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Mycobacteria Presence and Anti-Tuberculosis Drug Resistance Profile in Düzce Region: 18 Years of Experience

Düzce Bölgesinde Mikobakteri Varlığı ve Antitüberküloz İlaç Direnci Profili: 18 Yıllık Deneyim

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ABSTRACT

Aim: The aim of this study was to determine the frequency of *Mycobacterium tuberculosis* complex (MTBC) and non-tuberculous mycobacteria (NTM) growth among mycobacterial isolates collected over 18 years from patients suspected of having tuberculosis, and to evaluate the resistance rates of MTBC strains to first-line anti-tuberculosis drugs.
Material and Methods: A total of 17,199 clinical specimens, sent to the Mycobacteriology Laboratory, Department of Medical Microbiology, at Düzce University Health Application and Research Centre between 2004 and 2021 for suspected tuberculosis, were retrospectively analyzed in this study. To differentiate MTBC from NTM, the TBC Identification Test was performed to detect the MPT64 antigen of the MTBC. Ehrlich-Ziehl-Neelsen (EZN) staining method was used to detect the presence of acid-fast bacilli (AFB).
Results: Out of the samples with suspected tuberculosis, mycobacterial growth was detected in 896 (5.2%) cases. Among these, 824 (91.9%) were identified as MTBC, while 72 (8.1%) were classified as NTM. EZN staining revealed that 404 (45.1%) out of the 896 isolates were found AFB positive. Analysis of first-line anti-tuberculosis drug susceptibility in the 824 MTBC isolates revealed that 194 were resistant to at least one first-line anti-tuberculosis drug. Of these, 135 isolates showed resistance to a single drug, while 59 exhibited resistance to more than one drug.
Conclusion: Tuberculosis remains a significant public health problem both globally and in Türkiye. Regular investigation of local and regional growth patterns and resistance profiles is crucial for achieving the goals of tuberculosis elimination and eradication.
Keywords: Tuberculosis; non-tuberculous mycobacteria; anti-tuberculosis drug resistance.

ÖZ

Amaç: Bu çalışmanın amacı, tüberküloz şüphesi olan hastalardan 18 yıl boyunca toplanan mikobakteri izolatlarında *Mycobacterium tuberculosis* kompleks (MTBK) ve tüberküloz dışı mikobakteri (TDM) üreme sıklığını belirlemek ve MTBK olarak izole edilen suşların birinci basamak anti-tüberküloz ilaçlara direnç oranlarını değerlendirmektir.
Gereç ve Yöntemler: Bu çalışmada, Düzce Üniversitesi Sağlık Uygulama ve Araştırma Merkezi Tıbbi Mikrobiyoloji Anabilim Dalı Mikobakteriyoloji Laboratuvarı'na 2004 ve 2021 yılları arasında tüberküloz şüphesi ile gönderilen toplam 17.199 klinik örnek geriye dönük olarak analiz edildi. MTBK'yi TDM'den ayırt etmek için, MTBK'nin MPT64 antijenini tespit etmek amacıyla TBC Tanımlama Testi yapıldı. Asit-dirençli basillerin (ARB) varlığını tespit etmek amacıyla Ehrlich-Ziehl-Neelsen (EZN) boyama yöntemi kullanıldı.
Bulgular: Tüberküloz şüphesi ile gönderilen örneklerden 896 (%5,2) vakada mikobakteri üremesi tespit edildi. Bunlardan 824'ü (%91,9) MTBK olarak tanımlanırken 72'si (%8,1) TDM olarak sınıflandırıldı. EZN boyama sonucunda 896 izolatın 404'ünde (%45,1) ARB pozitifliği saptandı. 824 MTBK izolatında birinci basamak anti-tüberküloz ilaç duyarlılığının analizi, 194'ünün en az bir birinci basamak anti-tüberküloz ilaca dirençli olduğunu ortaya koydu. Bunlardan 135 izolat tek bir ilaca direnç gösterirken, 59'u birden fazla ilaca direnç göstermekteydi.
Sonuç: Tüberküloz, hem küresel olarak hem de Türkiye'de önemli bir halk sağlığı sorunu olmaya devam etmektedir. Tüberkülozun ortadan kaldırılması ve yok edilmesi hedeflerine ulaşmak için yerel ve bölgesel üreme kalıplarının ve direnç profillerinin düzenli olarak araştırılması hayati önem taşımaktadır.
Anahtar kelimeler: Tüberküloz; atipik mikobakteri; anti-tüberküloz ilaç direnci.

