

EVALUATION OF CLINICAL SPECIMENS
FOR EXTRAPULMONARY TUBERCULOSIS
FROM SAMSUN, TURKIYE: 2010–2022

Yeliz Tanrıverdi Çaycı✉, Safa Toy, Demet Gür Vural,
Kemal Bilgin, Canberk Çınar, Asuman Birinci

Received on January 20, 2026

Presented by B. Petrunov, Member of BAS, on April 28, 2026

Abstract

Tuberculosis is a multisystemic infectious disease that can affect all tissues and organs. Extrapulmonary tuberculosis (EPTB) is defined as the localization of the disease outside the lungs. This study included extrapulmonary samples (EP) sent to the Tuberculosis diagnostic laboratory of Ondokuz Mayıs University Faculty of Medicine between January 2010 and December 2022. In the laboratory, samples were subjected to Ehrlich–Ziehl–Neelsen (EZN) staining, culture on Löwenstein–Jensen (LJ) medium, BACTEC MGIT 960 system (BD, USA), and PCR (2010–2019 GeneXpert MTB/RIF, 2020–2022 BD MAX MDR-TB) for diagnosis. A total of 10 547 extrapulmonary samples were examined in the study. Growth was detected in 185 (1.75%) of the EP samples. *Mycobacterium tuberculosis* complex was identified in 181 of these samples, while growth of nontuberculous mycobacteria was observed in four samples. Of the samples with growth, 167 (90.27%) were isolated from the adult patient group; 18 (9.73%) were from the pediatric patient group. Looking at the distribution of clinics that sent the samples, it was found that 100 (54%) samples were sent from internal medicine clinics, 67 (36%) from surgical clinics, and 18 (10%) from pediatric clinics. Abscesses accounted for 37 (20%), surgical material 35 (19%), urine 28 (15%), thoracentesis fluid 20 (11%), cerebrospinal fluid 16 (9%), paracentesis fluid 8 (4%), pleural fluid 8 (4%), and others (pericardial fluid, peritoneal fluid, joint fluid, aspiration, exudate, etc.) 33 (18%). EZN positivity in these samples was found to be 18% (33/185). Tuberculosis PCR

was applied to 102 (102/185) of these EP samples, and PCR positivity was detected in 79 samples (78%). Of the 79 samples with detected PCR positivity, AFB positivity was also observed in 26 (33%). The detection rate of the *M. tuberculosis* complex in extrapulmonary specimens has been found to be quite low. Among extrapulmonary specimens, the highest positivity rate was observed in abscess specimens. The use of multiple diagnostic methods (culture + PCR) for detecting *M. tuberculosis* in extrapulmonary specimens may improve the detection rate.

Key words: extrapulmonary tuberculosis, *Mycobacterium tuberculosis*

Introduction. Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a multisystemic infectious disease that can affect all tissues and organs [1]. The vast majority of TB cases involve the lungs (pulmonary TB), and the primary mode of transmission and spread of the disease occurs through the lungs [2,3]. Pulmonary tuberculosis (PTB) is defined as the involvement of the lung parenchyma, trachea, and bronchial tree, and it constitutes the most common form of TB [4,5]. In contrast, cases that develop without lung parenchymal involvement, such as those with pleural effusion or intrathoracic lymph node enlargement, are classified as extrapulmonary tuberculosis (EPTB). When both forms are present, cases are reported to the World Health Organization (WHO) as pulmonary tuberculosis, with concurrent involvement noted separately [4]. According to international classifications, EPTB includes TB forms that affect extrapulmonary sites such as the pleura, lymph nodes, genitourinary system, skin, joints, bones, meninges, and brain parenchyma [6]. In this context, the detection of acid-fast bacilli (AFB) in specimens obtained from extrapulmonary sites or the presence of clinical findings consistent with TB supports a diagnosis of EPTB [7].

According to the WHO's 2024 Global TB Report, 8.2 million new TB cases were reported worldwide in 2023, the highest number of cases recorded since global surveillance began in 1995. This figure marks a significant increase from the 7.5 million cases reported in 2022. As the impact of the COVID-19 pandemic wanes, TB has once again become the single most deadly infectious disease globally. In 2023, an estimated 1.25 million people died from tuberculosis, almost double the number of deaths from HIV/AIDS [8].

