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Prevalence and Rifampicin Resistance Patterns in Extrapulmonary Tuberculosis at a Tertiary Care Centre in Western Maharashtra

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ABSTRACT

Background: Tuberculosis remains a major public health problem in India. Extrapulmonary tuberculosis (EPTB) poses considerable diagnostic challenges because of its paucibacillary nature and non-specific clinical presentation. Molecular diagnostic tools such as CBNAAT (Cartridge-Based Nucleic Acid Amplification Test) have improved detection rates in resource-limited settings. **Objectives:** To determine the prevalence of EPTB and rifampicin resistance among EPTB-positive cases. **Methods:** A laboratory-based descriptive cross-sectional study was conducted from January to December 2023 at the CBNAAT laboratory of a tertiary care teaching hospital in western Maharashtra. A total of 1419 extrapulmonary specimens were tested using the GeneXpert MTB/RIF assay (Cepheid, USA). Data were extracted from the GeneXpert software and analysed descriptively using SPSS version 22. Frequencies and percentages were calculated for categorical variables. **Results:** *Mycobacterium tuberculosis* (Mtb) was detected in 119 of 1419 (8.39%) extrapulmonary samples. Rifampicin resistance was found in 8 of 119 (6.72%) EPTB-positive isolates. Lymph nodes were the most commonly affected site (30.25%), followed by pus/abscess (25.21%) and pleural fluid (17.65%). Over 80% of EPTB cases demonstrated low or very low bacterial loads on semi-quantitative assessment. **Conclusion:** EPTB positivity is lower than pulmonary TB positivity. Lymphadenopathy constitutes the most common form of EPTB. Rifampicin resistance was detected in a notable proportion of EPTB-positive cases. The predominantly paucibacillary nature of EPTB continues to pose diagnostic difficulties.

KEYWORDS: CBNAAT, Extrapulmonary tuberculosis, GeneXpert, Rifampicin resistance, Lymph node tuberculosis, Paucibacillary

INTRODUCTION

Tuberculosis (TB) continues to be one of the leading infectious causes of morbidity and mortality worldwide. According to the World Health Organization (WHO) Global Tuberculosis Report 2024, an estimated 10.8 million individuals developed TB in 2023, with India accounting for approximately 26% of the global incident cases, the highest among all countries[1]. Although a gradual decline in TB incidence has been observed in India over the past decade, the disease still imposes a substantial burden on the healthcare system and remains a major public health priority.

Extrapulmonary tuberculosis (EPTB), although not a major contributor to disease transmission, is associated with

substantial morbidity and clinical complexity. Globally, 16% of all notified TB cases in 2023 were extrapulmonary, with higher proportions reported among people living with HIV (PLHIV)[1]. *Mycobacterium tuberculosis* can involve almost any organ system except for hair, nails, and teeth. EPTB is defined as tuberculosis affecting organs other than the lung parenchyma, including the pleura, lymph nodes, abdomen, genitourinary tract, meninges, and musculoskeletal system[2].

Diagnosing extrapulmonary tuberculosis is often difficult in routine clinical practice. The clinical presentation is usually non-specific and varies with the organ involved. Most extrapulmonary specimens are paucibacillary, which lowers the sensitivity of conventional tests such as smear microscopy and culture[3, 4]. This often results in delay in diagnosis and may lead to disease progression and complications. Radiological investigations are commonly used as an initial step. However, microbiological or molecular confirmation remains necessary wherever feasible.

Drug resistance adds further complexity to the management of extrapulmonary TB. Although the proportion of rifampicin resistance among EPTB cases is generally lower compared to pulmonary TB, its presence has important implications for treatment outcomes highlighting the need for routine molecular testing[5, 6]. In addition, the inherent diagnostic challenges of EPTB, particularly its paucibacillary nature and difficulty in specimen collection, may delay microbiological confirmation and consequently postpone detection of resistant disease[7, 8].