INTRODUCTION

Tuberculosis (TB) is one of the oldest infectious diseases, caused by *Mycobacterium tuberculosis* complex (MTBC), especially *M. tuberculosis* (1). Mycobacterial species that do not belong to this complex are referred to as non-TB mycobacteria (NTM), which are commonly found in the environment (2). While most NTM isolates are not clinically significant, recent studies have reported an increase in NTM infections, especially in the immunocompromised patients (3).

According to the World Health Organization (WHO), TB is the leading cause of death worldwide. Despite being both preventable and treatable, it still causes the death of approximately 1.5 million people every year (4).

The global effort to end the TB epidemic by 2030 was interrupted by the coronavirus disease 2019 (COVID-19) pandemic, resulting in a significant reduction in TB data. The number of newly reported TB cases dropped from 7.1 million in 2019 to 5.8 million in 2020, reverting to levels last seen in 2012. This reduction in access to TB diagnosis and treatment has caused an increase in deaths related to TB. In addition, it is estimated that these negative effects will worsen in the coming years (4).

Accurate and rapid diagnosis is the most important step in TB treatment and in preventing its transmission. While advancements in molecular methods have significantly improved TB diagnostics, the isolation of *M. tuberculosis* through culture remains the gold standard (5).

Delays in the diagnosis of TB and the emergence of drug-resistant strains pose significant challenges to eradicating the disease. Resistance to isoniazid and rifampicin, the most effective first-line treatments, is significantly concerning. Resistance to these drugs is classified as multidrug-resistant TB (MDR-TB). According to the WHO (4), an estimated half a million cases of MDR-TB have been reported in recent years, with 8.5% of these classified as extensively drug-resistant TB (XDR-TB).

This study aimed to determine the frequency of MTBC and NTM isolated from clinical samples with suspected TB over 18 years. Additionally, it sought to determine the resistance rates of MTBC species to first-line anti-TB drugs.

MATERIAL AND METHODS

A total of 17,199 clinical specimens sent to the Mycobacteriology Laboratory, Department of Medical Microbiology, at Düzce University Faculty of Medicine Health Application and Research Centre between 2004 and 2021 from various clinics and outpatient departments were retrospectively examined.

Lung specimens and non-sterile clinical specimens underwent homogenization and decontamination using conventional methods with N-acetyl-L-cysteine and 4% sodium hydroxide. Sterile samples, on the other hand, did not require decontamination. The processed samples were cultured on Löwenstein-Jensen (LJ) (Becton Dickinson, USA) as solid medium and Mycobacterium Growth Indicator Tube (BACTEC BD MGIT 320, Sparks, USA) as liquid medium following the manufacturer's instructions. Smear preparations were stained using Ehrlich-Ziehl-Neelsen (EZN) method and evaluated under a light microscope to detect the presence of acid-fast bacilli (AFB).

LJ media inoculated for bacterial growth were monitored weekly for up to eight weeks, and growth was evaluated accordingly. Similarly, the samples inoculated in the BACTEC MGIT BD 320 (Becton Dickinson, USA) automated system according to the manufacturer's recommendations were incubated for six weeks. Samples showing no growth by the end of these periods were considered negative. To differentiate MTBC from NTM, the TBC Identification Test (Becton Dickinson, USA) was performed to detect the MPT64 antigen of the MTBC, following the manufacturer's instructions (6). Drug susceptibility of the MTBC isolates, identified through differentiation against isoniazid (INH), streptomycin (SM), rifampicin (RIF), and ethambutol (EMB), was conducted using BACTEC MGIT 320 method with the SIRE kit containing first-line anti-TB drugs following the manufacturer's recommendations (5).

Prior to the study, permission was obtained from Düzce University Non-Interventional Ethics Committee, dated 25.07.2022, with reference number 138.

Statistical Analysis

The statistical analysis of the study was performed using IBM SPSS v.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) software. Categorical data were summarized as frequencies and percentages. Chi-square, Fisher's exact and Fisher-Freeman-Halton tests were used for data analysis. $p<0.05$ was considered statistically significant.