Although EPTB is a significant component of the disease burden, it often receives secondary attention in international control strategies and is not adequately addressed [2,3]. According to the WHO's 2020 Global TB Report, EPTB accounted for 16% of the 7.1 million TB cases reported in 2019, while in Türkiye specifically, this rate was reported as 35% [9]. This situation indicates that extrapulmonary forms constitute a significant share of the TB burden in our country.

The diagnosis of EPTB is often challenging due to its complex clinical presentation and the fact that specimens must be obtained through invasive procedures. Delays in EPTB diagnosis can lead to treatment problems and, indirectly, to increased mortality and morbidity. Therefore, the microbiological evaluation

of extrapulmonary samples is of critical importance for establishing an accurate diagnosis.

This study aims to evaluate extrapulmonary samples sent to our laboratory with a pre-diagnosis of tuberculosis between 2010 and 2022 and to determine the distribution of sample types of EPTB involvement.

Materials and methods. This retrospective, descriptive study included extrapulmonary clinical samples sent with a pre-diagnosis of tuberculosis to the Tuberculosis Diagnosis Laboratory at Ondokuz Mayıs University Faculty of Medicine between January 2010 and December 2022.

The samples included in the study were obtained from extrapulmonary sites: surgical materials ($n = 591$), pleural fluid ($n = 623$), thoracentesis ($n = 2241$), paracentesis ($n = 1327$), cerebrospinal fluid ($n = 1219$), urine ($n = 2704$), abscess ($n = 551$), and others – pericardial fluid ($n = 291$), peritoneal fluid ($n = 91$), joint fluid ($n = 106$), aspiration ($n = 81$), exudate ($n = 105$), bone marrow ($n = 54$), ascitic fluid ($n = 226$), wound ($n = 168$), body fluid ($n = 13$), puncture fluid ($n = 10$), dialysis fluid ($n = 69$), cyst fluid ($n = 27$), drain fluid ($n = 39$), and stool ($n = 11$), and were evaluated for diagnostic purposes in our laboratory.

The laboratory methods used for tuberculosis diagnosis were as follows:

- **Direct microscopy:** The Ehrlich–Ziehl–Neelsen (EZN) staining method was used to screen for acid-fast bacilli (AFB).
- **Culture:** All samples were cultured on Löwenstein–Jensen (LJ) solid medium. In addition, the BACTEC MGIT 960 system (BD, USA) was used as a liquid culture method.
- **Molecular diagnosis:** PCR-based molecular analysis was performed using the GeneXpert MTB/RIF (Cepheid, USA) system from 2010 to 2019 and the BD MAX MDR-TB (Becton Dickinson, USA) system from 2020 to 2022.

All samples were homogenized and decontaminated using the N-acetyl-L-cysteine (NALC)-NaOH method recommended by the Centers for Disease Control and Prevention (CDC). The samples were then evaluated for *Mycobacterium tuberculosis complex* (MTB) using Ehrlich–Ziehl–Neelsen (EZN) staining, Löwenstein–Jensen (LJ) solid media, and MGIT 960 liquid automated systems.

The susceptibility of the strains to first-line anti-tuberculosis (anti-TB) drugs was determined using automated liquid systems. Susceptibility tests were performed with the BACTEC MGIT 960 (BD, USA) automated liquid culture system. The resistance breakpoints were accepted as 1.0 µg/mL for rifampicin, 0.1 µg/mL for isoniazid, 1.0 µg/mL for streptomycin, and 5.0 µg/mL for ethambutol [15].

Data obtained from the clinical samples were retrospectively reviewed through the laboratory information system, and positivity rates and their distribution according to diagnostic methods were analyzed. The data collected for the study

were recorded in Microsoft Excel. Descriptive statistics for continuous variables, including the mean or median, standard deviation, minimum, and maximum values, were presented. Categorical variables were expressed as numbers (n) and percentages (%).

This study was approved by the Ondokuz Mayıs University Faculty of Medicine Clinical Research Ethics Committee with decision number KAEK 2025/473 on September 10, 2025.

Results. A total of 10 547 extrapulmonary (EP) clinical samples were examined in this study. Of these samples, culture positivity was detected in 185 (1.75%). Of the 185 samples with bacterial growth, 167 (90.27%) belonged to the adult patient group and 18 (9.73%) to the pediatric group.

