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Updates on clinical and molecular epidemiology of tuberculosis

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Updates on clinical and molecular epidemiology of tuberculosis

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Investigation of 3-year inpatient TB cases in Zunyi, China: Increased TB burden but improved bacteriological diagnosis

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Background: As one of the top three high tuberculosis (TB) burden countries, China is a country where the overall TB incidence continues to decline. However, due to its large population and area, the increased TB burden exists in regional areas.

Methods: This retrospective study analyzed local inpatient pulmonary TB cases in the Affiliated Hospital of Zunyi Medical University (AHZMU) from January 2016 to December 2018 in a high TB incidence and economically-less-developed area of China. Four methods, acid-fast bacilli stain, culture, Xpert and LAMP, were used to detect *Mycobacterium tuberculosis* (*M.tb*), while proportional method and Xpert were used to identify rifampicin-resistant TB (RR-TB). Case number, treatment history, *M.tb* confirmed TB and rifampicin resistant proportion were analyzed to investigate the local TB epidemic.

Results: Total 3,910 local inpatient cases with pulmonary TB were admitted to AHZMU during this study period. The annual numbers of total TB cases increased 26.4% (from 1,173 to 1,483), while new cases increased 29.6% (from 936 to 1,213) and RR-TB cases increased 2.7 times (from 31 to 84). Meanwhile, the percentage of previously treated cases declined from 20.2 to 18.2% and the *M.tb* confirmed TB proportion increased from 34.7 to 49.7%.

Conclusion: The elevated *M.tb* confirmed TB proportion and the declined percentage of previously treated cases indicated the improved TB diagnosis and treatment of AHZMU. However, the increasing number of total TB cases, new and RR-TB cases showed an upward trend and increased TB burden in a relatively underdeveloped area of China.

KEYWORDS

tuberculosis (TB), tendency, rifampicin-resistant TB, epidemic, diagnosis

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M.tb*), has been a serious threat to human health since ancient times. In the past decade, more than 10 million people have died from this disease worldwide. To end the global TB epidemic, the End TB strategy was mapped with the aim of reducing the global incidence by 90% by 2035 compared to the 2015 baseline. However, there were still 10 million people who developed TB disease in 2019 (1), besides that, the COVID-19 pandemic has impacted progress toward achieving the goals set in the END TB strategy.

Although China is one of the top three high TB burden countries, the TB incidence had continually declined from the year 2000 to 2019 (1), making important efforts to global TB control. Due to the diversity of economics, culture and geography in China, the regional variances in TB epidemic status also exist. Guizhou Province, located in Southwest China, an economically-less-developed area, is one of the regions with the most severe TB epidemic (2). The incidence rate of Guizhou Province is 134/100,000 population, ranking third in China, more than two times higher than the national average (63/100,000 population) (3). Zunyi City, the second largest city of Guizhou Province. This retrospective study analyzed the 3-years data of the local (Zunyi City) TB inpatients of the Affiliated Hospital of Zunyi Medical University (AHZMU), to investigate the current TB regional epidemic situation and risk factors for rifampicin (RIF) resistance. As a key designated hospital for TB diagnosis and treatment in Guizhou Province, the AHZMU is the main TB hospital in Zunyi. Therefore, the data of AHZMU can be an indicator of the local TB epidemic.

Materials and methods

Data collection

From January 1, 2016 to December 31, 2018, a total of 3,910 inpatient cases from Zunyi were diagnosed as pulmonary TB in AHZMU. Demographic and clinical data of patients were collected. The data collection and procedures were described previously (4). Any information related to patient identity was filtered to ensure the data used in this study were anonymous. In order to avoid duplicated cases, each patient was assigned a unique hospital admission number, which was used to organize all patient data. This study has been approved by the ethics committee of Zunyi Medical University.

Laboratory procedure

For TB or presumptive TB patients, sputum samples were collected for *M.tb* detection. The specimen procedure was performed according to the previous study (5). Bacteriological testing methods included acid-fast bacilli (AFB) stain, Löwenstein-Jensen (L-J) solid media culture, GeneXpert MTB/RIF (Xpert) (Cepheid Inc. USA) and Loopamp MTBC Detection Kit (LAMP) (Eiken Chemical Co. Japan). AFB stain and culture followed the WHO recommended procedures, while Xpert and LAMP were operated according to the instruction manual. To distinguish *M.tb* and non-tuberculosis mycobacteria (NTM), 2-thiophene carboxylic acid (TCH) and the p-nitrobenzoic acid (PNB) selective L-J media was used among cultural positive cases. And cases with identical sample that was AFB positive but molecular testing (Xpert or LAMP) negative, were not identified as *M.tb*. Cases with doubtful results required additional tests.

The RIF susceptibility testing of *M.tb* was conducted by traditional culture-based method and/or Xpert. Strictly according to the WHO recommended method, culture-based RIF susceptibility testing was proportional method, performed on L-J solid medium with a RIF concentration of 40 μg/ml. Cases with either phenotypic DST or Xpert of RIF resistant were identified as RIF resistance.

Clinical diagnosis of pulmonary TB

The clinical diagnostic criteria of pulmonary TB were judged by clinical features, imaging results and laboratory tests, which were according to the health industry standards of China (WS 288-2008 and WS 288-2017). NTM disease was excluded in this study which was diagnosed by bacteriological diagnosis and/or clinical evidence.

Literature review of rifampicin-resistant TB trends in China

To compare our result of RR-TB trend in other regions of China during the similar period, we reviewed articles published in recent years. We searched for eligible studies from PubMed and two Chinese Literature Databases (CNKI, https://www.cnki.net/; WANFANG, https://www.wanfangdata.com.cn/) by the following search terms: ("tuberculosis" or "TB") AND ("rifampicin" or "RIF") AND ("China" or "Chinese"). Studies targeting special populations, such as children or HIV-infected patients, were excluded. Only studies with multiple data of RR-TB proportions among years were included. The proportions of RR-TB at the beginning and end of each study were collected.

Statistics

Stata (version 16.0, StataCorp. USA) was used for statistical analysis. The trends of new and previously treated cases,

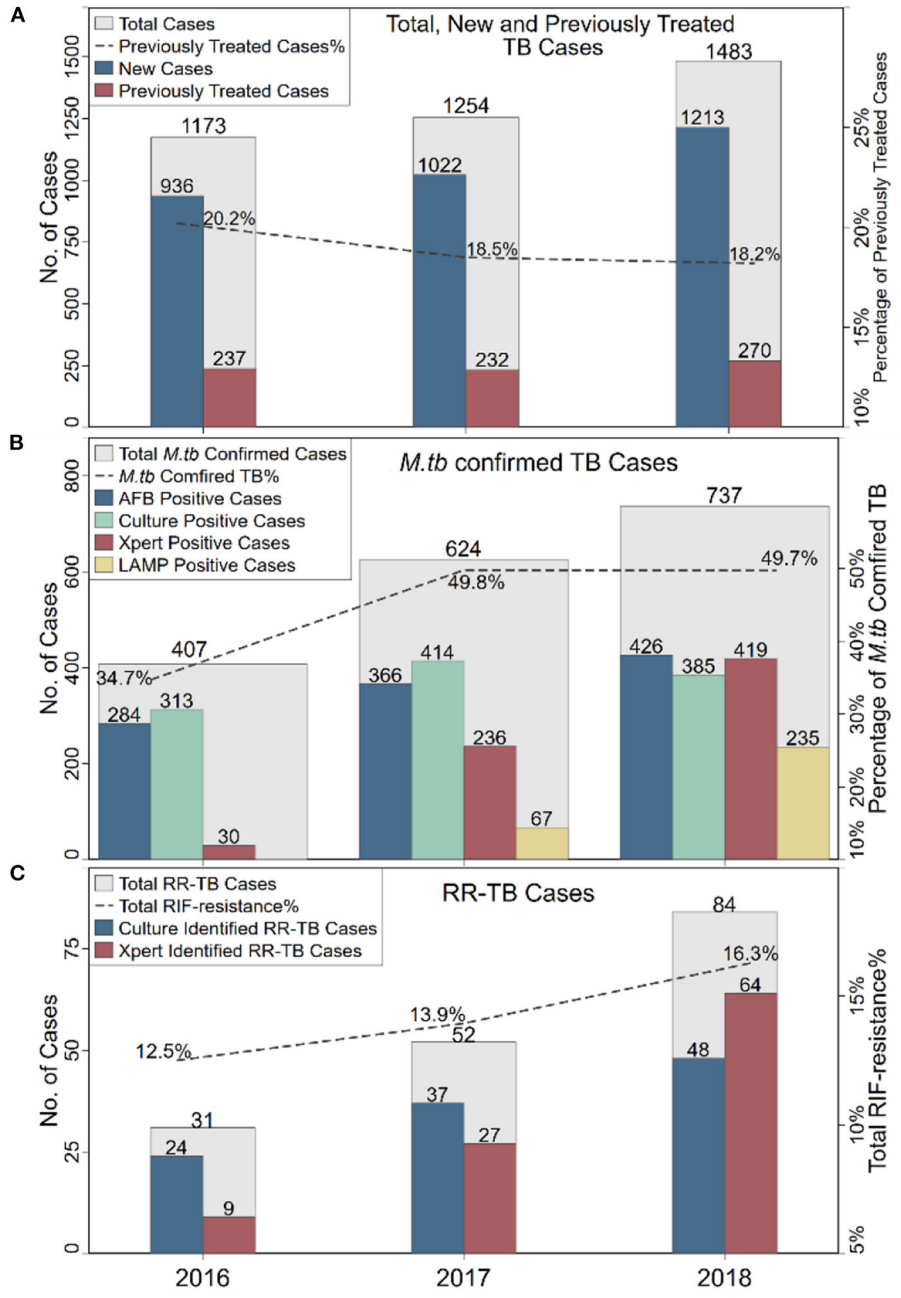

FIGURE 1
The annual distributions of TB cases **(A)**, *M.tb* confirmed TB cases **(B)** and RR-TB cases **(C)**. TB, tuberculosis; RR-TB, rifampicin-resistant TB; AFB, acid-fast bacilli.

M.tb positive and negative cases, rifampicin-susceptible and -resistant TB cases were analyzed by chi-square test. Since RIF susceptibility is important in treatment outcomes and TB control, multiple statistical approaches (6), logistic, Poisson, modified Poisson, and log-binomial regression, were applied to analysis of the characteristics associated with RIF resistance. The univariate and multivariate regression were used to calculate odds ratio (OR), adjusted odds ratio (AOR), prevalence ratio (PR), adjusted prevalence ratio (APR), and 95% confidence interval (CI) for factors associated with RR-TB.

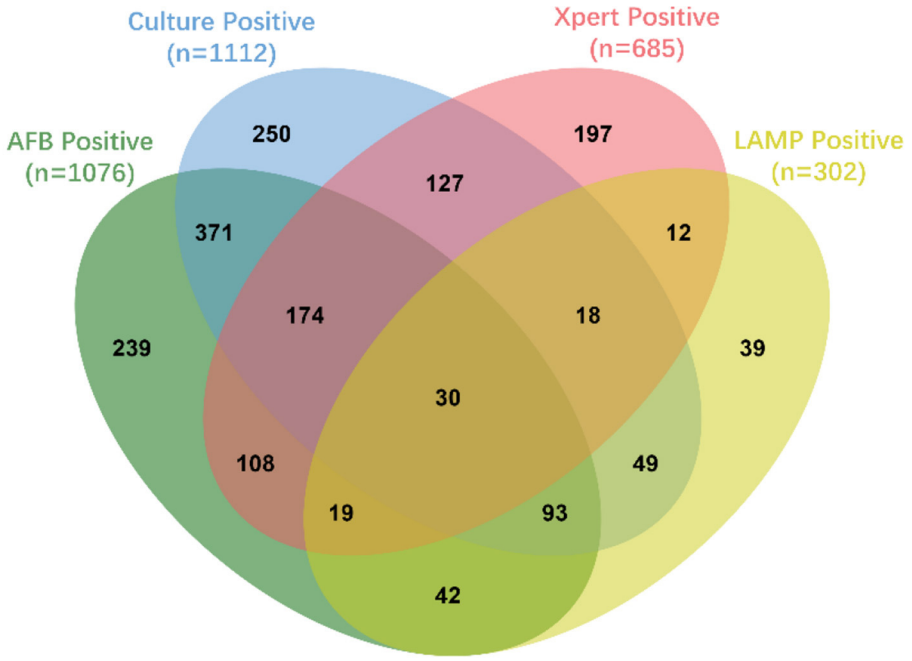

FIGURE 2
The Venn diagram of *M.tb* positive TB cases. This Venn diagram was created by jvenn web application (7). The oval-shaped areas and numbers indicated the *M.tb* positive TB cases that were detected by different methods, which green indicated acid-fast bacilli (AFB), blue indicated Löwenstein-Jensen (L-J) solid media culture, pink indicated GeneXpert MTB/RIF (Xpert) and yellow indicated Loopamp MTBC Detection Kit (LAMP).

Results

From 2016 to 2018, 3,910 inpatient cases from Zunyi were diagnosed as pulmonary TB in AHZMU. To investigate the local TB tendency, the annual distribution of TB cases was analyzed. The total number of TB inpatients increased 26.4%, from 1,173 cases in 2016 to 1,483 in 2018 (Figure 1A). The new TB cases increased 29.6%, from 936 to 1,213, which was a similar pattern to the overall TB cases. In contrast, the number of previously treated cases was relatively stable, and its percentage declined from 20.2 to 18.2%. No statistical significance ($P = 0.2026$) was revealed between new and previously treated cases among years (Supplementary Table 1).

Pathogen detection is an important criterion for TB diagnosis. In this investigation, four methods were used to detect *M.tb*. There was AFB stain, L-J solid media culture, Xpert and LAMP (since 2017). Almost every case (3,841/3,910, 98.2%) applied at least one of these four methods. A total of 1,768 cases were *M.tb* positive. More than half (1,043/1,768, 59.0%) of *M.tb* positive cases were verified by two or more methods (Figure 2). The overall *M.tb* confirmed TB proportion was 45.2% (1,768/3,910), which was increased from 34.7% in 2016 to 49.7% in 2018 (Figure 1B). There was a significant difference between *M.tb* positive and negative cases ($P < 0.0001$) among years (Supplementary Table 1).

In addition to the number of TB cases, the proportion of drug-resistant TB, especially the RR-TB, is also a key indicator of the TB burden. In this retrospective study, culture-based phenotypic drug-susceptibility test (DST) and/or molecular DST by Xpert were used to test RIF susceptibility. Among 1,112 *M.tb* culture positive cases, 60.1% (668/1,112) were subjected to phenotypic DST, of which 16.3% (109/668) were RR-TB. Among 685 Xpert *M.tb* positive cases, 14.6% (100/685) were RIF resistant. In total, 167 cases were RR-TB, of which 42 cases were identified by both phenotypic DST and Xpert. The number of RR-TB cases increased dramatically, which increased 2.71 times, from 31 in 2016 to 84 in 2018. Among all RIF susceptibility tested cases, the total RR-TB proportion was 14.7% (167/1,137), which increased from 12.5% in 2016 to 16.3% in 2018 (Figure 1C). However, no statistical significance ($P = 0.1456$) was revealed between rifampicin-susceptible and -resistant TB cases among years (Supplementary Table 1). To figure out whether this phenomenon of increased RR-TB proportion was unique in China. A total of 11 articles published in recent years with RR-TB proportions among years were found (8–18). There was a regional variation of RR-TB proportions in China (Figure 3). Interestingly, all four studies from the southeastern coast of China (one of the most developed areas of China) showed decreased or stable RR-TB proportions. On the contrary, among the rest studies (including this study), 75%

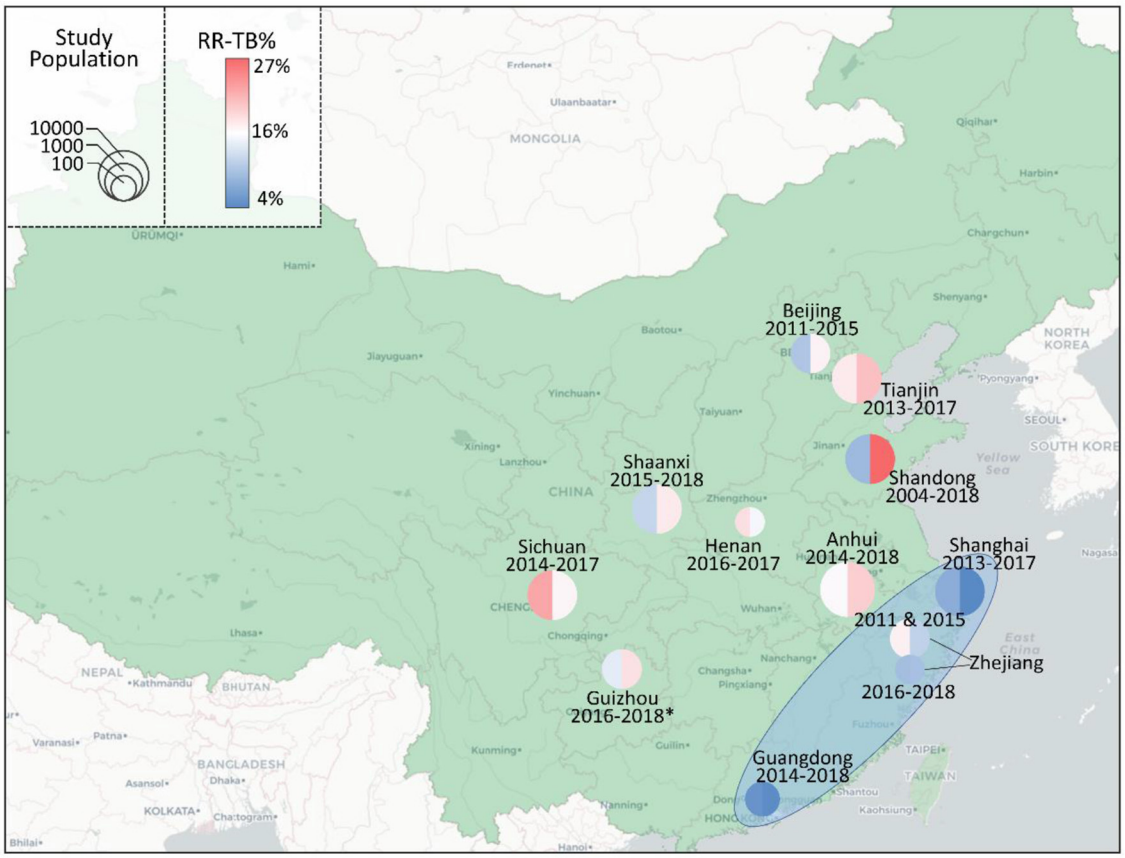

FIGURE 3
The proportions of RR-TB in different areas of China. The proportions of RR-TB at the beginning (left) and end (right) of each study were marked on the map of China by the color of the circle. A warmer color indicated a higher RR-TB proportion, while a cooler color indicated the opposite. The size of cycles indicated the size of the study population. The studied periods and the provinces were labeled next to the circles. The light blue oval area indicated the southeastern coast of China, where studies showed decreased or stable RR-TB proportions. The data of Anhui was obtained from Meng (8), Beijing from Zhang et al. (9), Guangdong from Han et al. (10), Guizhou from this study (*), Henan from Wang et al. (11), Shaanxi from Lei et al. (12), Shandong from Song et al. (13), Shanghai from Wang et al. (14), Sichuan from Zhou et al. (15), Tianjin from Bai et al. (16), Zhejiang (2011 and 2015) from Li et al. (17) and Zhejiang (2016–2018) from Zheng et al. (18).

showed upward trends in recent years. In order to identify the factors related to RIF resistance in this study, univariate and multivariate logistic regression were analyzed (Table 1). Previously treated patients had the highest odds (AOR 4.24, $P < 0.0001$) of being RR-TB compared to new patients, while patients over 60 years had a lower likelihood (AOR 0.43, $P = 0.012$) compared to those 20 years of age. Poisson, modified Poisson, and log-binomial regression also identified the same factors related to RIF resistance with minor differences in PR, Adjust PR and *P*-Value (Supplementary Table 2).

Discussion

As an important feature of TB epidemic status, the total number of pulmonary TB cases showed an upward trend in this study. Briefly, high TB transmission leads to more new TB cases, while poor treatment results in more previously treated cases. By analyzing the treatment history, we found that the increase in TB cases was mainly caused by new TB cases. In addition, both the number and proportion of RR-TB cases also increased. Unfortunately, this phenomenon of increased RR-TB proportion was not unique in China. Through literature review, we found that the increased RR-TB proportion was common in the middle and west of China. Our findings not only revealed the tendency of local TB epidemic in a relatively underdeveloped area of China, but also indicated that even in countries with continuously declined TB incidence rate, there is still a risk that the trends of TB epidemic will turn upward in some areas.

Nevertheless, there is some good news. During the 3-year-studying period, the *M.tb* confirmed TB proportion was elevated from 34.7 to 49.7%, and the percentage of previously treated cases declined from 20.2 to 18.2%, which indicated improvement in bacteriological TB diagnosis and treatment of AHZMU.

TABLE 1 Univariate and multivariate logistic regression analysis of characteristics associated with rifampicin resistance.

Characteristic	RIF susceptible		RIF resistant		Total	OR (95% CI)	*P*-Value	Adjust OR (95% CI)	*P*-Value[a]
	No.	%	No.	%					
Gender									
Male	612	85.2%	106	14.8%	718	1.00		1.00	
Female	358	85.4%	61	14.6%	419	0.98 (0.70–1.38)	0.925	1.04 (0.72–1.49)	0.829
Age									
≤20	108	86.4%	17	13.6%	125	1.00		1.00	
21–40	237	78.5%	65	21.5%	302	1.74 (0.98–3.11)	0.061	1.49 (0.82–2.72)	0.192
41–60	284	83.0%	58	17.0%	342	1.30 (0.72–2.33)	0.382	0.93 (0.50–1.72)	0.816
≥61	341	92.7%	27	7.3%	368	0.50 (0.26–0.96)	**0.037**	0.43 (0.22–0.83)	**0.012**
Treatment history									
New case	786	90.3%	84	9.7%	870	1.00		1.00	
Previously treated case	184	68.9%	83	31.1%	267	4.22 (2.99–5.95)	**<0.0001**	4.24 (2.97–6.05)	**<0.0001**
Total	970	85.3%	167	14.7%	1,137				

[a] *P*-values in bold denote statistical significance at the $P < 0.05$ level. RIF, rifampicin; CI, confidence interval; OR, odds ratio.

These improvements were potentially due to the application of molecular methods (Xpert and LAMP) and the accumulated clinical experience, which would have positive effects on local TB control. Nevertheless, hospitals are only one part of the TB control system. To control the local TB epidemic more effectively, more efforts need to be exerted, such as reducing the number of undetected TB patients and the risk of transmission, screening high-risk population, and monitoring close contacts.

Globally, the incidence rate of TB was reducing in both the high and low TB burden countries, such as China, India, and the United States (19). According to WHO's report, the TB incidence in China has dropped from 75/100,000 in 2011 (20) to 58/100,000 in 2019 (1). A similar decline has also been observed in many provinces and cities of China (21–24). However, this is not a universal phenomenon. In western China, one of the regions with the highest TB epidemic, Yang et al. (25) found an increased TB incidence in many areas from 8-year-data. In southwest China, our data also showed an upward trend of TB burden in Zunyi. These results indicated that in some regions of China, particularly the regions with high TB epidemic, the trends of TB epidemic might turn upward, which would be an obstruction to the national TB control and need to be paid more attention to.

RR-TB can cause serious consequences such as treatment failure, prolonged course of treatment, and high risk of relapse (26). Disturbingly, our data showed that the number of RR-TB in Zunyi rose from 31 to 84 in only three years of time. Although additional RR-TB cases were identified by Xpert, the number of RR-TB cases detected by phenotypic DST increased during the study period, suggesting that this increase in RR-TB was not only due to the application of the new detection method, but also to the presence of more RR-TB cases. In addition to the cases number, the percentage of RR-TB increased from 12.5 to 16.3%. This proportion would be a better indicator of RR-TB burden, which could reduce the disruption from additional methods or increased number of tested cases. Unfortunately, through literature review, we found that there was a general increase in RR-TB proportion in most regions except for the southeastern coast of China. Since the southeastern coast of China is one of the most developed regions in China, economic factors may be one of the reasons for the regional differences in RR-TB proportions. To treat RR-TB patients, more expensive anti-TB drugs and prolonged treatment are needed, which requires more public resources and places a heavier financial burden on patients.

One limitation of this investigation is that data was collected and analyzed only from hospitalized TB patients. Compared to outpatients, inpatient TB cases are under more serious TB conditions. It is possible that the proportion of inpatients with RR-TB is higher than the regional average. Nevertheless, the aim of this study was mainly to figure out the local epidemiological trends of TB in an underdeveloped region of China. As a 3,500-bed tertiary general hospital, AHZMU is one of the major medical centers of the healthcare system in Guizhou Province. Thus, the number of TB cases per year can be used as an indicator of TB burden. In addition, this hospital, designated by the provincial government, has been a main part of the regional referral system, specializing in the diagnosis and treatment of TB, particularly multidrug-resistant (resistance to both rifampicin and isoniazid) TB (MDR-TB) patients. Most patients with RR-TB require at least 1 month of inpatient treatment at AHZMU. Therefore, the increase in the proportion of RR-TB in this study could be an indicator of the rising trend of TB burden in Zunyi city.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

LC, XiaomW, and PX conceived and designed the study, contributed substantially to the preparation of tables and figures, and the critical revision of the manuscript. LC and PX wrote the draft manuscript. All authors were involved in collection and analysis or interpretation of the data.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.941183/full#supplementary-material

References

1. WHO. *Global Tuberculosis Report 2020*. Geneva, Switzerland: World Health Organization (2020).

2. Li Q, Liu M, Zhang Y, Wu S, Yang Y, Liu Y, et al. The spatio-temporal analysis of the incidence of tuberculosis and the associated factors in mainland China, 2009-2015. *Infect Genet Evol.* (2019) 75:103949. doi: 10.1016/j.meegid.2019.103949

3. Chen W, Xia Y, Li T, Chen H. Analysis for the global and China TB epidemic situation in 2015. *J Tuberc Lung Health.* (2016) 5:32–6.

4. Chen L, Fu X, Tian P, Li Q, Lei D, Peng Z, et al. Upward trends in new, rifampicin-resistant and concurrent extrapulmonary tuberculosis cases in northern Guizhou Province of China. *Sci Rep*. (2021) 11:18023. doi: 10.1038/s41598-021-97595-8

5. Lan Y, Li Y, Chen L, Zhang J, Zhang H. Drug resistance profiles and trends in drug-resistant tuberculosis at a major hospital in Guizhou Province of China. *Infect Drug Resist.* (2019) 12:211–9. doi: 10.2147/IDR.S188538

6. Fekedulegn D, Andrew M, Violanti J, Hartley T, Charles L, Burchfiel C. Comparison of statistical approaches to evaluate factors associated with metabolic syndrome. *J Clin Hypertens.* (2010) 12:365–73. doi: 10.1111/j.1751-7176.2010.00264.x

7. Bardou P, Mariette J, Escudie F, Djemiel C, Klopp C. jvenn: an interactive Venn diagram viewer. *BMC Bioinform.* (2014) 15:293. doi: 10.1186/1471-2105-15-293

8. Meng X. Drug resistance analysis of mycobacterium tuberculosis in Fuyang area from 2014 to 2018. *J Qiqihar Medical Univ.* (2019) 40:2318–20.

9. Zhang Z, Wu J, Wang L, Wang L, Han L, Ma S, et al. Genotyping and drug susceptibility of Mycobacterium tuberculosis isolated in Changping districtin Beijing, 2011-2015. *Chin J Epidemiol.* (2017) 38:1236–40. doi: 10.3760/cma.j.issn.0254-6450.2017.09.019

10. Han Z, Lin R, Zhu H. Analysis of drug sensitivity of mycobacterium tuberculosis from 2014 to 2018 in Huizhou city. *J Baotou Med College.* (2019) 35:51–2. doi: 10.16833/j.cnki.jbmc.2019.01.022

11. Wang Y, Liu T, Li H. Drug resistance analysis of *mycobacterium tuberculosis* in Hebi. *Henan J Prev Med.* (2020) 31:270–2. doi: 10.13515/j.cnki.hnjpm.1006-8414.2020.04.011

12. Lei Q, Wang H, Dang L, Zhu C, Lv X, Zhao Y. *Mycobacterium tuberculosis* drug resistance trend in a hospital of Shaanxi, 2015-2018. *Modern Prev Med.* (2020) 47:497–501.

13. Song WM, Li YF, Ma XB, Liu JY, Tao NN, Liu Y, et al. Primary drug resistance of mycobacterium tuberculosis in Shandong, China, 2004-2018. *Respir Res.* (2019) 20:223. doi: 10.1186/s12931-019-1199-3

14. Wang M, Li J, Zhang Y, Wang L, Yu C, Jiang Y, et al. The status and risk factors of drug resistance tuberculosis in Shanghai, 2013-2017. *Chin J Antituberculosis.* (2019) 41:1269–76.

15. Zhou M, Liu S, Li Q, Wang Q, Zhu M, Cao L, et al. Drug resistance characteristics of Mycobacterium tuberculosis isolates between 2014 and 2017 in Sichuan, China: a retrospective study. *PLoS ONE.* (2018) 13:e0209902. doi: 10.1371/journal.pone.0209902

16. Bai D, Kong W, Zhang L. Analysis of drug resistance of *Mycobacterium tuberculosis* during 2006-2017 in Tianjin. *Chin J Nosocomiol.* (2019) 29:346–50.

17. Li Q, Zhao G, Wu L, Lu M, Liu W, Wu Y, et al. Prevalence and patterns of drug resistance among pulmonary tuberculosis patients in Hangzhou, China. *Antimicrob Resist Infect Control.* (2018) 7:61. doi: 10.1186/s13756-018-0348-7

18. Zheng J, Gu M, Wang B, Xu Y, Sun Z. Analysis of the drug resistance of *Mycobacterium tuberculosis* from 2016 to 2018 in Ninghai County. *Chin J Health Lab Technol.* (2020) 30:2720-1+2724.

19. Cui Y, Shen H, Wang F, Wen H, Zeng Z, Wang Y, et al. A long-term trend study of tuberculosis incidence in China, India and United States 1992-2017: a joinpoint and age-period-cohort analysis. *Int J Env Res Public Health*. (2020) 11:17. doi: 10.3390/ijerph17093334

20. WHO. *Global Tuberculosis Report 2012*. Geneva, Switzerland: World Health Organization (2012).

21. Zheng Y, Zhang L, Wang L, Rifhat R. Statistical methods for predicting tuberculosis incidence based on data from Guangxi, China. *BMC Infect Dis*. (2020) 20:300. doi: 10.1186/s12879-020-05033-3

22. Liu K, Li T, Vongpradith A, Wang F, Peng Y, Wang W, et al. Identification and prediction of tuberculosis in Eastern China: analyses from 10-year population-based notification data in Zhejiang Province, China. *Sci Rep*. (2020) 10:7425. doi: 10.1038/s41598-020-64387-5

23. Liu Q, Li Z, Ji Y, Martinez L, Zia UH, Javaid A, et al. Forecasting the seasonality and trend of pulmonary tuberculosis in Jiangsu Province of China using advanced statistical time-series analyses. *Infect Drug Resist*. (2019) 12:2311–22. doi: 10.2147/IDR.S207809

24. Li T, Cheng Q, Li C, Stokes E, Collender P, Ohringer A, et al. Evidence for heterogeneity in China's progress against pulmonary tuberculosis: uneven reductions in a major center of ongoing transmission, 2005-2017. *BMC Infect Dis*. (2019) 19:615. doi: 10.1186/s12879-019-4262-2

25. Yang L, Li T, Chen W. Epidemic of pulmonary tuberculosis in minority ethnic areas included in health poverty alleviation project in four provinces in western China from 2011 to 2018. *Dis Surv*. (2020) 35:60–4. doi: 10.3784/j.issn.1003-9961.2020.01.014

26. Xu G, Liu H, Jia X, Wang X, Xu P. Mechanisms and detection methods of Mycobacterium tuberculosis rifampicin resistance: The phenomenon of drug resistance is complex. *Tuberculosis*. (2021) 128:102083. doi: 10.1016/j.tube.2021.102083

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Association of N6-methyladenosine readers' genes variation and expression level with pulmonary tuberculosis

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N6-Methyladenosine (m6A) is associated with many biological processes and the development of multiple diseases. The aim of this study was to analyze the association of m6A readers' genes variation, as well as their expression levels, with pulmonary tuberculosis (PTB). A total of 11 single-nucleotide polymorphisms (SNPs) in m6A readers' genes (i.e., *YTHDF1* rs6122103, rs6011668, *YTHDF2* rs602345, rs3738067, *YTHDF3* rs7464, rs12549833, *YTHDC1* rs3813832, rs17592288, rs2293596, and *YTHDC2* rs6594732, and rs2416282) were genotyped by SNPscan™ technique in 457 patients with PTB and 466 normal controls. The m6A readers' genes expression levels in peripheral blood mononuclear cells (PBMCs) from 78 patients with PTB and 86 normal controls were detected by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). There was no significant association between all SNPs in *YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2* genes and PTB susceptibility. The increased frequencies of *YTHDF2* rs3738067 GG genotype and *YTHDC1* rs3813832 CC genotype, C allele, were, respectively, found in PTB patients with hypoproteinemia and fever. *YTHDC2* rs6594732 variant was significantly associated with drug-induced liver damage and sputum smear-positive, and the rs2416282 variant was significantly associated with fever in patients with PTB. Compared with controls, the YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 mRNA levels were significantly decreased in PTB. Moreover, YTHDF1 level was negatively associated with erythrocyte sedimentation rate (ESR), and YTHDF3 and YTHDC1 levels were negatively related to alanine aminotransferase (ALT) in patients with PTB. Our results demonstrated that *YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2* genes SNPs did not contribute to PTB susceptibility, while their decreased levels in patients with PTB suggested that these m6A readers might play significant roles in PTB.

KEYWORDS

pulmonary tuberculosis, N6-methyladenosine, single-nucleotide polymorphisms, epidemiology, infectious diseases

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is a serious infectious disease with high morbidity and mortality. Pulmonary TB (PTB) is the most common and poses a serious threat to public health. China is still the third highest burdened country and accounts for 8.4% of the total global patients in 2019 (1). Studies had shown that the occurrence and development of TB was mainly determined by a complex interaction between multiple factors, including MTB strains and environmental and host genetic factors (2–4). Host genetics was confirmed to play an important role in determining disease progression and prognosis after MTB infection; hence, identifying the factors that influence disease susceptibility could provide important evidence for the design of effective control strategies. A considerable number of genetic variants for PTB susceptibility had been identified, while it could only account for part of the heritability of PTB (5–7).

Epigenetic modification also played an important role in the pathogenesis of PTB, and DNA methylation was an important epigenetic marker for the risk of several diseases (8, 9). Both cytosine and adenine could be methylated in DNA, resulting in N4-methylcytosine, 5-methylcytosine, and N6-methyladenosine (m6A) (10). m6A had critical modification effects on a variety of cytological processes, including nuclear export, splicing, translatability, and stability of mRNA, and was closely related to the pathogenesis of various diseases (11). Moreover, m6A methylation was found in the *MTB* genome, and DNA methylation could regulate the expression of genes related to the hypoxia survival of *MTB*; hence, m6A methylation was likely to be involved in the pathogenesis of PTB (12, 13). Disease-related genetic variations had been proved to influence m6A methylation by altering the RNA sequence of its target sites or key flanking nucleotides, suggesting that m6A-related single-nucleotide polymorphisms (SNPs) might influence the stability of mRNA, which might contribute to the development of human disease (14). Some studies had explored the potential association between genetic variation in the m6A-modified core genes and the risk of human diseases (15, 16).

N6-Methyladenosine-associated RNA binding proteins (readers), including YTH m6A RNA-binding protein 1 (YTHDF1), YTHDF2, YTHDF3, YTH domain-containing 1 (YTHDC1), and YTHDC2, played a key role in m6A modification by modulating mRNA fate (17, 18). Moreover, functional SNPs in *YTHDF1* might influence its expression and binding ability to m6A-modified RNA, which eventually affect tumorgenesis (19). However, the role of these m6A readers in PTB was still unclear. Thus, we performed this study to evaluate the associations of m6A readers' (YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2) gene variation and their expression levels with PTB susceptibility in a Chinese Han population.

Materials and methods

Study participants

In this study, we consecutively recruited a total of 923 subjects including 457 patients with PTB and 466 normal controls to analyze the association between *YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2* genes polymorphisms and PTB susceptibility. Then, 78 patients with PTB and 86 normal controls were enrolled to detect these genes levels. All patients with PTB were selected from the Department of Tuberculosis at Anhui Chest Hospital, and diagnosed by a specialist on the basis of these criteria as follows: suspicious clinical symptoms, chest radiography, sputum and/or bronchoalveolar lavage fluid MTB culture, microscopy for acid-fast bacilli, and effect of anti-TB treatment. The exclusion criteria for patients with PTB included HIV-positive, hepatitis, malignancy, and immune-compromised conditions. The normal controls were enrolled from health examination center in the same area and needed to be asymptomatic with sputum smear- and culture-negative, normal chest radiograph, and no history of TB. All patients with PTB and normal controls were the Chinese Han population, and no biological relationship was existed in these study subjects.

This study was approved by the Medical Ethics Committee of Anhui Medical University (20200250), and written informed consent was obtained from all subjects prior to the study. Then, the peripheral blood samples, demographic characteristics, clinical manifestations, and laboratory indicators were collected from study participants. The clinical manifestations of patients with PTB included fever, drug resistance, drug-induced liver injury (DILI), pulmonary infection, leukopenia, and sputum smears, and the laboratory indicators of patients with PTB included erythrocyte sedimentation rate (ESR), total bilirubin (TBIL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

SNP selection, DNA extraction, and genotyping

In this study, we screened several specific tagSNPs in each gene for genotyping. The tagSNPs were selected with a minor allele frequency (MAF) $\geq$ 0.05 in CHB, capturing all the common SNPs located in the chromosome locations of these m6A readers (*YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2*) and their flanking 2.0 kbp region by using genetic data of CHB from Ensembl genome browser 85 and CHBS_1000g. The selection was conducted using the linkage disequilibrium (LD) analysis according to r^2 threshold $>$ 0.8, and the Haploview 4.0 software (Cambridge, MA, USA). We also reviewed the existing studies regarding the association of these gene polymorphisms with disease susceptibility, and searched

other potentially functional SNPs. Finally, we selected two tagSNPs (rs6122103 and rs6011668) in *YTHDF1*, two tagSNPs (rs602345 and rs3738067) in *YTHDF2*, two tagSNPs (rs7464 and rs12549833) in *YTHDF3*, three tagSNPs (rs3813832, rs17592288, and rs2293596) in *YTHDC1*, and two tagSNPs (rs6594732 and rs2416282) in *YTHDC2* for genotyping.

The genomic DNA was extracted from the peripheral blood leukocytes by the Flexi Gene-DNA Kit (Qiagen, Valencia, CA). The SNPscan^{TM} technique, with technical support from the Center for Genetic & Genomic Analysis, Genesky Biotechnologies Inc. (Shanghai), was used for genotyping. Those individuals with a 100% genotyping success rate for the above SNPs were included in the final analysis.

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

The PBMCs were isolated from 5 ml peripheral blood and stored at −80°C until processed. Total RNA was extracted from PBMCs using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and the RNA concentration was detected with NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Next, the total RNA was reversely transcribed into cDNA by the PrimeScriptTM RT Reagent Kit (Takara Bio Inc., Japan).

In this study, the YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 expression levels in PBMC were measured by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) with SYBR Green (SYBR Premix Ex Taq II, Takara Bio Inc., Japan), and this experiment was carried out in duplicate by using QuantStudio 12K Flex Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Thermal cycling conditions were as follows: 95°C for 1 min, followed by 42 cycles at 95°C for 10 s, 60°C for 30 s, and 72°C for 1 min. The relative expression levels of these genes were calculated by using the $2^{-\Delta\Delta Ct}$ method normalized to an endogenous control, and the housekeeping gene β-actin was used as an internal control in the same sample.

Statistical analysis

All the statistical analysis was performed using SPSS version 23.0 and data were shown as frequency, percentage, mean ± standard deviation (SD), and median (quartile range) according to their types. The differences in these m6A readers' expression levels between two groups and three groups were, respectively, analyzed by the Mann–Whitney *U* test and Kruskal–Wallis *H* test, and correlation analysis was performed with Spearman's rank correlation coefficient test. We performed the Hardy–Weinberg equilibrium test in normal controls using chi-square (χ^2). The difference in each SNP genotype and allele frequency distribution between different groups was evaluated with χ^2; odds ratio (OR) and 95% confidence interval (CI) were determined by the logistic regression analysis. Two genetic models (dominant model and recessive model) were used for statistical analysis, and haplotype analysis was conducted using the SHEsis software (20). A *P*-value of < 0.05 was considered statistically significant.

Results

In the genotyping experiment, 457 patients with PTB consisted of 264 men and 193 women, with a mean age of 45.42 ± 17.74 years, and 466 controls consisted of 202 men and 264 women, with an average age of 43.43 ± 12.95 years (Supplementary Table S1). In the qRT-PCR experiment, the PTB group enrolled 51 men and 27 women, with a mean age of 49.83 ± 18.59 years, and 57 men and 29 women were included in the control group, with an average age of 48.47 ± 17.40 years (Supplementary Table S1).

Association of m6A readers' genes polymorphisms with the susceptibility for PTB

The allele and genotype frequencies of all SNPs in *YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2* genes are shown in Table 1, and the genotype distribution of all SNPs in controls was conformed to the Hardy–Weinberg equilibrium. There were no significant differences in allele and genotype distributions of *YTHDF11* rs6122103 and rs6011668 polymorphism between patients with PTB and controls (all *P*-values > 0.05). Similarly, we did not find any significant association between *YTHDF2* rs602345, rs3738067, *YTHDF3* rs7464, rs12549833, *YTHDC1* rs3813832, rs17592288, rs2293596, *YTHDC2* rs6594732, and rs2416282 polymorphisms and the risk of PTB. The association between these SNPs and PTB susceptibility under the dominant model and recessive model was also analyzed; however, no significant association was detected.

The results regarding the association between these SNPs and several clinical manifestations in patients with PTB suggested that *YTHDF2* rs3738067 GG genotype frequency was significantly increased in PTB patients with hypoproteinemia when compared to the patients without hypoproteinemia ($P = 0.009$), and *YTHDC1* rs3813832 CC genotype, C allele, was significantly related to the occurrence of fever in patients with PTB ($P = 0.044$ and $P = 0.027$, respectively) (Supplementary Table S2, Table 2). In the *YTHDC2* gene, the elevated frequencies of rs6594732 AA genotype and A allele were significantly associated with DILI in patients with PTB ($P = 0.033$ and $P = 0.008$, respectively), while the decreased

TABLE 1 Genotype and allele frequencies of m6A readers' genes in patients with PTB and normal controls.

SNP	Analyze model		PTB patients	Controls	*P*-value	OR (95 % CI)
YTHDF1						
rs6122103	Genotype	GG	214 (46.83)	201 (43.13)	Reference	
		GA	191 (41.79)	213 (45.71)	0.220	0.842 (0.640, 1.108)
		AA	52 (11.38)	52 (11.16)	0.775	0.939 (0.611, 1.444)
	Allele	G	619 (67.72)	615 (65.99)	Reference	
		A	295 (32.28)	317 (34.01)	0.428	0.949 (0.834, 1.080)
	Dominant model	GA+AA	243 (53.17)	265 (56.87)	Reference	
		GG	214 (46.83)	201 (43.13)	0.428	0.949 (0.834, 1.080)
	Recessive model	GA+GG	405 (88.62)	414 (88.84)	Reference	
		AA	52 (11.38)	52 (11.16)	0.428	0.949 (0.834, 1.080)
rs6011668	Genotype	CC	323 (70.68)	323 (69.31)	Reference	
		TC	127 (27.79)	132 (28.33)	0.793	0.962 (0.721, 1.284)
		TT	7 (1.53)	11 (2.36)	0.356	0.636 (0.244, 1.662)
	Allele	C	773 (84.57)	778 (83.48)	Reference	
		T	141 (15.43)	154 (16.52)	0.520	0.934 (0.757, 1.151)
	Dominant model	CC	323 (70.68)	323 (69.31)	0.651	1.020 (0.937, 1.110)
		TC+TT	134 (29.32)	143 (30.69)	Reference	
	Recessive model	TC+CC	450 (98.47)	455 (97.64)	Reference	
		TT	7 (1.53)	11 (2.36)	0.363	0.649 (0.254, 1.659)
YTHDF2						
rs602345	Genotype	CC	327 (71.55)	337 (72.32)	Reference	
		TC	114 (24.95)	117 (25.11)	0.978	1.004 (0.744, 1.355)
		TT	16 (3.50)	12 (2.58)	0.632	1.375 (0.640, 2.949)
	Allele	C	768 (84.03)	791 (84.87)	Reference	
		T	146 (15.97)	141 (15.13)	0.616	1.056 (0.854, 1.306)
	Dominant model	CC	327 (71.55)	337 (72.32)	0.796	0.989 (0.913, 1.072)
		TC+TT	130 (28.45)	129 (27.68)	Reference	
	Recessive model	TC+CC	441 (96.50)	454 (97.42)	Reference	
		TT	16 (3.50)	12 (2.58)	0.412	1.360 (0.650, 2.842)
rs3738067	Genotype	AA	254 (55.58)	259 (55.58)	Reference	
		GA	166 (36.32)	171 (36.70)	0.942	0.990 (0.752, 1.303)
		GG	37 (8.10)	36 (7.73)	0.851	1.048 (0.642, 1.711)
	Allele	A	674 (73.74)	689 (73.93)	Reference	
		G	240 (26.26)	243 (26.07)	0.928	1.007 (0.864, 1.174)
	Dominant model	AA	254 (55.58)	259 (55.58)	1.000	1.000 (0.891, 1.122)
		GA+GG	203 (44.42)	207 (44.42)	Reference	
	Recessive model	GA+AA	420 (91.90)	430 (92.27)	Reference	
		GG	37 (8.10)	36 (7.73)	0.835	1.048 (0.675, 1.628)
YTHDF3						
rs7464	Genotype	AA	249 (54.49)	246 (52.79)	Reference	
		GA	176 (38.51)	182 (39.06)	0.742	0.955 (0.728, 1.254)
		GG	32 (7.00)	38 (8.15)	0.473	0.832 (0.504, 1.375)
	Allele	A	674 (73.74)	674 (72.32)	Reference	
		G	240 (26.26)	258 (27.68)	0.491	0.949 (0.816, 1.102)
	Dominant model	AA	249 (54.49)	246 (52.79)	0.605	1.032 (0.915, 1.164)
		GA+GG	208 (45.51)	220 (47.21)	Reference	
	Recessive model	GA+AA	425 (93.00)	428 (91.85)	Reference	
		GG	32 (7.00)	38 (8.15)	0.509	0.859 (0.546, 1.350)

(Continued)

TABLE 1 (Continued)

SNP	Analyze model		PTB patients	Controls	*P*-value	OR (95 % CI)
rs12549833	Genotype	AA	199 (43.54)	192 (41.20)	Reference	
		AG	202 (44.20)	218 (46.78)	0.426	0.894 (0.679, 1.178)
		GG	56 (12.25)	56 (12.02)	0.867	0.965 (0.634, 1.469)
	Allele	A	600 (65.65)	602 (64.59)	Reference	
		G	314 (34.35)	330 (35.41)	0.635	0.970 (0.857, 1.099)
	Dominant model	AG+GG	258 (56.46)	274 (58.80)	Reference	
		AA	199 (43.54)	192 (41.20)	0.471	1.057 (0.909, 1.229)
	Recessive model	AG+AA	401 (87.75)	410 (87.98)	Reference	
		GG	56 (12.25)	56 (12.02)	0.912	0.997 (0.951, 1.046)
YTHDC1						
rs3813832	Genotype	TT	236 (51.64)	237 (50.86)	Reference	
		TC	190 (41.58)	197 (42.27)	0.816	0.969 (0.740, 1.267)
		CC	31 (6.78)	32 (6.87)	0.918	0.973 (0.575, 1.646)
	Allele	T	662 (72.43)	671 (72.00)	Reference	
		C	252 (27.57)	261 (28.00)	0.835	0.983 (0.850, 1.141)
	Dominant model	TT	236 (51.64)	237 (50.86)	0.812	1.015 (0.895, 1.152)
		TC+CC	221 (48.36)	229 (49.14)	Reference	
	Recessive model	TC+TT	426 (93.22)	434 (93.13)	Reference	
		CC	31 (6.78)	32 (6.87)	0.960	0.988 (0.613, 1.591)
rs17592288	Genotype	AA	417 (91.25)	432 (92.70)	Reference	
		AC	39 (8.53)	34 (7.30)	0.480	1.188 (0.736, 1.919)
		CC	1 (0.22)	0 (0)	1.000	—
	Allele	A	873 (95.51)	898 (96.35)	Reference	
		C	41 (4.49)	34 (3.65)	0.362	0.991 (0.973, 1.010)
	Dominant model	AA	417 (91.25)	432 (92.70)	0.415	1.200 (0.774, 1.860)
		AC+CC	40 (8.75)	34 (7.30)	Reference	
	Recessive model	AC+AA	456 (99.78)	466 (100.00)	Reference	
		CC	1 (0.22)	0 (0)	0.312	0.998 (0.994, 1.002)
rs2293596	Genotype	TT	300 (65.65)	309 (66.31)	Reference	
		TC	138 (30.2)	140 (30.04)	0.917	1.015 (0.764, 1.348)
		CC	19 (4.16)	17 (3.65)	0.682	1.151 (0.587, 2.257)
	Allele	T	738 (80.74)	758 (81.33)	Reference	
		C	176 (19.26)	174 (18.67)	0.748	1.031 (0.854, 1.246)
	Dominant model	TT	300 (65.65)	309 (66.31)	0.832	0.990 (0.902, 1.086)
		TC+CC	157 (34.35)	157 (33.69)	Reference	
	Recessive model	TC+TT	438 (95.84)	449 (96.35)	Reference	
		CC	19 (4.16)	17 (3.65)	0.689	1.140 (0.600, 2.165)
YTHDC2						
rs6594732	Genotype	CC	302 (66.08)	281 (60.30)	Reference	
		CA	137 (29.98)	166 (35.62)	0.063	0.768 (0.581, 1.015)
		AA	18 (3.94)	19 (4.08)	0.710	0.881 (0.453, 1.714)
	Allele	C	741 (81.07)	728 (78.11)	Reference	
		A	173 (18.93)	204 (21.89)	0.115	0.865 (0.722, 1.036)
	Dominant model	CC	302 (66.08)	281 (60.30)	0.069	1.096 (0.993, 1.210)
		CA+AA	155 (33.92)	185 (39.70)	Reference	
	Recessive model	CA+CC	439 (96.06)	447 (95.92)	Reference	
		AA	18 (3.94)	19 (4.08)	0.915	0.966 (0.514, 1.817)

(Continued)

TABLE 1 (Continued)

SNP	Analyze model		PTB patients	Controls	*P*-value	OR (95 % CI)
rs2416282	Genotype	AA	147 (32.17)	144 (30.90)	Reference	
		CA	217 (47.48)	246 (52.79)	0.329	0.864 (0.644, 1.159)
		CC	93 (20.35)	76 (16.31)	0.350	1.199 (0.819, 1.753)
	Allele	A	511 (55.91)	534 (57.30)	Reference	
		C	403 (44.09)	398 (42.70)	0.547	1.033 (0.930, 1.146)
	Dominant model	CA+CC	310 (67.83)	322 (69.10)	Reference	
		AA	147 (32.17)	144 (30.90)	0.679	0.982 (0.899, 1.072)
	Recessive model	CA+AA	364 (79.65)	390 (83.69)	Reference	
		CC	93 (20.35)	76 (16.31)	0.112	1.248 (0.949, 1.641)

TABLE 2 The positive findings of associations between m6A readers' genes polymorphisms and clinical features of patients with PTB.