RESULTS

In this study, a total of 17,199 clinical samples sent to the Mycobacteriology Laboratory, Department of Medical Microbiology, at Düzce University Faculty of Medicine Health Application and Research Centre between 2004 and 2021, with a preliminary diagnosis of TB, were analyzed. Only 896 (5.2%) of the clinical samples showed growth. Of these, 824 (91.9%) were identified as MTBC and 72 (8.1%) as NTM. Of the 824 MTBC isolates, while 743 (90.2%) were isolated from lung specimens, 81 (9.8%) from extra-pulmonary specimens. In contrast, 68 (94.4%) of the NTM strains were isolated from lung specimens and 4 (5.6%) from extra pulmonary specimens (Table 1).

Staining of the samples using EZN method revealed that 404 (45.1%) out of the 896 isolates were found AFB positive. Among the samples with MTBC, 397 (48.2%) were AFB positive and seven (9.7%) NTM isolates were AFB positive (Table 1). The AFB positivity rate in MTBC-positive samples was statistically significantly higher than that of NTM isolates ($p<0.001$).

The distribution of samples with mycobacterial growth according to patient gender was analyzed (Table 2). Among the patients with MTBC isolates, 546 (66.3%) were male and 278 (33.7%) were female, while 51 (70.8%) male and 21 (29.2%) female patients had NTM isolates. No statistically significant difference was found between genders in terms of MTBC and NTM growth in patients with mycobacterial growth ($p=0.430$).

Analysis of the distribution of identified MTBC isolates over the years revealed that the highest number of isolates was obtained in 2010. Meanwhile, the number of isolates significantly decreased during the COVID-19 pandemic, reaching a minimum, especially in 2021.

Table 1. Distribution of mycobacteria according to sample type and EZN staining result

	MTBC (n=824)		NTM (n=72)	
Sample	**N**	**AFB (+)**	**N**	**AFB (+)**
Phlegm	577	319 (38.7)	40	4 (5.6)
BAL	140	41 (4.9)	25	1 (1.4)
GA	30	11 (1.3)	3	0 (0.0)
Biopsy	22	12 (1.5)	3	1 (1.4)
Urine	11	7 (0.9)	-	-
Body fluid	25	4 (0.5)	-	-
Wound	18	2 (0.2)	1	1 (1.4)
CSF	1	1 (0.1)	-	-
Total	**824**	**397 (48.2)**	**72**	**7 (9.7)**

N: number of isolated samples, EZN: Ehrlich-Ziehl-Neelsen, MTBC: mycobacterium tuberculosis complex, NTM: non-tuberculosis mycobacteria, AFB: acid-fast bacilli, BAL: bronchoalveolar lavage, GA: gastric aspirat, CSF: cerebrospinal fluid

Table 2. Comparison of patients with MTBC and NTM isolates according to the gender

	MTBC (n=824)	**NTM (n=72)**	**p**
Gender, n (%)			
Female	278 (33.7)	21 (29.2)	0.430
Male	546 (66.3)	51 (70.8)	

MTBC: mycobacterium tuberculosis complex, NTM: non-tuberculosis mycobacteria

Table 3. Resistance status of MTBC isolates

		MTBC (n=824)	**Isolates with at least one resistance (n=194)**
Anti-TB Drugs	**n**	**%**	**%**
INH	119	14.4	61.3
RIF	24	2.9	12.4
SM	77	9.3	39.7
EMB	54	6.5	27.8
Single Drug	135	16.4	69.6
INH	74	8.9	38.1
RIF	5	0.6	2.6
SM	32	3.9	16.5
EMB	24	2.9	12.4
Two Drug	40	4.8	20.6
INH+RIF	7	0.8	3.6
INH+SM	18	2.2	9.3
INH+EMB	4	0.5	2.1
RIF+SM	1	0.1	0.5
RIF+EMB	1	0.1	0.5
EMB+SM	9	1.1	4.6
Tree Drug	17	2.1	8.8
INH+RIF+SM	3	0.3	1.5
INH+RIF+EMB	2	0.2	1.0
INH+SM+EMB	9	1.1	4.6
RIF+SM+EMB	3	0.3	1.5
Four Drug	2	0.2	1.0

MTBC: mycobacterium tuberculosis complex, TB: tuberculosis, INH: isoniazid, RIF: rifampicin, SM: streptomycin, EMB: ethambutol

Analysis of the first-line anti-TB drug susceptibility of 824 MTBC isolates revealed that 194 of these isolates were resistant to at least one first-line anti-TB drug. The number of isolates resistant to INH, RIF, SM, and EMB were 119, 24, 77, and 54, respectively. Among the resistant MTBC isolates, 135 were resistant to a single drug, while 59 were resistant to more than one drug. Of the latter group, 14 were found to be MDR-TB due to concurrent resistance to both INH and RIF (Table 3).

