|
| Ascitic Fluid | 74 | 5 (7%) |
| CSF | 91 | 7 (8%) |
| FNAC | 182 | 52 (29%) |
| Gastric Aspirate | 688 | 34 (5%) |
| Pleural fluid | 370 | 57 (15%) |
| Pus | 93 | 50 (54%) |
| Synovial fluid | 04 | 01 (25%) |
| Other body fluids | 21 | 02 (10%) |

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**Table 4: The segregation of EPTB positive as per the load of Mycobacterium tuberculosis complex detection in Xpert**

| EPTB detected Mycobacterial load | No. (%) | Rifampicin resistant | Rifampicin sensitive |
|---|---|---|---|
| High | 13 (6%) | 02 (15%) | 11 (85%) |
| Medium | 48 (23%) | 10 (21%) | 38 (79%) |
| Low | 88 (42%) | 05 (6%) | 83 (94%) |
| Very low | 59 (29%) | 06 (10%) | 53 (90%) |

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## Discussion

Conventional laboratory techniques like direct microscopy for the diagnosis of tuberculosis are far being more sensitive. Moreover, cultures are time-consuming, require biosafety measures, and need trained laboratory personnel. Molecular techniques have substantially changed the field of tuberculosis diagnosis and have been proven to yield rapid results while being highly sensitive[13]. The new Xpert assay tested in our study targets the rifampin resistance-associated rpoB gene region by PCR with three specific primers and combines the sensitive detection of M. tuberculosis DNA and determination of RIF resistance. RIF resistance is a predictor of MDR TB because resistance to RIF, in most instances, co-exists with resistance to INH. Rapid diagnosis of RIF resistance potentially allows TB patients to start on effective treatment much sooner than waiting for results from other types of drug susceptibility testing[12]. Furthermore, the hands-on time is short due to automation of bacterial lysis, DNA extraction, real-time PCR amplification, and amplicon detection in a single system. Numerous studies have assessed the yield of PCR techniques for the diagnosis of extra pulmonary tuberculosis [14,15].

In the present study, out of 1523 EPTB specimens, 208 were positive (13.7 %) for Mycobacterium tuberculosis complex were detected over a period of January 2022 to December 2022. Among the 208 positive Mycobacterium tuberculosis complex detected 23 (11 %) were rifampicin resistant whereas 185 (89 %) were sensitive to EPTB infections. The findings of the present study were lower in accordance to a study done by Workneh Korma et al [16] in Ethiopia and Surya Kant et al [17] in Northern India in which, the prevalence of rifampicin resistant EPTB infections were 22% and 39.1%, which were higher than our study.

The present study found that out of the 208 rifampicin resistant detected MTBC, 17% were males and 83 % were females. Rifampicin resistant Mycobacterium tuberculosis complex in EPTB infections were higher in females in our study. It may be due to sex hormones, genetic factors, and nutritional status play roles in this disparity that compared to study done by E. Gunes, D. Tatar, O. Gunes et al [18] in Izmir, Turkey that shows females preponderance of EPTB 53%.

Majority of our patients were in the age group 11-20 years i.e.11 (24%) followed 21-30 years 08 (16%) -mainly late childhood and in younger age group. Lower socio-economic class and contact with active TB cases were the most frequent risk factors. Severe nutritional anemia and severe acute malnutrition were the most common co morbid conditions for development of EPTB infection. Young age group and female gender were found to be risk factors for EPTB in developing countries[19].

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In the present study, it was found that maximum numbers of rifampicin resistant EPTB were recovered from specimens of pleural fluids i.e. 57 (15%). The findings of the above study are in accordance with a study done by Mustafa Kürşat et al [20] showed 41 % of pleural fluids specimens. However, a study from Hong Kong showed the most common sites were pleura, followed by lymph node[21.]

The present study showed that rifampicin resistance EPTB was highest in “Medium” level was 21 % while study done by Kanchan Ajbani,et [22] al showed 32 % in “Medium” level which was higher than our study.

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## Conclusion

The overall frequency of EPTB and rifampicin-resistance was 13.7 % and 11 %, respectively. In our study Xpert MTB/RIF gave the highest yield in pleural samples followed by FNACs and pus. The review shows that the incidence of extra pulmonary Rifampicin Resistant Tuberculosis has continued to become a serious public health problem. Rapid diagnosis of RIF resistance potentially allows EPTB patients to start on effective treatment much sooner than waiting for results from other types of drug susceptibility testing. For patients who are found to NOT have EPTB disease, rapid results from the Xpert MTB/RIF assay may contribute to cost savings by avoiding unnecessary treatment. Female gender was found to be significantly associated with EPTB. Increased awareness of risk factors may facilitate early case finding and better management outcomes for these patients. A continuous educational programme on infectious diseases and how to control them is desirable. Clinicians should request drug susceptibility testing for all patients with presumptive EPTB to detect drug resistance.

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