SNP	Allele	Clinical features	Group	Genotype			*P*-value	Allele		*P*-value
	(M/m)			MM	Mm	mm		M	m	
YTHDF2 rs3738067	A/G	Hypoproteinemia	+	39	9	7	0.009	87	23	0.216
			-	265	177	38		707	253	
YTHDC1 rs3813832	T/C	Fever	+	27	38	6	0.044	92	50	0.027
			-	209	152	25		570	202	
YTHDC2 rs6594732	C/A	DILI	+	39	29	6	0.033	107	41	0.008
			-	306	139	16		751	171	
YTHDC2 rs6594732	C/A	Sputum smear	+	106	44	2	0.039	256	48	0.020
			-	211	113	20		535	153	
YTHDC2 rs2416282	A/C	Fever	+	15	45	11	0.013	75	67	0.420
			-	132	172	82		436	336	

+, with; -, without.

frequencies of rs6594732 AA genotype and A allele were significantly associated with sputum smear-positive ($P = 0.039$ and $P = 0.020$, respectively). In addition, rs2416282 CC genotype frequency was significantly decreased in PTB patients with fever ($P = 0.013$).

Haplotype analysis

The haplotype of *YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2* genes was detected using the SHEsis software, and then the differences in these haplotype frequencies between patients with PTB and controls were compared. Three main haplotypes each (AC, GC, GT), (CA, CG, TG), (AA, AG, GA), and (AC, CA, CC) for YTHDF2, YTHDF1, YTHDF2, and YTHDF2, respectively, and four main haplotypes (CAT, TAC, TAT, TCT) for YTHDC1 were detected.

As shown in Table 3, we found that *YTHDC2* gene CC haplotype frequency was significantly higher in patients with PTB than in controls ($P = 0.033$). Meanwhile, the frequencies of other haplotypes were not statistically associated with PTB susceptibility.

m6A readers' expression levels in patients with PTB and normal controls

The mRNA expression levels of YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 in PBMCs from patients with PTB and normal controls by qRT-PCR were further detected. As shown in Figure 1, the YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 expression levels in patients with PTB were significantly lower than that in normal controls (all *P*-values < 0.05).

TABLE 3 Haplotype analysis of m6A readers' genes in patients with PTB and controls.

Haplotype	PTB patients	Controls	*P*-value	OR (95% CI)
YTHDF1 rs6122103-rs6011668				
AC	294.95 (32.3)	316.97 (34.0)	0.427	0.924 (0.762,1.122)
GC	478.05 (52.3)	461.03 (49.5)	0.223	1.120 (0.933,1.345)
GT	140.95 (15.4)	153.97 (16.5)	0.519	0.921 (0.718,1.182)
YTHDF2 rs602345-rs3738067				
CA	672.90 (73.6)	689.00 (73.9)	0.916	0.989 (0.803,1.217)
CG	95.10 (10.4)	102.00 (10.9)	0.714	0.946 (0.704,1.272)
TG	144.90 (15.9)	141.00 (15.1)	0.659	1.058 (0.822,1.362)
YTHDF3 rs7464-rs12549833				
AA	360.02 (39.4)	344.02 (36.9)	0.273	1.111 (0.920,1.340)
AG	313.98 (34.4)	329.98 (35.4)	0.635	0.955 (0.788,1.156)
GA	239.98 (26.3)	257.98 (27.7)	0.491	0.930 (0.757,1.143)
YTHDC1 rs3813832- rs17592288- rs2293596				
CAT	251.96 (27.6)	260.97 (28.0)	0.835	0.979 (0.798,1.200)
TAC	175.96 (19.3)	173.96 (18.7)	0.748	1.039 (0.823,1.311)
TAT	445.04 (48.7)	463.04 (49.7)	0.671	0.961 (0.801,1.154)
TCT	40.99 (4.5)	33.99 (3.6)	0.362	1.240 (0.780,1.973)
YTHDC2 rs6594732- rs2416282				
AC	172.99 (18.9)	202.70 (21.7)	0.128	0.838 (0.668,1.052)
CA	510.99 (55.9)	532.70 (57.2)	0.565	0.947 (0.788,1.139)
CC	230.01 (25.2)	195.30 (21.0)	0.033	1.266 (1.019,1.573)

frequency < 0.03 in both controls & PTB patients has been dropped.
Bold value means P < 0.05.

The correlation of these m6A readers' expression levels with several common clinical features of patients with PTB was also analyzed. The results showed that YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 mRNA levels were not associated with the occurrence of those clinical features, including fever, DILI, pulmonary infection, hypoproteinemia, leukopenia, sputum smear-positive, in patients with PTB (Supplementary Table S2). In addition, the expression level of YTHDF1 was negatively associated with ESR in patients with PTB ($P = 0.039$), and YTHDF3 and YTHDC1 levels were negatively associated with ALT ($P = 0.031$ and $P = 0.012$, respectively). However, there were no significant correlations of these m6A reader levels with TBIL and AST of patients with PTB (Table 4).

Associations between m6A readers' genes polymorphisms with their levels in patients with PTB

A total of 62 patients with PTB were included to analyze the associations between these m6A readers' genes variation and their expression levels. The results demonstrated that there might be some differences in the expression levels of these genes among different genotypes, but no difference reached a statistical significance (all *P*-values > 0.05) (Supplementary Table S3).

Discussion

RNA m6A modification was considered the most abundant, pervasive, and important chemical modification in eukaryotic RNAs and could affect all aspects of RNA metabolism, such as RNA transcription, processing, translation, and transportation (21, 22). The m6A modification process was accomplished by a series of proteins, which were mainly divided into "writers," "erasers," and "readers," according to their different roles (18). Due to the important role of RNA m6A modification in a variety of biological processes, it was reasonable to believe that genetic variation in m6A modified genes had been involved in the development of multiple human diseases. For example, genetic variations in m6A modification core genes had been shown to be associated with cancer susceptibility in many studies (16, 23). Recently, the association between m6A modification genes variation and PTB susceptibility had also been discussed. Previous studies have found that rs9939609 polymorphism in fat mass and obesity-associated protein (*FTO*), known as m6A demethylases, was associated with the risk of PTB (24, 25). However, research on m6A critical gene SNPs

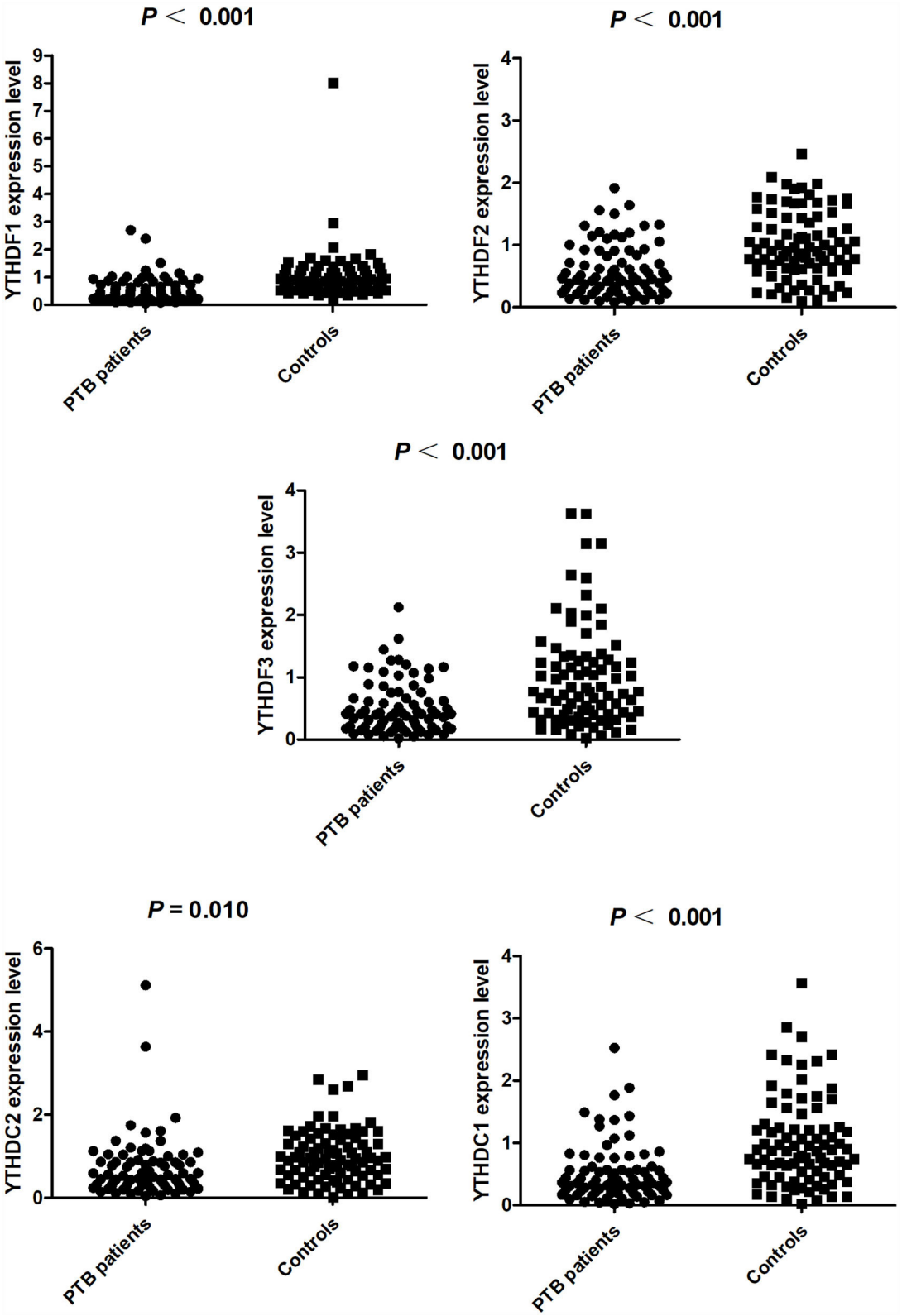

FIGURE 1
The m6A readers' expression levels in patients with PTB and normal controls.

TABLE 4 The correlation between m6A readers' genes expression levels and ESR, TBIL, ALT, and AST of patients with PTB.

Clinical parameters	YTHDF1 level		YTHDF2 level		YTHDF3 level		YTHDC1 level		YTHDC2 level	
	r_S	*P*–value	r_S	*P*–value	r_S	*P*–value	r_S	*P*–value	r_S	*P*–value
ESR	−0.238	0.039	−0.104	0.373	−0.031	0.788	−0.120	0.303	−0.127	0.274
TBIL	−0.050	0.671	−0.011	0.922	0.101	0.383	0.109	0.348	0.103	0.378
ALT	−0.011	0.923	−0.172	0.134	−0.245	0.031	−0.285	0.012	−0.175	0.128
AST	−0.040	0.729	−0.184	0.111	−0.153	0.187	−0.184	0.112	−0.158	0.173

r_S: Spearman's rank correlation coefficient.

on PTB risk was still at the primary stage. Therefore, we focused on the relationship between five m6A readers genes (*YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2*) SNPs and expression levels with PTB in this study.

The final consequences of m6A modification on mRNA fate were executed by "reader" proteins, and these readers' genes mainly included the YTH family (YTHDC1-2 and YTHDF1-3). The contribution of m6A readers' genes to cancer was widely studied, and multiple SNPs located in m6A readers' genes had been found to affect the risk of cancer (23, 26, 27). Liu *et al.* found that compared with the CC genotype, *YTHDF1* rs6011668 CT/TT genotype was associated with an increased risk of Wilms tumor in patients ≤18 months in stratification analysis, although this SNP might not contribute to the risk of Wilms tumor (26). The results by Zeng *et al.* demonstrated that *YTHDF2* rs3738067 A>G could decrease neuroblastoma risk in the Chinese children (27). First, we analyzed the association between *YTHDF1* rs6122103, rs6011668, *YTHDF2* rs602345, rs3738067, *YTHDF3* rs7464, and rs12549833 polymorphism and PTB susceptibility; however, no statistically significant findings were observed. In this sense, this viewpoint was providing evidence that these gene polymorphisms might not affect the susceptibility for PTB. In the progression of PTB, patients were usually accompanied by multiple complications and clinical manifestations, including fever, pulmonary infection, and drug resistance, which were also affected by genetic variation (6, 28). In this study, our results demonstrated that the *YTHDF2* rs3738067 GG genotype was significantly associated with the occurrence of hypoproteinemia in PTB. It was worth noting that the *YTHDF2* rs3738067 variant might be involved in the development of PTB, and this result would be verified and further explored in our future research.

Some studies have analyzed the relationship between *YTHDC1*, *YTHDC2* gene variation, and cancer susceptibility. *YTHDC1* rs3813832 TC genotype significantly reduced the susceptibility of neuroblastoma, and rs2293596 T>C polymorphism might contribute to hepatoblastoma susceptibly (29, 30). Another study suggested that the *YTHDC2* rs2416282 variant contributed to esophageal squamous-cell carcinoma risk by regulating YTHDC2 expression (31). Nonetheless, we failed to find any relationships between the selected SNPs in *YTHDC1* (rs3813832, rs17592288, and rs2293596), *YTHDC2* (rs6594732 and rs2416282), and PTB risk in this study. In addition, we found a statistically significant association between CC haplotype in *YTHDC2* and susceptibility to PTB, since haplotypes tended to have a stronger power of predicting disease-related genes than SNP (32). Hence, our results suggested a potential role of *YTHDF2* gene variation in PTB susceptibility, while the specific mechanisms needed to be further explored. Our results also showed that *YTHDC1* rs3813832 and *YTHDC2* rs2416282 variants were significantly related to fever and the *YTHDC2* rs6594732 variant was significantly associated with DILI and sputum smear-positive in patients with PTB. These clinical features could seriously affect the treatment and prognosis of patients with PTB, and the abovementioned SNPs were somewhat used to predispose the occurrence of these clinical features in patients with PTB. Therefore, we speculated that these findings might help to make more appropriate treatment choices for patients with PTB.

Interestingly, increasing evidence had indicated that the expression levels of m6A readers were closely related to the pathogenesis and progression of many diseases. A number of studies had shown that YTHDF1 was overexpressed in various cancers, including colorectal cancer, hepatocellular carcinoma, and breast cancer, and was closely related to the increased risk of these cancers (33–35). Decreased mRNA expression of YTHDF2 was found in patients with systemic lupus erythematosus and rheumatoid arthritis (RA) compared with controls, and YTHDF2 mRNA level in peripheral blood was a risk factor for RA by logistic regression analysis (36, 37). However, few studies had been conducted regarding m6A reader expression levels and the risk of PTB. Our study provided the first evidence that YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 mRNA levels in patients with PTB were significantly decreased than that in controls. This result showed that these m6A readers might be involved in PTB occurrence, and the decreased expression of YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 might be used as auxiliary indicators for PTB diagnosis. Then, we also investigated whether the expressions of these m6A readers in the peripheral blood of patients with PTB could reflect the clinical characteristics of this disease. We showed the expression

of peripheral blood YTHDF1 correlated with ESR, and YTHDF3 and YTHDC1 levels were negatively associated with ALT. These findings would help improve our understanding of m6A reader in the development of PTB.

Taken together, we have demonstrated in this study for the first time that *YTHDF1*, *YTHDF2*, *YTHDF3*, *YTHDC1*, and *YTHDC2* genes polymorphisms might not contribute to the susceptibility to PTB. However, several SNPs in *YTHDF2*, *YTHDC1*, and *YTHDF2* genes were significantly associated with some clinical features in patients with PTB. Moreover, decreased expression levels of YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 in patients with PTB indicated the important roles of these m6A readers in PTB, which might be considered auxiliary biomarkers for PTB diagnosis. It was worth noting that some limitations existed in this study. First, m6A readers' genes variation greatly modified the clinical manifestations of patients with PTB, especially drug resistance, but the mechanism remained unaccounted in this study. Second, this study only found the decreased levels of m6A readers' mRNA in PBMCs from patients with PTB, which should be verified at the protein level. Moreover, this study was only conducted in the Chinese Han population and the influence of m6A readers' genes variation and expression level on PTB needed to be confirmed in other ethnicities. Therefore, functional and replication studies with different ethnic groups and larger sample size were warranted to further explore the exact role of these m6A readers in PTB.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary files, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of Anhui Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

H-FP and T-PZ designed the study. H-ML and FT conducted the experiment. FT and L-JW participated in the collection of samples. QH performed the statistical analyses. H-ML and T-PZ drafted the manuscript. H-FP contributed to manuscript revision. All the authors approved the final submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.925303/full#supplementary-material

References

1. World Health Organization. Global Tuberculosis Report 2020. Available online at: https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf (accessed April 1, 2022).

2. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annu Rev Immunol.* (2013) 31:475–527. doi: 10.1146/annurev-immunol-032712-095939

3. Fox GJ, Redwood L, Chang V, Ho J. The effectiveness of individual and environmental infection control measures in reducing the transmission of mycobacterium tuberculosis: a systematic review. *Clin Infect Dis.* (2021) 72:15–26. doi: 10.1093/cid/ciaa719

4. Li HM, Wang LJ, Tang F, Pan HF, Zhang TP. Association of leptin and leptin receptor genes variants and pulmonary tuberculosis susceptibility, clinical manifestations in a Chinese population. *Microb Pathog.* (2022) 165:105499. doi: 10.1016/j.micpath.2022.105499

5. Varzari A, Deyneko IV, Tudor E, Grallert H, Illig T. Synergistic effect of genetic polymorphisms in TLR6 and TLR10 genes on the risk of pulmonary tuberculosis in a Moldavian population. *Innate Immun.* (2021) 27:365–76. doi: 10.1177/17534259211029996

6. Zhang TP, Chen SS, Zhang GY, Shi SJ, Wei L, Li HM. Association of vitamin D pathway genes polymorphisms with pulmonary tuberculosis susceptibility in a Chinese population. *Genes Nutr.* (2021) 16:6. doi: 10.1186/s12263-021-00687-3

7. Zheng R, Li Z, He F, Liu H, Chen J, Chen J, et al. Genome-wide association study identifies two risk loci for tuberculosis in Han Chinese. *Nat Commun.* (2018) 9:4072. doi: 10.1038/s41467-018-06539-w

8. Wang M, Kong W, He B, Li Z, Song H, Shi P, et al. Vitamin D and the promoter methylation of its metabolic pathway genes in association with the risk and prognosis of tuberculosis. *Clin Epigenetics.* (2018) 10:118. doi: 10.1186/s13148-018-0552-6

9. Chen KD, Huang YH, Ming-Huey Guo M, et al. The human blood DNA methylome identifies crucial role of beta-catenin in the pathogenesis of Kawasaki disease. *Oncotarget.* (2018) 9:28337–50. doi: 10.18632/oncotarget.25305

10. Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet.* (2008) 9:465–76. doi: 10.1038/nrg2341

11. Roignant JY, Soller MJY. m6A in mRNA: an ancient mechanism for fine-tuning gene expression. *Trends Genet.* (2017) 33:380–90. doi: 10.1016/j.tig.2017.04.003

12. Zhu L, Zhong J, Jia X, et al. Precision methylome characterization of Mycobacterium tuberculosis complex (MTBC) using PacBio single-molecule real-time (SMRT) technology. *Nucleic Acids Res.* (2016) 44:730–43. doi: 10.1093/nar/gkv1498

13. Shell SS, Prestwich EG, Baek SH, et al. DNA methylation impacts gene expression and ensures hypoxic survival of Mycobacterium tuberculosis. *PLoS Pathog.* (2013) 9:e1003419. doi: 10.1371/journal.ppat.1003419

14. Jiang S, Xie Y, He Z, Zhang Y, Zhao Y, Chen L, et al. m6ASNP: a tool for annotating genetic variants by m6A function. *Gigascience*. (2018) 7:35. doi: 10.1093/gigascience/giy035

15. Zhao H, Jiang J, Wang M, Xuan Z. Genome-Wide identification of m6A-associated single-nucleotide polymorphisms in colorectal cancer. *Pharmgenomics Pers Med.* (2021) 14:887–92. doi: 10.2147/PGPM.S314373

16. He J, Yuan L, Lin H, Lin A, Chen H, Luo A, et al. Genetic variants in mA modification core genes are associated with glioma risk in Chinese children. *Mol Ther Oncolytics.* (2021) 20:199–208. doi: 10.1016/j.omto.2020.12.013

17. Lan Q, Liu PY, Haase J, Bell JL, Huttelmaier S, Liu T. The critical role of RNA m(6)A methylation in cancer. *Cancer Res.* (2019) 79:1285–92. doi: 10.1158/0008-5472.CAN-18-2965

18. Chen XY, Zhang J, Zhu JS. The role of m(6)A RNA methylation in human cancer. *Mol Cancer.* (2019) 18:103. doi: 10.1186/s12943-019-1033-z

19. Liu J, Cheng J, Li L, Li Y, Zhou H, Zhang J, et al. YTHDF1 gene polymorphisms and neuroblastoma susceptibility in Chinese children: an eight-center case-control study. *J Cancer.* (2021) 12:2465–71. doi: 10.7150/jca.54496

20. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z. et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysisbio-xcn). *Cell Res.* (2009) 19:519–23. doi: 10.1038/cr.2009.33

21. Ke S, Alemu EA, Mertens C, Gantman EC, Fak JJ, Mele A, et al. A majority of m6A residues are in the last exons, allowing the potential for 3' UTR regulation. *Genes Dev.* (2015) 29:2037–53. doi: 10.1101/gad.269415.115

22. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, et al. 5' UTR m(6)A promotes cap-independent translation. *Cell.* (2015) 163:999–1010. doi: 10.1016/j.cell.2015.10.012

23. Ying P, Li Y, Yang N, Wang X, Wang H, He H, et al. Identification of genetic variants in mA modification genes associated with pancreatic cancer risk in the Chinese population. *Arch Toxicol.* (2021) 95:1117–28. doi: 10.1007/s00204-021-02978-5

24. Feng Y, Wang F, Pan H, Qiu S, Lü J, Wu L, et al. Obesity-associated gene FTO rs9939609 polymorphism in relation to the risk of tuberculosis. *BMC Infect Dis.* (2014) 14:592. doi: 10.1186/s12879-014-0592-2

25. Naderi M, Hashemi M, Dejkam N, Bahari G, Rezaei M, Taheri M. Association study of the FTO gene polymorphisms with the risk of pulmonary tuberculosis in a sample of Iranian population. *Acta Microbiol Immunol Hung.* (2017) 64:91–9. doi: 10.1556/030.64.2017.010

26. Liu Y, Lin H, Hua RX, Zhang J, Cheng J, Li S, et al. Impact of YTHDF1 gene polymorphisms on Wilms tumor susceptibility: a five-center case-control study. *J Clin Lab Anal.* (2021) 35:e23875. doi: 10.1002/jcla.23875

27. Zeng H, Li M, Liu J, Zhu J, Cheng J, Li Y, et al. YTHDF2 gene rs3738067 A>G polymorphism decreases neuroblastoma risk in Chinese children: evidence from an eight-center case-control study. *Front Med (Lausanne).* (2021) 8:797195. doi: 10.3389/fmed.2021.797195

28. Song J, Liu T, Zhao Z, Hu X, Wu Q, Peng W, et al. Genetic polymorphisms of long noncoding RNA RP11-37B2. 1 associate with susceptibility of tuberculosis and adverse events of antituberculosis drugs in west China. *J Clin Lab Anal.* (2019) 33:e22880. doi: 10.1002/jcla.22880

29. Li Y, Lu T, Wang J, Zhuo Z, Miao L, Yang Z, et al. YTHDC1 gene polymorphisms and neuroblastoma susceptibility in Chinese children. *Aging (Albany NY).* (2021) 13:25426–39. doi: 10.18632/aging.203760

30. Chen H, Li Y, Li L, Zhu J, Yang Z, Zhang J, et al. YTHDC1 gene polymorphisms and hepatoblastoma susceptibility in Chinese children: A seven-center case-control study. *J Gene Med.* (2020) 22:e3249. doi: 10.1002/jgm.3249

31. Yang N, Ying P, Tian J, Wang X, Mei S, Zou D, et al. Genetic variants in m6A modification genes are associated with esophageal squamous-cell carcinoma in the Chinese population. *Carcinogenesis.* (2020) 41:761–8. doi: 10.1093/carcin/bgaa012

32. Chen LZ, He CY, Su X, Peng JL, Chen DL, Ye Z, et al. SPP1 rs4754 and its epistatic interactions with SPARC polymorphisms in gastric cancer susceptibility. *Gene.* (2018) 640:43–50. doi: 10.1016/j.gene.2017.09.053

33. Bai Y, Yang C, Wu R, Huang L, Song S, Li W, et al. YTHDF1 regulates tumorigenicity and cancer stem cell-like activity in human colorectal carcinoma. *Front Oncol.* (2019) 9:332. doi: 10.3389/fonc.2019.00332

34. Zhao X, Chen Y, Mao Q, Jiang X, Jiang W, Chen J, et al. Overexpression of YTHDF1 is associated with poor prognosis in patients with hepatocellular carcinoma. *Cancer Biomark.* (2018) 21:859–68. doi: 10.3233/CBM-170791

35. Liu L, Liu X, Dong Z, Li J, Yu Y, Chen X, et al. N6-methyladenosine-related genomic targets are altered in breast cancer tissue and associated with poor survival. *J Cancer.* (2019) 10:5447–59. doi: 10.7150/jca.35053

36. Luo Q, Rao J, Zhang L, Fu B, Guo Y, Huang Z, et al. The study of METTL14, ALKBH5, and YTHDF2 in peripheral blood mononuclear cells from systemic lupus erythematosus. *Mol Genet Genomic Med.* (2020) 8:e1298. doi: 10.1002/mgg3.1298

37. Luo Q, Gao Y, Zhang L, Rao J, Guo Y, Huang Z, et al. Decreased ALKBH5, FTO, and YTHDF2 in peripheral blood are as risk factors for rheumatoid arthritis. *Biomed Res Int.* (2020) 2020:5735279. doi: 10.1155/2020/5735279

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A university-clustered tuberculosis outbreak during the COVID-19 pandemic in eastern China

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During the COVID-19 pandemic in 2020, a tuberculosis outbreak occurred in a university in eastern China, with 4,488 students and 421 staff on the campus. A 19-year-old student was diagnosed in August 2019. Later, the first round of screening was initiated among close contacts, but no active cases were found. Till September 2020, four rounds of screening were performed. Four rounds of screening were conducted on September 9, November 8, November 22-25 in 2019 and September 2020, with 0, 5, 0 and 43 cases identified, respectively. A total of 66 active tuberculosis were found in the same university, including 4 sputum culture-positive and 7 sputum smear-positive. The total attack rate of active tuberculosis was 1.34% (66/4909). The whole-genome sequencing showed that the isolates belonged to the same L2 sub-specie and were sensitive to all tested antituberculosis drugs. Delay detection, diagnosis and report of cases were the major cause of this university tuberculosis epidemic. More attention should be paid to the asymptomatic students in the index class. After the occurrence of tuberculosis cases in schools, multiple rounds of screening should be carried out, and preventive therapy should be applied in a timely manner.

KEYWORDS

tuberculosis, screening, whole-genome sequencing, preventive therapy, university

Introduction

Tuberculosis remains a global health problem with high morbidity and mortality (1). Although the elderly are at-risk groups for tuberculosis, teenagers and students can not be ignored (2). In recent years, the tuberculosis outbreak in schools has attracted wide attention (3–5). The number of student cases has been increasing, although the size of

the national tuberculosis epidemic is shrinking (2, 6). The high attack rate was probably due to the exposure to the source of infection in a closed environment.

The ability to discern and track individual *Mycobacterium tuberculosis* (*M.tb*) strains is of critical importance to identify the source of infection and routes of transmission. With the development of biological information and the increasing maturity of sequencing technology, the whole-genome sequencing (WGS) has provided powerful tools to track the transmission and identify the drug resistance of *M.tb* (7), showing its essential role in the tuberculosis outbreak investigation (8).

During the COVID-19 pandemic in 2020, a tuberculosis outbreak occurred in a university in eastern China. Since the initial case was found in 2019, due to various reasons, the epidemic lasted for a long time until the end of 2020. In order to comprehensively analyze the epidemic process of tuberculosis on campus, we used field investigation, infection screening, and laboratory examination, especially the application of WGS technology.

Materials and methods

Study subjects

The outbreak occurred in a university in Jiangsu province, China. This university is located in eastern China, with 4,488 students and 421 staffs. Tuberculosis was diagnosed according to the national guideline (9), mainly based on the epidemiological history, clinical manifestation, chest X-ray examination, and laboratory tests. The tuberculin skin test (TST) was used to screen for the person infected with *M.tb*. The skin test reaction was read between 48 and 72 h after administration by a trained health care worker. The reaction was measured in millimeters of the induration and divided into $\leq$5 mm, 5–10 mm, 10–15 mm, and $\geq$15 mm (10).

Close contacts screening

Close contacts referred to individuals who had direct contact with active tuberculosis patients. In general, all students and teachers should be included to the screening for at least once. The first round of screening covered all teachers and students in the index case class. The second round of screening included teachers and students staying in the same teaching building and living in the dormitories on the same floor, including those negative in the first round. The target population of the third screening were people who were not screened in the previous two rounds. All students and teachers in this university were screened in the fourth round.

Laboratory test

The Ziehl-Neelsen method was used for acid-fast staining and Lowenstein-Jensen solid medium was used for culture. GeneXpert MTB/RIF (Xpert, Cepheid, USA) was performed on collected sputum samples. The sputum sample was mixed with the sample processing solution in a ratio of 1:2. Then 2 ml of the mixture was drawn into the GeneXpert MTB/RIF reaction box, and finally, the reaction box was put into the detection system (11). Four types of first-line and two types of second-line antituberculosis drugs were used to perform phenotypic drug susceptibility tests (DSTs) (12). The critical concentrations was 40.0 mg/L for rifampin (RIF), 0.2 mg/L for isoniazid (INH), 2.0 mg/L for ethambutol (EMB), 4.0 mg/L for streptomycin (SM), 40.0 mg/L for capreomycin (CM), and 2.0 mg/L for levofloxacin (LFX).

Whole-genome sequencing analysis

Genomic DNA was extracted from cryopreserved strains and purified by Cetyltrimethylammonium Bromide (CTAB) method (13). Sequencing was performed on the Illumina Miseq (Illumina, San Diego, California) (14). The BBmap software was used to compare the sequencing reads with the reference of H37Rv (GenBank NC000962.3) (15). The minimum number of reads covering a site to be considered (default = 10), the minimum VCF variant call "quality" (default = 100). We used two online tools, "SAM-TB" (https://samtb.uni-medica.com/index) and "TB-Profiler" (https://tbdr.lshtm.ac.uk/), to obtain drug resistance and strain types from the raw sequence. Isolates with a difference of <10 pairs of single nucleotide polymorphisms (SNPs) were considered homologous (16).

Prophylactic therapy

Students and teachers with strongly positive TST received prophylactic therapy. All active tuberculosis patients were treated with a standardized first-line regimen (2 months of Isoniazid, Rifampicin, Ethambutol, and Pyrazinamide, plus 4 months of Isoniazid and Rifampicin).

Statistical analysis

All statistical analysis and graphing were performed through software R4.1.0 (https://www.r-project.org/). Categorical variables were expressed as percentages and analyzed using the chi-square test. -Three pies were used to compare the difference of developing active tuberculosis between index class and non-index class. A P value of 0.05 or less was considered statistically significant.

Results

Index case and the first round of screening

In August 2019, a 19-year-old student presented symptoms of paroxysmal cough, fever, sputum, and night sweats. She was diagnosed with tuberculosis pleurisy through a series of inspections and then reported to the local Center for Disease Control and Prevention (CDC). On September 9, the first round of tuberculosis screening was initiated among close contacts, including 73 students and 18 teachers. After TST and chest X-ray examinations, one person had an induration of 15 or more millimeters and no one had abnormal chest X-ray examinations.

Subsequent cases and close contacts screening

On November 8, the second student was diagnosed with pulmonary tuberculosis after seeking health care. Then local CDC performed the second screening from November 22 to November 25, including 1,109 students and 187 teachers staying in the same teaching building and living in the dormitories on the same floor. Among them, 45 had a TST induration of 15 or more millimeters, 6 had abnormal chest X-ray examinations, and 5 were diagnosed with active tuberculosis. Of 5 tuberculosis patients detected in the second round, 2 patients were asymptomatic in the first round and had negative TST and abnormal chest X-ray examinations.

On December 15, one student, who was strongly positive in the second round of screening, took the initiative to see a doctor and was diagnosed with pulmonary tuberculosis. Then, the local CDC conducted the third round of screening using TST and chest X-ray examination from December 16 to December 18. Among 3,634 close contacts, 564 had a TST induration $\geq$10 mm, and 155 had a TST induration $\geq$15 mm (Table 1). No one showed abnormal chest images.

From August 2019 to December 2019, the diagnosed cases had been reported to the infectious disease surveillance and the two rounds screening were given within 24 h upon reporting.

In early 2020, due to the emergence of COVID-19, all students left campus and studied online at home. During January 2020 and August 2020, 15 students scattered in various districts were diagnosed with tuberculosis in local hospitals. After students returned to school in September 2020, the fourth round of screening was performed for 4,909 subjects, of which 233 had a TST induration $\geq$15 mm and 45 had abnormal chest X-ray images. After a series of laboratory tests, a total of 43 students were diagnosed with tuberculosis.

Figure 1 showed the timeliness and delay in reporting of cases after diagnosis. From January 2020 to May 2020, 4 patients were not reported to the infectious disease surveillance system within 24 h. Meanwhile, during the COVID-19 pandemic, the public health where the students were domiciled didn't liaise with the CDC where the university was located. Due to the regulation and capacity constrains, timely and effective screening including TST and chest X-ray had not been carried out, which resulted in the delay diagnosis of asymptomatic stunents.

In total, 66 active tuberculosis patients were diagnosed in this university, of which 4 were sputum culture-positive, 7 were sputum smear-positive, and 18 were GeneXpert positive (Table 2). Phenotypic drug susceptibility tests showed that 4 confirmed isolates were susceptible to all anti-tuberculosis drugs. Three classes had highest numbers of patients and majority cases happened in grade 1.

Attack rates of active tuberculosis

The total attack rate of active tuberculosis was 1.34% (66/4909), of which 2.23% (34/1525) in male students and 0.95% (32/3384) in female students. In the index case class, the attack rate among teachers and students was 0 (0/18) and 52.05 (38/73) respectively (Table 3). Compared to the non-index class, there was a significantly higher prevalence of active tuberculosis in index class (41.76 vs. 0.58%, $P < 0.05$) (Figure 2).

WGS analysis and phylogenic tree

We successfully extracted DNA from four sputum culture-positive samples. WGS showed that the distance of SNPs between four isolates was <12. These four strains belonged to the L2, and no drug resistance-related genomic sites were found.

Treatment outcome

175 Students and 42 Teachers With Strongly Positive TST Were Given the Prophylactic Therapy. No Teachers and Students who Accepted Preventive Treatment Have Developed Active Tuberculosis.

Discussion

The incidence of tuberculosis among general populations is continuously declining in China, but the number of student cases is rising (6). School with high population densities can be a greenhouse of pathogens, and school outbreaks of tuberculosis have been reported frequently (3, 5, 17). This study described a school-clustered tuberculosis outbreak during the COVID-19 epidemic and highlighted the importance of interventional measures to prevent a larger scale transmission.

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TABLE 1 Four rounds of close contacts screening.

	Total	TST results				Abnormal chest X-ray (covered in previous round)	Suspected patients (covered in the previous round)	Confirmed patients (covered in the previous round)
		<5 mm	5–10 mm	10–15 mm	≥15 mm			
First round								
students	73	64	1	7	1	0 (0)	0 (0)	0 (0)
teachers/staff	18	13	4	1	0	0 (0)	0 (0)	0 (0)
Second round								
students	1,109	748	174	158	29	6 (2)	6 (2)	5 (2)
teachers/staff	187	128	19	24	16	0 (0)	0 (0)	0 (0)
Third round								
students	3,400	2,473	380	400	147	0 (0)	0 (0)	0 (0)
teachers/staff	234	196	21	9	8	0 (0)	0 (0)	0 (0)
Fourth round								
students	4,488	2,641	948	708	191	45 (30)	45 (30)	43 (28)
teachers/staff	421	245	72	62	42	0 (0)	0 (0)	0 (0)

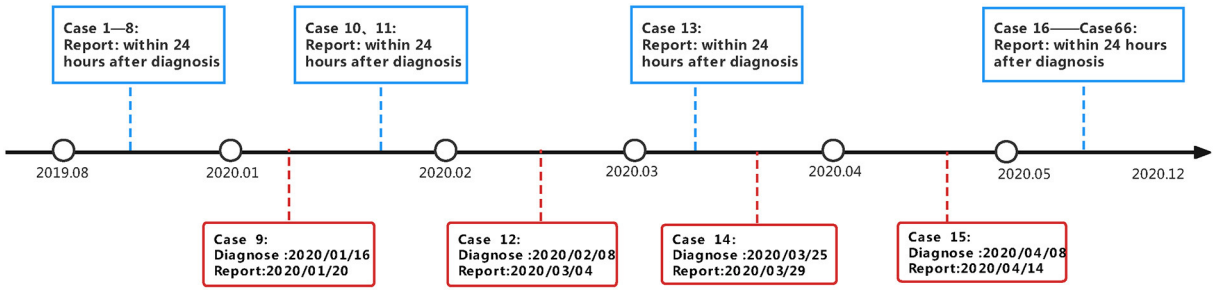

FIGURE 1
Timeline of close contacts screening and case finding in a school tuberculosis outbreak investigation.

There are several features that are worth consideration in this outbreak. First, delayed diagnosis is common in the school tuberculosis outbreaks (18). Before the detection of the first case, pathogen transmission may have been going on among students and teachers for quite a long time. Considering the window stage after infection, several screening and follow-up rounds are critical to identifying infected persons and active cases. Second, few cases were diagnosed because of active seeking health care due to the lack of apparent symptoms. As shown in Table 2, out of 66 active patients, 72.7% were detected through contact screening, and more than 45% had no tuberculosis-related symptoms. Third, during the pandemic, COVID-19 was the main concern in every clinic and may result in delaying the diagnosis and treatment of tuberculosis (19). Only 5 student patients were screened in the previous three rounds through TST and chest X-ray. Some of the asymptomatic students later developed active TB during the COVID-19. During this phase, some hospitals failed to report to the infectious disease surveillance system in a timely manner and the public health department took no actions due to the policy constrains, which caused further spread of this tuberculosis outbreak. One of our previous studies in Jiangsu Province showed that tuberculosis notifications dropped 52% during the COVID-19 pandemic in 2020 compared to 2015–2019, and the treatment completion and screening for drug resistance decreased continuously in 2020 (20). Regional lockdown, transportation restrictions, and school closures have all contributed to the delays in case detection and close contacts screening. Fourth, for fear of being stigmatized or affecting their studies, some students and their parents consciously do not report disease status and choose to go to school, resulting in continuous transmission among students.

TABLE 2 Demographic and clinical characteristics of the patients in a university, Jiangsu, China.

Characteristics	Patients	%
Age (years)		
18-	8	12.1
19-	34	51.5
20-	24	36.4
Gender		
Male	34	51.5
Female	32	48.5
Bacteriological diagnosis		
Positive	18	27.3
Negative	48	72.7
Sputum culture		
Positive	4	6.1
Negative	62	93.9
Sputum smear		
Positive	7	10.6
Negative	59	89.4
GeneXpert		
Positive	18	27.3
Negative	48	72.7
With diabetes		
Yes	0	0.0
No	100	100.0
Discovery method		
clinical consultation	18	27.3
contact screening	48	72.7
TB-related symptoms		
No cough	30	45.5
Cough	36	54.5

TABLE 3 Attack rates of active tuberculosis in this school tuberculosis outbreak, Jiangsu, China.

Group	No.at risk	Active TB	
		N	Attack rate (95%CI)
Total	4,909	66	1.34 (1.04, 1.70)
Teachers	421	0	0
Students	4,488	66	1.47 (1.14, 1.87)
Gender			
Male	1,525	34	2.23 (1.55, 3.10)
Femal	3,384	32	0.95 (0.65, 1.33)
Classes			
Index class	91	38	41.76 (31.50, 52.57)
Non-index class	4,818	28	0.58 (0.39, 0.84)
Teachers			
Index class	18	0	0
Non-index class	403	0	0
Students			
Index class	73	38	52.05 (40.04, 63.90)
Non-index class	4,415	28	0.63 (0.42, 0.92)

WGS has been promoted in the investigation of tuberculosis outbreaks to determine the genotypes of *M.tb* and drug resistance. A pilot study of the European surveillance system proved that it was feasible to monitor the occurrence of multidrug-resistant tuberculosis outbreaks and could better clarify the mode of cross-border transmission (21). Compared with traditional epidemiological methods, WGS is time-consuming and can quickly determine the source of infection and route of transmission (22). However, in many investigations of tuberculosis outbreaks, it is challenging to obtain *M.tb* strains for sequencing.

For high-burden countries, it is crucial to explore safe and effective preventive treatment tools and to establish an optimized LTBI management system (23). Currently, the state encourages tuberculosis preventive therapy for students with latent TB infection. The effectiveness of preventive treatment in high burden countries has not been determined, especially for China with high MDR prevalence. To date, few studies have evaluated the tuberculosis preventive therapy (TPT) effectiveness and the probability of treatment completion in adolescents in the tuberculosis outbreak (24, 25). The directly observed therapy (DOT) and full course management (FCM) were widely used in the preventive treatment of LTBI. A study in Dalian, China, showed that DOT is effective and plays an irreplaceable role in improving preventive treatment adherence and outcomes (26). In our study, no students who accepted preventive treatment have developed active tuberculosis, and follow-up is required to evaluate the effectiveness of tuberculosis preventive treatment. It is crucial to explore safe and effective preventive treatment tools and to establish an optimized LTBI management system (23).

There were several limitations in this study. First, the proportion of bacteriological evidence in tuberculosis cases is very small, and most of them are clinically diagnosed. Previous studies have shown a low rate of pathogenic positivity in the student population and the bacterial load in children's specimens is low and difficult to collect (27, 28). To improve the sensitivity of bacteriological testing, some researchers suggest the use of multiple sampling methods for microbiological diagnosis, including serial collection of biological samples such as gastric aspirates, nasopharyngeal aspirates, sputum, bronchoalveolar lavage, and lymph node aspirates and urine (29). However, in this study, we collected sputum from patients on a continuous basis and performed pictures and

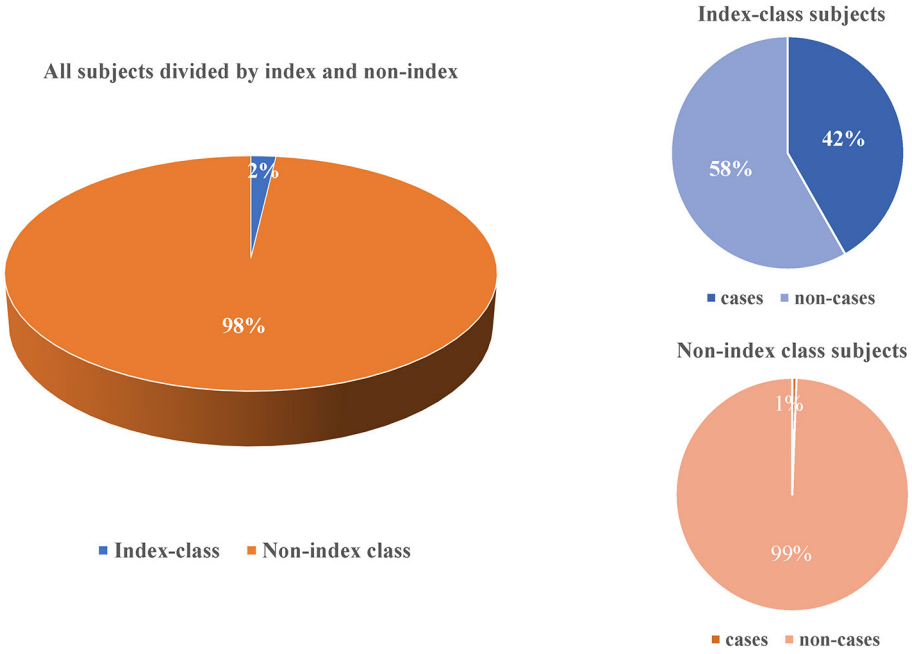

FIGURE 2
Comparison for active tuberculosis between teachers and students in the index class and non-index class in this outbreak.

cultures, which explained the low bacteriological examination. This is a common problem in most tuberculosis outbreak investigations, which has limited our understanding of the whole transmission network (30). Second, we did not follow those students who received preventive therapy for a long enough time. But we checked the national tuberculosis surveillance system in January 2022, and none of them was reported as active tuberculosis.

In conclusion, delay detection, diagnosis and report of cases were the major cause of this university tuberculosis outbreak. More attention should be paid to the asymptomatic students in the index class.After the occurrence of tuberculosis cases in schools, multiple rounds of screening should be carried out, and preventive therapy should be applied in a timely manner. WGS is the approach to support the transmission theory. This study could give a clear picture of transmission, screening-intervention, and the disruption of case finding routine due to COVID-19 pandemic and its negative impacts to TB control in university settings.

Data availability statement

The sequencing data presented in the study are deposited in the CNCB (China National Center for Bioinformation) repository, accession number PRJCA010507.

Ethics statement

The studies involving human participants were reviewed and approved by Nanjing Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JWu, LM, and JY conceived the study, analyzed the data, and drafted the manuscript. QL, JS, and YW participated in the study design. XD, PL, and LM implemented the field investigation and performed laboratory tests. JWa and WL participated in the study design and helped draft the manuscript. All authors contributed to the study and have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. WHO. *Global Tuberculosis Report 2021*. Geneva: WHO (2021).

2. Dodd PJ, Yuen CM, Sismanidis C, Seddon JA, Jenkins HE. The global burden of tuberculosis mortality in children: a mathematical modelling study. *Lancet Glob Health.* (2017) 5:e898–906. doi: 10.1016/S2214-109X(17)30289-9

3. Hou J, Pang Y, Yang X, Chen T, Yang H, Yang R, et al. Outbreak of mycobacterium tuberculosis beijing strain in a high school in Yunnan, China. *Am J Trop Med Hyg.* (2020) 102:728–30. doi: 10.4269/ajtmh.19-0533

4. You NN, Zhu LM Li GL, Martinez L, Lu W, Liu Q, et al. A tuberculosis school outbreak in china, 2018: reaching an often overlooked adolescent population. *Epidemiol Infect.* (2019) 147:e303. doi: 10.1017/S0950268819001882

5. Bao H, Liu K, Wu Z, Wang X, Chai C, He T, et al. Tuberculosis outbreaks among students in Mainland China: a systematic review and meta-analysis. *BMC Infect Dis.* (2019) 19:972. doi: 10.1186/s12879-019-4573-3

6. Wei S, Yunzhou R, Tao L, Xin D, Jiawen J, Yuying H, et al. Epidemiological characteristics of rifampicin-resistant tuberculosis in students — China, 2015–2019. *China CDC Weekly.* (2021) 3:549–52. doi: 10.46234/ccdcw2021.142

7. Meehan CJ, Goig GA, Kohl TA, Verboven L, Dippenaar A, Ezewudo M, et al. Whole genome sequencing of mycobacterium tuberculosis: current standards and open issues. *Nat Rev Microbiol.* (2019) 17:533–45. doi: 10.1038/s41579-019-0214-5

8. van der Werf MJ, Ködmön C. Whole-genome sequencing as tool for investigating international tuberculosis outbreaks: a systematic review. *Front Public Health.* (2019) 7:87. doi: 10.3389/fpubh.2019.00087

9. Wu X, Pang Y, Song Y, Dong W, Zhang T, Wen S, et al. Implications of a school outbreak of multidrug-resistant tuberculosis in Northern China. *Epidemiol Infect.* (2018) 146:584–8. doi: 10.1017/S0950268817003120

10. Nelson K. Tuberculin testing to detect latent tuberculosis in developing countries. *Epidemiology.* (2007) 18:348–9. doi: 10.1097/01.ede.0000259985.76928.64

11. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med.* (2010) 363:1005–15. doi: 10.1056/NEJMoa0907847

12. Grandjean L, Moore DA. Tuberculosis in the Developing World: Recent advances in diagnosis with special consideration of extensively drug-resistant tuberculosis. *Curr Opin Infect Dis.* (2008) 21:454–61. doi: 10.1097/QCO.0b013e32830ce783

13. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, et al. Strain identification of mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol.* (1993) 31:406–9. doi: 10.1128/jcm.31.2.406-409.1993

14. Wu X, Gao R, Shen X, Guo Y, Yang J, Wu Z, et al. Use of whole-genome sequencing to predict mycobacterium tuberculosis drug resistance in Shanghai, China. *Int J Infect Dis.* (2020) 96:48–53. doi: 10.1016/j.ijid.2020.04.039

15. He G, Li Y, Chen X, Chen J, Zhang W. Prediction of treatment outcomes for multidrug-resistant tuberculosis by whole-genome sequencing. *Int J Infect Dis.* (2020) 96:68–72. doi: 10.1016/j.ijid.2020.04.043

16. Chee CBE, Lim LKY, Ong RTH, Sng LH, Hsu LY, Lee VJM, et al. Whole genome sequencing analysis of multidrug-resistant tuberculosis in Singapore, 2006-2018. *Eur J Clin Microbiol Infect Dis.* (2021) 40:1079–83. doi: 10.1007/s10096-020-04100-6

17. Yu H, Zhang Y, Chen X, He G, Sun F, Li Y, et al. Whole-genome sequencing and epidemiological analysis of a tuberculosis outbreak in a high school of Southern China. *Infect Genet Evol.* (2020) 83:104343. doi: 10.1016/j.meegid.2020.104343

18. Zhang S, Li X, Zhang T, Fan Y, Li Y. The experiences of high school students with pulmonary tuberculosis in China: a qualitative study. *BMC Infect Dis.* (2016) 16:758. doi: 10.1186/s12879-016-2077-y

19. Can Sarinoglu R, Sili U, Eryuksel E, Olgun Yildizeli S, Cimsit C, Karahasan Yagci A. Tuberculosis and Covid-19: an overlapping situation during pandemic. *J Infect Dev Ctries.* (2020) 14:721–5. doi: 10.3855/jidc.13152

20. Huang HL, Huang WC, Lin KD, Liu SS, Lee MR, Cheng MH, et al. Completion rate and safety of programmatic screening and treatment for latent tuberculosis infection in elderly patients with poorly controlled diabetic mellitus: a prospective multicenter study. *Clin Infect Dis.* (2021). doi: 10.1093/cid/ciab209

21. Tagliani E, Anthony R, Kohl TA, de Neeling A, Nikolayevskyy V, Ködmön C, et al. Use of a whole genome sequencing-based approach for mycobacterium tuberculosis surveillance in Europe in 2017-2019: an ecdc pilot study. *The European respiratory journal.* (2021) 57(1). doi: 10.1183/13993003.02272-2020

22. Chen X, He G, Wang S, Lin S, Chen J, Zhang W. Corrigendum: evaluation of whole-genome sequence method to diagnose resistance of 13 anti-tuberculosis drugs and characterize resistance genes in clinical multi-drug resistance mycobacterium tuberculosis isolates from China. *Front Microbiol.* (2019) 10:2221. doi: 10.3389/fmicb.2019.02221

23. Cui X, Gao L, Cao B. Management of latent tuberculosis infection in china: exploring solutions suitable for high-burden countries. *Int J Infect Dis.* (2020) 92s:S37–40. doi: 10.1016/j.ijid.2020.02.034

24. Ayieko J, Abuogi L, Simchowitz B, Bukusi EA, Smith AH, Reingold A. Efficacy of isoniazid prophylactic therapy in prevention of tuberculosis in children: a meta-analysis. *BMC Infect Dis.* (2014) 14:91. doi: 10.1186/1471-2334-14-91

25. Gray DM, Zar H, Cotton M. Impact of tuberculosis preventive therapy on tuberculosis and mortality in HIV-infected Children. *Cochrane Database Syst Rev.* (2009) (1):Cd006418. doi: 10.1002/14651858.CD006418.pub2

26. Chen Q, Wang XM Qi Y, Liu XF, Jiang LP, Hou W, et al. The impact of directly observed therapy on preventive treatment for latent tuberculosis

infection among students in Dalian, China. *Biomed Environ Sci.* (2015) 28:611–5. doi: 10.3967/bes2015.085

27. Li H, Liu C, Liang M, Liu D, Zhao B, Shi J, et al. Tuberculosis outbreak in an educational institution in Henan Province, China. *Front Public Health.* (2021) 9:737488. doi: 10.3389/fpubh.2021.737488

28. Lamb GS, Starke JR. Tuberculosis in infants and children. *Microbiol Spectrum.* (2017) 5:5.2.21. doi: 10.1128/microbiolspec.TNMI7-0037-2016

29. Magee MJ, Salindri AD, Kyaw NTT, Auld SC, Haw JS, Umpierrez GE. Stress hyperglycemia in patients with tuberculosis disease: epidemiology and clinical implications. *Curr Diab Rep.* (2018) 18:71. doi: 10.1007/s11892-018-1036-y

30. Liu D, Huang F, Zhang G, He W, Ou X, He P, et al. Whole-genome sequencing for surveillance of tuberculosis drug resistance and determination of resistance level in China. *Clin Microbiol Infect.* (2021) 28:731.e9–731.e15. doi: 10.1016/j.cmi.2021.09.014

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Case report: A 9-year systematic treatment failure of a pulmonary tuberculosis patient

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Objective: To explore the reasons of failure in a case of pulmonary tuberculosis (PTB) after 9 years systematic treatment.