The evaluation of anti-TB resistance over the years revealed that the resistance rates for years 2016, 2017, and 2021 were not statistically significantly different from one another ($p=0.632$), but were significantly higher compared to the other years ($p<0.001$). Additionally, 2016 was the only year in which resistance to all four anti-TB drugs was detected (Table 4).

DISCUSSION

Tuberculosis (TB) can affect individuals of any age or gender. According to the WHO Global Tuberculosis Report 2021, 56% of all TB cases in 2020 were in males, while 33% were in females. The same report also indicated that TB is more prevalent among individuals between the ages of 25-34 (4). In the Turkish Tuberculosis Control Report, 58.5% of all TB patients were male, and 41.5% were female (7). Kirui et al. (8) analyzed 120 TB cases in Nairobi and reported that 73% of the patients were male, while 27% were female. In Türkiye, Yazısız et al. (9) analyzed data from 974 patients and reported that 74.5% of TB cases were male and 25.5% were female. In this study, the gender distribution of TB patients revealed that 66.3% were male and 33.7% were female. Therefore, the findings in this study align with the literature.

TB is a systemic disease that typically affects the lungs but can also involve other organs and tissues. In 2020, 4.8 million new pulmonary TB cases were diagnosed worldwide, accounting for nearly half of all TB cases (4). According to the Tuberculosis Control Report in Türkiye, pulmonary TB cases constituted 60.9% of all TB cases (7). A study by Yazısız et al. (9) showed that lung samples comprised the majority (92.1%) of specimens from which MTBC was isolated. In this study, 743 (90.2%) out of 824 MTBC strains were isolated from lung samples. When compared to the data worldwide and in Türkiye, the pulmonary TB rates observed in the present study were found to be similar.

In the diagnosis of TB, searching for AFB using the EZN staining method is a simple, rapid, and inexpensive method. Kivihya-Ndugga et al. (10) reported that 332 (59.9%) out of 554 samples from which MTBC was isolated were AFB positive. Similarly, Yıldıran et al. (11) reported that 1,221 (71.8%) out of 1,701 samples with MTBC isolates were AFB positive. Tarhan et al. (12) reported 89 (86.4%) out of 103 MTBC isolates cultured were AFB positive. In the current study, 397 (48.2%) MTBC isolates and 7 (9.7%) NTM isolates were found to be AFB positive. Notably, the low positivity rate of EZN staining in samples with NTM isolates is remarkable. In the surveyed literature, no data on EZN staining results for NTM isolates were found. In this study, the findings revealed a lower AFB positivity rate compared to existing literature. The reason for this discrepancy may be attributed to factors such as the type of sample material, smear thickness, bacilli concentration