The GeneXpert MTB/RIF assay, used under the CBNAAT platform, has been recommended by WHO and adopted under India's National Tuberculosis Elimination Programme (NTEP) as a frontline diagnostic tool. It enables simultaneous detection of *Mycobacterium tuberculosis* complex DNA and rifampicin resistance–conferring mutations within a short turnaround time. This makes it particularly useful in the diagnosis of extrapulmonary tuberculosis[3]. Evidence from Indian programmatic settings has also demonstrated its improved diagnostic yield across a wide range of extrapulmonary samples and its utility in routine clinical practice[5, 9].

Published data on EPTB prevalence, site distribution, and rifampicin resistance from tertiary care settings in western Maharashtra remain limited. The present study was therefore undertaken to determine the prevalence, site distribution and rifampicin resistance of EPTB, at a single NTEP-linked CBNAAT laboratory.

MATERIALS AND METHODS

Study Design and Setting: A laboratory-based, descriptive cross-sectional study was conducted at the CBNAAT laboratory of a tertiary care teaching hospital in western Maharashtra, India from January to December 2023. This laboratory functions under the National Tuberculosis Elimination Programme (NTEP) and follows its standardised diagnostic algorithms and quality assurance protocols.

Ethical Approval: The study was approved by the Institutional Ethics Committee (Approval No.: ND-0126006-06; Date: 28/05/2023). As the study involved retrospective analysis of routinely collected programme data, the committee granted a waiver of individual informed consent. Patient confidentiality and data anonymity were maintained throughout.

Study Population and Specimen Inclusion: Of 3,649 specimens received for CBNAAT testing during the study period, 1,419 were extrapulmonary specimens submitted from patients with presumptive EPTB. These specimens included lymph node aspirates, pleural fluid, ascitic fluid, cerebrospinal fluid (CSF), pus, urine, and tissue biopsies. Specimens were submitted by treating clinicians based on clinical, radiological, or biochemical suspicion of EPTB.

Inclusion criteria: All extrapulmonary specimens received for CBNAAT testing from presumptive EPTB cases during the study period.

Exclusion criteria: Specimens yielding an "Invalid," "Error," or "No Result" outcome on CBNAAT testing, and specimens that were inadequate in volume or improperly labelled.

Operational Definitions (as per NTEP)

- **Presumptive EPTB case:** A patient presenting with clinical features, imaging findings, or laboratory parameters suggestive of tuberculosis involving organs other than the lungs, warranting microbiological investigation.
- **MTB Detected:** Identification of *Mycobacterium tuberculosis* complex DNA by real-time PCR on the GeneXpert platform.
- **Rifampicin Resistance Detected:** Detection of mutations in the rpoB gene, indicative of rifampicin resistance, as reported by the Xpert MTB/RIF assay.
- **Semi-quantitative bacterial load:** Automatically categorised by the GeneXpert system as High, Medium, Low, or Very Low, based on the cycle threshold (Ct) values generated during amplification.

Specimen Collection and Transport: All specimens were collected by trained clinicians under aseptic conditions using appropriate containers. Specimens were

transported to the CBNAAT laboratory in accordance with NTEP specimen transport guidelines. Cold chain maintenance was ensured where required to preserve sample integrity.

CBNAAT Testing Procedure: CBNAAT testing was performed using the GeneXpert system (Cepheid, USA; Model: 838113) with Xpert MTB/RIF assay cartridges, following the SOP prescribed under NTEP. The assay simultaneously detects *Mycobacterium tuberculosis* complex DNA and rifampicin resistance-conferring mutations in the *rpoB* gene.

Quality Assurance: Internal quality control was ensured through two built-in controls within each cartridge: the Sample Processing Control (SPC), which verifies adequate sample processing and inhibitor removal; and the Probe Check Control (PCC), which confirms reagent integrity and fluorescence function prior to amplification. External quality assurance (EQA) was conducted under the NTEP framework through periodic proficiency testing panels and supervisory assessments by the designated State Reference Laboratory (SRL).