Methods: We extracted the patients' treatment history, drug susceptibility testing (DST), Computed tomography (CT) images, and sequenced the isolated strains by whole gene sequencing (WGS).

Results: Although most results of the phenotypical DSTs were consistent with the genotype DST, the occurrence of gene resistance to amikacin (AMK), capreomycin (CAP), moxifloxacin (MFX) was earlier than the phenotypical DST. Based on the continuously reversed results of phenotypical DSTs, CT images in different stages and WGS, it can be confirmed that the patient was infected with two different strains of Mycobacterium tuberculosis (M.TB). Moreover, severe cavities may be another factor leading to treatment failure.

Conclusion: Given the suggestive effect of genotype DST is earlier than the phenotypical DST, so genotype DST can play a better guiding role in patients with MDR-TB. Additionally, for patients who have not been cured for a long time, medication should be more cautious and the role of WGS in drug resistance surveillance should be fully utilized.

KEYWORDS

tuberculosis, treatment failure, whole genome sequencing, multidrug resistance, computed tomography-three dimensional

We report and discuss a case of pulmonary tuberculosis (PTB) with failure after 9 years (February 2012, to July 2021) systematic treatment. The immunodeficiency workup on the patient, a 29-year-old female, revealed normal immunoglobulin levels and a negative result for the anti-HIV human T-lymphotropic virus. In addition, further relevant analysis showed no evidence of other comorbidities including hepatitis, diabetes, and other respiratory diseases. By analyzing her treatment history, drug susceptibility testing (DST), Computed tomography (CT) images, and isolated strains' whole genome sequencing (WGS), we hope to identify the causes of treatment failure and seek expert guidance to improve her survival time and

quality of life (A detailed case report is provided in the Supplementary Appendix).

The case's whole treatment history can be divided into three stages: in the first stage (February 2012 to March 19, 2017), the patient was hospitalized in the local tuberculosis-designated hospital in February 2012 with her first pulmonary tuberculosis diagnosis and began to receive first-line antituberculosis regimen. After 1 year of treatment, she decided to stop treatment with improvement in March 2013. Only 2 months later, she was admitted to Beijing Chest Hospital due to the recurrence and deterioration of the previous clinical manifestations and then treated with para-aminosalicylate (PAS), rifabutin (RFB), pyrazinamide (PZA), amikacin (AMK), ethambutol (EMB), and levofloxacin (LFX) for 2 years but not cured. On March 19, 2017, CT images showed the lesions increased and progressed. Throughout this stage of therapy, DSTs results were absent. In the second stage (March 20, 2017, to June 12, 2019), the patient received standardized treatment for multidrug-resistant tuberculosis (MDR-TB) under DST results and was not cured. In this stage, based on the DST results, the patient was treated with TB sensitive drugs, including cycloserine (CS), linezolid (LZD), capreomycin (CM), moxifloxacin (MFX), clofazimine (CFZ), but the deterioration continued to progress. In addition, the CT images showed that two pre-existing independent thick-walled cavities 1 and 2 in the right upper lung had partial absorption. In the third stage (June 13, 2019, to July 23, 2021), the patient received Bedaquiline (BDQ) and Delamanid (DLM) combinated with the previous standardized treatment but was still not cured. In the absence of accessible therapeutic medications and after declining our surgical recommendations, the patient was gradually transitioned to palliative care. Interestingly, the DSTs results on June 13, 2019, and August 24, 2020, showed that LFX, protionamide (PTO) and isoniazid aminosalicylate (PA) changed from drug resistance to sensitivity. However, the subsequent five phenotypical DSTs results from October 30, 2020, to July 23, 2021, were similar to those in 2017. In addition, CT images presented those two cavities fused from connected locally to extensively. The fusion lesion infiltrated outward and deteriorated rapidly, resulting in the destruction of the entire right upper lobe (Figures 1A,B, Table 1).

Based on the continuously reversed results of phenotypical DSTs results and CT images in different stages, we speculate that the patient was infected with two different strains of *Mycobacterium tuberculosis* (*Mtb*). To verify our conjecture, the five existing sputum samples sampled in Apr 2017, Aug 2017, Jun 2019, Apr 2021, and May 2021 were sequenced by the whole genome (Details of WGS and Variant calling are provided in the Supplementary Appendix). All five strains belonged to lineage 2.3, differing by up to 196 SNPs, of which the strain in 2019 is a separate isolate (cluster 1), and the remaining four strains are from the same cluster (cluster 2). In view of the diversity of strains *in vivo* hardly exceeds 12 SNPs, (1) we speculate that this patient has mixed infection. The two clusters shared four mutations in *rpoB*, *fabG1_promoter*, *rpsL* and *gyrA*, which were associated with rifampicin (RFP), isoniazid (INH), streptomycin (SM) and fluoroquinolones resistance, respectively. Additionally, five independent mutations in cluster 1 were associated with first-line drug resistance (INH, EMB, PZA), consistent with the patient's first-stage treatment history. Given that drug selection pressure is the primary driver of resistance mutations in strains, (2) and no EMB and PZA related mutations were found in cluster 2, we speculate that cluster 1 was the first infected strain of this patient (Figures 1C,D). This was reflected in the emergence of mutations in *rrl*. Therefore, we speculated that the two strains coexisted in the lung for a long time, resulting in the failure of the patient's treatment.

Summarizing the above information, although most results of the phenotypical DSTs results were consistent with the genotype DST results, the occurrence of gene resistance to AMK, capreomycin (CAP), MFX was earlier than the phenotypical DST results, which indicates that the drug resistance mutations had gradually accumulated during the treatment, but the phenotypical DST results was not prompted in time. The treatment scheme was not changed in time, resulting in the gradual accumulation of resistance and treatment failure. In addition, with the advent of new drugs such as BDQ and DLM, the cure rate of multidrug-resistant TB can reach over 80% (3). However, this patient still failed to respond to treatment without resistance to BDQ and DLM, which may be related to severe cavities (4) The necrotic tissue in the cavities is not only the rich medium of *Mtb*, (5) but also the cavity walls composed of connective tissue may hinder the drugs' penetration, (6) which is easier to induce *Mtb* to produce resilience, tolerance and resistance.

This study has some limitations. First, the treatment failure in the first stage can be explained from the genotype resistance results of the strain in April 2017, but we have no way of knowing the resistance situation and evolution process of the strains before. Second, the early strains were not preserved during the 9-year treatment process, so the sequencing of infection of the two clusters cannot be accurately obtained and the cumulative changes of drug resistance genes could not be dynamically observed.

In this study, we found that genotype DST findings, which are sooner than phenotypical DST results, can provide superior treatment guidance for tuberculosis. In addition, mixed infection of *Mtb* leads to the emergence of heterogeneous flora, making the treatment more complex. Therefore, we should be more cautious when prescribing medication to patients who haven't

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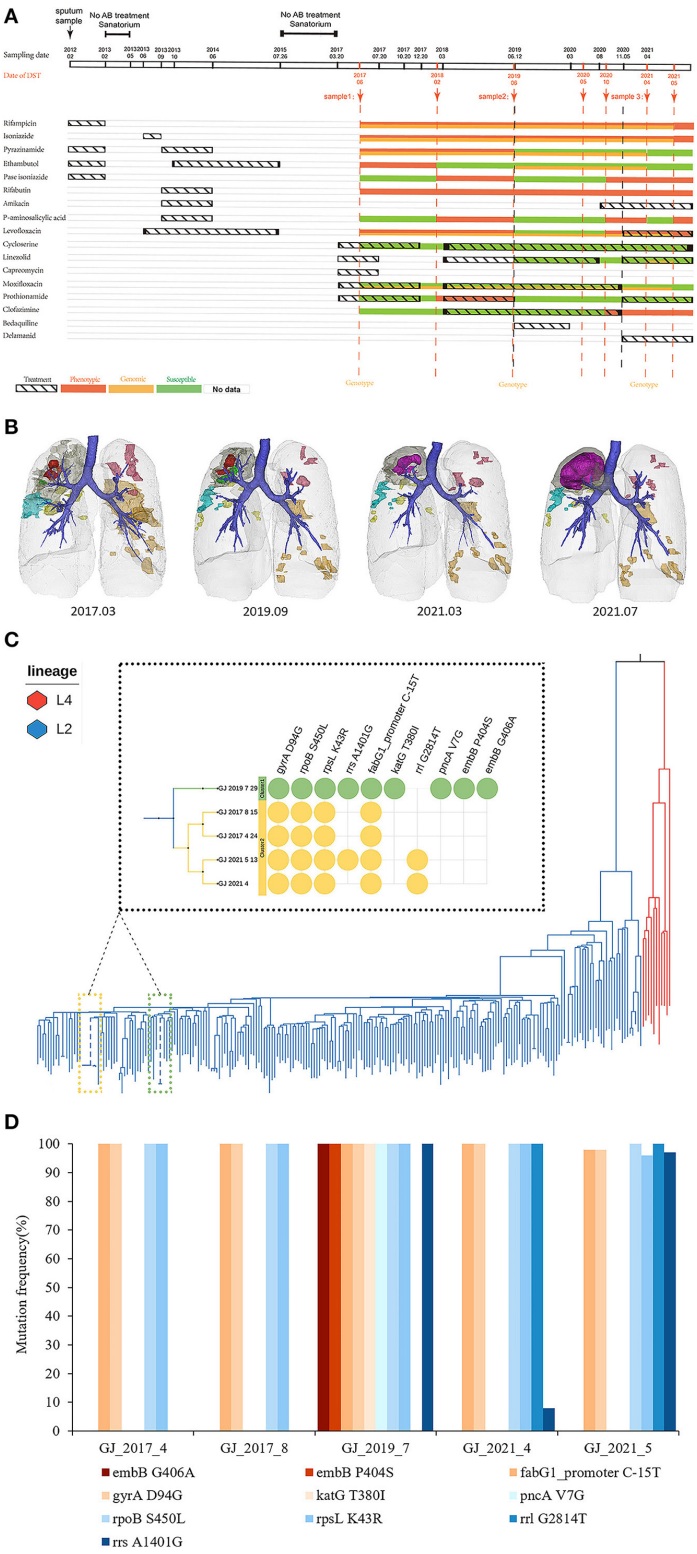

FIGURE 1
Treatment history, CT images, phylogenetics and drug resistance profiles, and unfixed mutation subgroups of drug resistance genes. **(A)** Treatment History. Anti-tuberculosis drugs used in different periods. **(B)** Three-dimensional visualization of CT scans demonstrates the longitudinal anatomical change in lung lesions. Lesions in different lung lobes are shown in different colors, and the trachea (blue) and pulmonary contours (light gray) are shown as references. There were two independent cavities 1 (red) and 2 (green) in the right upper lobe parenchymal abnormality (dark gray) in March 2017, which appeared partial absorption in September 2019, and subsequently fused extensively and infiltrated outward (magenta) from March to July 2021. Such rapid deterioration resulted in the destruction of the right upper lung lobe. All other lung lobar lesions showed a benign response to treatment or remained relatively stable. Lesions of the right middle lobe (cyan), right lower.

FIGURE 1 (Continued)
lobe (yellow), left upper lobe (pink), and left lower lobe (orange) are also shown in the **(B)**. **(C)** Whole-genome sequencing. Five strains belong to L2. Mutations that confer drug resistance are shown. FLUOROQUINOLONES (*gyrA*), RIFAMPICIN (*rpoB*), STREPTOMYCIN (*rpsL*), AMIKACIN (*rrs*), LINEZOLID (*rrl*), ISONIAZID (*fabG1_promoter*, *katG*), PYRAZINAMIDE (*pncA*), ETHAMBUTOL (*embB*). **(D)** Unfixed mutation subgroups of drug resistance genes. Resistance to amikacin developed in 2021. Percentages on the y axis are based on the number of genome-sequencing reads supporting the corresponding drug resistance-conferring mutation.

TABLE 1 The results of drug susceptibility test to 16 drugs from 2017 to 2021.

Date	RFP	INH	EMB	RFB	SM	AMK	PAS	LFX	CPM	MFX	PTO	CFZ	RFT	PI	CLR	KAN
2017.3.28	R	S	S	R	R	S	S	R	S	S	S	S	R	S	S	S
2017.4.13	R	S	S	R	R	S	S	R	S	S	S	S	R	S	S	S
2017.4.14	R	S	S	R	R	S	S	R	S	S	S	S	R	S	S	S
2017.4.18	R	S	S	R	R	S	S	R	S	S	S	S	R	S	S	S
2017.6.29	R	S	S	R	R	S	S	R	S	S	S	S	R	R	S	S
2017.6.30	R	S	S	R	R	S	S	R	S	S	S	S	R	R	S	S
2017.7.03	R	S	S	R	R	S	S	R	S	S	S	S	R	R	S	S
2018.2.8	R	R	S	R	R	S	R	R	S	S	R	S	R	R	S	S
2018.3.15	R	R	S	R	R	S	S	R	S	S	R	S	R	R	S	S
2019.6.13	R	R	S	R	R	S	S	S	S	S	S	S	R	S	S	S
2020.8.24	R	R	S	R	R	S	S	S	S	S	S	S	R	S	S	S
2020.10.30	R	R	S	R	R	S	R	R	S	S	S	R	R	R	R	R
2021.03.26	R	R	S	R	R	S	S	R	S	S	S	R	R	R	S	R
2021.03.26	R	R	S	R	R	S	S	R	S	S	S	R	R	R	S	R
2021.04.23	R	R	S	R	R	S	S	R	S	S	S	R	R	R	S	R
2021.07.23	R	R	S	R	R	R	R	R	R	S	S	R	R	R	S	R

RFP, rifampicin; INH, soniazid; EMB, ethambutol; RFB, rifabutin; SM, streptomycin; AMK, amikacin; PAS, para-aminosalicylate; LFX, levofloxacin; CPM, capreomycin; MFX, moxifloxacin; PTO, protionamide; CFZ, clofazimine; RFT, rifapentine; PI, isoniazid-aminosalicylate; CLR, clarithromycin; KAN, kanamycin; R, resistance; S, susceptible.

been healed for a long time and utilize WGS for drug resistance surveillance.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/bioproject/, PRJNA868171.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Chest Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

WL, MG, and HJ conceived, designed, and supervised the study. XW, JJ, FH, YX, and YG collected the data. CZ and JY analyzed WGS data. LQ analyzed CT data. CZ, JY, LQ, YG, and HM created the figures. HJ, CZ, LQ, and XW interpreted the findings. HJ wrote the drafts of the manuscript. WL and MG commented on and revised the drafts of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.966891/full#supplementary-material

References

1. Liu Q, Wei J, Li Y, Wang M, Su J, Lu Y, et al. Mycobacterium tuberculosis clinical isolates carry mutational signatures of host immune environments. *Sci Adv*. (2020) 6:eaba4901. doi: 10.1126/sciadv.aba4901

2. Nguyen QH, Contamin L, Nguyen TVA, Bañuls AL. Insights into the processes that drive the evolution of drug resistance in *Mycobacterium tuberculosis*. *Evol Appl*. (2018) 11:1498–511. doi: 10.1111/eva.12654

3. Liu Y, Gao M, Du J, Wang L, Gao J, Shu W, et al. Reduced susceptibility of *Mycobacterium tuberculosis* to Bedaquiline during antituberculosis treatment and its correlation with clinical outcomes in China. *Clin Infect Dis*. (2021) 73:e3391–e7. doi: 10.1093/cid/ciaa1002

4. Urbanowski ME, Ordonez AA, Ruiz-Bedoya CA, Jain SK, Bishai WR. Cavitary tuberculosis: the gateway of disease transmission. *Lancet Infect Dis*. (2020) 20:e117–28. doi: 10.1016/S1473-3099(20)30148-1

5. Sarathy JP, Dartois V. Caseum: a niche for *Mycobacterium tuberculosis* drug-tolerant persisters. *Clin Microbiol Rev*. (2020) 33:e00159–19. doi: 10.1128/CMR.00159-19

6. Strydom N, Gupta SV, Fox WS, Via LE, Bang H, Lee M, et al. Tuberculosis drugs' distribution and emergence of resistance in patient's lung lesions: a mechanistic model and tool for regimen and dose optimization. *PLoS Med*. (2019) 16:e1002773. doi: 10.1371/journal.pmed.1002773

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Whole-genome sequencing for surveillance of fluoroquinolone resistance in rifampicin-susceptible tuberculosis in a rural district of Shanghai: A 10-year retrospective study

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Background: Fluoroquinolones (FQs) are the most important second-line anti-tuberculosis (anti-TB) drugs, primarily used for the treatment of multidrug- or rifampicin-resistant TB (MDR/RR-TB). However, FQs are also commonly used to treat other bacterial infections. There are few published data on the rates of FQ resistance among rifampicin-susceptible TB.

Methods: We used whole-genome sequencing (WGS) to determine the prevalence of FQ resistance among rifampicin-susceptible TB in a rural district of Shanghai. This was a population-based retrospective study of all culture-positive pulmonary TB patients diagnosed in the Chongming district of Shanghai, China during 2009–2018.

Results: The rate of FQ resistance was 8.4% (29/345) among TB, 6.2% (20/324) among rifampicin-susceptible TB, and 42.9% (9/21) among MDR/RR-TB. Transmission of FQ-resistant strains was defined as strains differing within 12 single-nucleotide polymorphisms (SNPs) based on WGS. Among the rifampicin-susceptible TB, 20% (4/20) of FQ resistance was caused by the transmission of FQ-resistant strains and 45% (9/20) of FQ resistance was identified as hetero-resistance.

Conclusions: The prevalence of FQ resistance in rifampicin-susceptible TB was higher than expected in Shanghai. Both the transmission and the selection of drug-resistant strains drive the emergence of FQ resistance in rifampicin-susceptible TB isolates. Therefore, the WGS-based surveillance system for TB

should be urgently established and the clinical awareness of the rational use of FQs for respiratory infections should be enhanced to prevent the premature occurrence of FQ resistance.

KEYWORDS

fluoroquinolone resistance, whole-genome sequencing, rifampicin-susceptible tuberculosis, tuberculosis surveillance, hetero-resistance

Introduction

Fluoroquinolones (FQs) are the most important second-line anti-tuberculosis drugs. The World Health Organization (WHO) recommended moxifloxacin and levofloxacin as Group A agents for use in multidrug- or rifampicin-resistant tuberculosis (MDR/RR-TB) regimens because they can significantly reduce the risk of treatment failure or relapse and death in MDR/RR-TB (1). Moxifloxacin, a fourth-generation FQ, is currently being considered by the WHO for use in four-month regimens for drug-susceptible tuberculosis (TB) because of its excellent pharmacokinetics and drug-penetration into macrophages (2, 3). Thus, in addition to being the cornerstone of the regimens for MDR/RR-TB, FQs will be the key drugs in the shorter regimen for drug-susceptible TB in the future.

According to the technical specifications on TB prevention and control in China, FQs are still mainly used for the treatment of MDR/RR-TB. To date, few studies have investigated the prevalence of FQ resistance among rifampicin-susceptible TB, and the majority of these investigations indicate that FQ resistance in rifampicin-susceptible TB is uncommon (4, 5). However, FQs are a class of broad-spectrum antibiotics that differ from other anti-TB drugs, which are widely used to treat other bacterial infections, especially respiratory infections (6). In addition, several studies have shown that FQs have been extensively used in health facilities for the diagnostic treatment of patients with suspected TB and for the empirical treatment of TB patients without a drug susceptibility testing (DST) result (7, 8). The inappropriate use and the vital role of FQs in TB treatment bring our attention to the premature development of FQ resistance in rifampicin-susceptible *Mycobacterial tuberculosis* (MTB) isolates in TB high-burden settings.

Whole-genome sequencing (WGS) is promising to be an ideal tool for the surveillance of drug resistance in TB (9). Furthermore, WGS can offer information on hetero-resistance, resulting in more precise predictions of drug resistance phenotypes (10). Mutations in the *gyrA* and *gyrB* genes, which code for two subunits of DNA gyrase, have been identified as the main causes of FQ resistance in MTB (10, 11). The issue of hetero-resistance appears to be more frequent in FQ resistance (12, 13). Multiple large comparative studies have demonstrated that the accuracy of WGS in predicting phenotypic resistance to rifampicin, isoniazid, and FQs is high. The several databases of high-confidence resistance-conferring variants have also been developed (13–15).

In this study, we present the results from a WGS-based retrospective study of all MTB isolates from cases of pulmonary TB diagnosed in the Chongming district of Shanghai, China during 2009–2018. We aimed to determine the prevalence of FQ resistance, particularly among rifampicin-susceptible TB. Additionally, we identified the hetero-resistance in FQ resistance and quantified the FQ resistance due to the transmission of FQ-resistant strains.

Materials and methods

Study design and participants

This was a retrospective study that included all the culture-positive pulmonary TB patients who were reported by local designated hospitals in the Chongming district of Shanghai, China, between Jan 1, 2009, and Dec 31, 2018. According to the TB surveillance system in Shanghai, all local inhabitants aged 15 years or older with TB symptoms were referred to local TB designated hospitals for diagnosis, which involved the use of sputum smear and Lowenstein-Jensen (L-J) medium culture. All clinical isolates of pulmonary TB patients were submitted to Shanghai Municipal Center for Disease Control and Prevention (Shanghai CDC) TB reference laboratory for species identification and strain preservation. Demographic, clinical, and microbiological records were obtained from the national TB information management system.

WGS and bioinformatics analysis

Stored isolates were revived on L-J medium and inactivated in a water bath of 80°C in a biosafety laboratory. Genomic DNA was extracted and purified using the QIAamp DNA Mini Kit (Qiagen, Hilden, GER) and sequenced on the Hiseq 2500 platform (Illumina, San Diego, CA, USA) with an expected coverage of 100. Raw sequencing data was trimmed and filtered using fastp v0.23.1 (16). Paired-end reads were mapped to the reference genome H37Rv (GenBank NC_000962.3) with

BWA-MEM v0.7.17. Genetic variants, including SNPs and small insertions/deletions (indels), were called using SAMtools v1.6 and BCFtools v1.6 (17).

For WGS-based DST, WGS predictions of drug resistance phenotypes to drugs were based on a list of drug resistance-conferring mutations (13). The following 16 anti-TB drugs were tested: rifampicin, fluoroquinolones, isoniazid, ethambutol, pyrazinamide, streptomycin, ethionamide, amikacin, capreomycin, kanamycin, para-aminosalicylic acid, cycloserine, linezolid, bedaquiline, clofazimine, and delamanid. The allele frequency threshold of 10% was used to predict resistance. Hetero-resistance was defined based on the frequency of resistant alleles in the sequence reads <99% in this study.

To assess the transmission of FQ resistance, the fixed SNPs (frequency ≥ 75%), which were not in drug resistance-conferring mutations nor in PPE/PE-PGRS family genes (18), were used to calculate the pairwise SNP distances between FQ-resistant isolates. Clusters of isolates potentially consistent with recent transmission were identified using the genomic threshold of ≤ 12 SNPs (19). The strain lineages/sub-lineages were identified according to the SNP schemes previously established (20, 21).

A maximum likelihood (ML) phylogenetic tree of FQ-resistant isolates was inferred using RAxML-NG v1.0.2 (22) with the GTR+GAMMA model of nucleotide substitution and 100 bootstraps. The phylogenetic tree was visualized and annotated with iTol (https://itol.embl.de/).

Definitions of primary and acquired FQ resistance

Both primary and acquired FQ resistance were determined by the time of diagnosis, FQ resistance-conferring mutations, and genomic cluster. The primary FQ resistance (transmitted FQ resistance) was defined as the FQ resistance-conferring mutation shared by more than or equal to two strains in a genomic cluster with the removal of the FQ resistance-conferring mutation of the earliest onset strain among them; the remaining FQ resistance-conferring mutations were considered to be acquired FQ resistance.

Statistical analysis

All statistical analyses were done using IBM SPSS v.20 software. The Pearson's chi-squared or Fisher's exact test was used for comparison of categorical variables, such as demographic, bacteriological, and clinical characteristics. Ages were presented as the mean with a standard deviation. The Mann-Whitney *U*-test was used for the comparison of ages. A $P < 0.05$ was defined as significant.

Results

Characteristics of the patients and MTB isolates

Totally, 370 culture-positive pulmonary TB patients who were reported in the Chongming district between 2009 and 2018 were enrolled in this study. Of these patients, 25 (6.8%) were excluded from analysis due to strain contamination, recovery failure, or WGS failure. Among the remaining 345 patients, 283 (82%) were male, 297 (86.1%) were new TB cases, and the average age was 56 years (range 17 to 93 years). According to the results of WGS-based DST, 21 (6.1%) were diagnosed with MDR/RR-TB. MDR/RR-TB patients were more likely to have been previously treated for TB (47.6 vs. 11.7%; $p < 0{\cdot}0001$) than rifampicin-susceptible TB patients (Table 1).

Prevalence and mutation types of FQ resistance

Across the 345 isolates tested for WGS-based DST, 29 (8.4%) were resistant to FQ, including 20 rifampicin-susceptible isolates and 9 rifampicin-resistant isolates. The rate of FQ resistance was 6.2% (20/324) among rifampicin-susceptible TB and 42.9% (9/21) among MDR/RR-TB. The drug resistance profile and epidemiological information of 29 FQ-resistant TB patients were shown in the Supplementary Table S1.

On sequence analysis, nine rifampicin-resistant isolates harbored the FQ resistance-conferring mutations in the *gyrA* gene, including four *gyrA* D94A and five *gyrA* A90V. Seventeen rifampicin-susceptible isolates harbored diverse FQ resistance-conferring mutations, including nine *gyrA* D94G, four *gyrA* D94A, two *gyrA* D94Y, one *gyrA* A90V, and one *gyrA* S91P. In addition, three rifampicin-susceptible isolates harbored more than one FQ resistance-conferring mutation, including *gyrA* A90V+S91P+D94A, *gyrA* S91P+D94N, and *gyrA* D94G+*gyrB* T500N.

On allele frequency of drug resistance-conferring mutations, FQ hetero-resistance was only observed in the rifampicin-susceptible isolates. Among 20 rifampicin-susceptible isolates with FQ resistance-conferring mutations, 9 were identified as FQ hetero-resistance. The allele frequencies of FQ hetero-resistance ranged from 13.3 to 94.0%. Of the FQ hetero-resistant isolates, seven had a single unfixed mutation and two had multiple unfixed mutations in *gyrA*. The allele frequencies of FQ resistance-conferring mutations in 29 FQ-resistant isolates were shown in Figure 1B.

TABLE 1 Characteristics of MDR/RR-TB and rifampicin-susceptible TB patients in Chongming, Shanghai[a].

	MDR/RR-TB ($n = 21$)	Rifampicin-susceptible TB ($n = 324$)	p
Demographic factors			
Age (yr), mean ± SD	46 ± 20	57 ± 21	0.018*
Gender			0.391
Female	2 (9.5)	60 (18.5)	
Male	19 (90.5)	264 (81.5)	
Census register			1.000
Resident	19 (90.5)	285 (88.0)	
Migrant	2 (9.5)	39 (12.0)	
Clinical factors			
Case detection			0.106
Referral	16 (76.2)	186 (57.4)	
Clinical consultation	4 (19.0)	129 (39.8)	
Physical examination	1 (4.8)	9 (2.8)	
TB treatment history			<0.0001*
No	11 (52.4)	286 (88.3)	
Yes	10 (47.6)	38 (11.7)	
Pulmonary cavity			0.556
No	15 (71.4)	211 (65.1)	
Yes	6 (28.6)	113 (34.9)	
Positive sputum smear result			0.630
No	5 (23.8)	93 (8.7)	
Yes	16 (76.2)	231 (91.3)	
Bacteriological factors			
Genomic clustered			0.249
No	13 (93.1)	238 (73.5)	
Yes	8 (6.9)	86 (26.5)	
lineage			0.201
Non-Beijing	2 (9.5)	45 (13.9)	
Ancient Beijing	8 (38.1)	67 (20.7)	
Modern Beijing	11 (52.4)	212 (65.4)	

[a]Data are *n* (%); MDR, multidrug resistant; RR, rifampicin resistant; TB, tuberculosis.
*$P < 0.05$ is statistically significant.

Transmission of FQ-resistant isolates

A total of 11 FQ-resistant isolates were grouped into 5 genomic clusters defined as strains that differed by 12 or fewer SNPs, among which 7 were rifampicin-susceptible isolates and 4 were rifampicin-resistant isolates. Drug resistance mainly emerges in two ways: acquired drug resistance due to inadequate therapy, or primary drug resistance caused by the transmission of drug-resistant strains. Among the 11 FQ-resistant clustered isolates, all the paired strains carried the identical FQ resistance-conferring mutations except for one, which carried three unfixed mutations. Notably, we were able to observe the frequency dynamics of FQ resistance-conferring mutations in this recent transmission cluster (patient 12-0252 and patient 13-0580). Patient 12-0252 was diagnosed in 2012 with unfixed FQ resistance-conferring mutations (*gyrA* 50% A90V + 23.2% S91P + 13.5% D94A); the *gyrA* A90V mutation became fixed during the subsequent infection of patient 13-0580 (Figure 1). By comparing the transmission and resistance-conferring mutations of FQ-resistant isolates, we found that the FQ resistance of 20% (4/20) of rifampicin-susceptible isolates and 22.2% (2/9) of rifampicin-resistant isolates resulted from the transmission of FQ-resistant strains.

Discussion

The WGS of MTB is rapidly developing from being a scientific research tool to a drug resistance and epidemiological surveillance tool of TB for public health protection (9, 23). In the current study, we used WGS to retrospectively investigate the prevalence of FQ resistance and its transmission in all MTB isolates from pulmonary TB patients diagnosed in Chongming, Shanghai for 10 years. Our results showed that rifampicin-susceptible TB accounted for most (69%) of the cases of FQ-resistant TB overall. The prevalence of FQ resistance among rifampicin-susceptible TB was 6.1%, a high proportion (45%) of which was identified as hetero-resistance. By WGS analysis, the transmission of FQ-resistant strains resulted in 20% of FQ resistance in rifampicin-susceptible TB.

In our study population, more than two-thirds of the FQ resistance was detected in rifampicin-susceptible TB patients. It is well known that FQs are the core agents for MDR/RR-TB treatment. In China, moxifloxacin and levofloxacin are usually prescribed only to MDR/RR-TB patients. This prescription practice aligns with the technical specifications on TB prevention and control. As a result, FQ DST is not routinely performed on rifampicin-susceptible TB patients, and there are few surveillance data on the prevalence of FQ resistance in rifampicin-susceptible TB. The present study found that among rifampicin-susceptible TB patients in Shanghai, the prevalence of FQ resistance in MTB clinical isolates was higher (6.1%) than that reported in other countries, ranging from 0 to 4.4% (4, 5, 24, 25). China is one of the high TB burden countries, with a large number of active TB cases and latent TB infections. In addition, FQs are the most commonly prescribed antibiotics for respiratory infections in Shanghai (26). There is therefore a need to conduct surveillance of FQ resistance and FQ exposure in newly diagnosed TB patients in high TB burden countries.

Previous studies have shown that *gyrA* D94G and A90V are the most prevalent FQ resistance-conferring mutations, which have been reported to confer higher-level FQ resistance or lower fitness cost *in vitro* (27–32). All of the FQ resistance-conferring mutations occurring in rifampicin-resistant MTB in this study were D94G or A90V, suggesting that MTB carrying

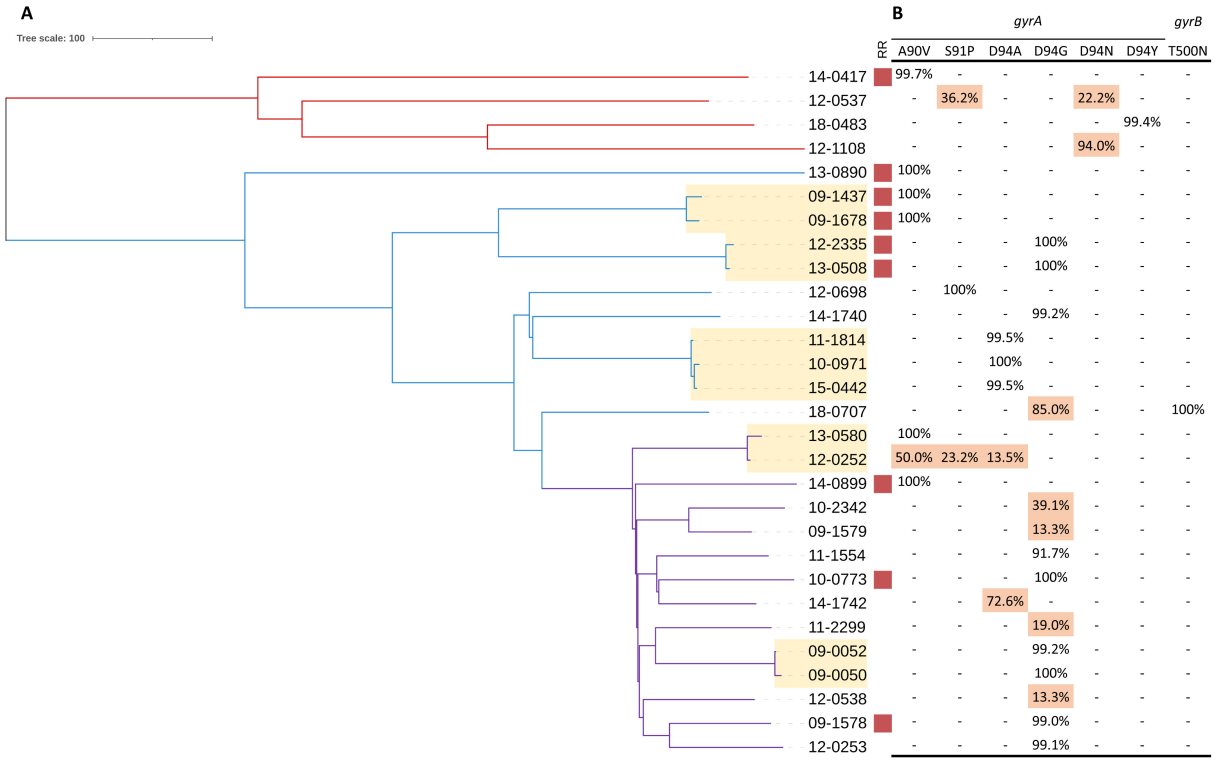

FIGURE 1
Phylogeny, clustering and FQ hetero-resistance profile of 29 FQ-resistant MTB isolates. **(A)** Red, blue and purple branches indicate Lineage 4, ancient Beijing and modern Beijing strains, respectively. Genomic-clustered strains differing by ≤ 12 SNPs are highlighted in yellow. Red square indicates the MTB isolate resistant to rifampicin. **(B)** Allele frequency of FQ resistance-conferring loci in *gyrA* and *gyrB*. Hetero-resistance mutations are highlighted in red.

multiple other drug resistance-conferring mutations might be more likely to acquire FQ resistance-conferring mutations with a lower fitness cost. The patterns of FQ resistance-conferring mutations occurring in rifampicin-susceptible MTB showed more diversity. All the FQ hetero-resistance was detected in rifampicin-susceptible MTB. Hetero-resistance is a crucial phase in the progression of an originally drug-susceptible MTB population becoming completely drug-resistant to a given drug during the course of an infection (32). Non-lethal drug concentration facilitates the emergence of drug mutations and the selection of mutations with a low fitness cost (33, 34). Based on these hypotheses, the features of the FQ resistance-conferring mutation in rifampicin-susceptible MTB imply that the FQ resistance in rifampicin-susceptible MTB might be induced by inefficient FQ therapy. Several studies have demonstrated that the proportion of TB patients exposed to FQs before TB diagnosis is high, due to the easy access and inappropriate use of FQs (7, 8, 35–37). Devasia et al. reported that more than 10 days of FQ exposure prior to TB diagnosis is a primary risk factor for FQ resistance (38). Meanwhile, MTB rapidly acquires FQ resistance during moxifloxacin monotherapy (39). In recent years, with the use of large-scale genotype-phenotype analyses, substantial improvements have been made in the correlation of genotype with resistance phenotype (9, 15). However, hetero-resistance limits the ability to detect drug resistance of rapid molecular assays (12). The variant allele frequencies provided by WGS could be used to identify hetero-resistance for better predictions of drug resistance phenotypes (10, 11). FQ hetero-resistance was found in nine rifampicin-susceptible MTB isolates in our investigation, with hetero-resistance frequencies ranging from 13.3 to 94.0% as identified by WGS. These findings have two important implications. First, WGS makes the detection of FQ resistance more sensitive, especially in hetero-resistant strains. Second, the real burden of FQ resistance in rifampicin-susceptible TB might be underestimated.

Transmission of drug-resistant strains is a major driver of the high prevalence of drug-resistant TB in China (19, 40). In this study, over 20% of FQ resistance was regarded as primary drug resistance caused by the transmission of FQ-resistant strains. The rates of primary drug resistance of FQ in rifampicin-susceptible and rifampicin-resistant MTB isolates were similar. Due to the massive migrant population in Shanghai and inadequate therapy, almost one-third of MDR-TB cases were attributed to recent transmission (19). Transmission of rifampicin-resistant strains also facilitated the spread of FQ resistance in this study. One unanticipated

finding was that a similar proportion of FQ resistance caused by transmission was observed in rifampicin-susceptible MTB isolates. A meta-analysis of nine studies concluded that empirical FQ prescriptions for respiratory infections are linked to delays in pulmonary TB diagnosis and treatment (35), and such delays might contribute to the transmission of TB (41). Intriguingly, our results displayed the transition of multiple unfixed FQ resistance-conferring mutations to a single fixed mutation during transmission, which suggests that *gyrA* A90V is likely a FQ resistance-conferring mutation with a low fitness cost *in vivo*.

Our study was limited by the retrospective study design so that the TB patients' information on FQ prescriptions prior to diagnosis, such as date, dosage, type of FQ, and days of supply, could not be obtained in this study. A prospective cohort study is warranted to further understand the source of FQ resistance in rifampicin-susceptible TB.

In summary, the prevalence of FQ resistance among rifampicin-susceptible TB was 6.1% in Shanghai, which was more than expected. The results of WGS analysis showed that half of the FQ resistance in rifampicin-susceptible TB was hetero-resistance and that the transmission of FQ-resistant strains also contributed to the emergence of FQ-resistant TB. Therefore, the WGS-based surveillance system for TB should be urgently established and the clinical awareness of the rational use of FQs for respiratory infections should be enhanced to prevent the premature occurrence of FQ resistance.

Data availability statement

The datasets presented in this study can be found online at: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA760838.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Shanghai Municipal Center for Disease Control and Prevention (No. 2020-14). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YZ and XiS designed the study, drafted, and revised the manuscript. CY, JL, and XuS did the laboratory work. YZ did the data analyses. YJ, QP, and XiS supervised the project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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References

1. WHO. *WHO Consolidated Guidelines on Tuberculosis, Module 4: Treatment - Drug-Resistant Tuberculosis Treatment*. Geneva: World Health Organization. (2020).

2. Dorman SE, Nahid P, Kurbatova EV, Phillips PPJ, Bryant K, Dooley KE, et al. Four-month rifapentine regimens with or without moxifloxacin for tuberculosis. *N Engl J Med.* (2021) 384:1705–18. doi: 10.1056/NEJMoa2033400

3. WHO. *Treatment of Drug-Susceptible Tuberculosis: Rapid Communication.* Geneva: World Health Organization. (2021).

4. Ho J, Jelfs P, Sintchenko V. Fluoroquinolone resistance in non-multidrug-resistant tuberculosis-a surveillance study in New South Wales, Australia, and a review of global resistance rates. *Int J Infect Dis.* (2014) 26:149–53. doi: 10.1016/j.ijid.2014.03.1388

5. Schwalb A, Cachay R, Meza E, Cáceres T, Blackman A, Maruri F, et al. Fluoroquinolone Susceptibility in First-Line Drug-Susceptible M. Tuberculosis Isolates in Lima, Peru. *BMC Res Notes.* (2021) 14:413. doi: 10.1186/s13104-021-05832-0

6. Andriole VT. The quinolones: past, present, and future. *Clin Infect Dis.* (2005) 41 Suppl 2:S113–9. doi: 10.1086/428051

7. Wang JY, Hsueh PR, Jan IS, Lee LN, Liaw YS, Yang PC, et al. Empirical treatment with a fluoroquinolone delays the treatment for tuberculosis and is associated with a poor prognosis in endemic areas. *Thorax.* (2006) 61:903–8. doi: 10.1136/thx.2005.056887

8. Hu Y, Mathema B, Wang W, Kreiswirth B, Jiang W, Xu B. Population-based investigation of fluoroquinolones resistant tuberculosis in rural eastern China. *Tuberculosis (Edinb).* (2011) 91:238–43. doi: 10.1016/j.tube.2011.03.001

9. Meehan CJ, Goig GA, Kohl TA, Verboven L, Dippenaar A, Ezewudo M, et al. Whole genome sequencing of mycobacterium tuberculosis: current standards and open issues. *Nat Rev Microbiol.* (2019) 17:533–45. doi: 10.1038/s41579-019-0214-5

10. Maruri F, Guo Y, Blackman A, van der Heijden YF, Rebeiro PF, Sterling TR. Resistance-conferring mutations on whole-genome sequencing of fluoroquinolone-resistant and -susceptible mycobacterium tuberculosis isolates: a proposed threshold for identifying resistance. *Clin Infect Dis.* (2021) 72:1910–8. doi: 10.1093/cid/ciaa496

11. Farhat MR, Jacobson KR, Franke MF, Kaur D, Murray M, Mitnick CD. Fluoroquinolone resistance mutation detection is equivalent to culture-based drug sensitivity testing for predicting multidrug-resistant tuberculosis treatment outcome: a retrospective cohort study. *Clin Infect Dis.* (2017) 65:1364–70. doi: 10.1093/cid/cix556

12. Eilertson B, Maruri F, Blackman A, Herrera M, Samuels DC, Sterling TR. High Proportion of Heteroresistance in Gyra and Gyrb in Fluoroquinolone-Resistant Mycobacterium Tuberculosis Clinical Isolates. *Antimicrob Agents Chemother.* (2014) 58:3270–5. doi: 10.1128/AAC.02066-13

13. Phelan JE, O'Sullivan DM, Machado D, Ramos J, Oppong YEA, Campino S, et al. Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs. *Genome Med.* (2019) 11:41. doi: 10.1186/s13073-019-0650-x

14. Walker TM, Kohl TA, Omar SV, Hedge J, Del Ojo Elias C, Bradley P, et al. Whole-genome sequencing for prediction of mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. *Lancet Infect Dis.* (2015) 15:1193–202. doi: 10.1016/S1473-3099(15)00062-6

15. Gröschel MI, Owens M, Freschi L, Vargas R Jr, Marin MG, Phelan J, et al. Gentb: a user-friendly genome-based predictor for tuberculosis resistance powered by machine learning. *Genome Med.* (2021) 13:138. doi: 10.1186/s13073-021-00953-4

16. Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one fastq preprocessor. *Bioinformatics.* (2018) 34:i884–i90. doi: 10.1093/bioinformatics/bty560

17. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve Years of Samtools and Bcftools. *GigaScience.* (2021) 10:giab008. doi: 10.1093/gigascience/giab008

18. Casali N, Broda A, Harris SR, Parkhill J, Brown T, Drobniewski F. Whole genome sequence analysis of a large isoniazid-resistant tuberculosis outbreak in london: a retrospective observational study. *PLoS Med.* (2016) 13:e1002137. doi: 10.1371/journal.pmed.1002137

19. Yang C, Luo T, Shen X, Wu J, Gan M, Xu P, et al. Transmission of multidrug-resistant mycobacterium tuberculosis in Shanghai, China: a retrospective observational study using whole-genome sequencing and epidemiological investigation. *Lancet Infect Dis.* (2017) 17:275–84. doi: 10.1016/S1473-3099(16)30418-2

20. Coll F, McNerney R, Guerra-Assunção JA, Glynn JR, Perdigão J, Viveiros M, et al. A robust Snp barcode for typing mycobacterium tuberculosis complex strains. *Nat Commun.* (2014) 5:4812. doi: 10.1038/ncomms5812

21. Luo T, Comas I, Luo D, Lu B, Wu J, Wei L, et al. Southern East Asian origin and coexpansion of mycobacterium tuberculosis Beijing family with han Chinese. *Proc Natl Acad Sci U S A.* (2015) 112:8136–41. doi: 10.1073/pnas.1424063112

22. Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. Raxml-Ng: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics.* (2019) 35:4453–5. doi: 10.1093/bioinformatics/btz305

23. Tagliani E, Anthony R, Kohl TA, de Neeling A, Nikolayevskyy V, Ködmön C, et al. Use of a whole genome sequencing-based approach for mycobacterium tuberculosis surveillance in Europe in 2017-2019: an ecdc pilot study. *Eur Respir J.* (2021) 57:2002272. doi: 10.1183/13993003.02272-2020

24. Ginsburg AS, Hooper N, Parrish N, Dooley KE, Dorman SE, Booth J, et al. Fluoroquinolone resistance in patients with newly diagnosed tuberculosis. *Clin Infect Dis.* (2003) 37:1448–52. doi: 10.1086/379328

25. Aydin Kayali R, Özkan SA, Biçmen C, Erer OF. The relation between the emergence of fluoroquinolone resistance and fluoroquinolone exposure in new cases of active pulmonary tuberculosis. *Turk Thorac J.* (2021) 22:45–9. doi: 10.5152/TurkThoracJ.2021.19128

26. Lin H, Dyar OJ, Rosales-Klintz S, Zhang J, Tomson G, Hao M, et al. Trends and patterns of antibiotic consumption in shanghai municipality, China: a 6 year surveillance with sales records, 2009-14. *J Antimicrob Chemother.* (2016) 71:1723–9. doi: 10.1093/jac/dkw013

27. Hu Y, Hoffner S, Wu L, Zhao Q, Jiang W, Xu B. Prevalence and genetic characterization of second-line drug-resistant and extensively drug-resistant mycobacterium tuberculosis in rural China. *Antimicrob Agents Chemother.* (2013) 57:3857–63. doi: 10.1128/AAC.00102-13

28. Li J, Gao X, Luo T, Wu J, Sun G, Liu Q, et al. Association of Gyra/B mutations and resistance levels to fluoroquinolones in clinical isolates of mycobacterium tuberculosis. *Emerg Microbes Infect.* (2014) 3:e19. doi: 10.1038/emi.2014.21

29. Avalos E, Catanzaro D, Catanzaro A, Ganiats T, Brodine S, Alcaraz J, et al. Frequency and geographic distribution of gyra and gyrb mutations associated with fluoroquinolone resistance in clinical mycobacterium tuberculosis isolates: a systematic review. *PLoS ONE.* (2015) 10:e0120470. doi: 10.1371/journal.pone.0120470

30. Aldred KJ, Blower TR, Kerns RJ, Berger JM, Osheroff N. Fluoroquinolone Interactions with mycobacterium tuberculosis gyrase: enhancing drug activity against wild-type and resistant gyrase. *Proc Natl Acad Sci U S A.* (2016) 113:E839–46. doi: 10.1073/pnas.1525055113

31. Huseby DL, Pietsch F, Brandis G, Garoff L, Tegehall A, Hughes D. Mutation supply and relative fitness shape the genotypes of ciprofloxacin-resistant Escherichia Coli. *Mol Biol Evol.* (2017) 34:1029–39. doi: 10.1093/molbev/msx052

32. Castro RAD, Ross A, Kamwela L, Reinhard M, Loiseau C, Feldmann J, et al. The genetic background modulates the evolution of fluoroquinolone-resistance in mycobacterium tuberculosis. *Mol Biol Evol.* (2020) 37:195–207. doi: 10.1093/molbev/msz214

33. Andersson DI, Hughes D. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resist Updat.* (2012) 15:162–72. doi: 10.1016/j.drup.2012.03.005

34. Dheda K, Lenders L, Magombedze G, Srivastava S, Raj P, Arning E, et al. Drug-penetration gradients associated with acquired drug resistance in patients with tuberculosis. *Am J Respir Crit Care Med.* (2018) 198:1208–19. doi: 10.1164/rccm.201711-2333OC

35. Chen TC, Lu PL, Lin CY, Lin WR, Chen YH. Fluoroquinolones are associated with delayed treatment and resistance in tuberculosis: a systematic review and meta-analysis. *Int J Infect Dis.* (2011) 15:e211–6. doi: 10.1016/j.ijid.2010.11.008

36. Migliori GB, Langendam MW, D'Ambrosio L, Centis R, Blasi F, Huitric E, et al. Protecting the tuberculosis drug pipeline: stating the case for the rational use of fluoroquinolones. *Eur Respir J.* (2012) 40:814–22. doi: 10.1183/09031936.00036812

37. Hicks LA, Bartoces MG, Roberts RM, Suda KJ, Hunkler RJ, Taylor TH Jr, et al. Us outpatient antibiotic prescribing variation according to geography, patient population, and provider specialty in 2011. *Clin Infect Dis.* (2015) 60:1308–16. doi: 10.1093/cid/civ076

38. Devasia RA, Blackman A, Gebretsadik T, Griffin M, Shintani A, May C, et al. Fluoroquinolone resistance in mycobacterium tuberculosis: the effect of duration and timing of fluoroquinolone exposure. *Am J Respir Crit Care Med.* (2009) 180:365–70. doi: 10.1164/rccm.200901-0146OC

39. Ginsburg AS, Sun R, Calamita H, Scott CP, Bishai WR, Grosset JH. Emergence of fluoroquinolone resistance in mycobacterium tuberculosis during continuously dosed moxifloxacin monotherapy in a mouse model. *Antimicrob Agents Chemother.* (2005) 49:3977–9. doi: 10.1128/AAC.49.9.3977-3979.2005

40. Yang C, Shen X, Peng Y, Lan R, Zhao Y, Long B, et al. Transmission of mycobacterium tuberculosis in China: a population-based molecular epidemiologic study. *Clin Infect Dis.* (2015) 61:219–27. doi: 10.1093/cid/civ255

41. Yuen CM, Amanullah F, Dharmadhikari A, Nardell EA, Seddon JA, Vasilyeva I, et al. Turning off the tap: stopping tuberculosis transmission through active case-finding and prompt effective treatment. *Lancet.* (2015) 386:2334–43. doi: 10.1016/S0140-6736(15)00322-0

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Molecular epidemiology of tuberculosis in the Somali region, eastern Ethiopia

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Background: Tuberculosis (TB) is one of the leading causes of morbidity and mortality in low-income countries like Ethiopia. However, because of the limited laboratory infrastructure there is a shortage of comprehensive data on the genotypes of clinical isolates of *Mycobacterium tuberculosis* (*M. tuberculosis*) complex (MTBC) in peripheral regions of Ethiopia. The objective of this study was to characterize MTBC isolates in the Somali region of eastern Ethiopia.