Table 4. Distribution of resistance in MTBC isolates according to years

Year	INH	RIF	SM	EMB	Two drug	Three drug	Four drug	Total
2004 (n=19)	4 (21.1)	2 (10.5)	0 (0.0)	1 (5.3)	1 (5.3)	1 (5.3)	0 (0.0)	4 (21.1)
2005 (n=41)	1 (2.4)	0 (0.0)	1 (2.4)	3 (7.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (12.2)
2006 (n=36)	0 (0.0)	1 (2.8)	1 (2.8)	3 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (13.9)
2007 (n=47)	5 (10.6)	2 (4.3)	3 (6.4)	4 (8.5)	3 (6.4)	0 (0.0)	0 (0.0)	16 (34.0)
2008 (n=42)	6 (14.3)	1 (2.4)	2 (4.8)	2 (4.8)	1 (2.4)	0 (0.0)	0 (0.0)	10 (23.8)
2009 (n=60)	11 (18.3)	4 (6.7)	8 (13.3)	4 (6.7)	5 (8.3)	4 (6.7)	0 (0.0)	14 (23.3)
2010 (n=102)	6 (5.9)	2 (1.9)	9 (8.8)	4 (3.9)	3 (2.9)	2 (1.9)	0 (0.0)	13 (12.7)
2011 (n=75)	8 (10.7)	2 (2.7)	8 (10.7)	5 (6.7)	3 (4.0)	2 (2.7)	0 (0.0)	13 (17.3)
2012 (n=65)	15 (23.1)	2 (3.1)	2 (3.1)	2 (3.1)	4 (6.2)	0 (0.0)	0 (0.0)	16 (24.6)
2013 (n=37)	2 (5.4)	0 (0.0)	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.1)
2014 (n=48)	6 (12.5)	2 (4.2)	8 (16.7)	0 (0.0)	3 (6.3)	0 (0.0)	0 (0.0)	13 (27.1)
2015 (n=37)	6 (16.2)	0 (0.0)	4 (10.8)	2 (5.4)	2 (5.4)	1 (2.7)	0 (0.0)	8 (21.6)
2016 (n=53)	19 (35.8)	5 (9.4)	11 (20.7)	9 (17.0)	5 (9.4)	4 (7.5)	2 (3.8)	**24 (45.3)**
2017 (n=44)	13 (29.5)	0 (0.0)	3 (6.8)	3 (6.8)	3 (6.8)	0 (0.0)	0 (0.0)	**18 (40.9)**
2018 (n=46)	3 (6.5)	0 (0.0)	7 (15.2)	5 (10.9)	2 (4.3)	0 (0.0)	0 (0.0)	13 (28.3)
2019 (n=33)	6 (18.2)	0 (0.0)	7 (21.2)	4 (12.1)	2 (6.1)	2 (6.1)	0 (0.0)	10 (30.3)
2020 (n=23)	4 (17.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (17.4)
2021 (n=16)	4 (25.0)	1 (6.3)	3 (18.8)	2 (12.5)	3 (18.8)	1 (6.3)	0 (0.0)	**5 (31.3)**
Total (n=824)	**119 (14.4)**	**24 (2.9)**	**77 (9.3)**	**54 (6.5)**	**40 (4.9)**	**17 (2.1)**	**2 (0.2)**	**194 (23.5)**

MTBC: mycobacterium tuberculosis complex, TB: tuberculosis, INH: isoniazid, RIF: rifampicin, SM: streptomycin, EMB: ethambutol

in the sample, and the experience of the person performing the microscopic examination.

Drug-resistant TB continues to be an important public health concern in Türkiye and many other countries worldwide. Detecting drug resistance in TB and initiating appropriate and effective treatment are critical steps in preventing infections and ensuring clinical recovery (5). Studies conducted in Türkiye and other countries have reported varying rates of resistance to anti-TB drugs. Arora et al. (13) evaluated 4,910 MTBC isolates and found that 3,941 isolates were resistant to at least one anti-TB drug. In the same study, resistance rates for INH, RIF, SM and EMB were reported as 8.3%, 0.44%, 0.16% and 1.9%, respectively. Similarly, He et al. (14) analyzed 13,486 TB cases in China and reported that 21.1% of the isolates were resistant to at least one first-line drug. Resistance rates for INH, RIF, SM and EMB were reported as 13.4%, 8.4%, 14.7% and 2.6%, respectively. In Türkiye, Yazısız et al. (9) investigated MTBC isolates at the Akdeniz University and reported that 26.7% were resistant to at least one of the first-line anti-TB drugs. In the aforementioned study, resistance rates for INH, RIF, SM and EMB were 9.1%, 1.3%, 4.4% and 0.5%, respectively. Yılmaz et al. (15) conducted a study in Erzurum and found that 19.6% of 419 MTBC strains were resistant to at least one drug. Analysis of resistance rates revealed that 1.9%, 4.1%, 11.7% and 3.6% for INH, RIF, SM and EMB, respectively. In the current study, among 824 MTBC strains isolated, 23.5% demonstrated resistance to at least one anti-TB drug. Resistance rates for INH, RIF, SM and EMB were 14.4%, 2.9%, 9.3% and 6.5%, respectively. Both in this study and in the previously mentioned literature, the most common resistance observed in MTBC isolates worldwide was against INH. This high resistance rate may be due to the frequent use of INH both in treatment and prophylaxis, poor patient compliance to treatment regimens, and geographical differences in drug use and access.