Data Collection and Statistical Analysis: Results were extracted from the GeneXpert software and matched with NTEP laboratory registers. All reportable outcomes were entered into the NIKSHAY portal. Data were entered and managed in Microsoft Excel 2021 and analysed using SPSS version 22. Categorical variables were summarised as frequencies and percentages. As the study was descriptive in design, inferential statistical testing was not performed.

RESULTS

A total of 3649 specimens were received for CBNAAT testing during the study period, of which 1419 (38.89%) were extrapulmonary specimens from patients with presumptive EPTB. *Mycobacterium tuberculosis* was identified in 119 of these 1419 specimens, giving a positivity rate of 8.39% among EPTB samples. Among all 437 MTB-positive specimens (pulmonary and extrapulmonary combined), 119 (27.32%) were extrapulmonary. Rifampicin resistance was detected in 8 of 119 (6.72%) EPTB-positive isolates [Table. 1].

Regarding the distribution of affected anatomical sites, lymph nodes were the most involved (36 cases, 30.25%), followed by pus/abscess specimens (30 cases, 25.21%) and pleural fluid (21 cases, 17.65%). Other sites included CSF (9 cases, 7.56%), miscellaneous specimens (10 cases, 8.40%), ascitic fluid (7 cases, 5.88%), tissue biopsy (3 cases, 2.52%), FNAC (2 cases, 1.68%), and urine (1 case, 0.84%) as shown in [Table. 2].

Analysis of semi-quantitative bacterial load data [Table. 3] showed that the majority of EPTB-positive specimens exhibited low (51 cases, 42.86%) or very low (49 cases, 41.17%) bacterial loads.

Month	EP Specimens (n)	MTB Detected (n)	Rif Sensitive (n)	Rif Resistant (n)
January	219	20	18	2
February	137	19	19	0
March	187	14	12	2
April	162	10	10	0
May	133	10	9	1
June	194	11	11	0
July	110	12	11	1
August	122	13	13	0
September	57	5	4	1
October	58	2	2	0
November	1	1	0	1
December	39	2	2	0
Total (n)	**1,419**	**119**	**111**	**8**
Percentage (of EP specimens)	100%	8.39%	93.28% of positives	6.72% of positives

Table 1: Monthly distribution of extrapulmonary specimens, MTB detection, and rifampicin resistance (January–December 2023)

Note: EP – extrapulmonary; Mtb – *Mycobacterium tuberculosis*; Rif – rifampicin

Specimen Site	MTB Detected (n)	Percent (of 119 positives)
Lymph Nodes	36	30.25%
Pus / Abscess	30	25.21%
Pleural Fluid	21	17.65%
CSF	9	7.56%
Miscellaneous	10	8.40%
Ascitic Fluid	7	5.88%
Tissue Biopsy	3	2.52%
FNAC	2	1.68%
Urine	1	0.84%
Total	**119**	**100%**

Table 2: Site-wise distribution of MTB-positive EPTB cases (n = 119)

Note: EPTB – extrapulmonary tuberculosis; Mtb – *Mycobacterium tuberculosis*; FNAC – fine needle aspiration cytology; CSF – cerebrospinal fluid

Bacterial Load Category	Specimens (n)	Percent (of 119)	Rif Resistant (n)
High	1	0.84%	0
Medium	18	15.12%	0
Low	51	42.86%	4
Very Low	49	41.17%	4
Total	**119**	**100%**	**8**

Table 3: Semi-quantitative bacterial load distribution and rifampicin resistance among EPTB-positive specimens (n = 119)

Note: Mtb – *Mycobacterium tuberculosis*; Rif – rifampicin; bacterial load categories are as automatically reported by the GeneXpert system

Medium load was recorded in 18 cases (15.12%), while only one sample (0.84%) had a high bacterial load.

Rifampicin resistance was distributed among low-load (4 cases) and very low-load (4 cases) specimens, with none in the high or medium load categories.