Methods: A cross-sectional study was conducted in three health institutions between October 2018 and December 2019 in the capital of Somali region. A total of 323 MTBC isolates (249 from pulmonary TB and 74 from extrapulmonary TB) were analyzed using regions of difference 9 (RD 9)-based polymerase chain reaction (PCR) and spoligotyping.

Results: Of the 323 MTBC isolates, 99.7% (95% CI: 99.1–100%) were *M. tuberculosis* while the remaining one isolate was *M. bovis* based on RD 9-based PCR. Spoligotyping identified 71 spoligotype patterns; 61 shared types and 10 orphans. A majority of the isolates were grouped in shared types while the remaining grouped in orphans. The *M. tuberculosis* lineages identified in this study were lineage 1, 2, 3, 4, and 7 with the percentages of 7.4, 2.2, 28.2, 60.4, and 0.6%, respectively. Most (87.9%) of the isolates were classified in clustered spoligotypes while the remaining 12.1% isolates were singletons. The predominant clustered spoligotypes identified were SIT 149, SIT 21, SIT 26, SIT 53, and SIT 52, each consisting of 17.6, 13.3, 8.4, 7.4, and 5%, respectively. Lineage 3 and lineage 4, as well as the age group (15–24), were associated significantly with clustering.

Conclusion: The MTBC isolated from TB patients in Somali region were highly diverse, with considerable spoligotype clustering which suggests active TB transmission. In addition, the Beijing spoligotype was isolated in relatively higher frequency than the frequencies of its isolation from the other regions

of Ethiopia warranting the attention of the TB Control Program of the Somali region.

KEYWORDS

molecular epidemiology, tuberculosis, spoligotyping, eastern Ethiopia, *M. tuberculosis* complex

Introduction

Tuberculosis (TB) is a bacterial infection caused by the *M. tuberculosis* complex (MTBC) and affects any part of the human body, although it most commonly affects the lungs. It is transmitted through inhalation and is a major cause of morbidity and one of the top causes of mortality worldwide. Until the coronavirus (COVID-19) pandemic, TB was the leading cause of mortality caused by a single infectious agent, surpassing HIV/AIDS. According to the latest report, WHO estimated 9.9 million cases of TB and 1.5 million deaths in 2020, and an additional 214,000 deaths resulting from TB disease among people living with HIV (1).

Genotyping approaches for *M. tuberculosis* have proved to be valuable in acquiring a better understanding of TB epidemiology, which is important for effective TB control strategies (2), such as, detecting distinct strains that spread in epidemics (3), identifying recurring TB attributable to external reinfection or relapse (4), and detecting laboratory cross-contamination (5). Furthermore, the establishment of a phylogenetic framework for *M. tuberculosis* due to variances in their genetic makeup has allowed researchers to investigate the public health consequences of different genotypes of MTBC such as transmission rate, drug resistance development, immunological responses, and disease severity (6).

In Ethiopia TB remains one of the leading public health concerns claiming the lives of thousands of Ethiopians every year. Ethiopia is among 30 countries with a high TB burden and 30 countries with a high TB/HIV burden throughout the world and has recently been removed from the WHO's list of 30 countries with a high multidrug resistant (MDR) TB burden (1). Previous molecular epidemiology studies in different regions of Ethiopia revealed that lineage 4 and lineage 3 were predominant, whereas lineage 1 and lineage 2 were the least common. Lineage 7 (Ethiopian) appeared to be geographically restricted to northern Ethiopia. The most prevalent clades/families found in the country were T, CAS, H, Manu, and Ethiopian, with Shared International Type (SIT) 149, SIT 53, SIT 25, SIT 37, and SIT 21 being the most common SITs (7).

TB has long been a focus of epidemiological studies in Ethiopia. Molecular genotyping approaches are currently being used in such studies to identify mycobacteria species, monitor recent TB transmission, and assess genotype diversity. However, our understanding of TB disease dynamics was limited due to a lack of comprehensive molecular epidemiological data from Ethiopia's peripheral regions, such as the Somali region. Furthermore, because of the inability to distinguish *M. bovis* from *M. tuberculosis* based on routine diagnosis, there is a lack of information regarding the relative contribution of zoonotic TB in pastoral settings, where people live in an environment that allows direct contact with animals or animal products. Therefore, the objective of this study is to investigate the molecular epidemiology of TB in the Somali region of eastern Ethiopia.

Methodology

Study area and setting

The study was carried out in health facilities in the Jigjiga city, the capital of the Somali region that is located at 626 km east of Addis Ababa, Ethiopia's capital. The Somali region shares borders with Somaliland, Somalia, Djibouti, and Kenya, as well as local borders with Oromia and Afar regions. More than 83% of the population lives in rural areas, mainly with pastoral or agropastoral livelihood, with livestock serving as the primary source of income. People in the Somali region and those in neighboring countries are ethnically, linguistically, and religiously similar and cross-border movement is common (8, 9). In the Somali region, long-running unrest and insecurity severely impeded the government's capacity to offer basic social services to rural populations (10).

TB patients were recruited from Abilelie Health Center, Karamara Regional Hospital, and Jigjiga University Sheik Hassan Yabare Referral Hospital; all of which are located in Jigjiga City (Figure 1). These hospitals were chosen as they represent the major TB diagnosis and treatment centers in the Region.

Study design and study subjects

A health institution based, cross-sectional study was conducted on 249 pulmonary and 74 extra-pulmonary TB patients visiting selected health facilities of Jigjiga, Somali region, between October 2018 and December 2019. The study subjects consisted of all consecutive, consenting, bacteriologically

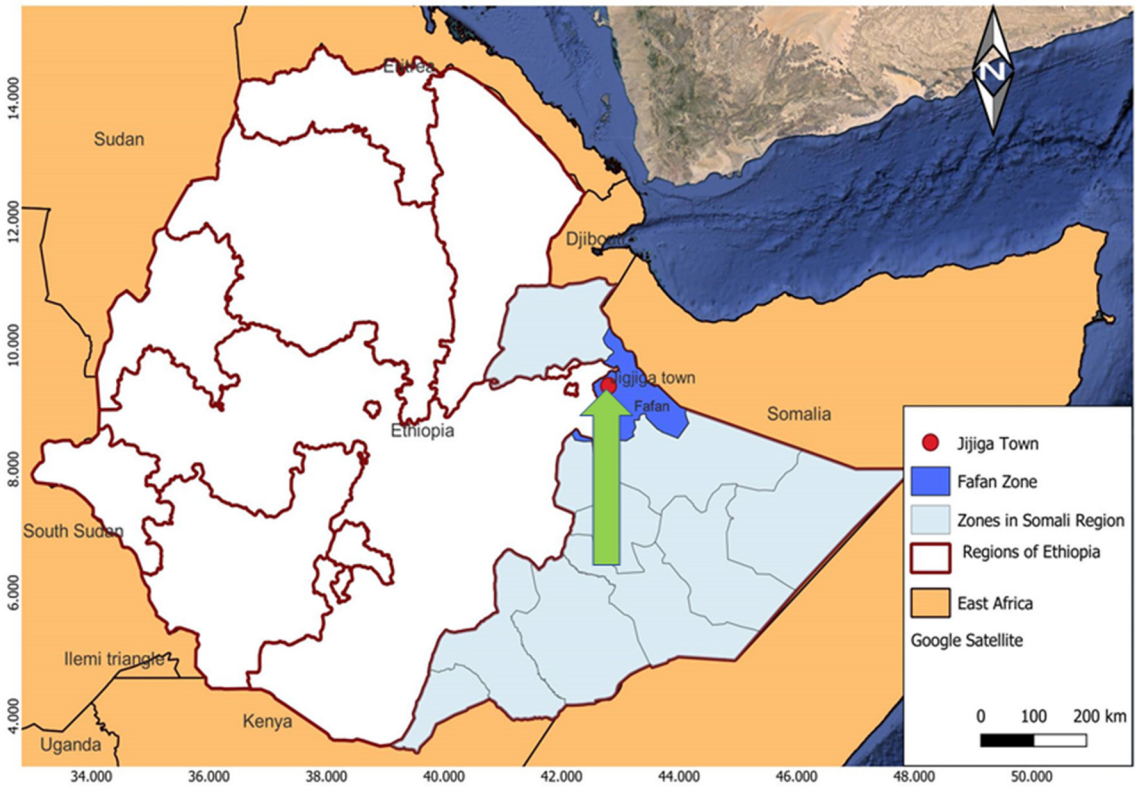

FIGURE 1
Map of the study area. The arrow indicates the City of Jigjiga that is indicated with a red filled circle. The patients were recruited from three health care provider clinics and hospitals, which are located in the Jigjiga City, namely the Abilelie Health center, Karamara Regional hospital, and Jigjiga University Sheik Hassan Yabare Referral Hospital.

confirmed pulmonary TB patients and extra-pulmonary TB patients aged ≥15 years who visited the selected public health institutions. Patients under the age of 15 and those who were unable to produce sputum were excluded from the study.

Sample collection and mycobacterial culture

Laboratory professionals collected sputum samples from each study participant, as per procedure indicated by WHO (11). If the samples tested positive for acid fast bacilli (AFB) using the Ziehl-Neelsen (ZN) staining method or Xpert MTB/RIF, the remaining portion were kept at −20°C at the sample collection site until transported to Aklilu Lemma Institute of Pathobiology (ALIPB) Addis Ababa University (AAU) TB laboratory. Fine Needle Aspiration (FNA) specimens were collected and evaluated by a pathologist. The first few drops of the aspirates were used for cytological diagnosis, and the rest were preserved at the sample collection site in sterilized phosphate buffer saline in the refrigerator at −20°C. All sputum specimens and FNA were transported in a packed ice box at +4°C to ALIPB TB laboratory, AAU for culture. At each health institute, qualified nurses and laboratory technologists filled out questionnaires to collect patient variables including socio-demographic factors.

The culturing of samples was conducted by following Petroff procedure at ALIPB, AAU (12). The specimens were decontaminated with a final maximum NaOH concentration of up to 2% by centrifuging at 3,000 rpm for 15 min with an equal amount of 4%t NaOH stock solution and sample. The sediment was neutralized with 2NHCl after the supernatant was discarded. The sediment was then inoculated into two conventional Löwenstein–Jensen (LJ) egg slant media, one of which contained 0.6% sodium pyruvate and the other 0.75% glycerol, and cultured for at least 8 weeks with weekly mycobacterial colony observation.

Molecular identification

DNA was extracted from the mycobacteria grown on LJ media. The bacterial colony from the LJ medium growth was taken and mixed with 200 μl of sterile distilled filtered water.

In a water bath, the bacteria and water mixture was heated to 80°C for 1 h (13).

RD9 typing was used to distinguish *M. tuberculosis* species from other members of the MTBC. To determine the presence or absence of RD9 deletion, PCR was performed using three primers: RD9flankF, RD9IntR, and RD9flankR. To identify isolates with deleted RD4, three RD4 primers were used: RD4flankF, RD4IntR, and RD4flankR. The gel was viewed using a Multi-Image Light Cabinet and evaluated based on molecular weight differences; *M. tuberculosis* was recognized by 396-bp intact RD9, whereas other MTBC was recognized by 575-bp deleted RD9. *M. bovis* can be distinguished from other MTBC members by deleted RD4 (446-bp), whereas the other MTBC members have intact RD4 (335-bp) (14).

Spoligotyping was used to characterize all isolates, as described previously by Kamerbeek, following the manufacturer's instructions (Mapmygenome, India) (15). Briefly, DR region was amplified with primers DRa (biotinylated at the 5 "end) and DRb, by Thermal Cycler PCR machine. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the direct repeat locus. Hybridizing DNA was detected by the enhanced chemiluminescence method and by exposure to X-ray film as specified by the manufacturer. The RD4 and RD9 typing was performed at ALIPB, AAU whereas spoligotyping was performed at Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia.

Quality control

The sterility of the all culture media was assessed by incubating for 48 h at 37 °C. For RD 9 based PCR and spoligotyping, positive and negative controls were present in each run. H37Rv and BCG strains were used as positive controls, and sterile molecular grade water was used as a negative control. Throughout the study period, a logbook was used to record every laboratory result. The collected data was checked for completeness, accuracy, and clarity before being analyzed and interpreted.

Identification of spoligotype patterns using SITVIT2 databases

The spoligotyping patterns were changed to binary and octal forms and submitted into an online spoligotype database to identify the SIT number, and the findings were compared to previously designated SIT numbers in the international spoligotyping database (SITVIT2 database) (Pasteur Institute of Guadeloupe), an upgraded version of the previously provided SITVITWEB database (http://www.pasteur-guadeloupe.fr:8081/SITVIT2/) (16). The "TBinsight" database (http://tbinsight.cs.rpi.edu/index.html) was used to designate major lineages. The international database www.mbovis.org was used to compare spoligotype patterns of *M. bovis* isolates.

Data analysis

All genotyping outputs from the computer analysis were imported into the SPSS 20 computer program and combined with socio-demographic variables. In this study, "clustered" meant two or more isolates with identical spoligotyping patterns, whereas "unique" meant isolates with no common patterns. To demonstrate the socio-demographic variables, descriptive statistics were used. Fisher's exact test was used to analyze the relationship between types of TB and the MTBC clades. The relationships between clustering and associated factors were analyzed using logistic regression analysis. The statistical test was considered significant at $p < 0.05$.

Ethical clearance

The study was approved by the ALIPB's Institutional Review Board (IRB), AAU Ref No. ALIPB/IRB/002/2017/18.Permission was obtained from the Somali region Health Bureau as well as each study site. Before the samples were collected, the patients were informed about the study and written consent to participate was obtained.

Results

Demographic characteristics of the study participants

Among the 323 culture positive TB patients included in this study, 64.4% (208/323) were male, 59.4% (192/323) were urban residents, and 77.1% (249/323) were pulmonary TB patients. Among culture positive extrapulmonary specimens, 95.9% (71/74) were collected from lymph nodes [with cervical lymph node 91.5% (65/71)], 4.0% (3/74) from skin lesion, and 1.3% (1/74) from breast abscess. The median age of the patients was 28 years (range 15–80 years) and 82% (265/323) were in the age group of 15–44 years (Table 1).

Identification MTBC species and spoligotype patterns

Mycobacterial growth was observed and confirmed by acid fast staining in 323 specimens. The RD9-based PCR analysis showed that 99.7% (322/323, CI: 99.1%-100%) of the isolates

TABLE 1 Factors associated with clustering of *M. tuberculosis* complex in Somali, Ethiopia.

		Clustered	Unique	AOR (95% CI)	*p*-value
Sex	Female	99 (86.1%)	16 (13.9%)	0.90 (0.41–1.95)	0.781
	Male	185 (88.9%)	23 (11.1%)	1	
Residence	Rural	112 (85.5%)	19 (14.5%)	0.73 (0.34–1.60)	0.436
	Urban	172 (89.6%)	20 (10.4%)	1	
Types of TB	Pulmonary TB	224 (90.0%)	25 (10.0%)	2.02 (0.81–4.99)	0.130
	Extra-pulmonary TB	60 (81.1%)	14 (18.9%)	1	
Lineage	Lineage 1	14 (58.3%)	10 (41.7%)	1	
	Lineage 3	88 (95.7%)	4 (4.3%)	15.26 (4.06–57.38)	**0.00006***
	Lineage 4	174 (88.8%)	22 (11.2%)	5.41 (2.00–14.60)	**0.001***
	Others	8 (72.7%)	3 (27.3%)	2.30 (0.43–12.24)	0.329
Age group	15–24	114 (91.2%)	11 (8.8%)	3.09 (1.13–8.41)	0.03
	25–34	74 (85.1%)	13 (14.9%)	1.47 (0.56–3.84)	0.43
	35–44	49 (92.5%)	4 (7.5%)	2.57 (0.72–9.16)	0.15
	≥45	47 (81%)	11 (19%)	1	

AOR, Adjusted odd ratio; CI, confidence interval. * statistically significant.

TABLE 2 Distribution of spoligotype clades between pulmonary and extrapulmonary TB patients in Somali region, Ethiopia.

	Pulmonary TB		Extrapulmonary TB		Total	
Clades	N (%)	95% CI	N (%)	95% CI	N (%)	95% CI
ATYPIC	0	0	1 (1.4)	0–4.1	1 (0.3)	0–0.9
Beijing	3 (1.2)	0–2.8	3 (4.1)	0–9.5	6 (1.9)	0.6–3.4
BOV_1	0	0	1 (1.4)	0–4.1	1 (0.3)	0–0.9
CAS	73 (29.3)	23.7–34.9	18 (24.3)	16.2–35.1	91 (28.2)	23.2–33.1
EAI	16 (6.4)	3.6–9.6	6 (8.1)	2.7–14.9	22 (6.8)	4.3–9.9
Ethiopian	0	0	2 (2.7)	0–6.8	2 (0.6)	0–1.5
Haarlem	23 (9.2)	5.6–13.3	5 (6.8)	1.3–13.8	28 (8.7)	6.2–11.8
LAM	15 (6)	3.2–9.2	5 (6.8)	1.3–13.8	20 (6.2)	3.7–9
MANU	2 (0.8)	0–2	0	0	2 (0.6)	0–1.5
S	1 (0.4)	0–1.2	0	0	1 (0.3)	0–0.9
T	105 (42.2)	36.1–47.8	29 (39.2)	28.4–50	134 (41.5)	36.2–46.7
Ural-1	0		1 (1.4)	0–4.1	1 (0.3)	0–0.9
X	3 (1.2)	0–2.8	0	0	3 (0.9)	0–2.2
Not defined	2 (0.8)	0–2	2 (2.7)	0–6.8	2 (0.6)	0–1.5
Unknown	6 (2.4)	0.8–4.4	1 (1.4)	0–4.1	7 (2.2)	0.6–4
Total	249 (100)		74 (100)		323 (100)	

CI, confidence interval.

had intact RD9 and were classified as *M. tuberculosis*, while 0.3% (1/323, CI: 0–0.9%) had RD9 and RD4 deleted PCR products and were classified as *M. bovis*.

The spoligotype analysis of 323 MTBC isolates based on the SITVIT2 database generated 71 different spoligotype patterns that belonged to 61 shared-types (SITs) with 96.3% (311/323) of the isolates and 10 orphan patterns with 3.7% (12/323) of the isolates. In the Mbovis.org database, the *M. bovis* spoligotype isolated from FNA was SB1942 spoligotype.

The analysis of isolates based on clades/families yields T (lineage 4) account for 41.5% (134/323), CAS (lineage 3) 28.2% (91/323), H (lineage 4) 8.7% (28/323), EAI (lineage 1) 6.8% (22/323), LAM (lineage 4) 6.2% (20/323), and Beijing (lineage 2) 1.9% (6/323) of the all isolates (Table 2). There is no statistically significant association between clade with forms of TB ($p =$

TABLE 3 Spoligotype patterns of clustered *M. tuberculosis* complex isolates ($n = 284$) TB patients in Somali region, Ethiopia.

Octal	SIT	Clade/Subclade	Major lineage	n (%)	Binary code
777000377760771	149	T3-ETH	Lineage 4	57 (17.6%)	■■■■■■■■■□□□□□□□□□■■■■■■■■■■■■■□□□□■■■■■■■
703377400001771	21	CAS1-Kili	Lineage 3	43 (13.3)	■■■□□□□■■□■■■■■■■■□□□□□□□□□□□□□□□□■■■■■■■■
703777740003771	26	CAS1-Delhi	Lineage 3	27 (8.4%)	■■■□□□□■■■■■■■■■■■■■■□□□□□□□□□□□□□■■■■■■■■■
777777777760771	53	T1	Lineage 4	24 (7.4%)	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■
777777777760731	52	T2	Lineage 4	16 (5%)	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□■■■
777737777760771	37	T3	Lineage 4	14 (4.3%)	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■
777777775720771	121	H3	Lineage 4	11 (3.4%)	■■■■■■■■■■■■■■■■■■■■■■■■■□■■■■□■□□□□■■■■■■■
677777607760771	20	LAM1	Lineage 4	10 (3.1%)	■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■
000000000003771	1	Beijing	Lineage 2	6 (1.9)	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■■
703777740003171	25	CAS1-Delhi	Lineage 3	6 (1.9)	■■■□□□□■■■■■■■■■■■■■■■□□□□□□□□□□□□□■■□□■■■■■
777775777760731	584	T2	Lineage 4	6 (1.9)	■■■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■□□□□■■■□■■■
000000007760771	4	Unknown	Lineage 4	5 (1.5%)	□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■□□□□■■■■■■■
477777277413771	10	EAI8-MDG	Lineage 1	5 (1.5%)	■□□■■■■■■■■■■■■■□■□■■■■■■■■□□□□■□■■■■■■■■■
777777774020771	47	H1	Lineage 4	5 (1.5%)	■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□■□□□□■■■■■■■
777777777413731	48	EAI1-SOM	Lineage 1	5 (1.5%)	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□■■■■■□■■■
703777700000771	1949	CAS	Lineage 3	5 (1.5%)	■■■□□□□■■■■■■■■■■■■■■□□□□□□□□□□□□□□□■■■■■■■
777777607760771	42	LAM9	Lineage 4	4 (1.2%)	■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■
777777777720771	50	H3	Lineage 4	4 (1.2%)	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■
777777777760711	78	T	Lineage 4	4 (1.2%)	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■□□■■
703777740000771	357	CAS1-Delhi	Lineage 3	3 (0.9%)	■■■□□□□■■■■■■■■■■■■■■□□□□□□□□□□□□□□□■■■■■■■
777777377760771	40	T4	Lineage 4	2 (0.6%)	■■■■■■■■■■■■■■■■■□■■■■■■■■■■■■■□□□□■■■■■■■
777777777720631	134	H3	Lineage 4	2 (0.6%)	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■□□■■■
703777740003471	247	CAS1-Delhi	Lineage 3	2 (0.6%)	■■■□□□□■■■■■■■■■■■■■■□□□□□□□□□□□□□■■□□■■■■
777756777760771	302	X1	Lineage 4	2 (0.6%)	■■■■■■■■■■■■■□■■■□■■■■■■■■■■■■■□□□□■■■■■■■
777777757413771	591	EAI6-BGD1	Lineage 1	2 (0.6%)	■■■■■■■■■■■■■■■■■■■■■□■■■■■□□□□■□■■■■■■■■■
777737747413771	726	EAI6-BGD1	Lineage 1	2 (0.6%)	■■■■■■■■■■■■□■■■■■■■■□□■■■■□□□□■□■■■■■■■■■
700000007177771	910	Ethiopian	Lineage 7	2 (0.6%)	■■■□□□□□□□□□□□□□□□□□□□□□■■■□□■■■■■■■■■■■■■
777727777760771	1547	T3	Lineage 4	2 (0.6%)	■■■■■■■■■■■■■□■□■■■■■■■■■■■■■■■■□□□□■■■■■■■
776737777760771	3137	T3	Lineage 4	2 (0.6%)	■■■■■■■■□■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■
177000377760771	3141	T3-ETH	Lineage 4	2 (0.6%)	□□■■■■■■■□□□□□□□□□■■■■■■■■■■■■■□□□□■■■■■■■

SIT, shared international types.

0.062). The major lineages including lineage 1, lineage 2, lineage 3, lineage 4, and lineage 7 were identified in proportions of 7.4, 2.2, 28.2, 60.4, and 0.6%, respectively. Lineage 4 was dominant and comprised of 61.8% (154/249) of pulmonary TB and 56.8% (42/74) of extrapulmonary TB isolates.

Clustered spoligotypes with the size of 2–57 isolates were identified and a majority of the isolates (87.9%; 284/323) (Table 3) were classified under the clustered spoligotypes. The remaining 39 (12.1%) isolates all exhibited unique patterns and thus were singletons (Table 4). The majority of clustered SIT's based on SITVIT classification belonged to the SIT 149 (17.6%), SIT 21 (13.3%), SIT 26 (8.4%), SIT 53 (7.43%), and SIT 52 (5%) which constitute 51.7% of the isolates (Table 3). Evaluation of the association of different factors with clustering demonstrated that lineage 3 and lineage 4, and age group (15–24) were significantly associated with clustering (Table 1).

The anti-TB drugs susceptibility testing was successfully done on 302 isolates using the MGIT960 system. The prevalence of resistance to at least one drug was 11.6%, while the prevalence of MDR-TB was 3.3%. The SIT 149 genotype was the most clustered spoligotype among MDR and significantly associated with both resistance to at least one drug and MDR. The study results have been described previously (17).

Discussion

Ethiopia has a high TB burden, thus identifying the most frequent MTBC genotypes spreading throughout the country from all types of TB and monitoring the spread of virulent genotypes or zoonotic-transmitted MTBC is crucial (1). To our knowledge this is the first study in the Somali region,

TABLE 4 Spoligotypes patterns of unique *M. tuberculosis* complex isolates ($n = 39$) from TB patients in Somali region, Ethiopia.

Octal	SIT	Sublineage	Lineage	N (%)	Binary code
477777777413071	11	EAI3-IND	Lineage 1	1 (0.3%)	
677737607760771	17	LAM2	Lineage 4	1 (0.3%)	
776377777760771	34	S	Lineage 4	1 (0.3%)	
777737777420771	35	Ural-1	Lineage 4	1 (0.3%)	
777737777720771	36	H3	Lineage 4	1 (0.3%)	
777777747413771	43	EAI6-BGD1	Lineage 1	1 (0.3%)	
777777777763771	54	Manu2	Lineage 1	1 (0.3%)	
777777607760731	60	LAM4	Lineage 4	1 (0.3%)	
477777377413771	109	EAI8-MDG	Lineage 1	1 (0.3%)	
777776777760771	119	X1	Lineage 4	1 (0.3%)	
703777700003771	142	CAS1-Delhi	Lineage 3	1 (0.3%)	
777777777413771	236	EAI5	Lineage 1	1 (0.3%)	
777777777700000	237	Unknown	Lineage 4	1 (0.3%)	
777777704020771	283	H1	Lineage 4	1 (0.3%)	
477776077411171	299	EAI3-IND	Lineage 1	1 (0.3%)	
703777740003071	381	CAS1-Delhi	Lineage 3	1 (0.3%)	
777777777760611	521	T1	Lineage 4	1 (0.3%)	
000000007720631	586	H3	Lineage 4	1 (0.3%)	
777600007413371	924	EAI5	Lineage 1	1 (0.3%)	
777777377760731	1077	T	Lineage 4	1 (0.3%)	
777777777413331	1183	EAI1-SOM	Lineage 1	1 (0.3%)	
777776607760771	1470	LAM9	Lineage 4	1 (0.3%)	
777377777761771	1516	Unknown	Unknown	1 (0.3%)	
601777606060771	1607	LAM11-ZWE	Lineage 4	1 (0.3%)	
777777777723771	1634	Manu2	Lineage 1	1 (0.3%)	
677777607560771	1755	LAM6	Lineage 4	1 (0.3%)	
703677740003171	2359	CAS1-Delhi	Lineage 3	1 (0.3%)	
000000000000000	2669	ATYPIC	Lineage 2	1 (0.3%)	
777717774020771	2866	H1	Lineage 4	1 (0.3%)	
777737377720771	3134	H3	Lineage 4	1 (0.3%)	
602773777774600	3750	BOV_1	M. bovis	1 (0.3%)	
777775745413771	Orphan	EAI6-BGD1	Lineage 1	1 (0.3%)	
777777177720731	Orphan	H3	Lineage 4	1 (0.3%)	
777774007760731	Orphan	LAM4	Lineage 4	1 (0.3%)	
000000020103771	Orphan	Not defined	Unknown	1 (0.3%)	
637700003760730	Orphan	Not defined	Lineage 4	1 (0.3%)	
777775477760731	Orphan	Not defined	Lineage 4	1 (0.3%)	
740000000000000	Orphan	Not defined	Unknown	1 (0.3%)	
777737767760771	Orphan	T3	Lineage 4	1 (0.3%)	

SIT, shared international types.

investigating MTBC isolates diversity in pulmonary TB and extrapulmonary TB patients.

In this study, MTBC were isolated from pulmonary TB and extrapulmonary TB and the majority of the isolates were *M. tuberculosis*. The role of *M. bovis* was minimal in causing human TB in the Somali region as only one isolate of *M. bovis* was isolated, which is consistent with the observations of the previous studies (18–21). The low rate of human infection by *M. bovis* might be due to the low prevalence of bovine TB in the Somali region. In support of this, Gumi et al. reported a low prevalence of bovine TB among Somali pastoral cattle in southeast Ethiopia (22). Furthermore, human infection requires

consumption of contaminated milk from cows with TB mastitis, which affects only about 1% of infected cattle (23, 24). However, Gumi et al. reported three cases of bovine TB among pulmonary TB patients in a study in the southeast Ethiopia pastoral area, suggesting the possibility of aerosol transfer from animal to human or human to human (25). But further study is needed to determine the true burden of zoonotic TB in Ethiopia, as could be a possible source of human TB (21, 25, 26). Thus, in order to end the TB epidemic by 2030, zoonotic TB must be incorporated in prevention efforts (27).

The predominant clades (91.3%) of *M. tuberculosis* isolated in this study belonged to the T, CAS, Haarlem, EAI, and LAM subfamilies. Similar findings were reported from different studies in Ethiopia (18, 28). SIT 149 (T3-ETH) was the most common T subclade identified in our study. Previous studies in Ethiopia also found a high prevalence of SIT 149 (T3-ETH), showing that SIT 149 (T3-ETH) is a dominant subclade in the country (18, 25, 26). As a result, TB epidemic in Ethiopia is mainly driven by the SIT 149 (T3-ETH) spoligotype that spreads across the country. Similar patterns have been found in other African nations where localized genotypes make up a higher proportion of *M. tuberculosis* spoligotypes circulating in those countries (29). The CAS clade was the second most common in our study, and has previously been reported in Ethiopia (30, 31). It is also abundant in Tanzania and Kenya, with SIT 21 (CAS1 KILI) being the most common clade (32).

In the current study, there was no difference in the genotype distribution between pulmonary TB and extrapulmonary TB patients. A previous study in Ethiopia found a similar genetic distribution between the two disease presentations (18). This could suggest that both pulmonary and extrapulmonary TB have similar transmission patterns in the community (23).

Interestingly, the frequency of isolation of the EAI ancestral clade (lineage 1) in this study was higher than the frequencies of its isolation by previously reported studies from the other parts of Ethiopia. Only a few previous studies in Ethiopia reported the EAI clade (25, 33). In the neighboring Somalia, 33.6% of isolates *M. tuberculosis* isolates were reported to be classified under the EAI clade (34), which could suggest that the pastoralists from both countries interact in the border areas on a regular basis as the Somali region of Ethiopia shares a border with Somaliland (Somalia) (9).

Beijing, Ethiopian, ATYPIC, MANU, X, Ural-1, and S genotypes constituted minority group that contributed for a pooled prevalence of 8.4%. The Beijing lineage is widespread in East Asia. In Africa, the Beijing lineage is most common in Southern Africa, although it can also be found sporadically in East Africa (29, 35). The Beijing (SIT 1) clade was detected in our study, and a similar frequency SIT 1 was observed by a study conducted in eastern Ethiopia (33). But the frequency of SIT 1 isolated by the present study was higher than its frequencies of isolation by previous studies from other parts of Ethiopia (7, 21, 28, 36, 37). The relative increase in the frequency of SIT 1 found in this study should considered a serious concern as the STI1 (Beijing) spoligotype is associated with virulence, transmissibility, and drug resistance (6). The prevalence of SIT 910 (lineage 7), which is geographically restricted to Ethiopia, was comparable to the proportion seen in TB patients from South Omo (28), but lower than the proportion found in TB patients from Northwest Ethiopia (38).

The overall clustering rate in this study was high and consistent with the clustering rates reported by the previous studies conducted in Ethiopia (13, 26, 31). A high rate of clustering could suggest active disease transmission in the area. The limited discriminating capacity of spoligotyping, on the other hand, should be noted, and further identification of the isolates using the genotyping method with a better discriminatory power is needed. Multivariable analysis of clustering and major lineages, indicated lineage 3 and lineage 4 were significantly associated with clustering compared with lineage 1 as a reference; clustering was also associated with the younger age group 15–24 compared with age group ≥45 as a reference. The association between the young age group and clustering might be attributed to greater social engagement in the young age group.

Although the low discriminatory power spoligotyping is acknowledged, the genotype data generated by the present study using a large number of *M. tuberculosis* isolates from the Somali region could be considered as useful input to the TB Control Program in the Somali region of Ethiopia, as well as for mapping the population dynamics of MTBC in Ethiopia.

Conclusion

The MTBC isolates identified from patients with TB in Somali region were highly diverse. The most common lineage types identified in this investigation were lineage 4 and lineage 3, both consisting of clustered spoligotypes, which could suggest the presence of active transmission. In addition, the Beijing spoligotype was isolated from the Somali region of Ethiopia in relatively higher frequency than the frequencies of isolations of the Beijing spoligotype from the other regions of Ethiopia warranting the attention of the TB Control Program of the Somali Region.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding author.

Ethics statement

The study was approved by the Institutional Review Board (IRB) of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University Ref No. ALIPB/IRB/002/2017/18. Permission

was requested from Ethiopian Somali Regional State Health Bureau and each study sites. The study was explained to the patients, and consent for participation was obtained prior to collecting the specimens. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GW contributed in designing of the study, data collection, analysis, and drafting of the manuscript. BG supervised the study and edited the manuscript. MG, BM, HS, AW, and WA contributed in the field data collection, culturing of samples, and drug sensitivity testing. RT and LC contributed in edition of the manuscript. GA contributed in conceptualizing and designing of the study, leading and supervision of GW, analysis of and interpretation of the result, and editing of the manuscript. All authors contributed to the manuscript for submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.960590/full#supplementary-material

References

1. WHO. *Global Tuberculosis Report 2021*. Geneva: WHO (2021).

2. Barnes PF, Cave MD. Molecular epidemiology of tuberculosis. *N Engl J Med.* (2003) 349:1149–56. doi: 10.1056/NEJMra021964

3. Valway SE, Sanchez MP, Shinnick TF, Orme I, Agerton T, Hoy D, et al. An outbreak involving extensive transmission of a virulent strain of *Mycobacterium tuberculosis*. *N Engl J Med.* (1998) 338:633–9. doi: 10.1056/NEJM199803053381001

4. Luzze H, Johnson D, Dickman K, Mayanja-Kizza H, Okwera A, Eisenach K, et al. Relapse more common than reinfection in recurrent tuberculosis 1–2 years post treatment in urban Uganda. *Int J Tubercul Lung Dis.* (2013) 17:361–7. doi: 10.5588/ijtld.11.0692

5. Small PM, McClenny NB, Singh SP, Schoolnik GK, Tompkins LS, Mickelsen PA. Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimize occurrence of false-positive cultures. *J Clin Microbiol.* (1993) 31:1677–82. doi: 10.1128/jcm.31.7.1677-1682.1993

6. Coscolla M, Gagneux S. Consequences of genomic diversity in *Mycobacterium tuberculosis*. *Semin Immunol.* (2014) 26:431–44. doi: 10.1016/j.smim.2014.09.012

7. Tulu B, Ameni G. Spoligotyping based genetic diversity of *Mycobacterium tuberculosis* in Ethiopia: a systematic review. *BMC Infect Dis.* (2018) 18:140. doi: 10.1186/s12879-018-3046-4

8. CSA E. *Population Projection of Ethiopia for all Regions at Wereda Level From 2014–2017*. Al Ain: Central Statistical Agency of Ethiopia (2013).

9. Getnet F, Demissie M, Worku A, Gobena T, Tschopp R, Farah AM, et al. Challenges in delivery of tuberculosis Services in Ethiopian pastoralist settings: clues for reforming service models and organizational structures. *BMC Health Serv Res.* (2021) 21:1–14. doi: 10.1186/s12913-021-06662-3

10. Gele AA, Bjune GA. Armed conflicts have an impact on the spread of tuberculosis: the case of the Somali Regional State of Ethiopia. *Confl Health.* (2010) 4:1–. doi: 10.1186/1752-1505-4-1

11. WHO. *Guidelines for Surveillance of Drug Resistance in Tuberculosis*. 5th ed. Geneva: WHO (2015).

12. WHO. *Laboratory Services in Tuberculosis Control. Part III. Culture*. Geneva: WHO (1998).

13. Bedewi Z, Worku A, Mekonnen Y, Yimer G, Medhin G, Mamo G, et al. Molecular typing of *Mycobacterium tuberculosis* complex isolated from pulmonary tuberculosis patients in central Ethiopia. *BMC Infect Dis.* (2017) 17:184. doi: 10.1186/s12879-017-2267-2

14. Berg S, Firdessa R, Habtamu M, Gadisa E, Mengistu A, Yamuah L, et al. The burden of mycobacterial disease in ethiopian cattle: implications for public health. *PLoS ONE.* (2009) 4:e5068. doi: 10.1371/journal.pone.0005068

15. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol.* (1997) 35:907–14. doi: 10.1128/jcm.35.4.907-914.1997

16. Couvin D, David A, Zozio T, Rastogi N. Macro-geographical specificities of the prevailing tuberculosis epidemic as seen through SITVIT2, an updated version of the *Mycobacterium tuberculosis* genotyping database. *Infect Genet Evolut.* (2019) 72:31–43. doi: 10.1016/j.meegid.2018.12.030

17. Worku G, Gumi B, Girma M, Mohammedbirhan B, Diriba G, Seid G, et al. Drug sensitivity of clinical isolates of *Mycobacterium tuberculosis* and its association with bacterial genotype in the Somali region, Eastern Ethiopia. *Front Public Health.* (2022) 10:942618. doi: 10.3389/fpubh.2022.942618

18. Firdessa R, Berg S, Hailu E, Schelling E, Gumi B, Erenso G, et al. Mycobacterial lineages causing pulmonary and extrapulmonary tuberculosis, Ethiopia. *Emerg Infect Dis.* (2013) 19:460–3. doi: 10.3201/eid1903.120256

19. Getahun M, Blumberg HM, Sinshaw W, Diriba G, Mollalign H, Tesfaye E, et al. Low prevalence of *Mycobacterium bovis* in Tuberculosis Patients, Ethiopia. *Emerg Infect Dis.* (2020) 26:613–5. doi: 10.3201/eid2603.190473

20. Yimer SA, Namouchi A, Zegeye ED, Holm-Hansen C, Norheim G, Abebe M, et al. Deciphering the recent phylogenetic expansion of the originally deeply rooted *Mycobacterium tuberculosis* lineage 7. *BMC Evol Biol.* (2016) 16:146. doi: 10.1186/s12862-016-0715-z

21. Hussien B, Zewude A, Wondale B, Hailu A, Ameni G. Spoligotyping of clinical isolates of *Mycobacterium tuberculosis* complex species in the oromia region of Ethiopia. *Front Public Health.* (2022) 10:808626–. doi: 10.3389/fpubh.2022.808626

22. Gumi B, Schelling E, Firdessa R, Erenso G, Biffa D, Aseffa A, et al. Low prevalence of bovine tuberculosis in Somali pastoral livestock, southeast Ethiopia. *Trop Anim Health Prod.* (2012) 44:1445–50. doi: 10.1007/s11250-012-0085-5

23. Grange JM, Zumla AI. Chapter 15-Human tuberculosis due to Mycobacterium bovis and related animal pathogens. In: *Tuberculosis*. Edinburgh: W.B. Saunders (2009). p. 146–53.

24. Ameni G, Tadesse K, Hailu E, Deresse Y, Medhin G, Aseffa A, et al. Transmission of *Mycobacterium tuberculosis* between farmers and cattle in central Ethiopia. *PLoS ONE.* (2013) 8:e76891. doi: 10.1371/journal.pone.0076891

25. Gumi B, Schelling E, Berg S, Firdessa R, Erenso G, Mekonnen W, et al. Zoonotic transmission of tuberculosis between pastoralists and their livestock in south-east Ethiopia. *Ecohealth.* (2012) 9:139–49. doi: 10.1007/s10393-012-0754-x

26. Belay M, Ameni G, Bjune G, Couvin D, Rastogi N, Abebe F. Strain diversity of *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients in afar pastoral region of Ethiopia. *Biomed Res Int.* (2014) 2014:238532. doi: 10.1155/2014/238532

27. WHO. *Roadmap for Zoonotic Tuberculosis*. Geneva: WHO (2017).

28. Wondale B, Keehwan K, Medhin G, Teklu T, Mohammed T, Tolosa S, et al. Molecular epidemiology of clinical *Mycobacterium tuberculosis* complex isolates in South Omo, Southern Ethiopia. *BMC Infect Dis.* (2020) 20:750. doi: 10.1186/s12879-020-05394-9

29. Brudey K, Driscoll JR, Rigouts L, Prodinger WM, Gori A, Al-Hajoj SA, et al. Mycobacterium tuberculosis complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* (2006) 6:23. doi: 10.1186/1471-2180-6-23

30. Yimer SA, Hailu E, Derese Y, Bjune GA, Holm-Hansen C. Spoligotyping of *Mycobacterium tuberculosis* isolates among pulmonary tuberculosis patients in Amhara Region, Ethiopia. *Apmis.* (2013) 121:878–85. doi: 10.1111/apm.12046

31. Mihret A, Bekele Y, Loxton AG, Jordan AM, Yamuah L, Aseffa A, et al. Diversity of *Mycobacterium tuberculosis* Isolates from new pulmonary tuberculosis cases in <city>Addis Ababa</city>, Ethiopia. *Tuberc Res Treat.* (2012) 2012:892079. doi: 10.1155/2012/892079

32. Mbugi EV, Katale BZ, Siame KK, Keyyu JD, Kendall SL, Dockrell HM, et al. Genetic diversity of *Mycobacterium tuberculosis* isolated from tuberculosis patients in the Serengeti ecosystem in Tanzania. *Tuberculosis.* (2015) 95:170–8. doi: 10.1016/j.tube.2014.11.006

33. Mekonnen A, Merker M, Collins JM, Addise D, Aseffa A, Petros B, et al. Molecular epidemiology and drug resistance patterns of *Mycobacterium tuberculosis* complex isolates from university students and the local community in Eastern Ethiopia. *PLoS ONE.* (2018) 13:e0198054–e. doi: 10.1371/journal.pone.0198054

34. Couvin D, Reynaud Y, Rastogi N. Two tales: Worldwide distribution of Central Asian (CAS) versus ancestral East-African Indian (EAI) lineages of *Mycobacterium tuberculosis* underlines a remarkable cleavage for phylogeographical, epidemiological and demographical characteristics. *PLoS ONE.* (2019) 14:e0219706. doi: 10.1371/journal.pone.0219706

35. Chihota VN, Niehaus A, Streicher EM, Wang X, Sampson SL, Mason P, et al. Geospatial distribution of *Mycobacterium tuberculosis* genotypes in Africa. *PLoS ONE.* (2018) 13:e0200632. doi: 10.1371/journal.pone.0200632

36. Taye H, Alemu K, Mihret A, Ayalew S, Hailu E, Wood JLN, et al. Epidemiology of *Mycobacterium tuberculosis* lineages and strain clustering within urban and peri-urban settings in Ethiopia. *PLoS ONE.* (2021) 16:e0253480–e. doi: 10.1371/journal.pone.0253480

37. Merid Y, Hailu E, Habtamu G, Tilahun M, Abebe M, Hailu M, et al. Molecular Epidemiology of *Mycobacterium tuberculosis* strains isolated from pulmonary tuberculosis patients in south Ethiopia. *J Infect Dev Ctries.* (2021) 15:1299–307. doi: 10.3855/jidc.14742

38. Tessema B, Beer J, Merker M, Emmrich F, Sack U, Rodloff AC, et al. Molecular epidemiology and transmission dynamics of *Mycobacterium tuberculosis* in northwest Ethiopia: new phylogenetic lineages found in northwest Ethiopia. *BMC Infect Dis.* (2013) 13:1. doi: 10.1186/1471-2334-13-131

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Association of *in vitro* fertilization with maternal and perinatal outcomes among pregnant women with active tuberculosis: A retrospective hospital-based cohort study

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Background: Study on effect of fertilization methods on maternal and perinatal outcomes with respect to TB during pregnancy was scarce. This study aimed to analyze maternal and perinatal outcomes in active TB cases after *in vitro* fertilization (IVF) treatment vs. normal pregnancy.

Methods: Clinical data of 80 pregnant women with active TB hospitalized at Shanghai Public Health Clinical Center between June 1st, 2014 and November 30th, 2020 were extracted and retrospectively analyzed. History of receiving IVF was recorded at admission and its association with maternal and perinatal outcomes were assessed using multivariable logistic regression models with adjustment for potential confounders.

Results: Of the 80 pregnant women with active TB, 28 (35.0%) received IVF treatment and 52 (65.0%) did not receive IVF treatment. After adjusting for potential confounders, receiving IVF was associated with worse maternal and perinatal outcomes, including maternal criticality (21.4 vs. 2.0%, adjusted OR = 28.3, $P = 0.015$), miliary TB (89.3 vs. 13.5%, adjusted OR = 75.4, $P < 0.001$), TB meningitis (32.1 vs. 7.7%, adjusted OR = 6.2, $P = 0.010$), and perinatal mortality (64.3 vs. 28.8%, adjusted OR = 9.8, $P = 0.001$).

Conclusion: The additional risk of TB to women receiving IVF treatment is a public health challenge specific to countries with a high tuberculosis burden. Increased awareness of latent tuberculosis infection in women receiving IVF treatment is needed.

KEYWORDS

tuberculosis, *in vitro* fertilization, immunity, miliary TB, perinatal outcome

Introduction

Tuberculosis (TB) is a communicable disease caused by *Mycobacterium tuberculosis* (MTB). Approximately 2 billion people were infected by MTB globally in 2020 and 5–10% will develop tuberculosis (TB) in their lifetime (1). It is estimated that about 217 thousand active TB cases among pregnancy women in 2011 (2). In high TB-burden regions, TB is considered as a leading non-obstetric cause of mortality in women of reproductive age (3, 4), and is also associated with adverse perinatal outcomes, including preterm birth, low birth weight, birth asphyxia and perinatal death. Studies has shown that pregnancy increases the susceptibility to new infections and reactivation of TB (3, 5), partly owing to the effect of endogenous progesterone (6, 7). Progestogens may suppress host immunity with a dose-dependent effect on the Th1/Th2 response and thus reduce T-cell proliferation (6). The host immune response, especially the equilibrium between T-helper 1 (Th1) and T-helper 2 (Th2) cells, is critical for determining the outcome of MTB infection or disease (8–10).

In vitro fertilization (IVF) is the most widely used assisted reproductive technology, and progestogen is administered during the IVF procedure (11). The progestogen dose used during IVF is approximately 2–4-fold or higher than that of the normal supplement administered to reduce the risk of preterm birth or increase maternal-fetal tolerance (12, 13). The development of TB after IVF treatment reportedly results in severe maternal complications (such as progressive respiratory failure, miliary TB, or tuberculous meningitis) and unfavorable perinatal outcomes (14–17). Therefore, it is reasonable to assume that exogenous progestogen used in IVF may induce excessive immunosuppressive effects and result in adverse consequences when overlapped with tuberculosis.

To date, the association between IVF treatment and the outcome of TB-in-pregnancy is unclear. No published study has compared complications or outcomes of TB patients receiving IVF with those not receiving IVF. To assess the association between IVF treatment and maternal and perinatal outcomes in active TB cases during pregnancy, we conducted a retrospective study based on registered data on electronic medical record (EMR) system.

Methods

Study design and participants

This retrospective cohort study was conducted at Shanghai Public Health Clinical Center (SHPHCC), the only inpatient center for pregnant women with tuberculosis in Shanghai. In the past decade, almost all patients with active TB during pregnancy or postpartum that require hospitalization service in Shanghai were registered at this center.

We screened all in-patients with TB-in-pregnancy record at SHPHCC from June 1, 2014 to November 30, 2020. Those who met inclusion and exclusion criteria were considered eligible. Inclusion criteria included: (1) 18–42 years old; (2) developed active TB during pregnancy or within 6 weeks after delivery. Exclusion criteria include the following: (1) those who have developed TB before pregnancy according to clinical assessment; (2) those who failed to receive routine anti-TB treatment for any reason, such as severe adverse effect of drugs, or rejected to receive anti-TB therapy; (3) those without complete medical records.

An active TB case was defined as both bacteriologically (a valid specimen with positive results on smear microscopy, culture, histopathology, or a Food and Drug Administration-approved nucleic acid amplification test, whichever was available) and clinically (diagnosed as TB with a decision to treat by experienced clinicians without a bacteriologically confirmation) positive (18).

Data collection

Demographic and clinical data of all eligible study participants were extracted from the electronic medical record (EMR) system of SHPHCC. These data included age, symptoms on admission (such as cough, fever, night sweats, weight loss, vaginal bleeding, pleural effusion, and progressive respiratory failure), TB diagnostic test results (sputum smear, sputum culture, interferon-gamma release assay, nucleic acid amplification tests), chest radiography findings, drug susceptibility tests results, anti-TB treatment regimen, whether HIV positive and CD4+ cell counts and maternal/perinatal outcomes. Data on dates of first TB-related symptoms, TB diagnosis, and pregnancy were extracted as well. The history of IVF treatment was determined by checking medical records or via a telephonic interview. If any clinical information was missing, we conducted a telephonic interview to collect the required data.

Definition of variables

Maternal outcomes include obstetric complications (whether to develop preeclampsia or criticality at the time of delivery to 6 weeks postpartum) and TB outcomes (whether to develop TB-related symptoms, systemic dissemination, or death). Maternal criticality is defined as a life-threatening condition, including acute respiratory distress syndrome, severe eclampsia, hemorrhage, or loss of consciousness, requiring comprehensive care and constant monitoring.

Perinatal outcomes include preterm birth rate, perinatal mortality, and TB outcome (whether to develop TB after birth).