The mean age of patients in the adult group was 49.2 years, with an age range of 18–83 years. The mean age of the 18 culture-positive patients in the pediatric group was 7.22 years, with ages ranging from 6 months to 17 years. Of the 167 adult patients, 80 were female and 87 were male. The mean age for female patients was 45.82 years (range: 18–81 years), and for male patients, it was 52.46 years (range: 18–83 years).

Of the 185 culture-positive samples, *Mycobacterium tuberculosis* complex (MTB) was isolated in 181, while nontuberculous mycobacterial growth was detected in four samples.

When the distribution of samples by the clinics they were sent from was examined, 54% ($n = 100$) came from internal medicine clinics, 36% ($n = 67$) from surgical clinics, and 10% ($n = 18$) from pediatric health and disease clinics.

The distribution of the 185 culture-positive EP samples by type is shown in Table 1.

Acid-fast bacilli (AFB) positivity was detected in 18% (33/185) of the culture-positive EP samples. Of these samples, 102 were tested with the tuberculosis PCR

T a b l e 1
The distribution of the 185 culture-positive EP samples

Sample type	Number (n)	Percentage (%)
Abscess	37	20
Surgical material	35	19
Urine	28	15
Thoracentesis fluid	20	11
Cerebrospinal fluid (CSF)	16	9
Paracentesis fluid	8	4
Pleural fluid	8	4
Other (pericardial fluid, peritoneal fluid, joint fluid, aspirate, exudate, etc.)	33	18

T a b l e 2

Detailed analysis of culture-positive EP samples

Sample type	Total culture positive (n)	AFB positive n(%)	PCR tested (n)	PCR positive n(%)	Culture isolate distribution
Abscess	37	12(32.4)	26	25(96.2)	37 <i>M. tuberculosis</i> complex (100%)
Surgical material	35	4(11.4)	18	12(66.7)	34 <i>M. tuberculosis</i> complex (97%), 1 NTM (3%)
Urine	28	10(35.7)	15	14(93.3)	26 <i>M. tuberculosis</i> complex (93%), 2 NTM (7%)
Thoracentesis fluid	20	1(5.0)	6	1(16.7)	19 <i>M. tuberculosis</i> complex (95%), 1 NTM (5%)
CSF	16	0	14	12(85.7)	16 <i>M. tuberculosis</i> complex (100%)

test, and 79 (78%) were found to be PCR-positive. Among the 79 PCR-positive samples, 26 (33%) were also AFB-positive.

The most frequently positive sample types from the 185 culture-positive samples – abscess, surgical material, urine, thoracentesis fluid, and CSF samples – were analyzed in detail (Table 2):

- **Abscess samples (n = 551):** A total of 551 abscess samples were sent to the laboratory during the specified period (2010–2022). AFB positivity was detected in 12 (32.4%) of the 37 total culture-positive abscess samples. PCR analysis was performed on 26 of these samples, with 25 (96.2%) yielding a positive result. The causative agent isolated from all abscess samples was identified as *M. tuberculosis*.
- **Surgical material samples (n = 591):** A total of 591 surgical material samples were submitted. AFB positivity was observed in 4 (11.4%) of the 35 culture-positive surgical materials. In this group, 12 out of 18 samples tested by PCR (66.7%) were found to be positive. Culture showed *M. tuberculosis* in 34 samples and nontuberculous mycobacterial growth in one sample.
- **Urine samples (n = 2704):** A total of 2704 urine samples were received by the laboratory. Of these, 28 were culture-positive. AFB positivity was determined in 10 (35.7%) of the culture-positive urine samples. Of the 15 samples that underwent PCR, 14 (93.3%) were positive; nontuberculous mycobacterial growth was also observed in two samples.
- **Thoracentesis fluid samples (n = 2241):** A total of 2241 thoracentesis samples were processed in the laboratory. Twenty of these samples were

culture-positive, and only 1 (5%) of these positive samples was AFB-positive. Of the six samples analyzed by PCR, only 1 (16.7%) yielded a positive result. Additionally, one nontuberculous mycobacterial growth was detected in this sample group.

- **Cerebrospinal fluid (CSF) samples ($n = 1219$):** During the same time period, 1219 CSF samples were processed in our laboratory. None of the 16 culture-positive CSF samples were AFB-positive. However, PCR was positive in 12 out of the 14 samples tested (85.7%). In all CSF samples, *M. tuberculosis* was isolated by culture.