DISCUSSION

The present study was conducted in a tertiary care NTEP-linked laboratory and evaluated extrapulmonary tuberculosis over a one-year period. Among 1419 extrapulmonary specimens tested, *Mycobacterium tuberculosis* was detected in 8.39% of samples. Extrapulmonary cases accounted for 27.32% of all microbiologically confirmed tuberculosis in the laboratory.

The proportion of EPTB observed in this study is higher than global estimates. WHO Global Tuberculosis Report 2024 indicates that extrapulmonary disease contributes to approximately 16% of notified cases worldwide[1] and it is 15% to 24% in India[10]. The higher proportion observed in this study is likely related to the referral pattern of a tertiary care centre. Patients presenting to such facilities often have diagnostic uncertainty or atypical manifestations, which increases the likelihood of extrapulmonary evaluation. Similar variability has been described in international studies, where the burden of EPTB differs across settings depending on epidemiological and health system factors[11-14].

The positivity rate among extrapulmonary specimens was relatively low. Such findings are expected in extrapulmonary tuberculosis, where bacillary load is often limited and sample quality may vary[8, 9].

Rifampicin resistance was detected in 6.72% of EPTB cases. This estimate is within the range reported in Indian studies. VidyaRaj *et al*. observed resistance in 4.83% of EPTB in a multicentric South Indian cohort[6], while Sidiq *et al*. reported higher resistance proportions from CBNAAT laboratories in Delhi[5]. Differences across studies may be influenced by variation in patient profile, including prior treatment exposure and referral bias, although such variables were not available in the present dataset.

Lymph node involvement was the most common presentation in this study, accounting for 30.25% of cases. This observation is in agreement with established literature, where lymph nodes are consistently reported as the predominant site of extrapulmonary tuberculosis[2, 5-7]. Rolo et al. reported a similar pattern in a European setting, with lymph node involvement forming the largest proportion of cases[11]. In the present study, pus and abscess samples constituted the second most common category, followed by pleural fluid. This distribution may partly be influenced by sampling practices, as superficial and accessible lesions are more likely to undergo microbiological testing.

More than 80% of positive specimens showed low or very low bacterial load on semi-quantitative assessment. Extrapulmonary tuberculosis is well recognized to be paucibacillary in nature[4, 9, 16]. Bacillary multiplication at these sites is often limited, and organisms tend to remain localised[7]. This has practical implications, as conventional methods such as smear microscopy frequently fail to detect disease. Molecular assays, including CBNAAT, therefore play an important role, particularly in smear-negative specimens[3, 9, 15, 16].

All rifampicin-resistant cases in this study were identified in specimens with low or very low bacterial load. No resistance was detected among medium or high bacillary categories. Current evidence does not clearly establish a relationship between bacillary load and drug resistance in extrapulmonary tuberculosis. Systematic reviews and diagnostic evaluations have noted variability in assay performance, particularly in specimens with very low bacillary load, where detection thresholds may influence results[17, 18]. This observation should be interpreted cautiously, as current evidence does not establish a clear association between bacillary load and drug resistance in extrapulmonary tuberculosis.

The study has certain limitations. It was conducted at a single centre over a limited duration, which restricts generalisability. Clinical variables such as age, sex, HIV status, and prior treatment history were not available, limiting subgroup analysis. Rifampicin resistance was assessed only through CBNAAT without culture-based confirmation. Patient outcomes were not evaluated. Despite these limitations, the study reflects routine diagnostic practices under NTEP conditions and provides relevant insights into extrapulmonary tuberculosis in a tertiary care setting.

CONCLUSION

Extrapulmonary tuberculosis formed a considerable share of confirmed cases (8.39%) in this tertiary care setting, while the overall positivity among extrapulmonary specimens remained low. Lymph nodes were more commonly involved site. Rifampicin resistance was present in a small proportion of cases. The predominance of low bacillary load reflects the diagnostic difficulty in extrapulmonary disease and supports the use of CBNAAT in routine practice. Further studies with clinical details and confirmatory testing would provide better clarity on disease patterns.

DISCLOSURE

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