Delay in diagnosis is quantified using the days from the first TB symptom to the diagnosis of TB. The time to the

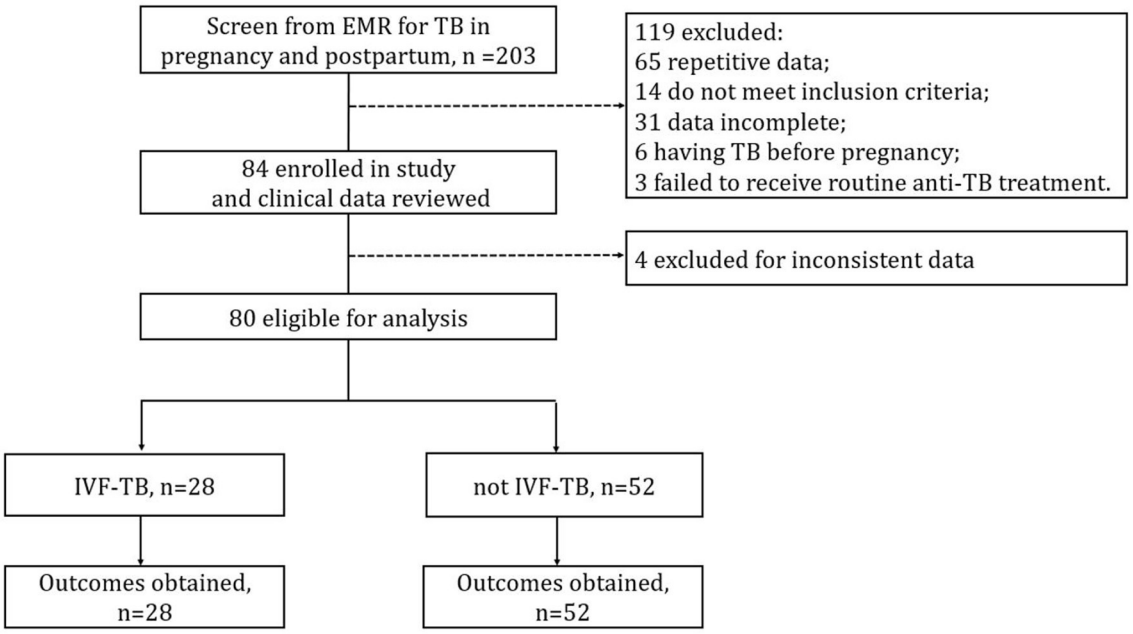

FIGURE 1
Flow chart of participants and data collection. EMR, electronic medical record; IVF, *in vitro* fertilization.

first TB symptom was calculated using gestational weeks from conception to the first TB symptom. Patients with a medical record of IVF treatment or confirmed history via the telephonic interview were categorized into the treatment group (IVF-TB) and those without a record or confirmation into the control group (not IVF-TB).

Ethics statement

This study procedure was reviewed and approved by the Ethics committee of SHPHCC (2020-S203-01) and a waiver of informed consent was obtained.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 22. Categorical variables are presented as percentage (%), and continuous variables are presented as mean ± standard deviation or median (interquartile range). Differences in categorial variables were analyzed using the chi-square test or Fisher's exact test, whereas differences in continuous variables were analyzed using Student's *t*-test or the Mann–Whitney U test. Logistic regression analysis was performed to determine the association between patient characteristics and each maternal complications, and between IVF treatment and each perinatal outcome. Crude and adjusted odds ratios and their 95% confidence intervals (CIs) were estimated. $P < 0.05$ indicated statistical significance in both univariate and multivariate analysis.

Results

Characteristics of participants

A total of 98 patients with TB during pregnancy or postpartum were hospitalized at SHPHCC during the study period. Of them, 80 had complete data and were included in the final analysis; 18 were excluded either owing to ineligibility or incomplete medical records (Figure 1). The 80 participants a mean age of 28.05 ± 4.81 years; 59 (73.8%) had bacteriologically confirmed TB, 7 (8.8%) reported a history of TB, 77 (77/79, 97.5%) tested positive on interferon-gamma release assays (IGRAs), and none tested positive for HIV. All participants received anti-TB treatment under experienced clinicians' guidance, and none were diagnosed with MDR-TB (resistant to both isoniazid and rifampicin). 28 (35.0%) patients reported a history of IVF treatment, and 67.9% (19/28) of them had experienced fallopian tube obstruction before IVF treatment.

The median time to the first TB symptom in IVF treatment group was less than that in control group (13 vs. 22 gestational weeks, $P < 0.001$). The CD4+ T cell count was lower in treatment group than in control group (337 ± 152 vs. 454

TABLE 1 Characteristics of study participants.

	IVF-TB	Non-IVF-TB	*P-value*
Cases (n)	28	52	
Age (years, mean ± SD)	29.46 ± 4.03	27.29 ± 5.05	0.053
Reasons for IVF (%)			
Fallopian tube obstruction	19 (67.9)	-	-
Male infertility	1 (3.6)	-	-
Unknown	8 (28.6)	-	-
Time to first TB symptom(s) onset (gestational weeks, median ± IQR)	13 (8-17.75)	22 (15-31.75)	<0.001
Delay in TB diagnosis (days, median ± IQR)	14.5 (7.25-30)	14 (7-28.5)	0.466
Onset postpartum TB (%)	1 (3.6)	5 (9.6)	0.659
History of TB (%)	4 (14.3)	3 (5.8)	0.232
Diagnosis of TB			
Bacteriologically confirmed TB (%)	22 (78.6)	37 (71.2)	0.597
Clinically diagnosed TB (%)	6 (21.4)	15 (28.8)	
Smear positive (%)	16 (57.1)	23 (44.2)	0.350
Culture positive (%)	18 (64.3)	32/51 (62.7)	1.000
IGRA positive (%)	28/28 (100)	49/51 (96.1)	0.537
CD4+ T cell count (cell/μL, mean ± SD)	337 ± 152 ($n = 15$)	454 ± 237 ($n = 32$)	0.086

IGRA, interferon-gamma release assay; IQR, interquartile range; TB, tuberculosis.

± 237, $P = 0.086$). There was no statistical difference in age, delay in diagnosis, TB history, and percentage of bacteriological confirmation between the two groups (Table 1).

Maternal outcomes in association with receiving IVF treatment

Obstetric complications, such as vaginal bleeding (46.4 vs. 1.9%, $P < 0.001$) and maternal criticality (21.4 vs. 2.0%, $P = 0.007$) were more common among participants receiving IVF treatment compared to those without receiving IVF. TB-related symptoms or complications were also more common in treatment group, miliary TB (89.3 vs. 13.5%, $P < 0.001$), TB meningitis (32.1 vs. 7.7%, $P = 0.008$), fever > 38.2°C (92.9 vs. 51.9%, $P < 0.001$), except pleural effusion (3.6 vs. 36.5%, $P = 0.001$). None TB-related maternal death was reported in this study.

After adjustment of potential confounders (age, delay in diagnosis, and MTB culture result), receiving IVF treatment was associated with a higher risk of vaginal bleeding (adjusted OR = 47.6), maternal criticality (adjusted OR = 28.3), fever > 38.2°C (adjusted OR = 16.7), cough (adjusted OR = 2.3), miliary TB (adjusted OR = 75.4), and TB meningitis (adjusted OR = 6.2) (Table 2).

Perinatal outcomes in association with receiving IVF treatment

Perinatal mortality was significantly higher in the treatment group (64.3 vs. 28.8%, $P = 0.008$). After adjusting for age, delay in diagnosis, and sputum MTB culture, IVF was significantly associated with increased infant mortality (adjusted OR = 5.8, $P = 0.002$). Fourteen participants chose to receive induced abortion. After induced abortion was excluded in the analysis, IVF was still associated with increased infant mortality (adjusted OR = 9.8, $P = 0.001$) (Table 3).

Discussion

IVF treatment is widely used to help with fertility and assist with the conception of a child. The risks of adverse perinatal outcomes associated with IVF treatment are generally mild, with a favorable success rate (19–21). However, TB infection, especially active TB may complicate this. Based on a retrospective study, we have proved that receiving IVF treatment is a potential risk factor associated with severe maternal complications and worse perinatal outcomes in TB-in-pregnancy patients. In areas with high tuberculosis prevalence, such as China and India, the number of pregnant women receiving IVF combined with latent tuberculosis infection should be large, and its potential risks should be paid enough attention to.

After adjusting for potential confounders, receiving IVF treatment was still associated with adverse perinatal outcomes. This effect was likely mediated by the shorter onset time and more maternal complications (the infant was more likely to be affected by TB before maturation). Spontaneous abortion accounted for 72.2% (13/18) of infant mortality cases in the treatment group. In these cases, vaginal bleeding (6/13, 46.2%) and fallopian tube obstruction (11/13, 84.6%) were common. Abdulhakim et al. reported that approximately 40% of infertility cases with a tuber factor were found to have genital TB (22). Therefore, some cases of spontaneous abortion may have been induced by the reactivation of tubal TB. Lin et al. reported that cured endometrial TB was also associated with a low live birth rate (23). We reviewed the medical records of all IVF-TB cases (including ultrasound, magnetic resonance imaging, computed tomography, and gynecologic examination report findings) to assess the

TABLE 2 Association between IVF status and maternal outcome among TB-in-pregnancy women.

	IVF-TB ($n = 28$)	Non-IVF-TB ($n = 52$)	OR*	95% CI	*P*-value (crude)	adjusted OR**	95% CI	*P*-value (adjusted)
Obstetric complications (%)								
Vaginal bleeding	13 (46.4)	1 (1.9)	44.2	5.3–366.0	<0.001	47.6	5.2–439.6	0.001
Maternal criticality	6 (21.4)	1 (2.0)	13.9	1.6–122.4	0.007	28.3	1.9–417.2	0.015
Preeclampsia	0 (0)	0 (0)	-		-	-		-
TB outcomes (%)								
Fever > 38.2°C	26 (92.9)	27 (51.9)	12	2.6–56.0	<0.001	16.7	3.2–87.7	0.001
Cough	21 (75.0)	30 (57.7)	2.2	0.8–6.1	0.149	2.3	0.8–6.7	0.135
Pleural effusion	1 (3.6)	19(36.5)	0.06	0.01–0.51	0.001	0.05	0.01–0.42	0.006
Miliary TB	25 (89.3)	7 (13.5)	53.6	12.7–225.7	<0.001	75.4	13.7–415.2	<0.001
TB meningitis	9 (32.1)	4 (7.7)	6	1.6–21.9	0.008	6.2	1.5–24.8	0.01
Death	0 (0)	0 (0)	-		-	-		-

*Odds ratio for IVF treatment vs. no IVF treatment by univariate analysis; **Multivariate regression was applied after adjusting for age, delay in diagnosis, and culture result.

TABLE 3 Association between IVF status and perinatal outcome among TB-in-pregnancy women.

	IVF-TB ($n = 28$)	Non-IVF-TB ($n = 52$)	OR*	95% CI	*P*-value (crude)	Adjusted OR**	95% CI	*P*-value (adjusted)
Mortality (%)	18 (64.3)	15 (28.8)	4.4	1.7–11.8	0.004	9.8	2.6–36.8	0.001
Spontaneous abortion	13 (46.4)	6 (11.5)	-		-	-		-
Induced abortion	5 (17.9)	9 (17.3)	-		-	-		-
Preterm birth (alive) (%)	2 (7.1)	3 (5.8)	-		-	-		-
Normal birth (%)	8 (28.6)	34 (65.4)	-		-	-		-
Developed TB after birth*** (%)	0 (0)	0 (0)	-		-	-		-

*Odds ratio for IVF treatment vs. no IVF treatment by univariate analysis; **Multivariate regression was applied after adjusting for age, delay in diagnosis, culture result, and induced abortion according to the patient's will. ***These data came from medical records or telephone review conducted at least 1 year after delivery.

possibility of pre-existed endometrial TB, but the evidence was quite limited.

This study's evidence suggests that women receiving IVF treatment may develop immunosuppression, but this evidence is still insufficient to establish that the adverse outcomes are attributable to IVF-related immunosuppression. In the IVF treatment group, 89.3% of the participants developed miliary TB, and the mean CD4+ T cell level was lower than control group (not statistically significant, likely due to the small sample size). As it is known, miliary TB is characterized by the dissemination of tiny tubercles to one or more organs of the body, often combined with systemic manifestations including high fever, respiratory failure, or meningitis. With a skewed shift toward Th2 in the Th1/Th2 response, TB reportedly tends to develop into a disseminated disease (10, 24, 25). The added progestogen used during IVF may induce increased immunosuppression to the Th1 response and reduce CD4+ T cell proliferation (6), and TB may amplify this side effect into a clinical phenomenon. IVF-TB patients are more likely to develop systemic symptoms and complications. In contrast, pleural effusion, which is considered as localized TB with Th1 predominance (10), was more often observed in control group (36.5 vs. 3.6%). These evidences suggest that IVF may be associated with a damaged host immunity against TB.

Another indirect evidence pointing to the association between IVF treatment and host susceptibility to TB is that most IVF-TB patients, rather the control group patients develop symptoms earlier (14.07 ± 7.38 vs. 22.73 ± 9.31 gestational weeks). In routine practice, progestogen supplementation is often discontinued at the 8–10th gestational week (11, 13), and a large proportion of patients develop symptoms a few weeks later. In contrast, this trend was not observed in the control group. This finding shows a clear intervention point for TB after IVF treatment: in a high-prevalence context, healthcare providers should screen for TB during the 8–22nd gestational week in women who underwent IVF treatment if they present with relevant symptoms. However,

over one third of not-IVF participants did not present typical TB symptoms. Performing laboratory tests to rule out TB should be more efficient. Pasipamire et al. reported that routine TB symptom screening is insufficient to rule out TB in pregnant and postpartum women (26). In our study, the routine TB tests, such as sputum acid-fast smear, MTB culture, or IGRAs yielded relatively higher positivity. The IGRAs should be considered as an ideal rule-out tool because almost all participants tested positive on IGRAs at admission.

In this study, participants without a record or confirmation of IVF history were categorized into the control group. If some participants did not report their IVF history, the results might bias towards the null hypothesis.

We adjusted for potential confounders as far as we were aware (2–5, 27). However, due to the limited sample size and retrospective design, it was difficult to adjust for all confounders and verify possible mediating effects (such as the worse perinatal outcome mediated by shorter onset time). Nonetheless, data on IVF-associated TB-in-pregnancy are scarce, and we consider this preliminary study valuable clinical evidence.

This study has some additional limitations. First, the current data do not reveal the number of participants latently infected before IVF treatment because TB screening was not routinely performed; thus, we cannot calculate the risk of new infection and reactivation of TB from receiving IVF treatment. Second, the route of administration and accurate dose of progestogens used in the IVF treatment were not well recorded, thus introducing potential confounders. If some participants in the treatment group did not receive sufficient progestogens, the results would have a negative bias. Third, participants enrolled in this study were all hospitalized patients; therefore, their clinical findings could be different from those of outpatients.

In conclusion, the influence of IVF treatment on host immunity against TB and other pathogens is not sufficiently assessed. Receiving IVF treatment may render women more vulnerable to TB and is associated with worse perinatal outcomes. Investigators should be aware of women preparing to receive IVF treatment in a high-burden TB context, evaluate TB risk, and administer prophylactic therapy for high-risk populations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Shanghai Public Health Clinical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

X-HL and S-HL: conception and design of study. LX and X-HL: acquisition of data. LX, PM, X-HL, Z-DH, and X-YF: analysis and/or interpretation of data.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. WHO, Global Tuberculosis Report 2020. *World Health Organization* (2020).

2. Sugarman J, Colvin C, Moran AC, Oxlade O. Tuberculosis in pregnancy: an estimate of the global burden of disease. *Lancet Glob Health*. (2014) 2:e710–6. doi: 10.1016/S2214-109X(14)70330-4

3. Mathad JS, Gupta A. Tuberculosis in pregnant and postpartum women: epidemiology, management, and research gaps. *Clin Infect Dis.* (2012) 55:1532–49. doi: 10.1093/cid/cis732

4. Orazulike NC, Alegbeleye JO, Obiorah CC, Nyengidiki TK, Uzoigwe SA. A 3-year retrospective review of mortality in women of reproductive age in a tertiary health facility in Port Harcourt, Nigeria. *Int J Womens Health.* (2017) 9:769–75. doi: 10.2147/IJWH.S138052

5. Jonsson J, Kühlmann-Berenzon S, Berggren I Bruchfeld J. Increased risk of active tuberculosis during pregnancy and postpartum: a register-based cohort study in Sweden. *Eur Respir J.* (2020). 55:1901886. doi: 10.1183/13993003.01886-2019

6. Lissauer D, Eldershaw SA, Inman CF, Coomarasamy A, Moss PA, Kilby MD. Progesterone promotes maternal-fetal tolerance by reducing human maternal T-cell polyfunctionality and inducing a specific cytokine profile. *Eur J Immunol.* (2015) 45:2858–72. doi: 10.1002/eji.201445404

7. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol.* (2010) 63:425–33. doi: 10.1111/j.1600-0897.2010.00836.x

8. Lienhardt C, Azzurri A, Amedei A, Fielding K, Sillah J, Sow OY, et al. Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity *in vivo*. *Eur J Immunol*. (2002). 32: 1605–13. doi: 10.1002/1521-4141(200206)32

9. Rook GA, Dheda K, Zumla A. Do successful tuberculosis vaccines need to be immunoregulatory rather than merely Th1-boosting? *Vaccine.* (2005) 23:2115–20. doi: 10.1016/j.vaccine.2005.01.069

10. Sharma SK, Mitra DK, Balamurugan A, Pandey RM. and Mehra N.K., Cytokine polarization in miliary and pleural tuberculosis. *J Clin Immunol.* (2002) 22:345–52. doi: 10.1023/A:1020604331886

11. Labarta E, Rodríguez C. Progesterone use in assisted reproductive technology. *Best Pract Res Clin Obstet Gynaecol.* (2020) 69:74–84. doi: 10.1016/j.bpobgyn.2020.05.005

12. Dodd JM, Jones L, Flenady V, Cincotta R, Crowther CA. Prenatal administration of progesterone for preventing preterm birth in women considered to be at risk of preterm birth. *Cochrane Database Syst Rev* 2013: Cd004947. doi: 10.1002/14651858.CD004947.pub3

13. Jiang L, Chen Y, Wang Q, Wang X, Luo X, Chen J, et al. A Chinese practice guideline of the assisted reproductive technology strategies for women with advanced age. *J Evid Based Med.* (2019) 12:167–84. doi: 10.1111/jebm.12346

14. Ye R, Wang C, Zhao L, Wu X, Gao Y, Liu H. Characteristics of miliary tuberculosis in pregnant women after in vitro fertilisation and embryo transfer. *Int J Tuberc Lung Dis.* (2019) 23:136–9. doi: 10.5588/ijtld.18.0223

15. Zhang X, Zhuxiao R, Xu F, Zhang Q, Yang H, Chen L, et al. Congenital tuberculosis after *in vitro* fertilization: suggestion for tuberculosis tests in infertile women in developing countries. *J Int Med Res.* (2018) 46:5316–21. doi: 10.1177/0300060518808179

16. Hongbo L, Li Z. Miliary tuberculosis after *in vitro* fertilization and embryo transplantation. *Afr Health Sci.* (2015) 15:701–4. doi: 10.4314/ahs.v15i2.50

17. Samedi V, Field SK, Al Awad E. Ratcliffe G, Yusuf K. Congenital tuberculosis in an extremely preterm infant conceived after in vitro fertilization: case report. *BMC Pregn Childbirth.* (2017) 17:66. doi: 10.1186/s12884-017-1256-1

18. WHO revised definitions and reporting framework for tuberculosis. *Euro Surveill.* (2013) 18: 20455. Available online at: http://www.euro.who.int/en/health-topics/communicable-diseases/t

19. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, et al. Reproductive technologies and the risk of birth defects. *N Engl J Med.* (2012) 366:1803–13. doi: 10.1056/NEJMoa1008095

20. Malizia BA, Hacker MR. and Penzias AS, Cumulative live-birth rates after *in vitro* fertilization. *N Engl J Med.* (2009) 360:236–43. doi: 10.1056/NEJMoa0803072

21. Seibel MM. A new era in reproductive technology. *In vitro* fertilization, gamete intrafallopian transfer, and donated gametes and embryos. *N Engl J Med.* (1988) 318:828–34. doi: 10.1056/NEJM198803313181306

22. Abdelrub AS, Al Harazi AH. Genital tuberculosis is common among females with tubal factor infertility: observational study, Alexandria. *J Med*. (2014) 51: 321–24. doi: 10.1016/j.ajme.2014.11.004

23. Lin MM, Yang W, Du XG, Song XL, Qiao J, Li R. Lower cumulative live birth rates in cured endometrial tuberculosis patients after one ART cycle including all subsequent frozen-thaw cycles: a matched-pair study. *Eur J Obstet Gynecol Reprod Biol X.* (2020) 6:100104. doi: 10.1016/j.eurox.2019.100104

24. Ayelign B, Negash M, Genetu M. Wondmagegn T, Shibabaw T. immunological impacts of diabetes on the susceptibility of mycobacterium tuberculosis. *J Immunol Res.* (2019) 2019:6196532. doi: 10.1155/2019/6196532

25. Sharma SK, Mohan A. Sharma A, *Mitra DK,* Miliary tuberculosis: new insights into an old disease. *Lancet Infect Dis.* (2005) 5:415–30. doi: 10.1016/S1473-3099(05)70163-8

26. Pasipamire M, Broughton E, Mkhontfo M, Maphalala G, Simelane-Vilane B, Haumba S. Detecting tuberculosis in pregnant and postpartum women in Eswatini. *Afr J Lab Med*. (2020) 9:837. doi: 10.4102/ajlm.v9i1.837

27. Sobhy S, Babiker Z, Zamora J, Khan KS, Kunst H. Maternal and perinatal mortality and morbidity associated with tuberculosis during pregnancy and the postpartum period: a systematic review and meta-analysis. *BJOG.* (2017) 124:727–33. doi: 10.1111/1471-0528.14408

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Transmission of multidrug-resistant tuberculosis in Beijing, China: An epidemiological and genomic analysis

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Background: Understanding multidrug-resistant tuberculosis (MDR-TB) transmission patterns is crucial for controlling the disease. We aimed to identify high-risk populations and geographic settings of MDR-TB transmission.

Methods: We conducted a population-based retrospective study of MDR-TB patients in Beijing from 2018 to 2020, and assessed MDR-TB recent transmission using whole-genome sequencing of isolates. Geospatial analysis was conducted with kernel density estimation. We combined TransPhylo software with epidemiological investigation data to construct transmission networks. Logistic regression analysis was utilized to identify risk factors for recent transmission.

Results: We included 241 MDR-TB patients, of which 146 (60.58%) were available for genomic analysis. Drug resistance prediction showed that resistance to fluoroquinolones (FQs) was as high as 39.74% among new cases. 36 (24.66%) of the 146 MDR strains were grouped into 12 genome clusters, suggesting recent transmission of MDR strains. 44.82% (13/29) of the clustered patients lived in the same residential community, adjacent residential community or the same street as other cases. The inferred transmission chain found a total of 6 transmission events in 3 clusters; of these, 4 transmission events occurred in residential areas and nearby public places. Logistic regression analysis revealed that being aged 25–34 years-old was a risk factor for recent transmission.

Conclusions: The recent transmission of MDR-TB in Beijing is severe, and residential areas are common sites of transmission; high levels of FQs drug resistance suggest that FQs should be used with caution unless resistance can be ruled out by laboratory testing.

KEYWORDS

transmission, multidrug-resistant tuberculosis, whole-genome sequencing, migrants, epidemiology

Introduction

Multidrug-resistant tuberculosis (MDR-TB) poses a serious threat to global tuberculosis control programs. According to the World Health Organization (WHO) report, there were an estimated 360,000 MDR-TB cases in 2020, but only 38% received treatment (1). MDR-TB is associated with a lower cure rates (15% vs. 4%), higher mortality (59% vs. 85%) and high treatment cost (5,659$/person) compared with drug-susceptible TB (1). China ranks second among the 27 countries most burdened by TB worldwide, contributing to 14% of all estimated MDR-TB cases (1). MDR-TB is thus a global problem and a major public health issue in China. Recent transmission is primarily responsible for driving the global endemic of MDR-TB (2–4). Therefore, understanding MDR-TB transmission patterns is crucial to informing public health efforts in carrying out effective interventions.

With the development of whole-genome sequencing technology, we can define a threshold for the number of genomic differences above which direct transmission is unlikely (5). This approach has the advantage of providing a simple way for judging transmission occurrence but cannot reconstruct an accurate transmission network. Most reconstructions rely heavily upon fieldwork data. However, due to the lack of investigation data and participant recall bias, identifying specific and accurate person-to-person transmission events is challenging. Nevertheless, it is possible to infer transmission from sequencing data using alternative approaches; several such methods have been proposed (6–8). Phylogenetic networks based on whole-genome sequencing can be used to identify putative source cases, super-spreaders, and transmission directions in the absence of comprehensive epidemiological data. Therefore, there has been increasing integration of genetic and epidemiological data to construct a more accurate transmission network and infer transmission dynamics (9).

Beijing is one of the most populous cities in the world with a resident population of 21.9 million. The city hosts large numbers of migrants from other parts of China, who account for around 38.5% of its population (10). Compared with other Chinese regions, the incidence of TB is relatively low, but slowly declining (11). Internal migrants are at a higher risk of TB (9, 12) and may spread the disease during their travels.

Understanding TB transmission is of great importance for identifying the origins of the disease and the population that is at risk of infection (13). However, the mechanisms involved in MDR-TB transmission in Beijing have not been investigated to date. We combined epidemiological, molecular genetics, and spatial analysis to investigate the transmission dynamics of MDR-TB in Beijing, China. We quantified MDR-TB recent transmission, and identified risk factors for transmission and high-risk geographic sites. Our findings provide a scientific basis for public health agencies to create more effective strategies for TB control.

Methods

Study design and population

Beijing is the capital of China and is divided into 16 districts. All individuals with suspected TB are referred to local designated hospitals for diagnosis and treatment. Drug resistance screening by GeneXpert technology is performed in all patients diagnosed with etiologically positive TB. All cases in Beijing confirmed to harbor rifampicin-resistant TB (RR-TB) are registered in the TB management information system. Sputum samples from these cases are collected for culture and drug susceptibility testing with rifampin and isoniazid to further identify patients with MDR-TB. All culture-positive strains are mainly stored in two designated hospitals (Beijing Chest Hospital and Beijing Institute of Tuberculosis Control). This observational study included all cases with culture-confirmed MDR-TB who were reported by the Beijing Chest Hospital during 2018–2020.

Whole-genome sequencing

Strains of MDR-TB were re-grown on Löwenstein-Jensen medium and their genomic DNA was extracted using the cetyl trimethyl ammonium bromide method (14). For each sample, a pair-end library was constructed and sequenced on an Illumina platform (Illumina), with an expected 250× coverage. Paired-end reads were mapped to the reference genome H37Rv (GenBank AL123456) with Bowtie2. The SAMtools/VarScan suite was used for SNP calling with a mapping quality > 30. Fixed SNPs (frequency $\geq 75\%$), excluding those present in genes associated with drug-resistance and repetitive regions of the genome (e.g., PPE/PE-PGRS family genes, phage sequence, insertion or mobile genetic elements), were used to calculate the pairwise SNP distances. A genomic cluster was defined as strains with a genetic distance of ≤ 12 SNPs, suggesting they were the result of a recent transmission (4). Strains that differed by >12 SNPs as compared with all other strains were defined as unique strains. The strains were classified into different lineages according to Coll et al. (15). L2 strains were classified into L2.1, L2.2 and L2.3 (16). The L2.3 represents "modern" Beijing and others are "ancient" Beijing. The drug-resistance profile was predicted for 14 anti-TB drugs based on the mutations reported to be associated with resistance (17). Phylogenetic trees were constructed using MEGA (version 7.0). Visualization of the bacteriological information was performed by using the Interactive Tree Of Life (https://itol.embl.de/). The sequencing data have been deposited with links to BioProject accession number PRJNA888557 in the National Center for Biotechnology.

Information BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

Epidemiological investigation

Consenting participants underwent a structured interview. Based on self-administered questionnaires, we collected data on close contacts, social contacts, and places they frequented in the 5 years preceding their MDR-TB diagnosis, including the detailed addresses of their residences, workplaces, and entertainment venues. In addition, we investigated changes in behavior following their diagnosis. Individuals with confirmed epidemiological links were defined as patients knew each other. Individuals with probable epidemiological links were defined as: cohabitating at the same address or complex or shared locations where transmission likely occurred, including in a neighborhood complex or street in the same district.

Statistical analysis

Participant demographic and clinical characteristics were collected from the TB management information system and the Beijing Chest Hospital clinical system. Statistical analyses were performed in R (version 4.1.1). The Chi-square test and Fisher's exact tests were used to compare differences between a cluster group and a unique group. Variables with p-value ≤ 0.5 on Chi-square test were entered into a multivariate logistic regression, which was used to assess possible associations of genomic clustering and estimate odds ratios (ORs) and 95% confidence intervals (CIs). Significance was determined by $p < 0.05$. Spatial analysis and visualization were performed in ArcGIS (version 10.2). We used kernel density estimation methods with Gaussian smoothing to analyze the aggregation of patients with MDR-TB.

We inferred transmission chains in clustered patients based on whole genome sequencing data. This study only inferred the transmission relationship for cluster size ≥ 4. We first used BEAST (version 2.6.6) to infer a timed phylogeny tree with the genomic sequencing data. This timed phylogeny tree was then used as input for the transmission tree inference using TransPhylo (https://github.com/xavierdidelot/TransPhylo) R package. We also predicted transmission probability based on the model (Supplementary materials).

Results

Study population and characteristics

9,012 individuals tested etiology-positive for Mycobacterium Tuberculosis (MTB) during 2018–2020, and 500 (5.55%) of these patients were classified as RR-TB by GeneXpert. After excluding culture-negative (12.80%), culture-positive non-RR-TB (8.6%), and missing culture or drug susceptibility (16.40%) cases, 311 (62.20%) individuals were confirmed to have RR-TB by phenotypic drug susceptibility testing; of these, 241 (77.49%) were MDR-TB. 159 (65.98%) MDR-TB cases were diagnosed and treated in the Beijing Chest Hospital and 146 (60.58%) had a clinical isolate suitable for analysis (Figure 1). The 146 individuals were representative of the whole diagnosed MDR-TB cohort in terms of age, sex, and internal migration status (defined as those who were not born in Beijing; home provinces were be inferred from the national identification number) (Supplementary Table 1).

The general characteristics of MDR-TB cases are summarized in Table 1. Among the 146 participants, 104 (71.23%) were male, with a median age of 44 years [interquartile range (IQR), 28–56 years]. 17.12% (25/146) were retired and 16.44% (24/146) were unemployed. New cases accounted for 52.74% (77/146) of all cases. 57(39.04%) patients were classed as internal migrants. Internal migrants with MDR-TB (31[27–43]) were significantly younger than MDR-TB local residents (53[36–59]) ($p < 0.001$). 50.88% (29/57) of internal migrants had resided in Beijing for <5 years (determined based on residence time at the address provided at the time of diagnosis). A greater proportion of migrants worked in retail (17.54% vs. 2.25%; $p < 0.001$).

Whole-genome sequencing analysis

A maximum likelihood phylogeny tree was constructed for the 146 MDR-TB strains (Figure 2). The average SNP pairwise distances between strains were 358 $\pm$ 243 SNPs (Supplementary Figure 1). Most of the MTB isolates belonged to lineage 2.3 ($n = 111$; 76.03%), followed by lineage 2.2 ($n = 30$; 20.55%), and lineage 4 ($n = 5$; 3.42%). The tree also displays the resistance profile for 11 anti-TB drugs based on the presence of validated resistance-conferring mutations (Supplementary Table 2). 54.79% (80/146) were classified as fluoroquinolones (FQs) resistant; among new cases of MDR-TB, the resistance ratio to any FQs was 39.74%. Considering that FQs resistance may be caused by the use of FQs between the time of diagnosis by GeneXpert and the sampling time of culture-positive strains, we descripted the sampling time distribution of the strains (Supplementary Figure 2). We found that the average time interval between diagnosis and sampling was 24 days. After excluding 21 (14.38%) cases with a delay > 30 days, the proportion of new cases harboring FQs resistance mutations was 40.29%, indicating that we could assume that any bias associated with sampling delay was minimal and could be disregarded. In addition, 43(29.45%) strains had compensatory mutations in rpoA, rpoB, or rpoC genes; rpoC V483G and rpoC V483A were the most frequent mutations (Supplementary Figure 3).

36 (24.66%) strains were grouped into 12 genome clusters, whose group sizes ranged from two to eight cases, suggesting recent transmission of MDR strains. The presence of MDR-TB among new cases suggested transmission of MDR strains. If the cases of MDR-TB among new cases were combined with those in

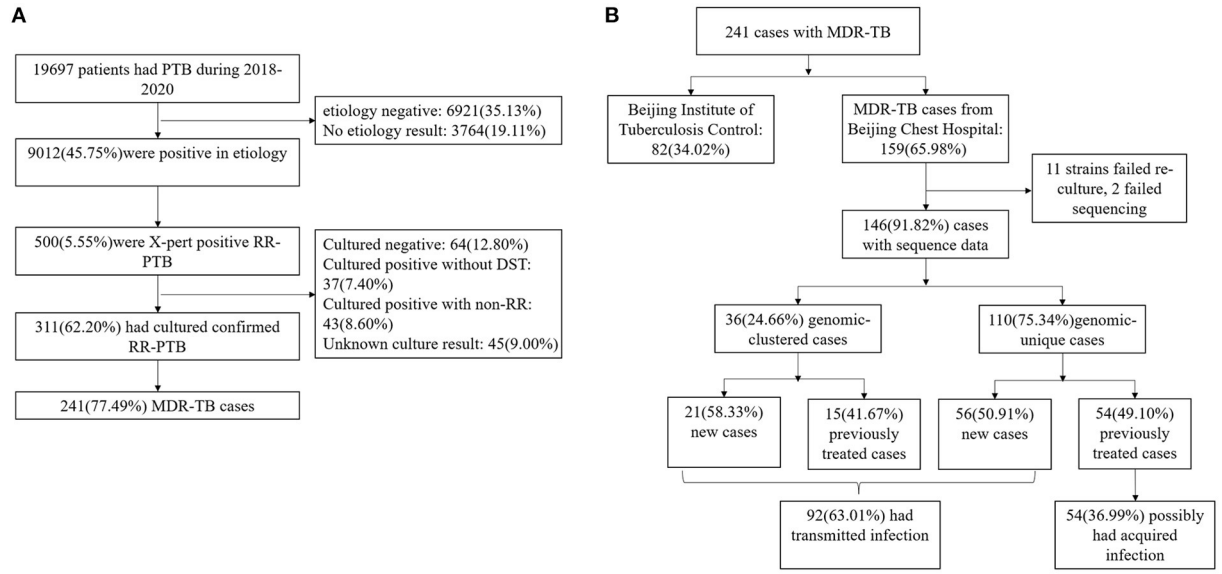

FIGURE 1
(A) Sample enrollment of MDR-TB. **(B)** Classification of MDR-TB based on treatment history and genomic analysis.

the genomic clusters, 63.01% (92/146) of cases were likely caused by the transmission of MDR strains (Figure 1B).

Geographic distribution of MDR-TB

The MDR-TB patient geographic distribution was heterogeneous. The majority of cases aggregated in central urban areas; Chaoyang District was the source of the largest number of cases, followed by Fengtai and Tongzhou Districts. Kernel density analysis of residents and internal migrants showed two distinct spatial distributions. Resident cases aggregated in central urban areas, while internal migrants were dispersed distributed around the urban area (Figure 3).

Of the 12 clusters, 7 were resident-only clusters and 2 were migrant-only clusters. 3 mixed clusters indicated that MDR-TB transmission had occurred between residents and migrants. We noted spatial aggregation within most resident-only clusters (3, 5, 6, 11, and 12) with hot spots in downtown and suburban areas. Individuals in migrant-only clusters (9, 10) and mixed clusters (1, 8), except for cluster 4 tended to reside in different districts (Figure 3D).

Epidemiologic survey of the study population

Of the 146 cases, there were nine deaths and 84 (57.53%) agreed to be interviewed by telephone or in person. We managed to establish probable epidemiological links for 15 cases, before their MDR-TB diagnosis. 9 individuals lived in the same community as other cases, and 15 had utilized the same entertainment venues, such as restaurants and supermarkets. Only 4 patients reported confirmed epidemiological links with others (all are friends). After the diagnosis of MDR-TB, 22.62% (19/84) continued to frequent public places and 9 were $\geq$ 55 years old. In the personal habit survey, 44.44% (24/54) stated that they would not cover their mouth during coughing and sneezing, and 16.32% (8/49) said they would expectorate sputum when necessary.

Inferring transmission chains of clustered cases

Overall, 29 (80.55%) of 36 individuals completed the survey, and seven refused or were lost to follow-up. Confirmed or probable epidemiological links were identified in 13 (44.82%) of 29 cases. 13 (44.82%) lived in the same or adjacent residential community, or on the same neighborhood street (distance $\leq$ 3 km), or shared public facilities such as restaurants. Eight of the clustered patients were internal migrants. Two cases came from the same county where the transmission likely occurred. Other migrants traveled to Beijing from different provinces, suggesting that local transmission likely occurred after arrival in Beijing (Supplementary Table 3).

We then focused on clusters 3, 4, and 12, each of which had a cluster size of $\geq$4, using TransPhylo. In cluster 3, Y1 and Y2 lived in neighborhood communities, and often attended the same restaurants near their homes.

TABLE 1 Demographic, clinical, and bacteriological characteristics of internal migrant patients and resident patients diagnosed in Beijing.

	Total ($n = 146$)	Migrant patients ($n = 57$)	Resident patients ($n = 89$)	*P*-value
Sex				0.980
Male	104 (71.23%)	41 (70.93%)	63 (70.79%)	
Female	42 (28.77%)	16 (28.07%)	26 (29.21%)	
Age group				<0.001
15–24	15 (10.27%)	9 (15.79%)	6 (6.74%)	
25–34	40 (27.40%)	26 (45.61%)	14 (15.73%)	
35–44	24 (16.44%)	9 (15.79%)	15 (16.85%)	
45–54	19 (13.01%)	6 (10.53%)	13 (14.61%)	
≥55	38 (26.03%)	7 (12.28%)	41 (46.07%)	
Residence years				<0.001
<5	29 (19.86%)	29 (50.88%)	0 (0.00%)	
5–10	20 (13.70%)	20 (35.09%)	0 (0.00%)	
≥10	97 (66.44%)	8 (14.04%)	89 (100.00%)	
Occupation				<0.001
Farmers	17 (11.64%)	5 (8.77%)	12 (13.48%)	
Workers	13 (8.90%)	3 (5.26%)	10 (11.24%)	
Retired people	24 (16.44%)	3 (5.26%)	21 (23.60%)	
Unemployed	25 (17.12%)	8 (14.04%)	17 (19.10%)	
Retail workers	12 (8.22%)	10 (17.54%)	2 (2.25%)	
Others	55 (37.67%)	28 (49.12%)	27 (30.34%)	
TB history*				0.486
New case	77 (52.74%)	33 (57.89%)	45 (50.56%)	
Previous treatment	69 (47.26%)	24 (42.11%)	44 (49.44%)	
Cough				0.259
Yes	87/116 (75.00%)	33/40 (82.50%)	54/76 (71.05%)	
No	29/116 (25.00%)	7/40 (17.50%)	22/76 (28.95%)	
Smear status				0.525
Positive	98 (67.12%)	36 (63.16%)	62 (69.66%)	
Negative	48 (32.88%)	21 (36.84%)	27 (30.34%)	
Putative compensatory mutation in rpoA, rpoB and rpoC				0.079
Yes	43 (29.45%)	22 (38.60%)	21 (23.60%)	
No	103 (70.55%)	35 (61.40%)	68 (76.40%)	
Genomic clustered				0.029
Yes	36 (24.65%)	8 (14.04%)	28 (31.46%)	
No	110 (75.34%)	49 (85.96%)	61 (68.54%)	

*Data from case management system of Beijing Chest Hospital.

We inferred that Y2 was a secondary case to Y1, with a transmission probability of 0.99. Individuals in cluster 4 lived in the neighborhood or the same communities, and Y6 and Y7 frequented restaurants near their homes. We found that Y5 first infected Y6, and Y6 further infected Y8 and his friend Y7. The probabilities of direct transmissions were > 0.6. The transmission chain provided supporting evidence for the epidemiological data. Cluster 12 included 8 cases; three lived in the same village and frequented the same restaurants. In addition, Y11 and Y15 worked on the same street, approximately 3.3 km apart. In the transmission chain of cluster 12, we confirmed the transmission relationship between Y11 and Y15. We also identified a casual transmission event between Y12 and Y16 without any apparent epidemiological link. Markedly, although there was an epidemiological link between Y10 and Y14, we found no direct transmission relationship, likely due to missing intermediary cases (Figure 4).

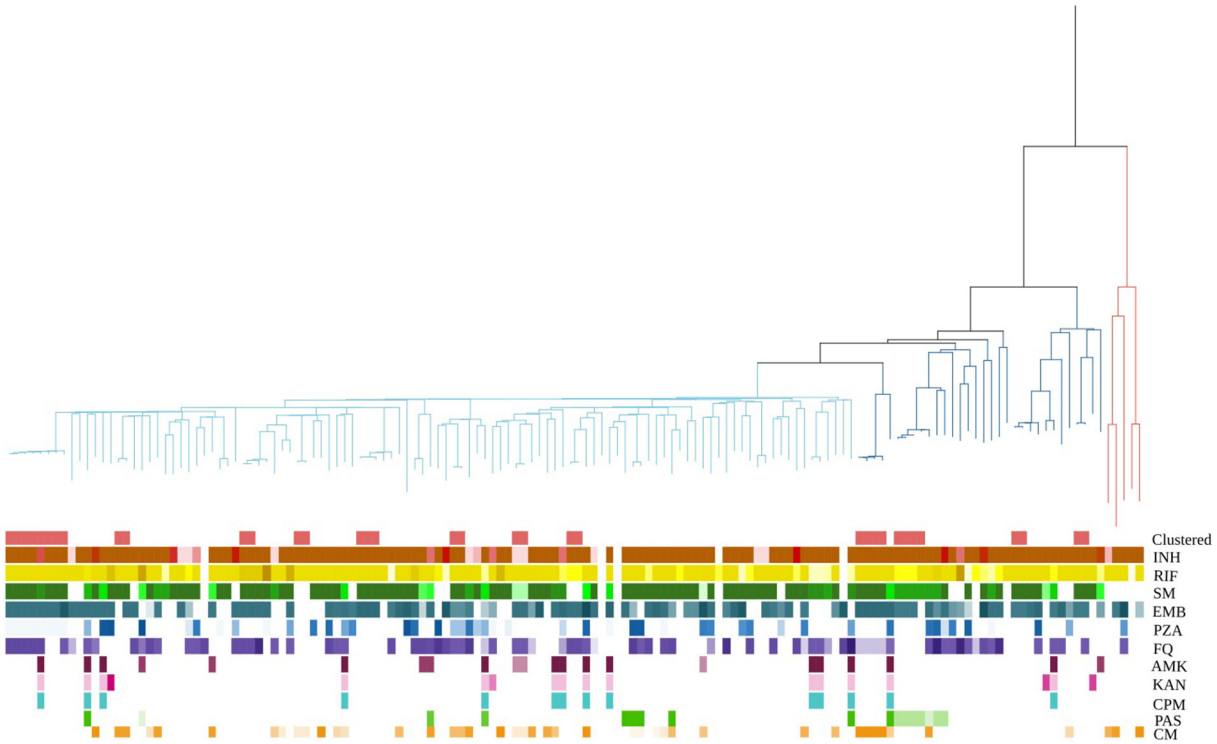

FIGURE 2
MDR-MTBC phylogeny and resistance mutations of isolates. First row showed whether it is clustered. Others show drug resistance associated mutations to first-line and second-line drugs (different mutations represented by different colors), and putative compensatory mutations in the RNA polymerase genes rpoA, rpoB and rpoC. MTBC lineage are differentiated into three clades. Red, blue, and cyan branches indicated lineage 4, ancient Beijing, and modern Beijing strains, respectively. INH, isoniazid; RIF, rifampicin; SM, streptomycin; EMB, ethambutol; Z PZA, pyrazinamide; FQ, fluoroquinolone; AMK, amikacin; KAN, kanamycin; CPM, capreomycin; PAS, para aminosalicylic acid; CM, compensatory mutations.

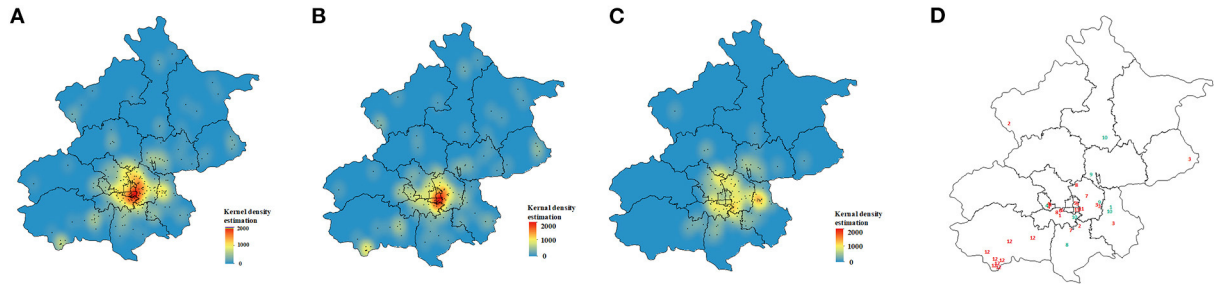

FIGURE 3
Spatial distribution of multidrug-resistant tuberculosis cases. **(A)** Kernel density estimation for all 241 patients, **(B)** resident patients and **(C)** migrant patients. **(D)** Spatial distribution of genetic clusters; red numbers represent resident patients and green numbers represent migrant patients.

Risk factors for recent transmission

We compared groups to identify the risk factors associated with the recent transmission of MDR-TB. The proportion of female was significantly higher among clustered cases than in unique cases (44.44% vs. 23.64%; $p = 0.029$), and residents had a higher proportion than internal migrants (31.04% vs. 14.64%; $p = 0.029$) (Supplementary Table 4). In the multivariate logistic analysis, the age group 25–34 had the highest risk for recent transmission (OR, 3.19; 95% CI, 1.14–9.35). As the number of cases was small, we did not identify any other significant factors (Table 2).

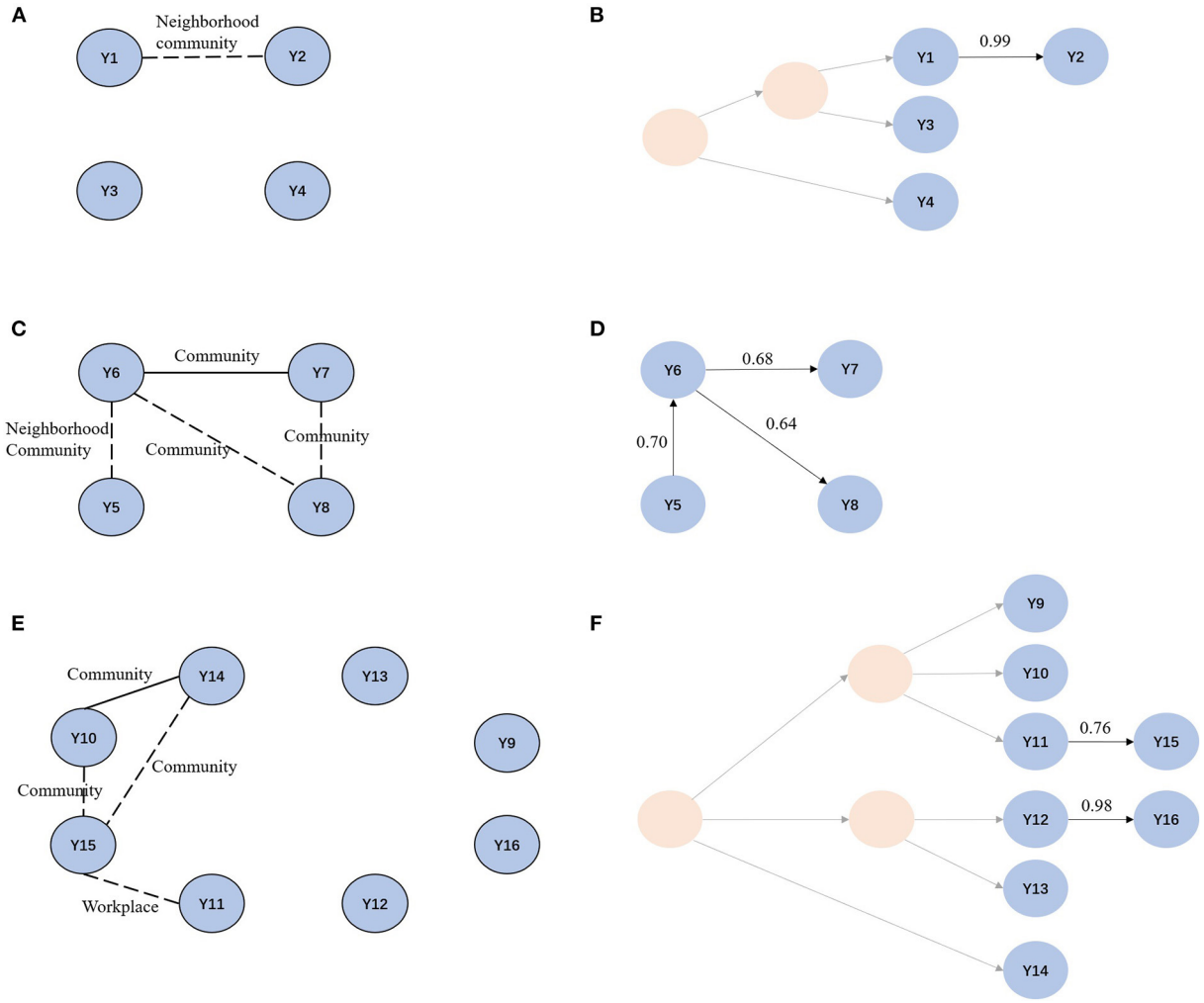

FIGURE 4
Social network and transmission chain for cluster 3, cluster 4 and cluster 12. **(A, C, E)** Social network for cluster 3, cluster 4, and cluster 12. Solid line indicates confirmed epidemiological links. Dotted line indicates probable epidemiological links. **(B, D, F)** Transmission chain for cluster 3, cluster 4 and cluster 12. Light yellow circles represent missing samples.

Discussion

We combined whole-genome sequencing, geospatial analysis, and epidemiological investigation to characterize the transmission of MDR-TB during a 3-year interval in Beijing, China. We inferred that approximately 63% of MDR-TB cases were due to transmission. 44.82% of the clustered patients were co-infected with others in residential communities and related public facilities. Based on reconstructed transmission networks, we identified 6 transmission events in three clusters; of these, four transmission events occurred in and near residential areas.

24.66% of our MDR-TB cases were attributable to recent transmission, a higher proportion than that reported in low-burden countries such as the US (18), but with little difference from Chinese regions such as Shenzhen (19) (25%) and Shanghai (4) (32%). The WHO pointed out that the detection rate of MDR-TB in China (27%) was much lower than the global average (44%) (1), suggesting that many cases are undetected and treated. This further implies that the transmission of MDR-TB is likely more extensive than seen in the present study.

Fluoroquinolones are one of the key antituberculosis drugs for MDR/RR-TB treatment (20). We found resistance to any FQs of up to 40% among new MDR-TB cases in our study. The proportion was higher than the national average (1, 21). But others have reported an even higher resistance to the FQs drug moxifloxacin at 53.9%, in the eastern region of China (22). FQs are broad-spectrum antibiotics and one of the most commonly prescribed classes of antibiotics in China, patients may be exposed to FQs to treat other microbial infections leading to resistance acquisition. It has been reported that Beijing is one

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TABLE 2 Multivariable logistic regression on the risk factors of multidrug resistance clustering.

	Total	Multivariable OR (95% CI)	*p*-value
Sex			
Male	104 (71.23%)	Ref	–
Female	42 (28.77%)	2.19 (0.90–5.32)	0.081
Age group			
25–34	40 (27.40%)	3.19 (1.14–9.35)	0.028
others	106 (72.60%)	Ref	–
Residence years			
<10	49 (33.56%)	Ref	–
≥10	97 (66.44%)	3.38 (0.738–23.23)	0.225
Birth in Beijing	89 (60.95%)	1.53 (0.28–12.21)	0.643
Farmers	17 (11.64%)	2.50 (0.75–8.11)	0.126
Smear positive	98 (67.12%)	1.95 (0.76–5.43)	0.179

of the largest regions for antibiotic use in China (23), which creates a greater risk for resistance to FQs in TB patients in this region. However, in most areas of China, including Beijing, drug susceptibility testing is only performed for parts of FQs drugs (22). Therefore, we caution that the use of FQs drugs as part of second-line regimens should be approached with caution, and FQs drugs susceptibility testing, especially molecular drug susceptibility testing, should be recommended in the national tuberculosis guidelines.