Upon examining the distribution of the four samples exhibiting non-tuberculous mycobacterial (NTM) growth, we discovered that they consisted of two urine samples, one surgical specimen, and one thoracentesis fluid. Among these, one NTM was identified by the National Tuberculosis Reference Laboratory as *Mycobacterium kansasii*, while the remaining samples were deemed contaminated and not identified.

Antibiotic susceptibility testing was performed on 101 of the 185 culture-positive samples. The BACTEC MGIT 960 system (BD, USA), an automated liquid-media-based culture method, was used to determine susceptibility. The test examined susceptibility to the first-line drugs: isoniazid, rifampicin, streptomycin, and ethambutol.

According to the results, resistance was detected in nine samples for streptomycin, six for isoniazid, six for ethambutol, and three for rifampicin among the 101 samples tested for susceptibility. Isoniazid and rifampicin resistance, which is indicative of multidrug resistance, was found in three samples. Additionally, one sample was found to be resistant to all first-line drugs. The three samples with multidrug resistance were from abscess, surgical material, and exudate.

Discussion. In this study, data from 10 547 extrapulmonary samples sent to the Ondokuz Mayıs University Medical Microbiology laboratory between 2010 and 2022 were evaluated. Culture positivity was found to be 1.75%, and a total of 185 samples were analyzed in detail. The findings were compared with the literature in terms of sample type distribution, diagnostic method efficacy, and mycobacterial species distribution.

The most common sample type with culture positivity in our study was abscess material ($n = 37$), followed by surgical material ($n = 35$), urine ($n = 28$), thoracentesis fluid ($n = 20$), and cerebrospinal fluid ($n = 16$). The positivity rates for these sample types show similarities to a study conducted in Mersin, where the rates for lymph node biopsy (34.7%), urine (19.8%), and pleural fluid (18.8%) were reported [10]. Similarly, a study in the Thrace region reported pleural fluid and lymph node biopsies as the most frequent positive samples [11]. Another study in Van province reported soft tissue and lymph node samples as being predominant [3]. A study from India indicates that the highest extrapulmonary tuberculo-

sis (EPTB) positivity rates are found in abscesses (47.06%), lymph node biopsies (38.37%), and pleural fluid (12.45%) [12]. These differences can be attributed to geographic characteristics, patient profiles, and sample collection methods.

Positivity for acid-fast bacilli (AFB) using classical microscopic methods like the EZN staining method was found to be low, making diagnosis difficult, especially in samples with low bacillary load such as cerebrospinal fluid and pleural fluid. In contrast, the sensitivity of PCR-based molecular methods was found to be very high and played a decisive role in the diagnosis of these samples. In a study from India using the cartridge-based nucleic acid amplification test (CB-NAAT), positivity was reported as 37.6% for abscess samples and 9.67% for CSF samples [13]. In our study, PCR positivity was detected in 85.7% of CSF samples and 96.2% of abscess samples. This demonstrates that molecular methods provide a significant advantage in terms of sensitivity, especially for samples with a low bacillary load.

In the culture-positive samples obtained in this study, *Mycobacterium tuberculosis complex* grew in 97.8% of cases, while NTM were found in only 2.2%. Among the samples with NTM growth, only one was identified as *Mycobacterium kansasii*, and the other three could not be identified at the species level. These rates are consistent with the studies from Thrace and Mersin, which noted the limited capacity of classical culture methods for NTM diagnosis. Higher rates of NTM have been reported when using next-generation sequencing (NGS) techniques [14].

Among the 101 samples that underwent drug susceptibility testing, isoniazid resistance was detected in 5.9% and rifampicin resistance in 3.0%. Isoniazid + rifampicin resistance, which is defined as MDR-TB, was observed in three samples (3.0%). In a study from India, this rate was given as 8.2%. It is known that general MDR-TB rates in Türkiye range between 2–4% [7]. The early diagnosis and appropriate treatment of MDR-TB are of great importance for public health.

The strengths of our study include its long 13-year follow-up period, large sample size, and the analysis of various sample types. This enabled statistical analysis even for rare sample types and allowed for a comparison between culture and molecular methods. The exclusion of patient clinical data and treatment responses from the analysis limits the clinical correlation of the diagnostic methods. Therefore, it is recommended that future prospective, multicentre studies be conducted to evaluate the clinical accuracy of molecular diagnostic methods.