MDR-TB is a condition where MTBC is resistant to INH and RIF, the two most effective first-line anti-TB drugs, with or without resistance to other anti-TB drugs. Globally, the prevalence of MDR-TB among newly diagnosed TB cases ranges from 3-4%, while it increases to 18-21% among previously treated TB patients (4). In a meta-analysis conducted in Pakistan, Molla et al. (16) showed that the prevalence of MDR-TB was 4% in new TB cases and 21%. Similarly, Yılmaz et al. (15) evaluated 419 MTBC strains in Erzurum, Türkiye, and found an MDR-TB prevalence rate of 3.6% which aligns with the national average in Türkiye. In the present study, the MDR-TB prevalence among MTBC strains was shown to be 1.6%. Compared to global and regional data, including data in Türkiye, MDR-TB prevalence rate in the present study is relatively lower. Moreover, the findings in this study show variations in TB resistance over the years. Specifically, resistance rates in 2016, 2017 and 2021 were statistically significantly higher compared to other years, while no significant differences were observed among themselves. These findings are consistent with previous studies conducted in Türkiye. This study suggests that incorporating these resistance rates to anti-TB drugs into treatment planning for TB patients would be beneficial. In addition, obtaining epidemiological data and understanding resistance rates in the laboratory, is necessary for evaluating regional, national, and international progress in the battle against TB and reaching potential targets.

Although NTMs are typically acquired from the environment, with no evidence of person-to-person transmission, their clinical importance has grown in recent years. Moreover, the pathogenic roles of NTMs have been well established and they are known to cause serious clinical results, especially in immunocompromised individuals (17). The incidence, prevalence and strain distribution of NTM cases are highly variable among different geographical locations (3). However, accurately determining the prevalence and incidence of NTM infections remains challenging, as the microbiological isolation of these microbes does not always correspond to clinical infection. Recent guidelines recommend the use of clinical, radiographic, and microbiological criteria for the diagnosis of NTM-related lung disease. In a study by Özen et al. (5) involving 31,017 samples, 5.1% of the samples submitted with suspicion of TB were reported as MTBC, while 1.1% were identified as NTM. Similarly, Martínez González et al. (18) analyzed the distribution of NTM isolates according to sex and found that isolates were three times more common in males than females. In another study conducted in Japan, it was reported that there was a significant increase in lung disease cases due to NTM (19). In this study, 4.8% of the mycobacteria isolated were identified as MTBC and 0.4% as NTM, with 70.8% of the NTM strains isolated from male patients and 29.2% from female patients, primarily from respiratory tract samples. When compared to the existing literature, the MTBC and NTM rates in this study were found to be consistent with previously reported findings. The detection of NTMs in Düzce region emphasizes the clinical importance and frequency of these isolates. Notably, this is the first time infections caused by NTMs have been identified from clinical samples in Düzce region, which constitutes a strength of this study. However, the lack of relevant data on whether guidelines for differentiating NTMs as causative agents or contaminants were applied by clinics represents a limitation. Therefore, this study recommends more comprehensive, collaborative studies with clinical facilities to address this concern.

CONCLUSION

The findings in this study indicate that TB remains a significant public health concern in Düzce region, which can be classified as endemic for TB. Notably, resistance rates in 2016, 2017, and 2021 were significantly higher compared to other years, highlighting periods of increased TB burden. Interestingly, the number of TB isolates identified dropped to a minimum in 2021, coinciding with the onset of the COVID-19 pandemic. This demonstrates the impact of global health crises such as COVID-19 on the surveillance and management of other infectious diseases. Such health crises can unknowingly lead to a reemergence of neglected diseases, emphasizing the need for sustained focus on the control of TB. Moreover, this study highlights the significance of whether the NTMs detected represent infections or mere contaminants. Future research should assess the adherence of clinicians to diagnostic guidelines to ensure accurate identification and management of NTMs. The biggest obstacle in global TB control programs is the increasing prevalence of drug-resistant bacilli. In addition, the lack of new TB drugs intensifies this problem. To meet the elimination and eradication targets for TB by the WHO, it is necessary to prioritize prevention, diagnosis, and treatment algorithms. This includes investigating local and regional resistance profiles, focusing on research for new drugs, and ensuring that global infections, such as the COVID-19 pandemic, do not reduce the importance of eradicating other infectious diseases.

Ethics Committee Approval: The study was approved by the Non-Interventional Health Research Ethics Committee of Düzce University (25.07.2022, 138).

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