Internal migrants are thought to play an important role in TB transmission (24–26). For example, TB in the Baoan District of Shenzhen principally stems from the reactivation of infections acquired by migrants in their home provinces (27). However, our results showed that the clustering rate among local residents was higher than among internal migrants, suggesting that the former played a major role in MDR-TB transmission, especially in the central urban area of Beijing. We speculated that this is attributable to local living conditions and the structure of the population. First, the central urban area had a higher population density and housing costs than the surrounding areas (10), resulting in a large crowded living environment, that likely contributed to recent transmission. In addition, most residents in the central area were middle-aged and elderly, and had more limited social circles and places of recreation; this may explain why recent transmission primarily occurred among residents of the central area. Compared with the spatial aggregation of resident-only clusters in urban centers, we found more dispersed spatial patterns for migrant-only and mixed clusters, which means that transmission events could occur through more casual contact in some settings, such as on public transportation, or in entertainment venues and hospitals (28).

Close household contact was a significant risk factor for TB transmission, but most TB cases were not attributable to household contacts in high-incidence settings (29). None of the patients in this study were in close household contact with TB patients. However, 44.82% of the patients lived in the same or adjacent residential community or street with other individuals. In addition, we identified six transmission events by reconstructing transmission networks; four occurred in and near residential areas. This suggested that residential areas and nearby public places were vitally important for TB transmission. A study of TB in Shanghai reached similar conclusions that transmission events were observed in game rooms near residential areas (4). As such, we need to place a greater emphasis on residential communities as potential areas of TB transmission, because the majority of individuals exposed to small risks can account for more cases than a few individuals exposed to large risks. However, residential communities, including supermarkets, restaurants, entertainment facilities, etc., are locations in which the implementation of prevention and control measures is logistically difficult if not impossible. Therefore, the need for further research on the characteristics of TB transmission in residential communities remains.

Patient management is an important component of disease control and prevention because the probability of transmission between a susceptible and an infectious person is significantly influenced by the behaviors of the infected individual. For example, covering of the mouth and nose during breathing, coughing, or sneezing can reduce TB transmission in gathering places (30). However, in our survey, 44% of our cohort reported inappropriate behaviors such as coughing without covering their mouths and spitting in public. These behaviors and relatively lax self-isolation protocols can increase the risk of infection of uninfected people. Therefore, we need to improve the education of patients and the wider public to help control transmission.

Our study has some limitations. First, our ability to infer MDR-TB transmission patterns in Beijing depended on how

complete our sampling of MTB isolates was during the study. Not all registered cases were culture-positive and we only included 60% of MDR-TB patients in Beijing. The absence of some cases might have masked some transmission events and epidemiological relationships. Second, the retrospective study design and limited timeframe made it difficult to fully cover all MDR-TB cases and strains in the population. Third, we might have missed cases among migrants who returned to their home province for treatment, potentially leading to an underestimation of the role of the migrants in disease transmission.

Taken together, the transmission of MDR-TB played an important role in the burden of MDR-TB. We found that residential areas were common transmission sites, and further work is needed to properly understand the characteristics of TB transmission in community settings. In a setting with high-level FQs drug resistance in MDR-TB patients, FQs should be used with caution when treating drug-resistant patients unless susceptibility can be confirmed by molecular or phenotypic methods, to reduce the burden of MDR-TB.

Data availability statement

The datasets presented in this study can be found in the National Center for Biotechnology Information BioProject Database (https://www.ncbi.nlm.nih.gov/bioproject/), accession number PRJNA888557.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Review Board of Beijing Chest Hospital (No. LW2022-005). The patients/participants provided their written informed consent to participate in this study.

Author contributions

WL and XH conceived, designed, and supervised the study. XH, ZG, and HZ collected the data. JY and LQ cleaned the data. JY and CZ analyzed the data. JY wrote the drafts of the manuscript. WL, HJ, and QG interpreted the findings, commented, and revised the drafts of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1019198/full#supplementary-material

References

1. World Health Organization. *Global Tuberculosis Report 2020*. Geneva: World Health Organization (2020).

2. Kendall EA, Fofana MO, Dowdy DW. Burden of transmitted multidrug resistance in epidemics of tuberculosis: a transmission modelling analysis. *Lancet Respir Med.* (2015) 3:963–72. doi: 10.1016/S2213-2600(15)00458-0

3. Shah NS, Auld SC, Brust JC, Mathema B, Ismail N, Moodley P, et al. Transmission of extensively drug-resistant tuberculosis in South Africa. *N Engl J Med.* (2017) 376:243–53. doi: 10.1056/NEJMoa1604544

4. Yang C, Luo T, Shen X, Wu J, Gan M, Xu P, et al. Transmission of multidrug-resistant Mycobacterium tuberculosis in Shanghai, China: a retrospective observational study using whole-genome sequencing and epidemiological investigation. *Lancet Infect Dis.* (2017) 17:275–84. doi: 10.1016/S1473-3099(16)30418-2

5. Walker TM, Lalor MK, Broda A, Ortega LS, Morgan M, Parker L, et al. Assessment of Mycobacterium tuberculosis transmission in Oxfordshire, UK, 2007–12, with whole pathogen genome sequences: an observational study. *Lancet Respir Med.* (2014) 2:285–92. doi: 10.1016/S2213-2600(14)70027-X

6. Klinkenberg D, Backer JA, Didelot X, Colijn C, Wallinga J. Simultaneous inference of phylogenetic and transmission trees in infectious disease outbreaks. *PLoS Comput Biol.* (2017) 13:e1005495. doi: 10.1371/journal.pcbi.1005495

7. Didelot X, Fraser C, Gardy J, Colijn C. Genomic infectious disease epidemiology in partially sampled and ongoing outbreaks. *Mol Biol Evol.* (2017) 34:997–1007. doi: 10.1093/molbev/msw275

8. Xu Y, Cancino-Munoz I, Torres-Puente M, Villamayor LM, Borras R, Borras-Manez M, et al. High-resolution mapping of tuberculosis transmission: whole genome sequencing and phylogenetic modelling of a cohort from Valencia Region, Spain. *PLoS Med.* (2019) 16:e1002961. doi: 10.1371/journal.pmed.1002961

9. Yang C, Lu L, Warren JL, Wu J, Jiang Q, Zuo T, et al. Internal migration and transmission dynamics of tuberculosis in Shanghai, China: an epidemiological, spatial, genomic analysis. *Lancet Infect Dis.* (2018) 18:788–95. doi: 10.1016/S1473-3099(18)30218-4

10. Beijing Municipal Bureau of Statistics. *Beijing Statistical Yearbook 2021*. Beijing: Beijing Municipal Bureau of Statistics (2021).

11. Jiang H, Liu M, Zhang Y, Yin J, Li Z, Zhu C, et al. Changes in incidence and epidemiological characteristics of pulmonary tuberculosis in Mainland China, 2005–2016. *JAMA Netw Open.* (2021) 4:e215302. doi: 10.1001/jamanetworkopen.2021.5302

12. Shen X, Xia Z, Li X, Wu J, Wang L, Li J, et al. Tuberculosis in an urban area in China: differences between urban migrants and local residents. *PLoS ONE.* (2012) 7:e51133. doi: 10.1371/journal.pone.0051133

13. Churchyard G, Kim P, Shah NS, Rustomjee R, Gandhi N, Mathema B, et al. What we know about tuberculosis transmission: an overview. *J Infect Dis.* (2017) 216:S629–35. doi: 10.1093/infdis/jix362

14. Schiebelhut LM, Abboud SS, Daglio LG, et al. A comparison of DNA extraction methods for high-throughput DNA analyses. *Mol Ecol Resour.* (2016) 17:721–9. doi: 10.1111/1755-0998.12620

15. Coll F, McNerney R, Guerra-Assuncao JA, Glynn JR, Perdigao J, Viveiros M, et al. A robust SNP barcode fortyping Mycobacterium tuberculosis complex strains. *Nat Commun.* (2014) 5:4812. doi: 10.1038/ncomms5812

16. Luo T, Comas I, Luo D, Lu B, Wu J, Wei L, et al. Southern East Asian origin and coexpansion of Mycobacterium tuberculosis Beijing family with Han Chinese. *Proc Natl Acad Sci U S A.* (2015) 112:8136–41. doi: 10.1073/pnas.1424063112

17. Walker TM, Miotto P, Köser CU, Fowler PW, Knaggs J, Iqbal Z, et al. The 2021 WHO catalogue of Mycobacterium tuberculosis complex mutations associated with drug resistance: a genotypic analysis. *Lancet Microbe.* (2022) 3:e265–73. doi: 10.1016/S2666-5247(21)00301-3

18. Moonan PK, Teeter LD, Salcedo K, Ghosh S, Ahuja SD, Flood J, et al. Transmission of multidrug-resistant tuberculosis in the USA: a cross-sectional study. *Lancet Infect Dis.* (2013) 13:777–84. doi: 10.1016/S1473-3099(13)70128-2

19. Jiang Q, Liu Q, Ji L, Li J, Zeng Y, Meng L, et al. Citywide transmission of multidrug-resistant tuberculosis under China's rapid urbanization: a retrospective population-based genomic spatial epidemiological study. *Clin Infect Dis.* (2020) 71:142–51. doi: 10.1093/cid/ciz790

20. World Health Organization. *WHO Consolidated Guidelines on Tuberculosis. Module 4: Treatment - Drug-Resistant Tuberculosis Treatment*. Geneva: World Health Organization (2020).

21. Zhao Y, Xu S, Wang L, Chin DP, Wang S, Jiang G, et al. National survey of drug-resistant tuberculosis in China. *N Engl J Med.* (2012) 366:2160–70. doi: 10.1056/NEJMoa1108789

22. Xia H, Zheng Y, Liu D, Wang S, He W, Zhao B, et al. Strong increase in moxifloxacin resistance rate among multidrug-resistant mycobacterium tuberculosis isolates in China, 2007 to 2013. *Microbiol Spectr.* (2021) 9:e0040921. doi: 10.1128/Spectrum.00409-21

23. Wushouer H, Zhou Y, Zhang X, Fu M, Fan D, Shi L, et al. Secular trend analysis of antibiotic utilisation in China's hospitals 2011-2018, a retrospective analysis of procurement data. *Antimicrob Resist Infect Control.* (2020) 9:53. doi: 10.1186/s13756-020-00709-6

24. Kamper-Jørgensen Z, Andersen AB, Kok-Jensen A, Kamper-Jorgensen M, Bygbjerg IC, Andersen PH, et al. Migrant tuberculosis: the extent of transmission in a low burden country. *BMC Infect Dis.* (2012) 12:60. doi: 10.1186/1471-2334-12-60

25. Wang W, Wang J, Zhao Q, Darling ND, Yu M, Zhou B, et al. Contribution of rural-to-urban migration in the prevalence of drug resistant tuberculosis in China. *Eur J Clin Microbiol Infect Dis.* (2011) 30:581–6. doi: 10.1007/s10096-010-1125-6

26. Pareek M, Greenaway C, Noori T, Munoz J, Zenner D. The impact of migration on tuberculosis epidemiology and control in high-income countries: a review. *BMC Med.* (2016) 14:48. doi: 10.1186/s12916-016-0595-5

27. Yang T, Wang Y, Liu Q, Jiang Q, Hong C, Wu L, et al. A population-based genomic epidemiological study of the source of tuberculosis infections in an emerging city: Shenzhen, China. *Lancet Reg Health West Pac.* (2021) 8:100106. doi: 10.1016/j.lanwpc.2021.100106

28. Yates TA, Khan PY, Knight GM, Taylor JG, McHugh TD, Lipman M, et al. The transmission of Mycobacterium tuberculosis in high burden settings. *Lancet Infect Dis.* (2016) 16:227–38. doi: 10.1016/S1473-3099(15)00499-5

29. Martinez L, Shen Y, Mupere E, Kizza A, Hill PC, Whalen CC. Transmission of mycobacterium tuberculosis in households and the community: a systematic review and meta-analysis. *Am J Epidemiol.* (2017) 185:1327–39. doi: 10.1093/aje/kwx025

30. Fox GJ, Redwood L, Chang V, Ho J. The effectiveness of individual and environmental infection control measures in reducing the transmission of mycobacterium tuberculosis: a systematic review. *Clin Infect Dis.* (2021) 72:15–26. doi: 10.1093/cid/ciaa719

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Untargeted metabolomics of pulmonary tuberculosis patient serum reveals potential prognostic markers of both latent infection and outcome

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Currently, there are no particularly effective biomarkers to distinguish between latent tuberculosis infection (LTBI) and active pulmonary tuberculosis (PTB) and evaluate the outcome of TB treatment. In this study, we have characterized the changes in the serum metabolic profiles caused by *Mycobacterium tuberculosis* (Mtb) infection and standard anti-TB treatment with isoniazid–rifampin–pyrazinamide–ethambutol (HRZE) using GC-MS and LC-MS/MS. Seven metabolites, including 3-oxopalmitic acid, akeboside ste, sulfolithocholic acid, 2-decylfuran (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol, d-(+)-camphor, and 2-methylaminoadenosine, were identified to have significantly higher levels in LTBI and untreated PTB patients (T0) than those in uninfected healthy controls (Un). Among them, akeboside Ste and sulfolithocholic acid were significantly decreased in PTB patients with 2-month HRZE (T2) and cured PTB patients with 2-month HRZE followed by 4-month isoniazid-rifampin (HR) (T6). Receiver operator characteristic curve analysis revealed that the combined diagnostic model showed excellent performance for distinguishing LT from T0 and Un. By analyzing the biochemical and disease-related pathways, we observed that the differential metabolites in the serum of LTBI or TB patients, compared to healthy controls, were mainly involved in glutathione metabolism, ascorbate and aldarate metabolism, and porphyrin and chlorophyll metabolism. The metabolites with significant differences between the T0 group and the T6 group were mainly enriched in niacin and nicotinamide metabolism. Our study provided more detailed experimental data for developing laboratory standards for evaluating LTBI and cured PTB.

KEYWORDS

latent TB infections (LTBI), tuberculosis, biomarkers, metabolomics, GC-MS, LC-MS/MS

Introduction

Tuberculosis (TB) is one of the most common communicable diseases worldwide, until the coronavirus, TB was the main cause of death from a single infectious agent. According to the WHO Global TB Report 2021, about 1.5 million people died from TB in 2020. A quarter of the global population was latently infected with *Mycobacterium tuberculosis* (Mtb) but remains asymptomatic, 5–15% of those individuals are likely to develop active TB (1). Early diagnosis has been recognized as the pillar to achieve the end of the TB epidemic; thus, the development of accurate, rapid, and easy diagnostic tools to improve diagnosis is required urgently.

Currently, the diagnosis of TB cases mainly depends on medical history, physical examination, imageological examination, and other laboratory tests. The traditional methods demonstrate some limitations in determining whether patients with TB are cured, as well as in identifying TB from latent tuberculosis infection. About 85% of TB patients can be successfully treated with a standardized 6-month anti-TB treatment regimen (2-month intensive phase plus a 4-month continuation phase) (2). However, due to the lack of a rapid and accurate method to evaluate the anti-TB efficacy, ∼14% of discharged patients are not completely cured (3). A major cause resulting from interrupted treatment is the development of drug-resistant TB, it also increases the risk of TB transmission and spread in a community (4). Besides the cured TB patients, no well-validated or specific biomarkers can differentiate effectively latent tuberculosis infection from active TB. The WHO recommends that an interferon (IFN)-γ release assay (IGRA) or a tuberculin skin test (TST) can be used to screen for TB infection (5). However, neither the IGRAs nor the TST can discriminate between latent tuberculosis infection and active TB (5–7). Consequently, a panel of rapidly measured biomarkers with high diagnostic accuracy is crucial for global TB control.

Metabolites are small molecules that represent ongoing biological processes and may provide insights into the mechanisms that underlie the disease process as well as disease progression (8). Due to their special characteristics, metabolites have become potential disease biomarkers for disease (9, 10). In recent years, metabonomics has been widely used in disease research because it provides a more precise method to detect changes in metabolism (11). In a recent study, a 4-differential metabolite in combination can be used as a potential biomarker to cure TB (3). In further a prospective multisite study across Subsaharan Africa, a trans-African metabolic biosignature for TB was found to predict the progression of TB at 69% sensitivity and 75% specificity on blinded test samples and in external data sets. Among the main analytical methods, mass spectrometry (MS) displays its high sensitivity and high throughput and has been widely used for identifying specific metabolites (13, 14). Currently, metabolomics technology is still not perfect, it is not yet possible to detect all compounds with one technology and different detection platforms are required. LC-MS/MS is commonly used for detailed analysis of natural compounds, such as serum, plasma, urine, and disease samples. GC-MS has an advantage over LC-MS/MS in the analysis of volatile and thermally stable metabolites. Therefore, LC-MS/MS combined with GC-MS could identify significantly altered metabolites as comprehensively as possible.

Here, we performed a well-powered, untargeted TB-associated serum metabolomics assessment by integrating the GC-MS and LC-MS/MS assays. We investigated the impact of Mtb infection on the serum metabolome and characterized the changes induced by front-line TB antibiotics on the composition of the serum metabolome at different time points, to provide a set of candidates for predicting the Mtb infection and potential outcome.

Materials and methods

Subjects in this study and sample collection

The serum samples used in this study were collected from the Major Infectious Disease Prevention and Control of the National Science and Technique Major Project and preserved by the Biobank of the Center for Tuberculosis Control of Guangdong Province. The volunteers in this study were enrolled and subjected to the analysis using the IFN-γ release assay (QuantiFERON-TB Gold In-Tube (QFT), Qiagen, CA, USA) along with clinical, microbiological, and radiographical examinations. The criteria for enrollment were as follows: (1) patients with active TB (ATB group in this study) showed clinical and radiographical features of tuberculosis and were confirmed by sputum smear or culture. Moreover, they did not receive anti-TB treatment before sample selection. (2) Standard anti-TB treatment includes RIF, INH, PZA, and EMB for 2 months, followed by RIF and INH for additional 4 months. After 6 month-treatment, PTB patients were considered cured if they meet the following conditions: chest X-ray or CT examination of TB symptoms disappeared, negative sputum test or sputum smear positive TB patients turned negative and symptoms improved. All active TB patients receive periodic follow-up appointments while on treatment. Exclusion criteria included a history of antibiotic or probiotic treatment more than 1 week within the previous 8 weeks. (3) All the controls (IGRA- and IGRA+) were with TB-resembling coughing symptoms and normal X-rays but were culture-negative and without other clinical TB symptoms and TB contact history. The clinical characteristics of the groups are given in Table 1 and Supplementary Table 1. All subjects with diabetes mellitus, HIV infection, hepatitis B, metabolic diseases, autoimmune diseases, and malignant tumors were excluded. Peripheral blood samples were obtained by venipuncture from all subjects and collected

in an aseptic vacuum blood collection tube and stored at 2∼8°C. The serum was centrifugated, collected, and stored in an −80°C refrigerator for cryopreservation. This study was performed in compliance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Center for Tuberculosis Control of Guangdong Province, China. Written informed consent was obtained from all subjects before blood sample collection.

LC-MS/MS metabolite extraction

Extraction of serum metabolites for LC-MS/MS analysis was performed as previously described (15). Briefly, another 100 μL of the above serum samples were taken and mixed with 400 μL prechilled methanol by well vortexing. The samples were incubated on ice for 5 min and then were centrifuged at 15,000 rpm, 4°C for 5 min. Some of the supernatants were diluted to a final concentration containing 60% methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube with a 0.22 μm filter and then were centrifuged at 15,000 *g*, 4°C for 10 min. A total of 60 μL of each sample were pipetted and mixed to form a QC sample. Finally, the filtrate was injected into the LC-MS/MS system analysis.

GC-MS metabolite extraction

Metabolite extraction for GC-MS analysis was performed according to previously published procedures with some modifications (16). A total of 100 μL serum sample was mixed with 300 μL prechilled methanol and 10 μL fluorophenylalanine, followed by vortexing and ultrasound concussion. The supernatant was carefully pipetted into a 1.5 mL EP tube (5). All the samples were dried completely in the vacuum concentrator without heating. A total of 60 μL Methoxyamination hydrochloride (20 mg/mL in pyridine) was added and incubated for 30 min at 80°C. A total of 80 μL of the BSTFA [N,O-Bis(trimethylsilyl) trifluoroacetamide] regent (1% TMCS, v/v) (Trimethylchlorosilane) was added and incubated at 70°C for 1.5 h. All the samples were analyzed by a gas chromatography system coupled with a Pegasus HT time-of-flight mass spectrometer (GC-MS). A total of 60 μL of each sample were pipetted and mixed to form a QC sample.

LC-MS/MS analysis

LC-MS/MS analysis was operated in both positive and negative ion modes with the parameters optimized according to previously published procedures with some modifications (17). LC-MS/MS analyses were performed using a Vanquish UHPLC system (Thermo Fisher) coupled with an Orbitrap Q Exactive series mass spectrometer (Thermo Fisher). Samples were injected into the Hyperil Gold column (100 × 2.1 mm, 1.9 μm) using a 16-min linear gradient at a flow rate of 0.2 mL/min. The eluents for the positive polarity mode were eluent A (0.1% FA in Water) and eluent B (Methanol). The eluents for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9.0) and eluent B (Methanol). The solvent gradient was set as follows: 1.5 min, 2% B; 12.0 min, 100% B; 14.0 min, 100% B; 14.1 min, 2% B; 16 min, 2% B. The flow rate was 0.2 mL/min. Q Exactive mass series spectrometer was operated in positive/negative polarity mode with a spray voltage of 3.2 kV, a capillary temperature of 320°C, a sheath gas flow rate of 35 arb, and an aux gas flow rate of 10 arb.

GC-MS analysis

Agilent 7,890 gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer was used for GC-MS analysis (16). The system utilized a DB-5MS capillary column coated with 5% diphenyl cross-linked with 95% dimethylpolysiloxane (30 m × 250 μm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). A 1 μL aliquot of the analyte was injected in splitless mode. Helium was used as the carrier gas, the front inlet purge flow was 3 mL/min, and the gas flow rate through the column was 1 mL/min. The initial temperature was kept at 50°C for 1 min, then raised to 310°C at a rate of 20°C min^{-1}, then kept for 6 min at 310°C. The injection, transfer line, and ion source temperatures were 280, 280, and 250°C, respectively. The energy was −70eV in electron impact mode. The mass spectrometry data were acquired in full-scan mode with the *m/z* range of 50–500 at a rate of 12.5 spectra per second after a solvent delay of 4.78 min.

Data processing and analysis

The raw data files of LC-MS/MS and GC-MS were processed by the software Compound Discoverer 3.1 (CD) (18) and Chroma TOF (V4.3X, LECO) (19), respectively. Initially, the data were performed peak alignment after filtrating by retention time and mass-to-charge ratio. Next, the exact molecular mass of the compounds utilized was determined by the mass-to-charge ratio in the high-resolution XIC charts. Meanwhile, the molecular formulas were predicted based on the mass deviation and adduct ion information. The metabolites in the biological system were identified by matching the fragment ion, collision energy, as well as other information of each compound, respectively, in the mzCloud database and LECO-Fiehn Rtx5 database. Subsequently, the compounds in the QC sample with a value of Coefficient of Variance (CV) <30% were applied as the final identification results for subsequent analysis. The Pearson correlation coefficient between

TABLE 1 Characteristic of pulmonary TB patients during the therapy, latent tuberculosis infection and healthy controls.

	Un	LT	T0	T2	T6	χ^2	*P*-value
Age, years range (mean ± SD)	20–53 34.8 ± 10.35	18–57 38.0 ± 10.64	18–59 35.76 ± 12.61	18–59 35.76 ± 12.6	18–59 35.76 ± 12.6		0.710[a]
Total Subjects (male)	15 (8)	16 (11)	17 (13)	17 (13)	17 (13)	1.967	0.374[b]

Un, Healthy controls; T0, Untreated PTB; T2, PTB with anti-TB chemotherapy for 2 months; T6, Cured PTB with anti-TB chemotherapy for 6 months; LT, Latent tuberculosis infection. Given that T0, T2, and T6 belonged to the same group of patients, the statistical analysis of age and sex differences among the multiple groups presented here was performed in Un, LT, and T0.

[a]One-way ANOVA test.

[b]Chi-square test.

QC samples was calculated based on the peak area value, the higher the correlation of QC samples, the better the stability of the whole detection process and the higher the data quality. The multivariate statistical methods, such as PCA and Partial Least Squares Discrimination Analysis (PLS-DA), were used to perform dimensionality reduction and regression analysis on the multi-dimensional data based on preserving the original information to the greatest extent. To annotate the function and classification of the identified metabolites, the databases KEGG (http://www.genome.jp/kegg/), HMDB (http://www.hmdb.ca/), and LIPIDMAPS (http://www.lipidmaps.org/) (20) were used in this study. Student's *t*-test was performed for parametric data between two groups. The statistical difference among multiple groups was analyzed using analysis of variance (ANOVA) for parametric data and the Kruskal-Wallis test for non-parametric data. A Chi-square test was performed for the composition ratios. The receiver operating characteristic curve (ROC) was drawn using SPSS (version 26.0, USA) software. $P \leq 0.05$ was considered significant.

Results

In this research, we enrolled 17 pulmonary TB patients without HRZE treatment (T0 group) and 31 healthy controls, including 15 volunteers without Mtb (Un group) and 16 volunteers with latent Mtb infection (LT group), to study the relationship between tuberculosis and serum metabolites. Furthermore, to detect the effect of periodic HRZE treatment, we tracked the serum metabolome changes in these TB patients with standard anti-TB therapy for 2 months (T2) and 6 months (T6), respectively. Detailed characteristics of recruited participants are shown in Table 1 and Supplementary Table 1.

Raw data pre-processing

We adopted an untargeted metabolomic approach using GC-MS and LC-MS/MS to cohorts of our serum samples. To study the variation of metabolites among different groups, we filtered out annotated peaks in the alignment table that presented in <50 % of the samples in each group. After this filtering, a total of 2,563 annotated peaks were commonly detected by all three platforms, while 1,456 annotated peaks by LC-MS/MS (+), 862 annotated peaks by LC-MS/MS (-), and 245 annotated peaks by GC-MS. These peaks were aligned using KEGG, HMDB, and LIPID MAPS databases to annotate the functional characteristics and classification of different metabolites. Data containing respective RT, relative log intensities, the variable importance in projection (VIP), and *P*-value was used for subsequent group comparisons.

Active TB can affect human serum metabolome

To assess the effect of Mtb infection on serum metabolome, the metabolites in serum samples were analyzed among the Un, LT, and T0 groups. To separate the important features of significant differences between these three groups, the PLS-DA model was used to eliminate the overfitting of test models and evaluate the statistical significance of the models. The PLS-DA score plots showed that the characteristics of metabolites were able to clearly distinguish the T0 group from the LT group (Figures 1A,E,I) and the Un group (Supplementary Figure 1). The results of the permutation test of the PLS-DA model showed that the LT group and Un group (Figures 1B,F,J) demonstrated a robust prediction performance and no overfitting phenomenon, as well as in the LT group and the T0 group (Supplementary Figure 1).

To further determine which metabolites were significantly affected by active Mtb, we integrated the LC-MS/MS and GC-MS metabolomics data to investigate significant features among the Un group, LT group, and T0 group. Differential metabolites were defined as those that showed a fold change >2.0 or <0.50 in relative abundance and a *p*-value < 0.05. Based on these criteria, there were 160 metabolites with different abundance between the LT group and the T0 group (Supplementary Table 2), and 254 metabolites between the T0 group and Un group (Supplementary Table 3), respectively. In comparison between the LT group the and T0 group, 77 metabolites were upregulated (>2-fold) and 83 metabolites were downregulated (<0.50-fold) in the LT group. There were

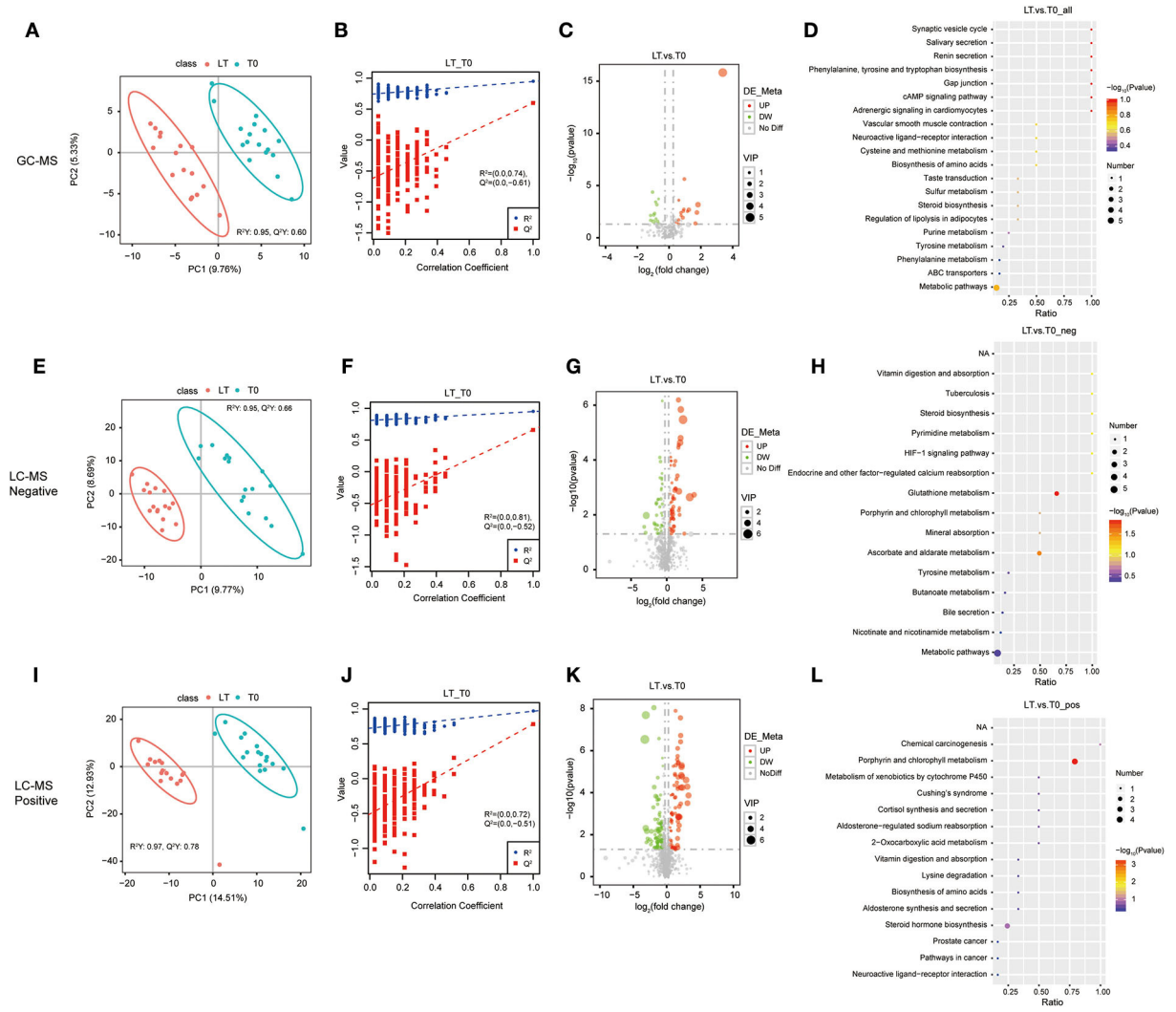

FIGURE 1
Identification of serum metabolites between latent tuberculosis infection (LT) and the untreated PTB group (Un). The PLS-DA model for the LT/Un group in GC-MS **(A)**, LC-MS/MS (−) **(E)**, and LC-MS/MS (+) **(I)**. The permutation test results for the LT/Un group in GC-MS **(B)**, LC-MS/MS (−) **(F)**, and LC-MS/MS (+) **(J)**. Volcanic map of differential metabolites for the LT/Un group in GC-MS **(C)**, LC-MS/MS (−) **(G)**, and LC-MS/MS (+) **(K)**. The abscissa: the fold change of LT/Un group (base 2 logarithm). The ordinate: the *P*-value of LT/Un group (base 10 logarithm). Red: significantly upregulated metabolites. Green: significantly downregulated metabolites. Gray: non-significant differential metabolites. Pathway analysis of differential metabolites for the LT/Un group in GC-MS **(D)**, LC-MS/MS **(-)** **(H)**, and LC-MS/MS (+) **(L)**.

124 upregulated metabolites (>2-fold) and 128 downregulated metabolites (<0.5-fold) in the T0 group when compared with the Un group. The differential metabolites between the LT group and the T0 group (Figures 1C,G,J), the Un group, and the T0 group (Supplementary Figure 1) were further illustrated in a volcano plot. Finally, we performed the pathway enrichment analysis for the selected metabolites. Metabolic pathway analysis [GC-MS, LC-MS/MS (+) and LC-MS/MS (−)] shows that differential metabolites are grouped in glutathione metabolism (p-value = 0.0145), ascorbate and aldarate metabolism (p-value =0.0279), and porphyrin and chlorophyll metabolism (p-value = 0.0004) between LT group and T0 group (Figures 1D,H,K,L and Supplementary Table 8). Besides, porphyrin and chlorophyll metabolism, bile secretion (p-value = 0.0006), primary bile acid biosynthesis (p-value = 0.0307), and mineral absorption (p-value = 0.0453), cholesterol metabolism (p-value =0.0453) were also significantly enriched between Un group and T0 group (Supplementary Figure 2 and Supplementary Table 8). These results further confirm that differential metabolites in serum have the potential to predict Mtb infection.

Anti-TB therapy results in some significant changes in serum metabolome

To explore the relevance of serum metabolome with HRZE treatment, and the temporal evolution of the selected predictors, longitudinal data were acquired from 17 TB patients with standard therapy. Similarly, the PLS-DA score map (Figure 2) revealed that the T6 group can be demarcated from the T0 group. The permutation test showed that the performance of PLS-DA model data of the T0 group and the T6 group was consistent with that of the standard parameters (Figure 2), as well as the T0 group and the T2 group (Supplementary Figure 2). Therefore, it can be effectively and reliably applied to detect differences in metabolic profiles related to the potential outcome. Student's *t*-test was used to screen differential metabolites between T0 groups and T6 groups. Comparing the T0 group to the T6 group, we found that 18 features were screened by GC-MS, 216 features by LC-MS/MS (+), and 127 features by LC-MS/MS (–) (Supplementary Table 4), including 150 upregulated and 211 downregulated compounds. Selected compounds were displayed in the form of volcano plots (Figures 2C,G,K). Between the T0 groups and T2 groups, 13

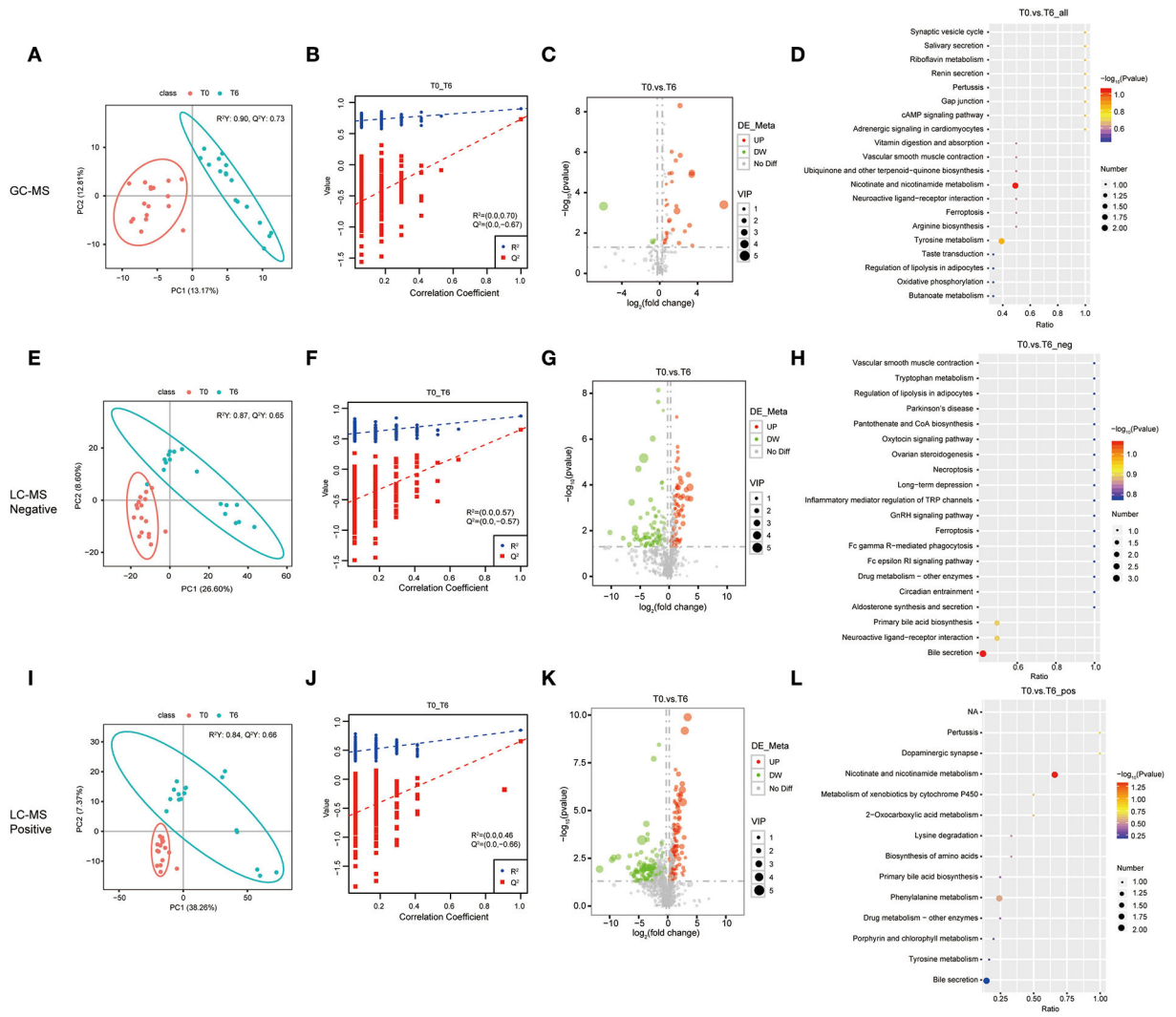

FIGURE 2
Identification of serum metabolites between the cured PTB group (T6) and the untreated PTB group (T0). The PLS-DA model for the T0/T6 group in GC-MS **(A)**, LC-MS/MS (–) **(E)**, and LC-MS/MS (+) **(I)**. The permutation test results for theT0/T6 group in GC-MS **(B)**, LC-MS/MS (–) **(F)**, and LC-MS/MS (+) **(J)**. Volcanic map of differential metabolites for the LT/Un group in GC-MS **(C)**, LC-MS/MS (–) **(G)**, and LC-MS/MS (+) **(K)**. The abscissa: the fold change of LT/Un group (base 2 logarithm). The ordinate: *P*-value of T0/T6 group (base 10 logarithm). Red: significantly upregulated metabolites. Green: significantly downregulated metabolites. Gray: non-significant differential metabolites. Pathway analysis of differential metabolites for the T0/T6 group in GC-MS **(D)**, LC-MS/MS (–) **(H)**, and LC-MS/MS (+) **(L)**.

features were screened by GC-MS, 70 features by LC-MS/MS (+), and 63 features by LC-MS/MS (−) (Supplementary Table 5), among which, 76 were upregulated and 70 were downregulated. Metabolic pathways enrichment is shown in Figure 2 between the T0 group and the T6 group, differential metabolites were mainly enriched in nicotinate and nicotinamide metabolism and bile secretion pathway. Besides, some amino acids, such as tyrosine metabolism, and phenylalanine metabolism were also significantly enriched.

Screening of differential metabolites as potential biomarkers

Next, the abnormally abundant metabolites in the T0 group compared with the other two groups were sought through screening all differential metabolites (FC $>$ 2.0, VIP $>$ 1.0, and $P < 0.05$) in the Un group, the LT group, and the T0 group. Comparison of T0 with the other two groups (LT vs. T0 and Un vs. T0) showed that the relative amount of 72 overlapping metabolites had changed dramatically in the serum of T0 patients. Table 2 shows the top 20 differential metabolites between the three groups, and these are listed in order of fold change and significance level. The total list of metabolites can be found in Supplementary Table 6. Furthermore, we screened seven metabolites that presented the same trends when compared with three groups, including sulfolithocholic acid, 2-decylfuran, (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol, D-(+)-camphor, 2-methylaminoadenosine, 3-oxopalmitic acid, and akeboside Ste (Figure 3).

The discriminative ability of these selected metabolites was evaluated by ROC curves. The seven differential metabolites mentioned above were used as input variables for multiple logistic regression analysis. The ROC curves of seven differential metabolites for distinguishing LT from T0 or Un are shown in Figure 4. When distinguishing the LT group from the T0 group, except that 2-methylaminoadenosine exhibited excellent efficiency with AUC values of 0.860 (95% CI 0.729–0.991), the remaining six differential metabolites had limited efficacy (AUC $<$ 0.8) as a biomarker. When distinguishing the LT group from the Un group, 2-decylfuran, d-(+)-camphor, akeboside ste, and sulfolithocholic acid showed excellent performance with AUC of 0.900 (95% CI 0.795–1.000), AUC of 0.900 (95% CI 0.794–1.000), AUC of 0.958 (95% CI 0.883–1.000), and AUC of 1 (95% CI 1.000–1.000), respectively. Then, we further used multiple logistic regression to analyze the efficacy of the seven metabolite combination. ROC analysis showed that results were very good, indicating that these seven combinations could be used to represent the most suitable biomarker group for the differentiation of PTB patients from the healthy controls.

In the same way, we are looking forward to screening differentially abundant metabolites as potential biomarkers for the cured PTB group and the untreated PTB group. We sought the metabolites which expressed abnormally in T0 but recovered after anti-tuberculosis treatment by investigating all differential metabolites among the Un/T0 group, the T0/T2 group, and the T0/T6 group. A total of 18 overlapping metabolites were significantly differentially expressed in the serum of T0 patients (Table 3). In addition, we also noticed that after treatment, especially 6-month anti-TB therapy, the seven serum metabolites expressed abnormally in T0 (Figure 3) significantly decreased and were close to the level of the healthy group, further demonstrating the potential of these metabolites as TB-related biomarkers (Supplementary Figure 3).

Discussion

WHO's End TB Strategy calls for the early diagnosis of TB, highlighting the critical role of laboratories in the post-2015 era in rapidly and accurately detecting TB (21). However, there is a lack of a gold standard for diagnosing latent tuberculosis infection, nor is there a uniform laboratory specification for the discharge of TB patients (6). Consequently, the screening of new diagnostic biomarkers has the potential to improve diagnostic accuracy and may provide unified diagnostic criteria for latent tuberculosis infection and the discharge of TB patients.

It is worth noting that metabolites represent the effects of cell viability and external exposure; therefore, the wealth of small-molecule metabolite data represented by individual metabolomes can generate key pathological insights. Metabolomics has also been widely used in TB over the past few years (12, 22–24). In 2014 and 2015, Mrinal et al. (25) and Sebabrata et al. (26) reported the application of liquid chromatography–mass spectrometry and gas chromatography mass spectrometry methods to identify the metabolites in urine samples of TB patients, respectively. Subsequently, many studies based on LC-MS approaches reported small molecule metabolites can be used as biomarkers for pulmonary TB in plasma, such as L-Histidine, arachidonic acid, biliverdin, L-cysteine-glutathione disulfide, Xanthine, 4-Pyridoxate, and D-glutamic acid (3). However, these studies only used LC-MS to explore the role of differential metabolites in plasma in PTB, it is not yet possible to detect all compounds with LC-MS.

To identify more metabolites, we screened differential metabolites in the serum of untreated PTB patients, 2-month-treated PTB patients, cured TB patients, latent tuberculosis infection, and healthy controls by GC-MS and LC-MS/MS. The GC-MS method has increased sensitivity to detect very small volatile or semi-volatile compounds in biological samples. To our knowledge, there have been few previous untargeted metabolomics studies of serum samples from TB patients that combined GC-MS and LC-MS/MS, especially for evaluating the efficacy of anti-TB treatment. Seven previously unreported metabolites were observed in latent tuberculosis

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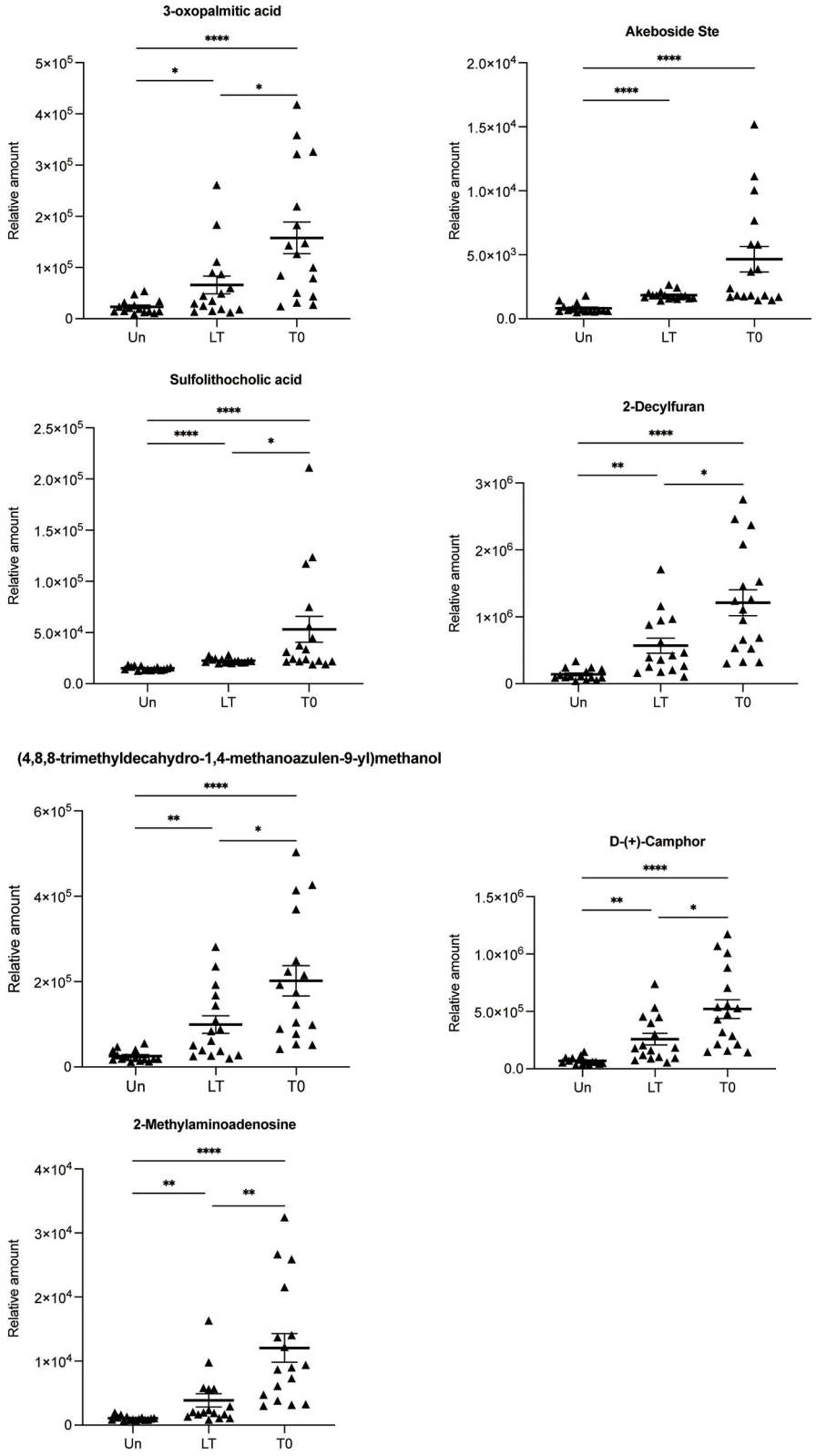

FIGURE 3
Relative abundance of seven differential metabolites 3-oxopalmitic acid, akeboside ste, sulfolithocholic acid, 2-decylfuran, (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl) methanol, d-(+)-camphor, and 2-methylaminoadenosine. The relative abundance of each metabolite in the serum from the untreated PTB (T0) and latent tuberculosis infection (LT) was significantly higher than that of the healthy control (Un) using the Kruskal–Wallis test and corrected for multiple comparisons by controlling the False Discovery Rate. *$q < 0.05$; **$q < 0.01$; ****$q < 0.0001$.

TABLE 2 Top 20 differential serum metabolites of T0 patients identified by LC-MS/MS and GC-MS, compared to LT and Un individuals.

No	Name_des	Un/T0			LT/T0			Trend
		Fold change	*p*-value	VIP	Fold change	*P*-value	VIP	
1	2-Methylaminoadenosine	0.08869288	7.6354E-10	4.01165828	0.32183336	0.00014766	3.15312305	↑
2	Carmustine	0.08972882	6.1405E-07	5.36149848	0.10362071	2.9201E-07	6.7564025	↑
3	2-Mercaptobenzothiazole	0.09599409	4.2994E-08	4.79467767	0.1142865	2.0319E-08	5.98713527	↑
4	2-Decylfuran	0.11522464	7.6919E-10	3.85942897	0.47028435	0.00494403	2.08491179	↑
5	(4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol	0.12689437	5.1544E-09	3.50374746	0.49206327	0.01133179	2.00191124	↑
6	D-(+)-Camphor	0.13339036	8.9712E-10	3.47389398	0.49728562	0.00673748	1.95060669	↑
7	3-oxopalmitic acid	0.14779064	8.9333E-07	3.10115364	0.41789084	0.00686639	2.40303662	↑
8	Pivagabine	0.15975548	0.03742746	1.41310153	0.13634797	0.0153759	2.26464813	↑
9	Thiolutin	5.76453916	1.1133E-06	4.72982861	4.86432478	3.3855E-06	6.19438115	↓
10	Akeboside Ste	0.17761794	5.0832E-07	2.72736425	0.39555739	0.00583788	1.56591184	↑
11	Benzquinamide	5.43411528	0.00023544	2.28109023	9.63323124	0.00013558	4.18646907	↓
12	(9cis)-Retinal	0.19175918	0.00014079	2.39998295	0.3651436	0.04168866	1.81045086	↑
13	Vitamin C	4.85885066	2.1287E-05	3.37433699	3.66201592	2.3383E-05	4.36822211	↓
14	2,3,-dihydroxybenzoylserine	0.20816884	2.7158E-06	2.63686566	0.27423189	5.4365E-05	3.04325781	↑
15	Dcebio	4.72077327	4.1241E-07	3.46112538	3.916837	1.4456E-06	4.4541779	↓
16	Dexamethasone tebutate	4.61437664	1.4492E-11	2.72745654	4.24282632	3.7766E-07	3.25382949	↓
17	Maleic acid	4.37259767	6.7671E-05	2.8486705	3.83535948	1.5916E-05	3.99476391	↓
18	Methyprylon	0.23096774	0.03419834	1.21199933	0.21277934	0.02037897	1.79394108	↑
19	Seratrodast	4.1954909	0.00421687	1.79337805	8.50637327	5.1876E-05	4.29763145	↓
20	Methional	0.2479882	1.7002E-08	2.68873807	0.26589941	8.9786E-09	3.51678769	↑

infection, including 3-oxopalmitic acid, akeboside Ste, sulfolithocholic acid, 2-Decylfuran, (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol, D-(+)-Camphor, and 2-methylaminoadenosine. In this study, seven metabolites were increased in the serum of latent tuberculosis infection and untreated TB patients. It has been reported that in the inflammatory response, the palmitic acid and its derivatives in the endoplasmic reticulum (ER), on the one hand, can increase the reactive oxygen species (ROS) generation, leading to cell death; on the other hand, they also drive the activation of NF-κB and NLRP3, facilitating the release of proinflammatory cytokine by monocytes/macrophages (27, 28). José Marcos Sanches et al. had also shown that certain potential lipid biomarkers in macrophages, such as palmitic acid and PE (16:0/0:0), are released after NLRP3 activation that can modulate the inflammatory responses in the damaged tissue (29). 3-oxopalmitic acid is oxo-fatty acid comprising palmitic acid (PA) having an oxo group at the 3-position, an intermediate in fatty acid biosynthesis, and has functional parent palmitic acid. Our study also showed that 3-oxopalmitic acid was upregulated after Mtb infection, further indicating that Mtb may depend on fatty acid metabolism to maintain chronic infection.