In conclusion, the type of sample and the diagnostic method used are among the most critical determinants of success in the diagnosis of extrapulmonary tuberculosis. Molecular methods, in particular, provide higher sensitivity compared to classical methods, especially in samples with low bacillary loads. However, culture still maintains its place as the gold standard in the diagnostic process.

REFERENCES

- [1] FITZGERALD D. W., T. R. STERLING, D. W. HAAS (2021) *Mycobacterium tuberculosis*. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 9th edn, Elsevier, 2985–3021.
- [2] AYED H. B., M. KOUBAA, C. MARRAKCHI et al. (2018) Extrapulmonary tuberculosis: Update on the epidemiology, risk factors and prevention strategies, *Int. J. Trop. Dis.*, **1**(1), 1–6.
- [3] BINICI İ., F. KAYA (2019) Evaluation of extrapulmonary tuberculosis cases in Van region, *Van Med. J.*, **26**(2), 200–205, (in Turkish).
- [4] Republic of Turkey Ministry of Health, General Directorate of Public Health, Tuberculosis Control in Turkey 2021 Report, Publication No. 1274, Ankara, 2023.
- [5] TANYEL E., A. DEVECI, L. ŞENSOY et al. (2023) Evaluation of adult extrapulmonary tuberculosis patients, *Klimik J.*, **36**(1), 52–57, (in Turkish).
- [6] MAURYA D. P., Y. SULTANA, M. SHARMA (2010) Extrapulmonary tuberculosis – its management and control, *Curr. Drug Ther.*, **5**(1), 67–74.
- [7] Republic of Turkey Ministry of Health, Tuberculosis Control Department Report, Publication No. 1059, Ankara, 2015.
- [8] World Health Organization (2024) Global tuberculosis report 2024, Geneva, WHO, Licence: CC BY-NC-SA 3.0 IGO.
- [9] World Health Organization (2020) Global tuberculosis report 2020, Geneva, WHO, Licence: CC BY-NC-SA 3.0 IGO.
- [10] ASLAN G., B. GÜLTEPE, G. EMEKDAŞ, L. BEYTUR (2017) The role of molecular methods in the diagnosis of extrapulmonary tuberculosis, *Microbiol. Bull.*, **51**(4), 390–400, (in Turkish).
- [11] TOPÇU A., B. TIRYAKI, A. KARA (2020) Evaluation of patients diagnosed with extrapulmonary tuberculosis in the Thrace region, *Trakya University Faculty of Medicine Journal*, **37**(1), 43–49, (in Turkish).
- [12] VIDYARAJ C. K., M. J. VADAKUNNEL, B. R. MANI et al. (2025) Prevalence of extrapulmonary tuberculosis and factors influencing successful treatment outcomes among notified cases in South India, *Sci Rep.*, **15**(1), 8290, <https://doi.org/10.1038/s41598-025-92613-5>.
- [13] KASHYAP B., K. SARKAR, S. LNU et al. (2024) Evaluating diagnostic performance of extrapulmonary tuberculosis using cartridge-based nucleic acid amplification test assay: a retrospective cross-sectional study at a tertiary health care setup in India, *Eur. Med. J.*, **9**(4), 89–96, <https://doi.org/10.33590/emj/KBFU2758>.
- [14] GOU B., C. ZHANG, Z. SONG et al. (2025) Targeted next-generation sequencing for rapid tuberculosis detection: a systematic review and meta-analysis, *Clin. Chim. Acta*, **577**, 120469, <https://doi.org/10.1016/j.cca.2025.120469>.
- [15] Clinical and Laboratory Standards Institute (2011) Performance Standards for Antimicrobial Susceptibility Testing, 21st Informational Supplement, CLSI Document M100-S21, Wayne, 2011.

Department of Medical Microbiology, Faculty of Medicine, Ondokuz Mayıs University, 55270 Atakum/Samsun, Türkiye

e-mails: yeliztanriverdi@gmail.com, safatoy55@gmail.com, demet.gur@yandex.com, kemal.bilgin@omu.edu.tr, cinarcanberk57@gmail.com, asumanbirinci@yahoo.com