Two secondary metabolites (2-methylaminoadenosine and 2-decylfuran) showed higher expression in latent tuberculosis infection and untreated TB patients compared with the healthy group. 2-methylaminoadenosine is a purine nucleoside. Currently, the natural products and derivatives of purine nucleosides have been developed as drugs for their unique biochemical properties and capabilities (30). 2-decylfuran is a member of the class of furans that is furan in which the hydrogen at position 2 is replaced by a decyl group. Furan exerts its antibacterial activity through selective inhibition of microbial growth and modification of enzymes (31). Our study showed that the 2-methylaminoadenosine and 2-decylfuran content increased rapidly after Mtb infection, but recovered after 2/6 months of anti-tuberculosis treatment. In most cases, secondary metabolites are metabolically or physiologically non-essential metabolites that may serve a role as defense or signaling

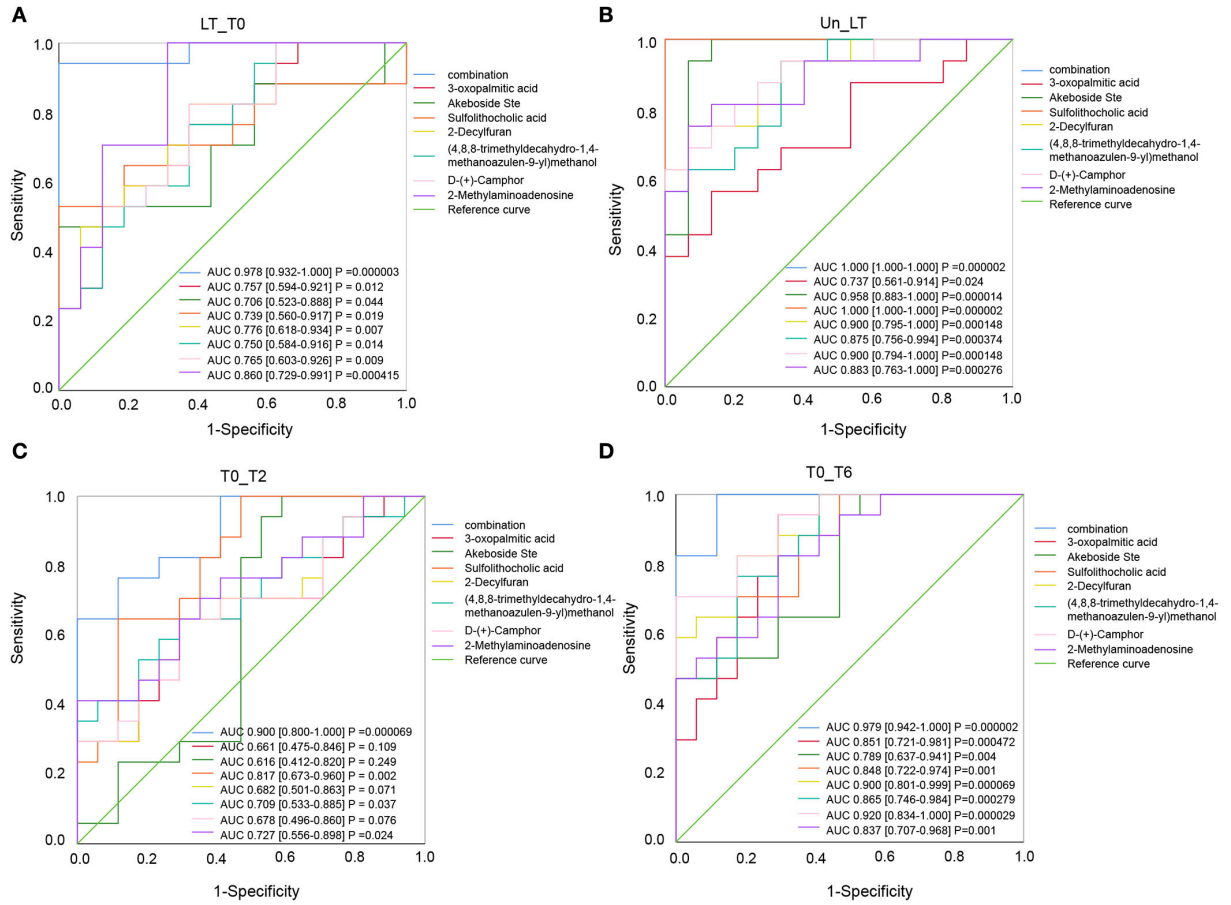

FIGURE 4
ROC curves are used to identify differential metabolites. The curve analysis of the seven metabolites shows that the combined diagnostic model performed well in distinguishing LT from T0 (AUC = 0.978) **(A)** and Un (AUC = 1.000) **(B)**. Compared with group T2 **(C)**, the combined diagnostic model could better distinguish T0 from T6 (AUC = 0.979) **(D)**.

molecules. This indicated that 2-methylaminoadenosine and 2-decylfuran are closely related to the survival of Mtb; therefore, we suspect that these two metabolites reflect the concentration of Mtb, *in vivo* after the intensive treatment phase. Camphor is a cyclic monoterpene ketone, which has been widely used as a chiral, enantiopure starting material in natural product synthesis (32). The study reported that a series of new amidoalcohols and amido diols were designed on the base of the camphor scaffold and evaluated for their *in vitro* activity against Mtb strains, and they showed 25 times higher activity than the classical anti-TB drug ethambutol (33). Our study showed that the D-(+)-camphor content increased rapidly after being infected with Mtb, indicating that camphor as a carrier has a strong bactericidal effect. Akeboside Ste is a triterpenoid, the studies reported that triterpenoids have various pharmacological activities, including anti-inflammatory, anti-allergic, anti-microbial, anti-angiogenic, etc. (34). Mtb stimulates the body's metabolism to produce akeboside Ste. As an anti-bacterial active substance, akeboside Ste would fight against tuberculosis quickly once Mtb is released from the body. Sulfolithocholic acid is the sulfated product of lithocholic acid, a secondary bile acid produced by microbiota (35). Recent reports suggest that sulfolithocholic acid is a potential marker for pancreatic fat (36). Our study showed that the level of akeboside Ste and sulfolithocholic acid significantly increased in latent tuberculosis infection and untreated TB patients, but both returned to a normal level after 2/6 months of TB treatment. This indicated that akeboside Ste and sulfolithocholic acid may be associated with the severity of Mtb infection and could reflect the change in the body's immunity to Mtb. Based on a literature review very few articles have been published on (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol. Studies showed that through physiological and metabolic clearance mechanisms, methanol remains at a low physiological level in healthy people, but increased levels of methanol were detected in the blood of patients with nervous systems disorders and the

TABLE 3 Metabolites with significant changes in the relative amount identified by LC-MS/MS and GC-MS among the Un group, T0 group, T2 group, and T6 group.

No.	Differential Metabolites	Un/T0			T0/T2			T0/T6		
		FC	*p*-value	VIP	FC	*p*-value	VIP	FC	*p*-value	VIP
1	(2R)-2-Hydroxy-3-(phosphonooxy)propyl (11Z)-11-docosenoate	2.23074	4.00E-11	1.48559	0.48008	0.01504	1.00738	0.26091	0.00981	1.62940
2	Akeboside ste	0.17762	5.08E-07	2.72736	2.23626	0.020102	1.27419	2.88922	0.00117	1.22118
3	Alpha-ketoglutaric acid	0.00919	0.0004	5.63162	80.47541	0.00060	6.11196	108.83	0.00040	5.01282
4	Benzylamine	0.41113	2.88E-05	1.54977	2.19781	7.67E-05	1.63210	2.4323	2.88E-05	1.44356
5	Cellobiose	0.09573	1.03E-05	3.59862	10.44709	1.03E-05	4.04631	10.44666	1.03E-05	3.32044
6	Dehydrocholic acid	0.35737	0.01569	1.580281	3.09395	0.002455	2.65723	3.78539	0.00337	2.26530
7	Guanidinosuccinic acid	0.22328	1.43E-06	2.65049	2.72994	7.46E-05	2.39798	4.47863	1.43E-06	2.47196
8	Levoglucosan	0.16025	0.00827	1.94374	6.24032	0.00827	2.14569	6.2403	0.00827	1.71159
9	Lyxonic acid, 1,4-lactone	0.31000	2.58E-06	2.06159	3.22580	2.58E-06	2.36436	3.2258	2.58E-06	1.90368
10	Medroxyprogesterone 17-acetate	2.32418	0.00807	1.49834	8.76635	3.20E-10	6.07807	7.59242	6.58E-10	4.51366
11	Monostearin	0.22807	4.95E-09	2.833215	2.07154	0.00015	2.29247	4.3846	4.95E-09	2.59887
12	Onapristone	0.38163	0.00130	1.65142	7.81206	3.15E-09	5.08336	10.66641	1.29E-10	4.48954
13	Oxamide	0.11328	0.00415	2.49446	8.82767	0.00415	2.82369	8.8278	0.00415	2.29857
14	Oxibendazole	0.02766	2.06E-16	6.29175	0.04339	0.00041	3.81391	0.02537	1.99E-05	3.30990
15	Paliperidone	0.39303	0.03442	1.24023	4.20549	0.00045	2.41296	3.35587	0.00211	2.05907
16	Sulfolithocholic acid	0.28332	7.03E-05	1.75722	2.55579	0.00303	1.58137	2.83680	0.00071	1.16347
17	Sunitinib	6.41533	0.04036	1.92767	2.75971	0.00555	2.27649	3.13116	0.00191	1.66104
18	Tributyl citrate acetate	4.02752	0.03744	1.67060	2.28837	0.00515	1.87899	2.08951	0.02348	1.08023

elderly (37). Our study showed that (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol was upregulated after Mtb infection and recovered after curing TB, suggesting a disruption of the genetic and biochemical mechanisms that are responsible for maintaining low methanol levels.

In conclusion, comparative serum metabolome analysis using GC-MS and LC-MS/MS demonstrated that differences do exist among untreated TB patients, two-month treated PTB patients, cured TB, latent tuberculosis infection, and healthy subjects. 3-oxopalmitic acid, akeboside Ste, sulfolithocholic acid, 2-decylfuran, (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol, D-(+)-camphor, and 2-methylaminoadenosine may serve as potential biomarkers for latent tuberculosis infection and cured TB patients. These metabolites may reflect the severity of MTB infection in patients with TB and the strength of the body's immune defense. New metabolites from latent tuberculosis infection presented in this study, to our knowledge, have not been reported before. Moreover, these metabolites may also provide a promising approach to predict a good therapeutic outcome. Whether these metabolites add value to the prediction of latent tuberculosis infection and cured TB patients will require further study and validation in separate cohorts. Our study provided more detailed experimental data for developing laboratory standards for evaluating latent tuberculosis infection and cured PTB.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The study was approved by the Ethics Committee of the Center for Tuberculosis Control of Guangdong Province, China. Written informed consent was obtained from all subjects prior to blood sample collection.

Author contributions

YL, WWe, and QT conceived, designed, and supervised the overall study. WWe, XW, and QT acquired funding, supervised, and administered the project. XW and WWe coordinated the study. XW, YL, and WWa collected the samples and the clinical supervision. YZ, JW, and LX processed the samples and performed the experiments. CZ, MY, XC, JZ, and LC analyzed the data. ZW, WWe, and XW wrote the article. JZ revised the article. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.962510/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Identification of serum metabolites between uninfected healthy control (Un) and the untreated PTB group (T0). The PLS-DA model for the Un/T0 group in GC-MS **(A)**, LC-MS/MS (+) **(E)** and LC-MS/MS (−) **(I)**. The permutation test results for the Un/T0 group in GC-MS **(B)**, LC-MS/MS (+) **(F)** and LC-MS/MS (−) **(J)**; Volcanic map of differential metabolites for the Un/T0 group in GC-MS **(C)**, LC-MS/MS (+) **(G)** and LC-MS/MS (−) **(K)**. The abscissa: the fold change of Un/T0 group (base 2 logarithm). The ordinate: the *P*-value of Un/T0 group (base 10 logarithm). Red: significantly upregulated metabolites. Green: significantly downregulated metabolites. Gray: non-significant differential metabolites. Pathway analysis of differential metabolites for the Un/T0 group in GC-MS **(D)**, LC-MS/MS (+) **(H)**, and LC-MS/MS (−) **(L)**.

SUPPLEMENTARY FIGURE 2
Identification of serum metabolites between the untreated PTB group (T0) and the 2-month treated PTB group (T2). The PLS-DA model for the Un/T0 group in GC-MS **(A)**, LC-MS/MS (+) **(E)**, and LC-MS/MS (−) **(I)**. The permutation test results for the T0/T2 group in GC-MS **(B)**, LC-MS/MS (+) **(F)**, and LC-MS/MS (−) **(J)**. Volcanic map of differential metabolites for the T0/T2 group in GC-MS **(C)**, LC-MS/MS (+) **(G)**, and LC-MS/MS (−) **(K)**. The abscissa: the fold change of the T0/T2 group (base 2 logarithm). The ordinate: the *P*-value of the T0/T2 group (base 10 logarithm). Red: significantly upregulated metabolites. Green: significantly downregulated metabolites. Gray: non-significant differential metabolites. Pathway analysis of differential metabolites for the T0/T2 group in GC-MS **(D)**, LC-MS/MS (+) **(H)**, and LC-MS/MS (−) **(L)**.

SUPPLEMENTARY FIGURE 3
Changes in relative quantitative values of seven differential metabolites 3-oxopalmitic acid, akeboside ste, sulfolithocholic acid, 2-decylfuran, (4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl)methanol, d-(+)-camphor, and 2-methylaminoadenosine in the serum from healthy controls (Un and LTBI), the untreated PTB (T0), 2-month treated PTB (T2) and 6-month treated PTB. The *q*-value was calculated using the Kruskal–Wallis test and corrected for multiple comparisons by controlling the False Discovery Rate. $^{*}q < 0.05$; $^{**}q < 0.01$; $^{***}q < 0.001$.

SUPPLEMENTARY FIGURE 4
The flowchart of the subjects' screening and enrolling in this study. To study the relationship between tuberculosis and serum metabolome, we recruited 147 subjects that include 52 uninfected (Un), 40 LTBI, and 55 active TB patients without anti-TB therapy (T0). In addition, to detect the effect of periodic HRZE treatment on the serum metabolome, we tried to track TB patients in the T0 group over the course of anti-TB treatment, and finally succeeded in obtaining serum samples from 17 patients with complete standard treatment nodes, viz. 2 months (T2) and 6 months (T6). Next, we screened 17 volunteers of similar age to these 17 patients in each of the Un and LTBI groups. Two serum samples from the Ungroup and one serum from the LTBI group were found to have hemolysis during metabolite extraction and were discarded. Finally, 82 serum samples were used for the metabolome experiment.

SUPPLEMENTARY TABLE 1
Characteristics of the individuals in this study.

SUPPLEMENTARY TABLE 2
Serum metabolites identified between the LT group and the T0 group. The Variable Importance in the Projection (VIP) value of the first principal component of the PLS-DA model is used, and the VIP value represents the contribution rate of metabolite differences in different groups; The ratio of the mean values of all biological replicates in the comparison group; combined with the *P*-value of *T*-test to find differentially

expressed metabolites, set the threshold to VIP > 1.0, the difference fold FC ≥ 2 or FC ≤ 0.5, and *P*-value < 0.05.

SUPPLEMENTARY TABLE 3
Serum metabolites identified between the Un group and the T0 group. The Variable Importance in the Projection (VIP) value of the first principal component of the PLS-DA model is used, and the VIP value represents the contribution rate of metabolite differences in different groups; The ratio of the mean values of all biological replicates in the comparison group; combined with the *P*-value of *T*-test to find differentially expressed metabolites, set the threshold to VIP > 1.0, the difference fold FC ≥ 2 or FC ≤ 0.5, and *P*-value < 0.05.

SUPPLEMENTARY TABLE 4
Serum metabolites identified between the T0 group and the T6 group (FC > 2.0, VIP > 1.0 and *P* < 0.05).

SUPPLEMENTARY TABLE 5
Serum metabolites identified between the T0 group and the T2 group (FC > 2.0, VIP > 1.0 and *P* < 0.05).

SUPPLEMENTARY TABLE 6
The total list of differential serum metabolites of T0 patients identified by LC-MS/MS and GC-MS, compared to LT and Un individuals (FC > 2.0, VIP > 1.0 and *P* < 0.05).

SUPPLEMENTARY TABLE 7
The relative amount of seven potential biomarkers shown in Figure 3 and Supplementary Figure 3.

SUPPLEMENTARY TABLE 8
KEGG pathway enriched by differential metabolites.

SUPPLEMENTARY TABLE 9
The function and classification of identified metabolites in this study were annotated by the HMDB database.

SUPPLEMENTARY TABLE 10
The function and classification of identified metabolites in this study were annotated by the KEGG database.

SUPPLEMENTARY TABLE 11
The function and classification of identified metabolites in this study were annotated by the LIPID MAPS database.

References

1. Wong YJ, Noordin NM, Keshavjee S, Lee S. Impact of latent tuberculosis infection on health and wellbeing: a systematic review and meta-analysis. *Eur Respir Rev.* (2021) 30:200260. doi: 10.1183/16000617.0260-2020

2. Ruan QL, Yang QL, Sun F, Liu W, Shen YJ, Wu J, et al. Recurrent pulmonary tuberculosis after treatment success: a population-based retrospective study in China. *Clin Microbiol Infect.* (2022) 28:684–9. doi: 10.1016/j.cmi.2021.09.022

3. Yi WJ, Han YS, Wei LL, Shi LY, Huang H, Jiang TT, et al. l-Histidine, arachidonic acid, biliverdin, and l-cysteine-glutathione disulfide as potential biomarkers for cured pulmonary tuberculosis. *Biomed Pharmacother.* (2019) 116:108980. doi: 10.1016/j.biopha.2019.108980

4. Tschampl CA, Garnick DW, Zuroweste E, Razavi M, Shepard DS. Use of transnational services to prevent treatment interruption in tuberculosis-infected persons who leave the United States. *Emerg Infect Dis.* (2016) 22:417–25. doi: 10.3201/eid2203.141971

5. Zhou G, Luo Q, Luo S, Teng Z, Ji Z, Yang J, et al. Interferon-gamma release assays or tuberculin skin test for detection and management of latent tuberculosis infection: a systematic review and meta-analysis. *Lancet Infect Dis.* (2020) 20:1457–69. doi: 10.1016/S1473-3099(20)30276-0

6. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, et al. Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. *Clin Microbiol Rev.* (2014) 27:3–20. doi: 10.1128/CMR.00034-13

7. Tang J, Huang Y, Cai Z, Ma Y. Mycobacterial heparin-binding hemagglutinin (HBHA)-induced interferon-gamma release assay (IGRA) for discrimination of latent and active tuberculosis: a systematic review and meta-analysis. *PLoS ONE.* (2021) 16:e0254571. doi: 10.1371/journal.pone.0254571

8. Glotfelty LG, Wong AC, Levy M. Small molecules, big effects: microbial metabolites in intestinal immunity. *Am J Physiol Gastrointest Liver Physiol.* (2020) 318:G907–11. doi: 10.1152/ajpgi.00263.2019

9. Gouveia MJ, Brindley PJ, Santos LL, Correia DCJ, Gomes P, Vale N. Mass spectrometry techniques in the survey of steroid metabolites as potential disease biomarkers: a review. *Metabolism.* (2013) 62:1206–17. doi: 10.1016/j.metabol.2013.04.003

10. Song Z, Wang H, Yin X, Deng P, Jiang W. Application of NMR metabolomics to search for human disease biomarkers in blood. *Clin Chem Lab Med.* (2019) 57:417–41. doi: 10.1515/cclm-2018-0380

11. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. *Nat Rev Mol Cell Biol.* (2016) 17:451–9. doi: 10.1038/nrm.2016.25

12. Weiner JR, Maertzdorf J, Sutherland JS, Duffy FJ, Thompson E, Suliman S, et al. Metabolite changes in blood predict the onset of tuberculosis. *Nat Commun.* (2018) 9:5208. doi: 10.1038/s41467-018-07635-7

13. Heaney LM. Applying mass spectrometry-based assays to explore gut microbial metabolism and associations with disease. *Clin Chem Lab Med.* (2020) 58:719–32. doi: 10.1515/cclm-2019-0974

14. Kim YM, Heyman HM. Mass spectrometry-based metabolomics. *Methods Mol Biol.* (2018) 1775:107–18. doi: 10.1007/978-1-4939-7804-5_10

15. Suarez-Diez M, Adam J, Adamski J, Chasapi SA, Luchinat C, Peters A, et al. Plasma and serum metabolite association networks: comparability within and between studies using NMR and MS profiling. *J Proteome Res.* (2017) 16:2547–59. doi: 10.1021/acs.jproteome.7b00106

16. Nishiumi S, Suzuki M, Kobayashi T, Yoshida M. Differences in metabolite profiles caused by pre-analytical blood processing procedures. *J Biosci Bioeng.* (2018) 125:613–8. doi: 10.1016/j.jbiosc.2017.11.011

17. Wu Z, Wei W, Zhou Y, Guo H, Zhao J, Liao Q, et al. Integrated quantitative proteomics and metabolome profiling reveal MSMEG_6171 overexpression perturbing lipid metabolism of *Mycobacterium smegmatis* leading to increased vancomycin resistance. *Front Microbiol.* (2020) 11:1572. doi: 10.3389/fmicb.2020.01572

18. Zullig T, Zandl-Lang M, Trotzmuller M, Hartler J, Plecko B, Kofeler HC. A metabolomics workflow for analyzing complex biological samples using a combined method of untargeted and target-list based approaches. *Metabolites.* (2020) 10:342. doi: 10.3390/metabo10090342

19. Yuan F, Kim S, Yin X, Zhang X, Kato I. Integrating two-dimensional gas and liquid chromatography-mass spectrometry for untargeted colorectal cancer metabolomics: a proof-of-principle study. *Metabolites.* (2020) 10:343. doi: 10.3390/metabo10090343

20. Brown M, Dunn WB, Dobson P, Patel Y, Winder CL, Francis-McIntyre S, et al. Mass spectrometry tools and metabolite-specific databases for molecular identification in metabolomics. *Analyst.* (2009) 134:1322–32. doi: 10.1039/b901179j

21. Ruiz P, Causse M, Vaquero M, Gutierrez JB, Casal M. Evaluation of a new automated Abbott RealTime MTB RIF/INH assay for qualitative detection of rifampicin/isoniazid resistance in pulmonary and extra-pulmonary clinical samples of Mycobacterium tuberculosis. *Infect Drug Resist.* (2017) 10:463–7. doi: 10.2147/IDR.S147272

22. Huang H, Shi LY, Wei LL, Han YS, Yi WJ, Pan ZW, et al. Plasma metabolites Xanthine, 4-Pyridoxate, and d-glutamic acid as novel potential biomarkers for pulmonary tuberculosis. *Clin Chim Acta.* (2019) 498:135–42. doi: 10.1016/j.cca.2019.08.017

23. Frediani JK, Jones DP, Tukvadze N, Uppal K, Sanikidze E, Kipiani M, et al. Plasma metabolomics in human pulmonary tuberculosis disease: a pilot study. *PLoS ONE.* (2014) 9:e108854. doi: 10.1371/journal.pone.0108854

24. Collins JM, Walker DI, Jones DP, Tukvadze N, Liu KH, Tran VT, et al. High-resolution plasma metabolomics analysis to detect Mycobacterium tuberculosis-associated metabolites that distinguish active pulmonary tuberculosis in humans. *PLoS ONE.* (2018) 13:e0205398. doi: 10.1371/journal.pone.0205398

25. Das MK, Bishwal SC, Das A, Dabral D, Badireddy VK, Pandit B, et al. Deregulated tyrosine-phenylalanine metabolism in pulmonary tuberculosis patients. *J Proteome Res.* (2015) 14:1947–56. doi: 10.1021/acs.jproteome.5b00016

26. Mahapatra S, Hess AM, Johnson JL, Eisenach KD, DeGroote MA, Gitta P, et al. A metabolic biosignature of early response to anti-tuberculosis treatment. *BMC Infect Dis.* (2014) 14:53. doi: 10.1186/1471-2334-14-53

27. Korbecki J, Bajdak-Rusinek K. The effect of palmitic acid on inflammatory response in macrophages: an overview of molecular mechanisms. *Inflamm Res.* (2019) 68:915–32. doi: 10.1007/s00011-019-01273-5

28. Robblee MM, Kim CC, Porter AJ, Valdearcos M, Sandlund KL, Shenoy MK, et al. Saturated fatty acids engage an IRE1alpha-dependent pathway to activate the NLRP3 inflammasome in myeloid cells. *Cell Rep.* (2016) 14:2611–23. doi: 10.1016/j.celrep.2016.02.053

29. Sanches JM, Branco LM, Duarte G, Oliani SM, Bortoluci KR, Moreira V, et al. Annexin A1 regulates NLRP3 inflammasome activation and modifies lipid release profile in isolated peritoneal macrophages. *Cells-Basel.* (2020) 9:926. doi: 10.3390/cells9040926

30. Besada P, Costas T, Teijeira M, Teran C. Synthesis and cytostatic activity of purine nucleosides derivatives of allofuranose. *Eur J Med Chem.* (2010) 45:6114–9. doi: 10.1016/j.ejmech.2010.09.046

31. Alizadeh M, Jalal M, Hamed K, Saber A, Kheirouri S, Pourteymour FTF, et al. Recent updates on anti-inflammatory and antimicrobial effects of furan natural derivatives. *J Inflamm Res.* (2020) 13:451–63. doi: 10.2147/JIR.S262132

32. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M. Camphor (Cinnamomum Camphora), a traditional remedy with the history of treating several diseases. *Int J Case Rep Images.* (2013) 4:86–9. doi: 10.5348/ijcri-2013-02-267-RA-1

33. Stavrakov G, Valcheva V, Philipova I, Doytchinova I. Novel camphane-based anti-tuberculosis agents with nanomolar activity. *Eur J Med Chem.* (2013) 70:372–9. doi: 10.1016/j.ejmech.2013.10.015

34. Das BK, Swamy AV, Koti BC, Gadad PC. Experimental evidence for use of *Acorus calamus* (asarone) for cancer chemoprevention. *Heliyon.* (2019) 5:e01585. doi: 10.1016/j.heliyon.2019.e01585

35. Hofmann AF. Detoxification of lithocholic acid, a toxic bile acid: relevance to drug hepatotoxicity. *Drug Metab Rev.* (2004) 36:703–22. doi: 10.1081/DMR-200033475

36. Wu ZE, Fraser K, Kruger MC, Sequeira IR, Yip W, Lu LW, et al. Untargeted metabolomics reveals plasma metabolites predictive of ectopic fat in pancreas and liver as assessed by magnetic resonance imaging: the TOFI_Asia study. *Int J Obes.* (2021) 45:1844–54. doi: 10.1038/s41366-021-00854-x

37. Dorokhov YL, Shindyapina AV, Sheshukova EV, Komarova TV. Metabolic methanol: molecular pathways and physiological roles. *Physiol Rev.* (2015) 95:603–44. doi: 10.1152/physrev.00034.2014

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High diversity of clinical *Mycobacterium intracellulare* in China revealed by whole genome sequencing

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Mycobacterium intracellulare is the most common cause of nontuberculous mycobacterial lung disease, with a rapidly growing prevalence worldwide. In this study, we performed comparative genomic analysis and antimicrobial susceptibility characteristics analysis of 117 clinical *M. intracellulare* strains in China. Phylogenetic analysis showed that clinical *M. intracellulare* strains had high genetic diversity and were not related to the geographical area. Notably, most strains (76.07%, 89/117) belonged to *Mycobacterium paraintracellulare* (MP) and *Mycobacterium indicus pranii* (MIP) in the genome, and we named them MP-MIP strains. These MP-MIP strains may be regarded as a causative agent of chronic lung disease. Furthermore, our data demonstrated that clarithromycin, amikacin, and rifabutin showed strong antimicrobial activity against both *M. intracellulare* and MP-MIP strains *in vitro*. Our findings also showed that there was no clear correlation between the *rrs*, *rrl*, and DNA gyrase genes (*gyrA* and *gyrB*) and the aminoglycosides, macrolides, and moxifloxacin resistance, respectively. In conclusion, this study highlights the high diversity of *M. intracellulare* in the clinical setting and suggests paying great attention to the lung disease caused by MP-MIP.

KEYWORDS

***Mycobacterium intracellulare*, drug resistance profile, nontuberculous mycobacterial lung disease, *Mycobacterium indicus prani*, whole genome sequencing**

Introduction

Mycobacterium intracellulare (*M. intracellulare*), a major species of the *Mycobacterium avium* complex (MAC), is the leading cause of nontuberculous mycobacterial lung disease worldwide (1, 2). It can cause lung illness in both immunocompetent and immunosuppressed patients, showing common respiratory

symptoms such as cough, sputum, and weight loss (3). *M. intracellulare* is ubiquitous in the environment, such as in water and soil. Some studies showed that residential environments like bathroom or drinking water could be the sources of infection (4). In recent years, the incidence of *M. intracellulare* infections is growing, causing widespread concern and attention (2). Researchers in Korea retrospectively investigated data on *Mycobacterium species* over 13 years in their country, showing that the most common species was *M. intracellulare* (50.6%) (5). A national survey of nontuberculous mycobacteria pulmonary disease in China showed that 34.1% of the strains belong to MAC, of which *M. intracellulare* is the most common and distributed widely (6). Thus, accurate identification of the *M. intracellulare* from patients and timely treatment are particularly important.

Mycobacterium indicus pranii (MIP) has been considered a non-pathogenic and cultivable organism with immunomodulatory characteristics, which has therapeutic value in the treatment of leprosy (7, 8). Its taxonomic characterization showed high sequence identity (>99%) to *M. intracellulare* based on the most common housekeeping genes as well as similar phenotypic characteristics, such as the negative urease (9). *Mycobacterium yongonense* and *Mycobacterium chimaera* are opportunistic pathogens, which could cause pulmonary infections in humans, usually in immunocompromised patients and in patients with underlying respiratory diseases (10, 11). To date, MIP, *Mycobacterium yongonense* and *Mycobacterium chimaera* have been regarded as *M. intracellulare subsp. intracellulare* (12), *Mycobacterium intracellulare subsp. yongonense* and *Mycobacterium intracellulare subsp. chimaera*, respectively, being the subspecies of *M. intracellulare*. *Mycobacterium paraintracellulare* (MP) is an independent species in the NCBI database, but previous reports showed that *M. paraintracellulare* should be reclassified into *M. intracellulare* at the subspecies level with high sequence similarity (average nucleotide identity ≥98%) (13, 14).

Lately, some studies have revealed that MIP could cause NTM pulmonary disease in clinical trials, and some strains were misdiagnosed as *M. intracellulare* because of the high similarity in clinical diagnosis (15, 16). A recent study about the genomic analysis of *M. intracellulare* and related species isolates showed that clinical *M. intracellulare* strains have been separated into two major groups: the typical *M. intracellulare* (TMI) group and the *M. paraintracellulare - M. indicus pranii* (MP-MIP) group (12). Thus, MIP should be considered a cause of pulmonary disease in humans with pre-existing lung diseases, such as tuberculosis and bronchiectasis (16).

Antimicrobial susceptibility information is considered critical for the successful and appropriate treatment of pulmonary illnesses (17, 18). Official clinical practice guidelines suggest that macrolide, ethambutol, and rifamycin (or rifabutin) should be included in treatment regimens for MAC infections, and amikacin or streptomycin may be added to the treatment regimens if the patient is macrolide-resistant or requires more aggressive therapy (18). However, the subspecies of MAC strains exhibit various drug susceptibility patterns. Researchers have investigated the differences in drug susceptibility of the subspecies strains, such as *M. intracellulare*, *Mycobacterium avium*, *Mycobacterium intracellulare subsp. chimaera*, and *Mycobacterium colombiense* (19, 20), but there is little information available regarding the drug susceptibility of MP and MIP.

In this study, we compared the genomics of 117 clinical strains that were previously identified with *M. intracellulare* to comprehend the genetic diversity and similarities of clinically isolated *M. intracellulare* strains in China. In addition, we further investigated the antimicrobial susceptibility characteristics of the strains, especially for the MP and MIP, which could increase the body of available MIC data and provide the basis for clinical treatment.

Materials and methods

Isolates collection and identification

A total of 117 clinical strains that were previously identified as *M. intracellulare* were randomly selected from the nontuberculous mycobacteria database of the national tuberculosis reference laboratory in China (6). The species of nontuberculous mycobacteria strains were identified by MALDI-TOF MS after four weeks grown on Lowenstein Jensen media and sequencing their 16S ribosomal genes. In this study, the subspecies were confirmed by the average nucleotide identity (ANI) and phylogenetic analysis based on whole genome sequence (WGS). The ANI was calculated by an ANI Calculator online (https://www.ezbiocloud.net/tools/ani). The pairwise ANI values were determined by pyani (https://github.com/widdowquinn/pyani) and visualized using the heatmap of the R package. The *M. intracellulare* ATCC 13950 (NC_016946.1), MIP MTCC 9506 (NC_018612.1), *M. intracellulare subsp. yongonense* 05-1390 (NC_021715.1), *Mycobacterium paraintracellulare* MOTT64 (NC_016948.1), and *M. intracellulare subsp. chimaera* DSM 44623(NZ_CP015278.1) were set as the subspecies reference genomes.

Antimicrobial susceptibility testing

According to the Clinical and Laboratory Standards Institute (CLSI) standard guideline, the antimicrobial susceptibility testing in this study was performed using the Sensititre™ SLOWMYCOI panel (21). It included 13 antimicrobials: clarithromycin (CLR), amikacin (AN), moxifloxacin (MXF), linezolid (LNZ), ciprofloxacin (CIP), doxycycline (DO), ethambutol (EMB), rifampicin (RIF), rifabutin (RFB), sulfamethoxazole (SXT), ethionamide (ETH), isoniazid (INH), and streptomycin (SM). The resistance

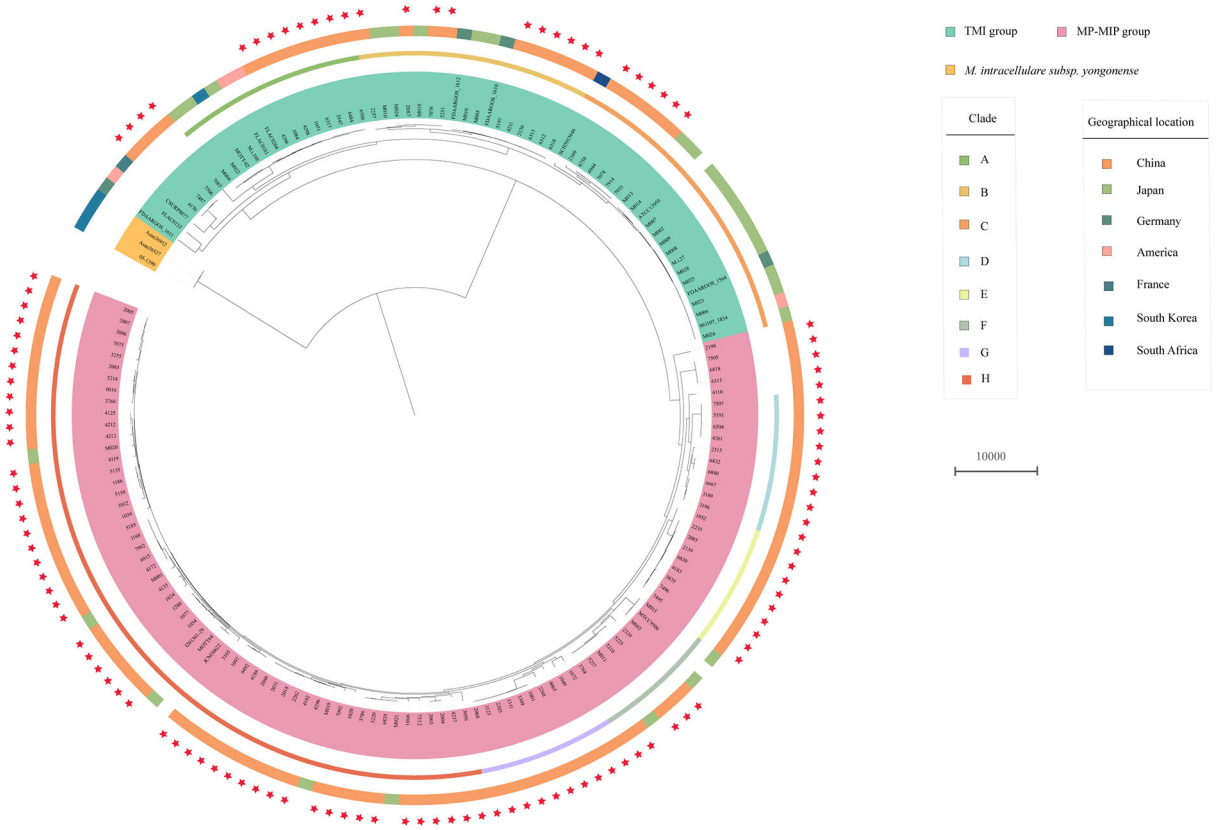

FIGURE 1
Phylogenetic tree of the 117 clinical *M. intracellulare* strains in this study, 30 *M. intracellulare* strains, and 14 related strains from the NCBI genome database. The tree based on core SNPs was constructed by RAxML with GTR+G model, with 1000 bootstrap replicates. The group type, clade, and geographic location of the strains are shown on the tree (from inner to outer circles), according to the color legend shown on the right. The red stars on the outside represent the strains in this study.

breakpoints were determined as previously described according to CLSI standards (6).

DNA extraction and sequencing

The 117 strains were cultured on Lowenstein Jensen media and genome DNA was extracted following the protocol of the cetyltrimethylammonium bromide (CTAB) method (22). Whole-genome sequencing was performed on the Illumina Hiseq PE150 platform by Annoroad (Beijing, China). The paired-end reads were examined using FastQC (v0.11.9) and trimmed using Trimmomatic (v0.39) (23). The genome sequences were assembled into a number of scaffolds by SPAdes (24). And the quality of the assemblies was evaluated using QUAST (v5.0.2).

Phylogenetic analysis

To get a better understanding of the population structure of *M. intracellulare*, we downloaded the publicly available genomes of 30 *M. intracellulare*, 2 MIP, 3 *Mycobacterium intracellulare subsp. Yongonense* and 9 *Mycobacterium paraintracellulare* from the NCBI public database (Supplementary Table S1). Single nucleotide polymorphisms (SNPs) were extracted by snippy pipeline (v4.3.6) with the reference genome ATCC 13950 (NC_016946.1) (https://github.com/tseemann/snippy). The maximum-likelihood phylogenetic tree based on the core SNPs was constructed by RAxML-NG, using 1000 bootstrap iterations and the GTR+G model. The genome comparison of the two strains was calculated by the ANI online tools (https://www.ezbiocloud.net/tools) (25).

Statistical analysis

All statistical analyses were performed by SPSS v18.0 software (SPSS Inc. USA), Chi-square test or Fisher exact test was used for categorical data. $P < 0.05$ was considered statistically significant.

Results

The species re-identification

In our study, 117 clinical *M. intracellulare* strains have been re-identified by the average nucleotide identity (ANI) of the whole genome sequence, and the strains are separated into two major groups (Supplementary Figure S1). The reference strains *M. intracellulare* ATCC 13950 and *M. paraintracellulare* MOTT64, and *M. indicus pranii* MTCC 9506 belong to two different groups, respectively. According to the recent study on genetic comparisons of *M. intracellulare* (12), the two groups are defined as the typical *M. intracellulare* (TMI) group and the *M. paraintracellulare-M. indicus pranii* (MP-MIP) group. However, it is noted that only 23.93% (28/117) of the strains were identified as typical *M. intracellulare*, and most strains (76.07%, 89/117) belong to the MP-MIP group, suggesting the strains of the MP-MIP group may be common in clinical isolates in China.

Phylogenetic analysis

The phylogenetic tree also shows that the 117 strains were divided into two major groups: TMI group and MP-MIP group (Figure 1). The population structure of enrolled strains is in line with the genomic analysis of the *M. intracellulare* in previous reports. In typical *M. intracellulare* (TMI) group, we can see that there are three main clades (clade A, B, C) and every clade, including the strains from different countries, show the clustering of strains was not influenced by the geographical location. Additionally, we discovered that *M. intracellulare* strains had a significant level of genetic diversity, the previously registered strains belong to different clades, such as ATCC13950, M.i.198, and FDAARGOS_1612. In the MP-MIP group, the strains can be classified into five clades (clade D-H), and most strains in our study belong to clade H. The MP reference strain MOTT64 clustered with other 6 strains (1280, 1077, 1034, 1029, 3105, and JCM30622) is in clade H. The MIP reference strain ATCC9506 is a member of clade F, which consists of 9 phylogenetically closely related strains including M003. However, the majority of strains of the MP-MIP group were divided into various clades and clusters, indicating the considerable genetic variability of the genome. Interestingly, we found a public strain of MIP (NFDAARGOS_1610, NZ_CP089222.1) from Germany belonging to the TMI group in our study. By comparing two genome sequences, the ANI identity of this strain with the reference ATCC 13950 (NC_016946.1) and ATCC 9506 (NC_018612.1) is 99.43 and 98.74%, respectively. Thus, this strain may be more related to *M. intracellulare*.

Antimicrobial susceptibility profiles

Antimicrobial susceptibilities and MIC range of 28 *M. intracellulare* strains and 89 MP-MIP strains in this study were shown in Table 1 and Figure 2. Clarithromycin was found to be the most effective antibiotic against typical *M. intracellulare* (96.43%) and MP-MIP (97.75%) strains. The MIC_{50} and MIC_{90} were 2 μg/ml and 4 μg/ml, respectively. Amikacin was highly active against the *M. intracellulare* (92.86%) and MP-MIP (89.89%) strains. Rifabutin also

TABLE 1 The antimicrobial susceptibilities and minimum inhibitory concentrations (MICs) of *M. intracellulare* and MP-MIP strains.

	Critical concentrations (μg/ml)			*M. intracellulare*			MP-MIP			χ^2	P-value
Agents	S	I	R	MIC50	MIC90	R (*n*,%)	MIC50	MIC90	R (*n*,%)		
CLR	8	16	32	2	4	1 (3.57)	2	4	2 (2.25)	–	0.564*
AN	16	32	64	8	32	2 (7.14)	16	64	9 (10.11)	0.010	0.922
MXF	1	2	4	2	4	13 (46.43)	4	8	55 (61.80)	2.067	0.151
LNZ	8	16	32	32	64	16 (57.14)	32	64	50 (56.18)	0.008	0.929
CIP	1	2	4	>16	>16	27 (96.43)	>16	>16	78 (87.64)	0.960	0.327
DO	1	2–4	8	>16	>16	27 (96.43)	>16	>16	87 (97.75)	–	0.563*
EMB	2	4	8	4	16	9 (32.14)	4	16	39 (43.82)	1.200	0.273
RIF	1		2	4	8	26 (92.86)	8	8	82 (92.13)	0.000	>0.999
RFB	2		4	0.5	2	2 (7.14)	0.5	2	7 (7.87)	0.000	>0.999
SXT	2/38		4/76	2	8	11 (39.29)	2	8	38 (42.70)	0.102	0.750
ETH				>20	>20	-	>20	>20	-		
INH				>8	>8	-	>8	>8	-		
SM				32	>64	-	32	>64	-		

*Indicating *P* value was calculated by Fisher exact test.

shows good activity against the *M. intracellulare* (92.86%) and MP-MIP (92.13%) strains. We also found that most strains are resistant to ciprofloxacin, doxycycline, and rifampicin. The resistant rates of clarithromycin, linezolid, doxycycline, and rifampicin for *M. intracellulare* are higher than MP-MIP strains with no significant difference. However, ethionamide, isoniazid, and streptomycin have no breakpoint established by CLSI, and the MIC_{90} for ethionamide, isoniazid, and streptomycin in this study were >20, >8, and >64 μg/ml, respectively. In addition, we have performed a comparative analysis of drug resistance in different clade strains and have not found a correlation between the clades and drug resistance (Supplementary Figure S2, Supplementary Table S2).

Mutations profiling

In our analysis, the 16S rRNA gene (*rrs*) sequences for amikacin-resistant and amikacin-susceptible strains were identical, which was responsible for amikacin resistance (26). A previous study has described that mutations in 23S rRNA gene (*rrl*) could lead to clarithromycin resistance (27). There are some nucleotide changes in the *rrl* gene, but they are unrelated to drug resistance (Supplementary Table S3). We also investigated the relationship between moxifloxacin resistance and gyrA or gyrB mutation. The peptide sequences of GyrA and GyrB were identical for *M. intracellulare* ATCC 13950 and MIP MTCC 9506, but there were more peptide substitutions in GyrA and GyrB for MP-MIP strains,

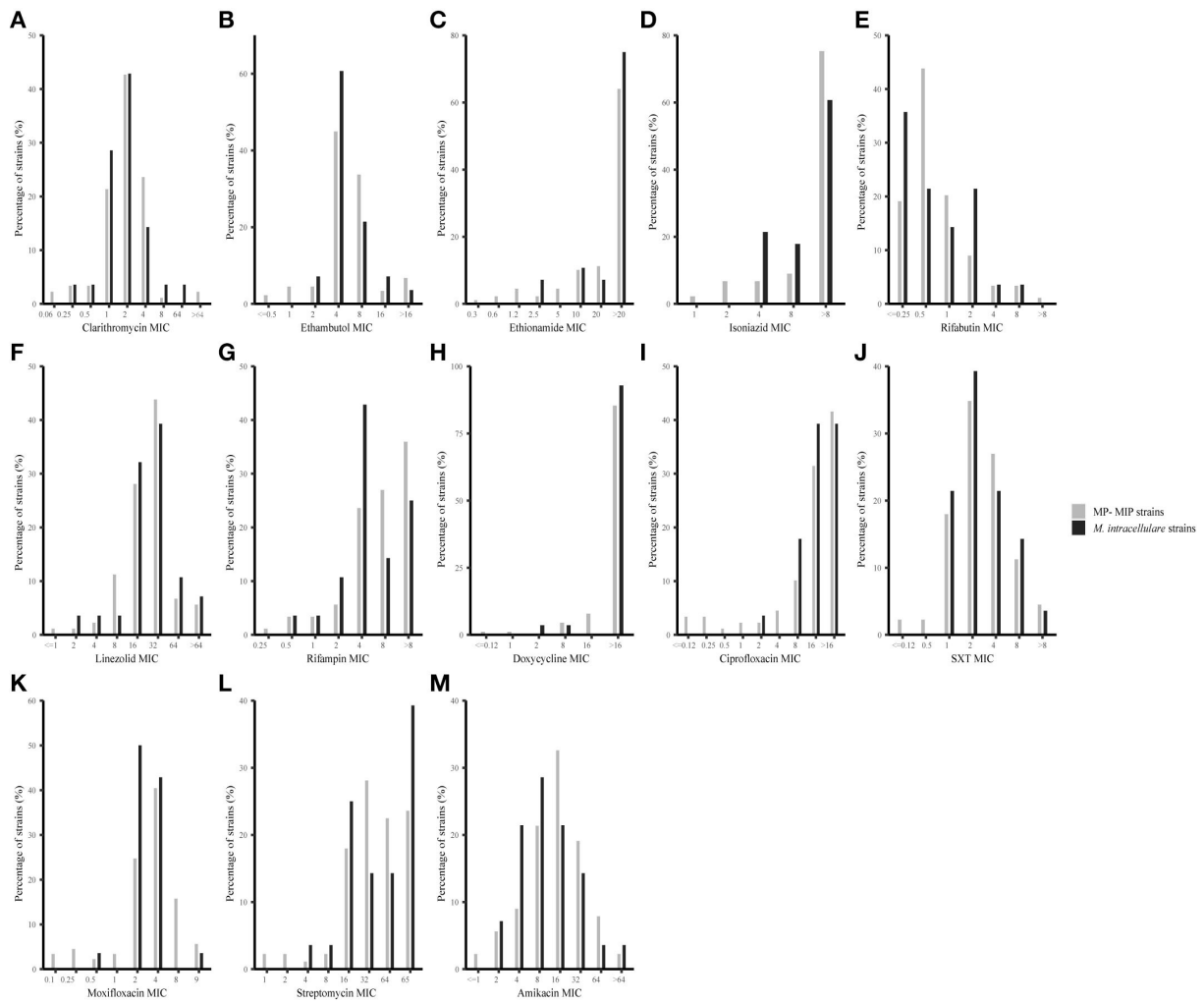

FIGURE 2
MIC distributions for 28 *M. intracellulare* and 89 MP-MIP strains in this study. The vertical axis of each graph represents the number of strains corresponding to each horizontal coordinate as a percentage of the total number of strains. The black bar and gray bar represent *M. intracellulare and* MP-MIP strains, respectively. **(A–M)** Showed MIC distributions of clarithromycin, ethambutol, ethionamide, isoniazid, rifabutin, linezolid, rifampicin, doxycycline, ciprofloxacin, SXT, moxifloxacin, streptomycin, and amikacin, respectively.

especially for the GyrB. In comparison to the reference *M. intracellulare* ATCC 13950, 85.39% (76/89) of the MP-MIP isolates had Arg222Lys mutations in gyrA, and 24 and 10 MIP isolates had Glu594Asp and Lys167Gln substitution in GyrB, respectively (Supplementary Table S4), but we have not found any moxifloxacin resistance-associated mutations in GyrA or GyrB, which suggest that mechanisms other than gyrA and gyrB mutations might have contributed to moxifloxacin resistance.

Discussion

M. intracellulare is one of the most common causes of NTM lung disease worldwide, and it has been isolated from clinical pulmonary disease in many areas of China (6, 28). MIP, MP, and *M. intracellulare* are very closely related in the genome. In this study, we analyzed the genome of 117 clinical strains that were previously identified as *M. intracellulare* and presented the phenotypic resistance profile of these strains.

By comparing the genome of clinical *M. intracellulare* strains, we revealed that *M. intracellulare* in China could be classified into two major groups: TMI group and MP-MIP group. This result is supported by a recent report about a genome analysis of *M. intracellulare*, which presented convincing evidence that MP and MIP should be regarded as variants of *M. intracellulare* (12). In our study, 76.07% of strains belong to the MP-MIP group, suggesting that the majority of *M. intracellulare* strains in China could be MP-MIP strains in the genome, and these strains should be considered potential causative agents of pulmonary diseases. With genetic sequencing increasingly affordable, the MP-MIP group strains can be detected more frequently in the future. Though variable numbers of tandem repeats (VNTR) analysis has been a highly discriminatory tool in molecular epidemiology analysis, it is unable to classify the *M. intracellulare* and related strains such as MP and MIP (29). A previous study found that there are 4% of *M. intracellulare* isolates that have been identified as MIP by sequence-based typing analyses (15, 16). Thus, the identification of *M. intracellulare* and related strains should be addressed by multigene sequence analysis or comparative genomic analysis (12, 16). In addition, more research on the pathogenesis of the MP-MIP group is needed, as well as comparisons with *M. intracellulare*.

Our results show that the genetic characteristics of clinical isolates of *M. intracellulare* are not related to geographical location, which is consistent with the previous reports with VNTR analyses (29, 30). In contrast to *M. intracellulare*, the genetic characteristics and molecular epidemiology of clinical strains of the MP-MIP group are poorly understood. Alexander et al. suggested that MIP is a strain of *M. intracellulare* and it is more likely to have specific transposons acquisition and inversion events (31). Our results showed genetic diversity in *M. indicus pranii*, which should be further explored in future studies.

As we all know, few studies reported antimicrobial susceptibility profiles of MP-MIP strains. Our study compared the antimicrobial susceptibilities profile between MP-MIP and *M. intracellulare* strains against 13 drugs, but no statistically significant differences were observed (Table 1). To date, only macrolides have been demonstrated to have a link between *in vitro* susceptibility and clinical responses in patients with MAC lung disease (32). Among the 13 antimicrobials, clarithromycin showed the best activity *in vitro* against *M. intracellulare* isolates, and amikacin has a low resistance rate in our study, which is in line with other studies (33, 34). Rifabutin also has good activity *in vitro* against *M. intracellulare* isolates. Previous studies found that rifabutin is efficacious in multidrug MAC therapy regimens, and it also affects the metabolism and levels of clarithromycin less than rifampin and is generally used to treat disseminated MAC disease (35). Van Ingen et al. did a pharmacokinetic/pharmacodynamic study about the treatment of MAC pulmonary disease, which showed that rifabutin could increase macrolide serum concentrations, especially azithromycin, but rifampin exhibited the opposite (36). Therefore, some experts suggest that rifampin could be replaced with rifabutin in the treatment of MAC infection. The ethambutol and moxifloxacin resistance rates in our strains are much lower than those previously reported in Shanghai, China (20).

Previous studies showed that mutations in the *rrs* and *rrl* genes are associated with aminoglycoside and macrolide resistance, respectively (26, 27). However, none of the tested *M. intracellulare* and MP-MIP strains in our study harbored mutations in the *rrs* genes. This result may be related to the level of drug resistance, as Su-Young Kim et al. showed that mechanisms of high-level resistance to amikacin in MAC isolates involve *rrs* mutations (37). We have not found nucleotide changes in the *rrl* gene related to macrolides resistance, which may be caused by an unknown molecular mechanism. Mutations in gyrA and gyrB are not associated with moxifloxacin resistance in this study, which is consistent with the previous study about *mycobacterium avium* complex isolates (38), suggesting that other mechanisms contribute to moxifloxacin resistance.

A few limitations in this research warrant mention. First, the strains we selected may have sampling bias, resulting in the proportion of the MP-MIP strains being higher than those in previous reports. Therefore, a larger study with more clinical samples of *M. intracellulare* strains is warranted to confirm our findings. Second, this study lacks the clinical background information of the strains, which limited our ability to determine the severity of the disease caused by MP-MIP strains.

Conclusion

In the present study, we found that clinical *M. intracellulare* strains in China were highly diverse. The phylogenetic analysis found that the *M. intracellulare* strains belong to two major groups: the *M. intracellulare* group and the MP-MIP group, and 76.07% of strains belong to the MP-MIP group. Our finding suggested MP-MIP strains should be considered as a causative agent of severe and chronic lung disease, and its pathogenicity needs to be investigated further. In addition, our data demonstrate no difference in drug susceptibility profiles between *M. intracellulare* and MP-MIP strains. Clarithromycin, amikacin and rifabutin showed strong antimicrobial activity *in vitro* against both *M. intracellulare* and MP-MIP. However, this study showed that there was no clear correlation between the *rrs*, *rrl*, DNA gyrase genes (*gyrA* and *gyrB*) and the aminoglycosides, macrolides, and moxifloxacin resistance, respectively, indicating other mechanisms might have been involved in drug resistance.

Data availability statement

The datasets presented in the study are deposited in the NCBI BioProject repository with accession number PRJNA890446.

Author contributions

ZS, AM, ZL, and YZ contributed to study design, data analysis, and manuscript writing. YW, WH, and DL participated in the study design, data collection, and analysis. PH, CL, XZ, and BZ conducted laboratory testing. HX and SW revised and polished the manuscript. All the authors have read the final version of the manuscript and have approved it.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.989587/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Pairwise comparison of ANIs of 117 clinical *M. intracellulare* strains in this study. The *M. intracellulare* ATCC 13950, MIP MTCC 9506, *M. intracellulare subsp. yongonense* 05-1390, *Mycobacterium paraintracellulare* MOTT64 and *M. intracellulare subsp. chimaera* DSM 44623 were set as the subspecies reference genomes. The ANI value and the strain type legend are shown on the right.

SUPPLEMENTARY FIGURE S2
Phylogenetic tree of the 28 *M. intracellulare* strains and 89 MP-MIP strains in this study. The tree based on core SNPs was constructed by RAxML with a GTR model, with 1000 bootstrap replicates. The group type, clades, and drug resistance profile of the strains are shown on the tree (from inner to outer circles), according to the color legend shown on the right.

SUPPLEMENTARY TABLE S1
The public genomes of 30 *M. intracellulare* and 14 related strains were used in this study.

SUPPLEMENTARY TABLE S2
The antimicrobial susceptibilities and minimum inhibitory concentrations (MICs) of strains in different clades.

SUPPLEMENTARY TABLE S3
The 23S RNA mutation of 28 *M. intracellulare* and 89 MP-MIP strains in this study.

SUPPLEMENTARY TABLE S4
The GyrA and GyrB mutation of 28 *M. intracellulare* and 89 MP-MIP strains in this study.

References

1. Kwon YS, Koh WJ. Diagnosis and treatment of nontuberculous mycobacterial lung disease. *J Korean Med Sci.* (2016) 31:649–59. doi: 10.3346/jkms.2016.31.5.649

2. Shah NM, Davidson JA, Anderson LF, Lalor MK, Kim J, Thomas HL, et al. Pulmonary Mycobacterium avium-intracellulare is the main driver of the rise in non-tuberculous mycobacteria incidence in England, Wales and Northern Ireland, 2007-2012. *BMC Infect Dis.* (2016) 16:195. doi: 10.1186/s12879-016-1521-3

3. Koh WJ, Jeong BH, Jeon K, Lee NY, Lee KS, Woo SY, et al. Clinical significance of the differentiation between Mycobacterium avium and Mycobacterium

intracellulare in M avium complex lung disease. *Chest.* (2012) 142:1482–8. doi: 10.1378/chest.12-0494

4. Nishiuchi Y, Iwamoto T, Maruyama F. Infection Sources of a Common Non-tuberculous Mycobacterial Pathogen, Mycobacterium avium Complex. *Front Med.* (2017) 4:27. doi: 10.3389/fmed.2017.00027

5. Ahn K, Kim YK, Hwang GY, Cho H, Uh Y. Continued upward trend in non-tuberculous mycobacteria isolation over 13 years in a tertiary care hospital in Korea. *Yonsei Med J.* (2021) 62:903–10. doi: 10.3349/ymj.2021.62.10.903

6. Liu CF, Song YM, He WC, Liu DX, He P, Bao JJ, et al. Nontuberculous mycobacteria in China: incidence and antimicrobial resistance spectrum from a nationwide survey. *Infect. Dis Pover.* (2021) 10:59. doi: 10.1186/s40249-021-00844-1

7. Rahman SA, Singh Y, Kohli S, Ahmad J, Ehtesham NZ, Tyagi AK, et al. Comparative analyses of nonpathogenic, opportunistic, and totally pathogenic mycobacteria reveal genomic and biochemical variabilities and highlight the survival attributes of Mycobacterium tuberculosis. *mBio.* (2014) 5:e02020. doi: 10.1128/mBio.02020-14

8. Sharma P, Mukherjee R, Talwar GP, Sarathchandra KG, Walia R, Parida SK, et al. Immunoprophylactic effects of the anti-leprosy Mw vaccine in household contacts of leprosy patients: clinical field trials with a follow up of 8-10 years. *Lepr Rev.* (2005) 76:127–43. doi: 10.47276/lr.76.2.127

9. Castejon M, Menéndez MC, Comas I, Vicente A, Garcia MJ. Whole-genome sequence analysis of the Mycobacterium avium complex and proposal of the transfer of Mycobacterium yongonense to Mycobacterium intracellulare subsp. yongonense subsp nov. *Int J System Evolut Microbiol.* (2018) 68:1998–2005. doi: 10.1099/ijsem.0.002767

10. Riccardi N, Monticelli J, Antonello RM, Luzzati R, Gabrielli M, Ferrarese M, et al. Mycobacterium chimaera infections: An update. *J Infect Chemother.* (2020) 26:199–205. doi: 10.1016/j.jiac.2019.11.004

11. Tortoli E, Mariottini A, Pierotti P, Simonetti TM, Rossolini GM. Mycobacterium yongonense in pulmonary disease, Italy. *Emerg Infect Dis.* (2013) 19:1902–4. doi: 10.3201/eid1911.130911

12. Tateishi Y, Ozeki Y, Nishiyama A, Miki M, Maekura R, Fukushima Y, et al. Comparative genomic analysis of Mycobacterium intracellulare: implications for clinical taxonomic classification in pulmonary Mycobacterium avium-intracellulare complex disease. *BMC Microbiol.* (2021) 21:103. doi: 10.1186/s12866-021-02163-9

13. Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T, Kyrpides NC, et al. Genome-based taxonomic classification of the phylum actinobacteria. *Front Microbiol.* (2018) 9:2007. doi: 10.3389/fmicb.2018.02007

14. van Ingen J, Turenne CY, Tortoli E, Wallace RJ, Brown-Elliott BA. A definition of the Mycobacterium avium complex for taxonomical and clinical purposes, a review. *Int J Syst Evol Microbiol.* (2018) 68:3666–77. doi: 10.1099/ijsem.0.003026

15. Kim SY, Shin SH, Moon SM, Yang B, Kim H, Kwon OJ, et al. Distribution and clinical significance of Mycobacterium avium complex species isolated from respiratory specimens. *Diagn Microbiol Infect Dis.* (2017) 88:125–37. doi: 10.1016/j.diagmicrobio.2017.02.017

16. Kim SY, Park HY, Jeong BH, Jeon K, Huh HJ Ki CS, Lee NY, et al. Molecular analysis of clinical isolates previously diagnosed as Mycobacterium intracellulare reveals incidental findings of "Mycobacterium indicus pranii" genotypes in human lung infection. *BMC Infect Dis.* (2015) 15:406. doi: 10.1186/s12879-015-1140-4

17. Wassilew N, Hoffmann H, Andrejak C, Lange C. Pulmonary Disease Caused by Non-Tuberculous Mycobacteria. *Respiration.* (2016) 91:386–402. doi: 10.1159/000445906

18. Daley CL, Iaccarino JM, Lange C, Cambau E, Wallace RJ, Andrejak C, et al. Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline. *Clin Infect Dis.* (2020) 71:e1–e36. doi: 10.1093/cid/ciaa241

19. Maurer FP, Pohle P, Kernbach M, Sievert D, Hillemann D, Rupp J, Hombach M, Kranzer K. Differential drug susceptibility patterns of Mycobacterium chimaera and other members of the Mycobacterium avium-intracellulare complex. *Clin Microbiol Infect.* (2019) 25:379.e1–379.e7. doi: 10.1016/j.cmi.2018.06.010

20. Wang W, Yang J, Wu X, Wan B, Wang H, Yu F, et al. Difference in drug susceptibility distribution and clinical characteristics between Mycobacterium avium and Mycobacterium intracellulare lung diseases in Shanghai, China. *J Med Microbiol.* (2021) 70:001358. doi: 10.1099/jmm.0.001358

21. Woods GL, Brown-Elliott BA, Conville PS, Desmond EP, Hall GS, Lin G, et al. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes*. Wayne (PA): Clinical and Laboratory Standards Institute.

22. Larsen MH, Biermann K, Tandberg S, Hsu T, Jacobs WR. Genetic Manipulation of Mycobacterium tuberculosis. *Curr Protocols Microbiol.* (2007) 6:10A—2. doi: 10.1002/9780471729259.mc10a02s6

23. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* (2014) 30:2114–20. doi: 10.1093/bioinformatics/btu170

24. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol.* (2013) 20:714–37. doi: 10.1089/cmb.2013.0084

25. Yoon SH, Ha SM, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek.* (2017) 110:1281–6. doi: 10.1007/s10482-017-0844-4

26. Brown-Elliott BA, Iakhiaeva E, Griffith DE, Woods GL, Stout JE, Wolfe CR, et al. In vitro activity of amikacin against isolates of Mycobacterium avium complex with proposed MIC breakpoints and finding of a 16S rRNA gene mutation in treated isolates. *J Clin Microbiol.* (2013) 51:3389–94. doi: 10.1128/JCM.01612-13

27. Mougari F, Loiseau J, Veziris N, Bernard C, Bercot B, Sougakoff W, et al. Evaluation of the new GenoType NTM-DR kit for the molecular detection of antimicrobial resistance in non-tuberculous mycobacteria. *J Antimicrob Chemother.* (2017) 72:1669–1677. doi: 10.1093/jac/dkx021

28. Tan Y, Deng Y, Yan X, Liu F, Tan Y, Wang Q, et al. Nontuberculous mycobacterial pulmonary disease and associated risk factors in China: A prospective surveillance study. *J Infect.* (2021) 83:46–53. doi: 10.1016/j.jinf.2021.05.019

29. Lari N, Rindi L. High genetic heterogeneity of Mycobacterium intracellulare isolated from respiratory specimens. *BMC Microbiol.* (2022) 22:5. doi: 10.1186/s12866-021-02426-5

30. Ichikawa K, van Ingen J, Koh WJ, Wagner D, Salfinger M, Inagaki T, et al. Genetic diversity of clinical Mycobacterium avium subsp. hominissuis and Mycobacterium intracellulare isolates causing pulmonary diseases recovered from different geographical regions. *Infect Genet Evol.* (2015) 36:250–5. doi: 10.1016/j.meegid.2015.09.029

31. Alexander DC, Turenne CY. "Mycobacterium indicus pranii" is a strain of Mycobacterium intracellulare. *MBio.* (2015) 6:e00013. doi: 10.1128/mBio.00013-15

32. Tanaka E, Kimoto T, Tsuyuguchi K, Watanabe I, Matsumoto H, Niimi A, et al. Effect of clarithromycin regimen for Mycobacterium avium complex pulmonary disease. *Am J Respir Crit Care Med.* (1999) 160:866–72. doi: 10.1164/ajrccm.160.3.9811086

33. Zheng HW, Pang Y, He GX, Song YY, Zhao YL. Comparing the Genotype and Drug Susceptibilities between Mycobacterium avium and Mycobacterium intracellulare in China. *Biomed Environ Sci.* (2017) 30:517–25.

34. Renvoisé A, Bernard C, Veziris N, Galati E, Jarlier V, Robert J. Significant difference in drug susceptibility distribution between Mycobacterium avium and Mycobacterium intracellulare. *J Clin Microbiol.* (2014) 52:4439–40. doi: 10.1128/JCM.02127-14

35. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med.* (2007) 175:367–416. doi: 10.1164/rccm.200604-571ST

36. van Ingen J, Egelund EF, Levin A, Totten SE, Boeree MJ, Mouton JW, et al. The pharmacokinetics and pharmacodynamics of pulmonary Mycobacterium avium complex disease treatment. *Am J Respir Crit Care Med.* (2012) 186:559–65. doi: 10.1164/rccm.201204-0682OC

37. Kim SY, Kim DH, Moon SM, Song JY, Huh HJ, Lee NY, et al. Association between 16S rRNA gene mutations and susceptibility to amikacin in Mycobacterium avium Complex and Mycobacterium abscessus clinical isolates. *Sci Rep.* (2021) 11:6108. doi: 10.1038/s41598-021-85721-5

38. Kim SY, Jhun BW, Moon SM, Shin SH, Jeon K, Kwon OJ, et al. Mutations in gyrA and gyrB in moxifloxacin-resistant Mycobacterium avium complex and mycobacterium abscessus complex clinical isolates. *Antimicrob Agents Chemother.* (2018) 62:e00527–18. doi: 10.1128/AAC.00527-18

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Bibliometric analysis of tuberculosis molecular epidemiology based on CiteSpace

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Background: Tuberculosis is a communicable disease that is a major cause of ill health. Bibliometrics is an important statistical methodology used to analyze articles and other publications in the literature study. In this study, publications on molecular epidemiology were analyzed using bibliometric analysis. The statistical analysis of influential publications, journals, countries and authors was first conducted.

Methods: The Web of Science database was searched for publications on the molecular epidemiology of tuberculosis with the keywords "tuberculosis" and "molecular epidemiology" in the title. The number of publications, citation analysis, co-authorship of the author, institution and country, keyword co-occurrence, and reference co-citations were analyzed.

Results: A total of 225 journal articles were retrieved. The mean citation was 37.72 per article and 292.69 per year. The annual publications on molecular epidemiology fluctuated within a certain range in the past. Journal of Clinical Microbiology is the most published journal with 33 articles. RASTOGI N is the most prolific author with 11 articles. The top 1 research institution is Inst Pasteur Guadeloupe. Stratified by the number of publications, the USA was the most prolific country. It also cooperates closely with other countries. Burstness analysis of references and keywords showed that the developing research trends in this field mainly focused on "genetic diversity" and "lineage" during the past decade.

Conclusion: The annual publications on tuberculosis molecular epidemiology fluctuated within a specific range in the past decade. The USA continues to dominate research output and funding. The exchange of expertise, ideas, and technology is of paramount importance in this field. More frequent and deeper cooperation among countries or institutions will be essential in the future.

KEYWORDS

tuberculosis, molecular epidemiology, bibliometrics, CiteSpace, Web of Science

Introduction

Tuberculosis (TB) remains a serious global public health threat asserted by the World Health Organization (WHO) in 2021. There were an estimated 9.9 million new cases of TB worldwide in 2020. The number of TB cases in China was 842,000, ranks second in the world flowing India (1). Molecular epidemiology of tuberculosis is based on genotyping technology and combines traditional epidemiology with emerging biotechnology to elucidate the occurrence, development, epidemic, drug resistance, and transmission rules of tuberculosis at the molecular level (2).

To have a more comprehensive understanding of the prevalence and transmission of tuberculosis and to formulate effective control measures, new theories and methods are needed to apply to tuberculosis research. Bibliometrics is a discipline that uses mathematics, statistics and other measurements to examine the quantitative relationship and development law of all knowledge carriers, such as documents and document information systems, and to explore the dynamic characteristics of science. As a relatively new literature research method, bibliometric analysis can evaluate the development trend of research activities by qualitative and quantitative methods according to the information provided by the literature database, grasp the development of a particular field and provide information for comparing the contributions of different levels (3). The bibliometric method can be used to analyse number of publications to efficiently find influential publications, authors, journals, organizations and countries. At present, there are a lot of software tools proposed to develop science mapping analysis (4–6). CiteSpace is a bibliometric tool based on the principle of "co-citation analysis theory" to reveal new technologies, hotspots, and trends in the medical field (7). It can perform bibliometric analysis on a specific area and draw a series of visual knowledge maps to explore the key paths and frontier developments in the evolution of scientific research fields.

In this paper, a bibliometric method is used to conduct a statistical analysis of English literature related to molecular epidemiology, and CiteSpace software is used to visualize the statistical results, which can help scholars quickly understand the research status and hotspots and provides new ideas and directions for studying the epidemic and transmission laws of tuberculosis.

Data and methods

Data collection

We used the keywords "tuberculosis" and "molecular epidemiology" in the title to retrieve manuscripts published from the Web of Science Core Collection from the establishment of the database to 2022. We collected 225 manuscripts and downloaded all information on 15 August 2022. The inclusion criteria were: (1) peer-reviewed published original articles; (2) reviews; (3) language in English, excluding meetings, letters, editorial material, books, and abstract.

Data analysis and visualization

Annual publications, citations, funding agencies and source journals were imported into Microsoft Excel 2016 for analysis and visualization. Other information from 225 manuscripts was converted to a text file and then imported into CiteSpace 6.1R3 Basic. We analyzed co-authorship of the author, institution and country, keyword co-occurrence, and reference co-citations. The main parameters were set as follows: the time slicing was 1 year per slice from January 1993 to August 2022. The selection criteria were g-index and the scale factor was $k = 25$.

Results

Annual publications and citations

There were 197 articles and 28 reviews published between 1993 and 2022. The summed citations were 8,488. The average number of citations per item was 37.72 and the average number of citations per year was 292.69 (Figure 1).

The first article focus on the molecular epidemiology of tuberculosis was published in 1993. From 1993 to 2003, the number of publications increased year by year. The number of citations also increased fast, showing a rapid development trend. From 2004 to 2013, the research superheat in this field declined, but still showed an overall volatile increased trend. There were two peaks in 2008 and 2013, and the number of citations in this field reached a peak of 491 in 2014. The annual publications declined since 2014. In 2018 and 2021, there were two publication peaks, with 13 and 10 articles published, respectively, and the number of citations was declining year by year.

Journal analysis

Articles in this field are mainly published in 92 journals, of which the productivity and average impact factors in the past 5 years of the top 10 active journals are shown in Table 1. Journal of Clinical Microbiology is the most published journal in this field with 33 articles. The average impact factor of the journal in the past 5 years is 8.075, and an impact factor in 2021 is 11.677. The second-ranked journal is the International Journal of Tuberculosis and Lung Disease, with a total of 22 published articles, with an average impact factor of 2.669 in the past 5 years and an impact factor of 3.427 in 2021.

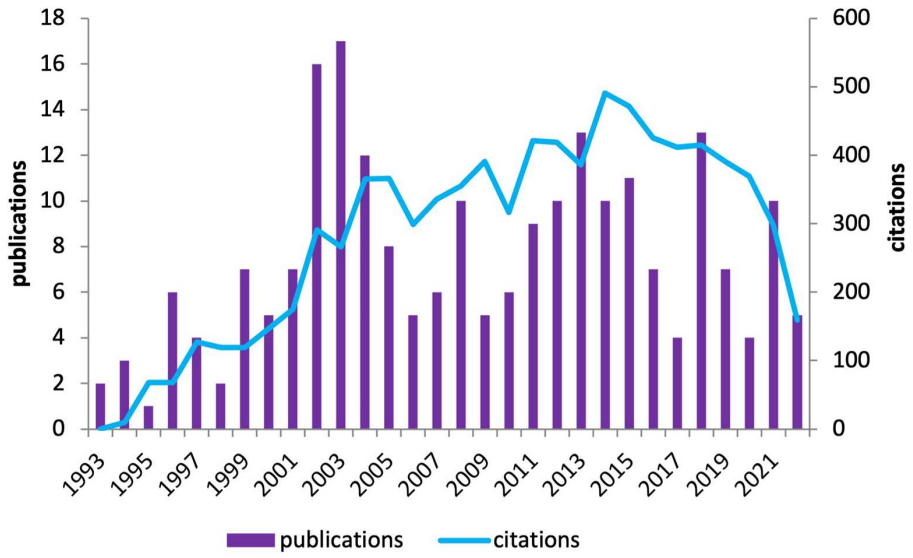

FIGURE 1
Times cited and publications on TB molecular epidemiology over time.

TABLE 1 Productivity and average impact factors (in the past five years) of top 10 active journals.

Journals	Numbers	Impact factor
Journal of clinical microbiology	33	8.075
International journal of tuberculosis and lung disease	22	2.669
Plos ONE	16	4.069
Infection genetics and evolution	12	3.656
BMC infectious diseases	10	3.714
Clinical microbiology and infection	8	10.298
Emerging infectious diseases	7	10.717
Tuberculosis	5	2.973
Clinical infectious diseases	4	15.446
Journal of infection	4	19.923

TABLE 2 Top 10 funding agencies of tuberculosis molecular epidemiology.

Funding agencies	Numbers
United States department of health human services	24
National institutes of health Nih Usa	23
European commission	15
Nih national institute of allergy infectious diseases Niaid	15
Nih fogarty international center Fic	6
Wellcome trust	5
Canadian institutes of health research Cihr	4
Conselho nacional de desenvolvimento cientifico e tecnologico Cnpq	4
Medical research council Uk Mrc	4
Uk research innovation Ukri	4

Funding agencies analysis

Table 2 shows top 10 of the most distributed funding agencies of tuberculosis molecular epidemiology. America plays an important role in supporting to research on tuberculosis molecular epidemiology with 24 studies and 23 studies supported by United States Department Of Health Human Services and National Institutes Of Health besides two funding agencies in the United States. Agencies in Europe take second place including three agencies in Britain and 1 agency of European Union, which support more than 28 projects. In this field, Canadian Institutes of Health Research in Canada and Conselho Nacional De Desenvolvimento Cientifico E Tecnologico in Brazil subsidize four researches, respectively.

Co-author, co-institution, and co-country analysis

Figures 2–4 show the cooperation network clustering map of the author, institution, and nation in the field of tuberculosis molecular epidemiology, respectively. The nodes in the figures represent the authors, institutions, or countries. The larger the node, the more published articles are. The lines between the

nodes demonstrate the cooperative relationship between them. The colors of the nodes, lines, and cluster outlines in Figures 2, 3 are categorized by year. Purple indicates a distant age and yellow indicates a relatively recent age. The color of the node in Figure 4 shows the year, and the color of the connection line and cluster outline are used to distinguish different clusters. The nodes of the same cluster denote that their research fields are similar, and # are the cluster names extracted from the analyzed literature. Cluster names were extracted from the titles of the included literature using the log-likelihood ratio (LLR) method.

The top 5 prolific authors in the field of TB molecular epidemiology are RASTOGI N (11), DROBNIEWSKI F (10), ASEFFA A (5), AMENI G (5), and NIEMANN S (5). The numbers in parentheses are the corresponding publications. Figure 2 shows the top two authors who have published more papers in each cluster. The node of RASTOGI N is the largest and cooperates closely with other authors. It forms a complex academic cooperation network with COUVIN D (4) and others by working on the molecular epidemiology of tuberculosis in Baja California Mexico. In recent years, BORRONI E (2) and CIRILLOD (2) have collaborated on a consensus set.

The top 5 institutions in the field of tuberculosis molecular epidemiology and their publications shown in parentheses are Inst Pasteur Guadeloupe (12), Inst Pasteur (11), Natl Inst Publ Hlth & Environm (9), Ctr Dis Control & Prevent (7), Minist Hlth (6). Figure 3 shows the top two institutions with more publications in each cluster. Represented by Inst Pasteur Guadeloupe and Inst Pasteur, the largest academic cooperation system in this field has been formed in recent years to jointly carry out research related to gene expert remnant in the early years, Natl Inst Publ Hlth & Environm, Ctr Dis Control & Prevent, and Minist Hlth formed the second largest cooperative system to jointly study risk factors.

Figure 4 shows that USA has published a maximum of 45 papers and cooperated with ETHIOPIA (10). FRANCE ranks second in the number of published papers with 28 papers. At the same time, it has formed the largest cooperative group in this field with PEOPLESRCHINA (11), ITALY (9), and other countries. ENGLAND is the third country with 26 articles published and cooperated with RUSSIA (7), NORWAY (7), and other countries.

Co-occurring keywords analysis

By extracting co-occurring keywords from the selected literature, it can reflect the development trend and research hotspots of a certain field. In this study, 391 co-occurring keywords were extracted from the keywords of the 225 included literature. Ten clusters marked with # were extracted from the title of the included literature by LLR method cluster analysis, and the connecting line in the figure indicates that two keywords appear together in the same article. Figure 5 shows the top 10 high-frequency co-occurrence keywords except for search terms and the co-occurrence frequency is shown in parentheses, which are strain (78), transmission (62), fragment length polymorphism (37), new york city (34), identification (31) infection (31), complex (29), genetic diversity (28), is 6,110 (27), breakout (25). The color of the nodes in the figure is distributed by year, purple is the oldest, and yellow is the newest; red is the outbreak keyword in this field, which means it is a research hotspot in this field. It can be observed from Table 3 that the frequency of genetic diversity suddenly increased between 2007 and 2017, and it is the co-occurrence keyword with the strongest outbreak intensity in the field of TB molecular epidemiology research. Lineage has gradually increased in frequency since 2012, with an outbreak intensity of 4.67, ranking fourth, and the outbreak continues to this day. Figure 5 also shows the migration and changes of the 10 cluster names and the content of research hotspots over time.

Co-citation references analysis

Co-citation analysis of references is the most distinctive feature of CiteSpace. The more documents in the cluster, the more important it represents the clustering field. Through the span of each document, we can see the rise, prosperity, and decline of a particular clustering research field. A total of 4,964 references were included in the 225 articles. Figure 6 extracts 825 of the co-cited documents as nodes to draw a sequence diagram. The 14 cluster names marked with # shown in the figure are the basic knowledge of TB molecular epidemiology and its deduction process over time. The top 10 references by co-citation frequency, author, publication journal and year, and co-citation frequency information are shown in Table 4.

Figure 6 shows that since 1993, the research articles concern on # mycobacterium tuberculosis strain published by Alland D (1994) and Vanembden JDA (1993) in the journals NEW ENGL J MED and J CLIN MICROBIOL, respectively, have been widely cited, with a total of 36 and 28 citations. Subsequently, # methodological problem appeared in a large number of articles that were cited, among which Small PM, (1994), Kamerbeek J (1997), Van SOOLINGEND, (1999) published articles in NEW ENGL J MED, J CLIN MICROBIOL and J INFECT DIS, respectively, which became high Cited Articles. # Genetic diversity also appeared in a large number of cited articles from 2004 to 2016, which were published in J CLIN MICROBIOL and BMC MICROBIOL by Brudey K (2006) and Supply P (2006), respectively. Since 2013, # systematic review and #genexpert remnant-related articles are an important research basis for the molecular epidemiology of tuberculosis. The research related to # northern india since 2019 also provides important research information and helps in this field.

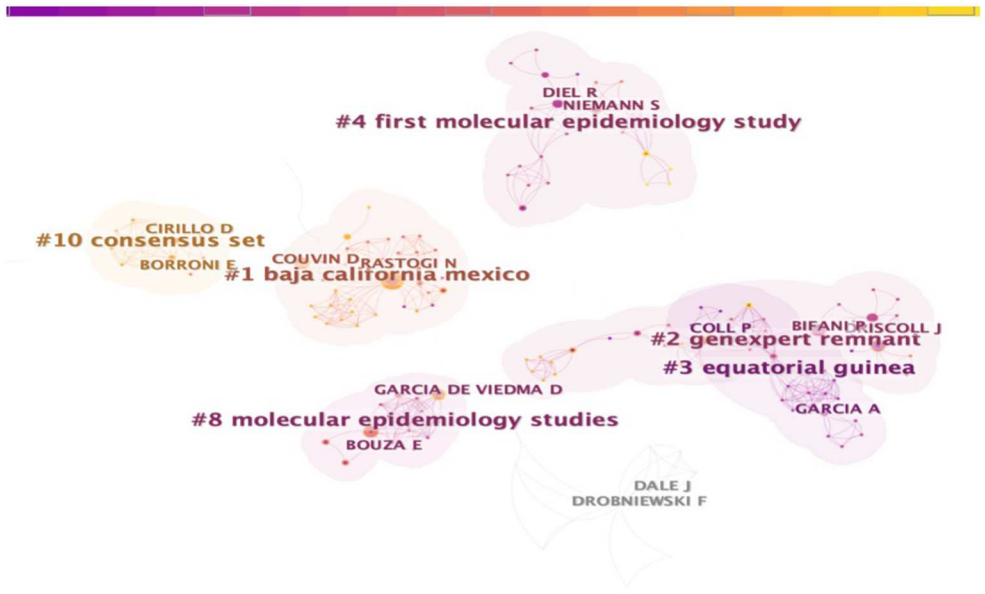

FIGURE 2
Clustered network of co-author analysis on tuberculosis molecular epidemiology.

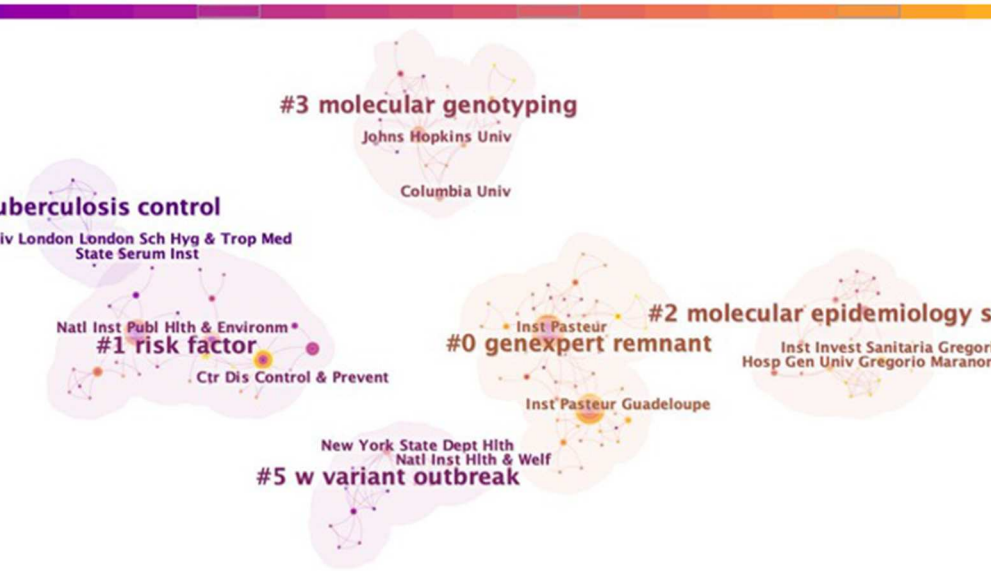

FIGURE 3
Clustered network of co-institution analysis on tuberculosis molecular epidemiology.

Discussion

Based on the multidimensional quantitative analysis of the TB molecular epidemiology literature published from the establishment of the Web of Science database to the present, it can be noticed that the number of TB molecular epidemiology studies had gradually increased before 2010s. Although the number of related articles has shrunk in recent years, it is prospected that articles associated with treatment and prevention of tuberculosis may spring up with the

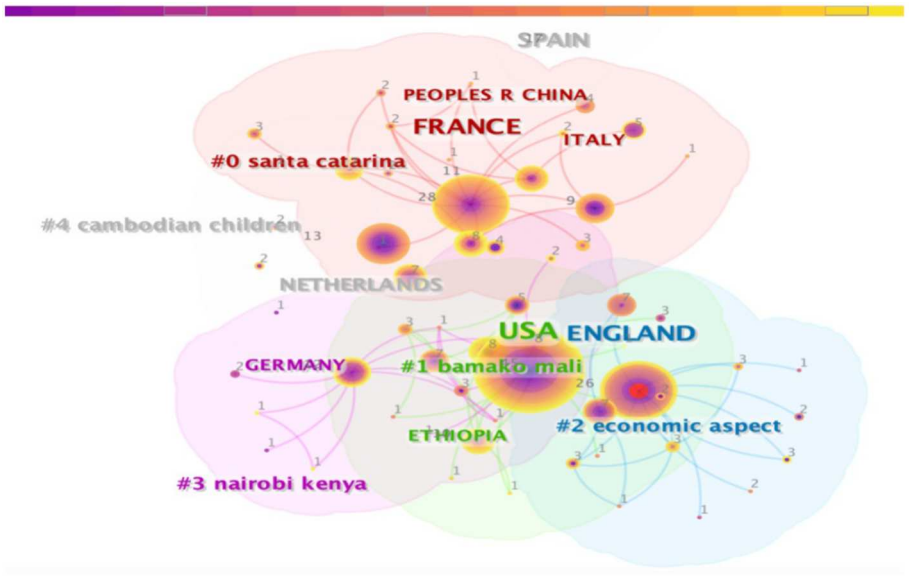

FIGURE 4
Clustered network of co-country analysis on tuberculosis molecular epidemiology.

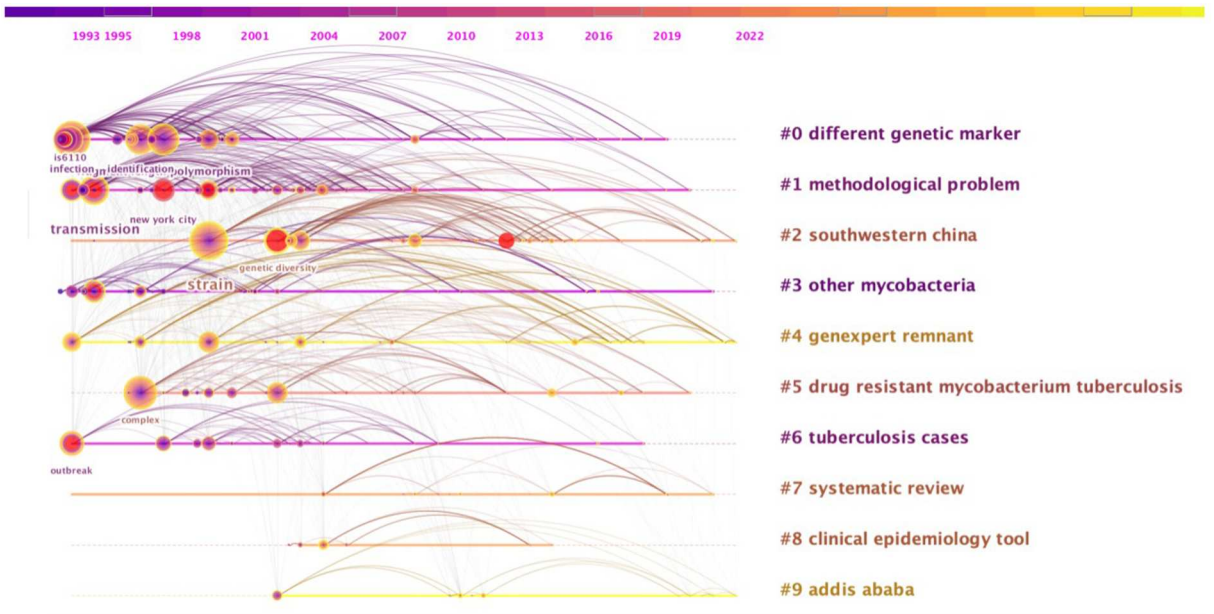

FIGURE 5
Clustered analysis of co-occurring keywords on tuberculosis molecular epidemiology.

development of molecular epidemiology and innovation of methodological problem.

In terms of the number of papers of institutions and countries and the frequency of citations, authors and institutions from developed countries in Europe and the United States have carried out more researches on the molecular epidemiology of tuberculosis, and their influence is also relatively huge. It is connected with not only economic factors but also epidemiological characteristics of tuberculosis. For the developed countries, there are definitely sufficient funds,

TABLE 3 Top 10 keywords with the strongest citation bursts of tuberculosis molecular epidemiology.

Keywords	Strength	Begin	End	1993–2022
Human immunodeficiency virus	4.96	1993	2001	
Tool	4.42	1993	2001	
Element	4.31	1993	2001	
Outbreak	3.91	1993	2002	
New york city	6.22	1998	2006	
Transmission	3.69	1998	2004	
Sanfrancisco	4.30	2002	2006	
Pulmonary tuberculosis	3.58	2004	2006	
Genetic diversity	6.27	2007	2017	
Lineage	4.67	2012	2022	

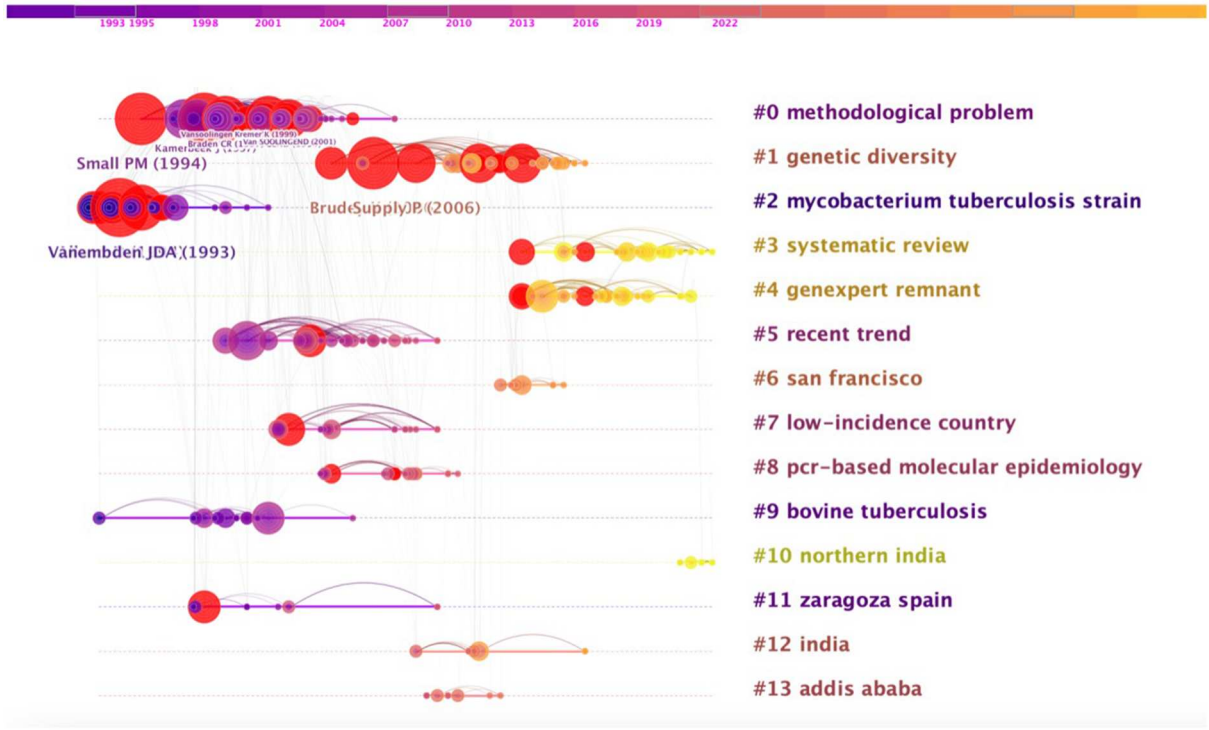

FIGURE 6
Clustered analysis of co-citation references on tuberculosis molecular epidemiology.

qualified talent, and advanced equipment to support the research. Even more, the prevalence of tuberculosis is low and not many new positive cases of TB are discovered every year in various developed countries. Then they have the capacity to explore all of them. Furthermore, mainly family transmission is convenient for carrying out related research (8–10). However, for the developing countries, it is just the opposite with a high burden of tuberculosis but lack funds, technology and equipment. As a result, control of tuberculosis might deteriorate and it becomes a vicious cycle because of the imbalance. Our research shows that there is close cooperation among countries both in developed and developing countries. Developed countries provide funding and technical support in low-income developing countries with a high TB burden. In this regard, developing countries should strive for international cooperation and financial support including some non-governmental organization funds, such as Global Fund Project and Gates Foundation Tuberculosis Prevention and Control projects,

TABLE 4 Top 10 co-cited references of tuberculosis molecular epidemiology.

Title	Author	Journal	Year	Citations
Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiologic methods	D Alland	N Engl J Med.	1994	36
The epidemiology of tuberculosis in San Francisco. A population-based study using conventional and molecular methods	Small PM	N Engl J Med.	1994	35
Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology	J Kamerbeek	J Clin Microbiol.	1997	30
Mycobacterium tuberculosis complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology	Brudey K	BMC Microbiol.	2006	28
Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology	van Embden JD	J Clin Microbiol.	1993	24
Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis.	Supply P	J Clin Microbiol.	2006	22
Molecular epidemiology of tuberculosis in the Netherlands: a nationwide study from 1993 through 1997	van Soolingen D	J Infect Dis.	1999	21
Interpretation of restriction fragment length polymorphism analysis of Mycobacterium tuberculosis isolates from a state with a large rural population.	Braden CR	J Infect Dis.	1997	19
Molecular epidemiology of tuberculosis and other mycobacterial infections: main methodologies and achievements.	Van Soolingen D	J Intern Med.	2001	18
Predominance of a single genotype of Mycobacterium tuberculosis in countries of east Asia.	van Soolingen D,	J Clin Microbiol.	1995	17

which will help the development of scientific research and reduce this global health threat.

We have acknowledged some authoritative writers in the field of tuberculosis molecular epidemiology, such as RASTOGI N, D Alland, D, Small PM, etc, through the distribution of literature authors and citation analysis. The top two cited articles are published in the New England Journal of Medicine in 1994 by D Alland and Small PM. These two articles used DNA fingerprinting combined with traditional epidemiological methods to investigate the pathogenesis of recurrent pulmonary tuberculosis in the New York City community and the transmission mechanism and risk factors of tuberculosis in the city of San Francisco. It has been recognized by scholars to solve the problem that traditional tuberculosis epidemiology cannot determine whether tuberculosis patients are recently infected or reactivated latent infection and (11, 12). The important authors play a decisive role in promoting the development of the discipline and opening up the depth and breadth of the research field. Follow-up research in the field of TB molecular epidemiology can also be achieved by paying attention to the above-mentioned core authors.

A review of the relevant published literatures shows that molecular epidemiology is used to study tuberculosis susceptibility, the occurrence and spread of the disease, the relationship between infection and the incubation period of onset, the ratio of endogenous recurrence and exogenous relapse, whether different strains have different pathological manifestations, transmission patterns and differences in susceptibility of anti-tuberculosis drugs, frequency of mixed infections, risk factors for morbidity in different populations, monitoring of laboratory testing errors, etc. It plays an important role in the fields of preventive medicine and basic medicine, and even big data analysis of public health events. There are currently five molecular epidemiological methods used in MTB research: (1) Typing method based on a variable number of tandem repeats of MTB scattered repeat units (MIRU-VNTR); (2) spacer oligonucleotide typing method (Spoligotyping); (3) insertion sequence 6,110 (IS6110) restriction fragment length polymorphism (IS6110-RFLP); (4) single nucleotide polymorphism (SNP); (5) whole genome sequencing (WGS) technology. Among them, WGS has the advantages of high throughput, high accuracy, and convenience. It can obtain the whole genome sequence information of Mycobacterium tuberculosis, which can not only identify the species but also analyze the phylogenetic relationship between MTB, infection sources, and individual process of dissemination (13–19). Current molecular epidemiological surveillance methods also have their own shortcomings. For example, the

detection process is slow due to dependence on MTB culture. Some method needs expensive equipment, large data analysis and standardization in technical expertise. Therefore, it is foreseeable that the continuous improvement and innovation of methods on molecular epidemiology of tuberculosis will be the focus and difficulty of research.

This study has certain limitations. First, the literature included in this study was all in English, and there was a language bias. Moreover, some recently published high-quality literature may be cited infrequently due to the short time of publication, which may lead to certain discrepancies between the research results and the real situation. Citation analysis is based on the Web of Science Core Collection, which may miss some important documents indexed by other databases, leading to biased results. Bibliometrics and visual analysis in medical research requires more comprehensive and up-to-date data and to overcome the adverse effects of the language and time of publication of the literature.

Conclusion

At present, American and European countries are still in a dominant position in the field of molecular epidemiology of tuberculosis. The relevant literatures they published are among the top in the world in terms of quantity and quality. In recent years, the research has focused on the genetic polymorphisms and family characteristics of Mycobacterium tuberculosis for a long time. With the progress of molecular epidemiology and innovation of methodological problem, more articles associated with treatment and prevention of tuberculosis may be published in the future. More frequent and deeper cooperation among countries or institutions is essential. This paper reflects the development of the molecular epidemiology of tuberculosis through the perspective of bibliometrics. It has specific guidance and reference significance for institutions and scholars who carry out tuberculosis-related research.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Z-yG and DC conceived and designed the analysis. M-qZ and X-xL wrote the paper. SL colledcted the data. Z-yR contributed data and analysis tools. RX performed the analysis and provided other contribution. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. *Global Tuberculosis Report*. (2021). Available online at: www.who.int/publications/i/item/9789240037021: 2022 (accessed March 15, 2022).

2. Ravansalar H, Tadayon K, Ghazvini K. Molecular typing methods used in studies of Mycobacterium tuberculosis in Iran: a systematic review. *Iran J Microbiol*. (2016) 85:338–46.

3. Pu Q-H, Lyu Q-J, Su H-Y. Bibliometric analysis of scientific publications in transplantation journals from Mainland China, Japan, South Korea and Taiwan between 2006 and 2015. *BMJ Open*. (2016) 68:e011623. doi: 10.1136/bmjopen-2016-011623

4. Yu D, Wang W, Zhang W, Zhang S. A bibliometric analysis of research on multiple criteria decision making. *Curr Sci*. (2018) 1144:747–58. doi: 10.18520/cs/v114/i04/747-758

5. Yu D, Kou G, Xu Z, Shi S. Analysis of collaboration evolution in AHP research: 1982–2018. *Int J Inf Technol Decis Mak*. (2021) 2001:7–36. doi: 10.1142/S0219622020500406

6. Yu D, Xu Z, Wang W. Bibliometric analysis of fuzzy theory research in China: A 30-year perspective. *Knowl Based Syst*. (2018) 141:188–99. doi: 10.1016/j.knosys.2017.11.018

7. Lin H, Wang X, Huang M, Li Z, Shen Z, Feng J, et al. Research hotspots and trends of bone defects based on Web of Science: a bibliometric analysis. *J Orthopaedic Surg Res*. (2020) 15:463. doi: 10.1186/s13018-020-01973-3

8. vanDeutekom H, Gerritsen JJJ, vanSoolingen D, vanAmeijden EJC, vanEmbden JDA, Coutinho RA. Molecular epidemiological approach to studying the transmission of tuberculosis in Amsterdam. *Clin Infect Dis*. (1997) 255:1071–7. doi: 10.1086/516072

9. Agasino CB PdLA, Jasmer RM, Small PM. Epidemiology of Mycobacterium tuberculosis strains in San Francisco that do not contain IS6110. *Int J Tuberc Lung Dis*. (1998) 26:518–20.

10. Pfyffer GE, Strassle A, Rose N, Wirth R, Brandli O, Shang H. Transmission of tuberculosis in the metropolitan area of Zurich: a 3 year survey based on DNA fingerprinting. *Euro Resp J*. (1998) 114:804–8. doi: 10.1183/09031936.98.11040804

11. Alland D, Kalkut GE, Moss AR, McAdam RA, Hahn JA, Bosworth W, et al. Transmission of Tuberculosis in New-York-City - an Analysis by DNA-Fingerprinting and Conventional Epidemiologic Methods. *N Engl J Med.* (1994) 33024:1710–6. doi: 10.1056/NEJM199406163302403

12. Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, et al. The Epidemiology of tuberculosis in San-Francisco - a population-based study using conventional and molecular methods. *N Engl J Med.* (1994) 33024:1703–9. doi: 10.1056/NEJM199406163302402

13. Zhou F, Olman V, Xu Y. Barcodes for genomes and applications. *BMC Bioinformatics*. (2008) 9:546. doi: 10.1186/1471-2105-9-546

14. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Ruesch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. *J Clin Microbiol.* (2006) 4412:4498–510. doi: 10.1128/JCM.01392-06

15. Pang Y, Zhou Y, Wang S, Lu J, Lu B, He G, et al. A novel method based on high resolution melting (HRM) analysis for MIRU-VNTR genotyping of Mycobacterium tuberculosis. *J Microbiol Methods.* (2011) 863:291–7. doi: 10.1016/j.mimet.2011.05.016

16. Vluggen C, Soetaert K, Groenen G, Wanlin M, Spitaels M, de Onate WA, et al. Molecular epidemiology of Mycobacterium tuberculosis complex in Brussels, 2010–2013. *Plos ONE.* (2017) 122:e0172554. doi: 10.1371/journal.pone.0172554

17. Romero B, Aranaz A, Bezos J, Alvarez J, de Juan L, Javed MT, et al. Drug, susceptibility of Spanish Mycobacterium tuberculosis complex isolates from animals. *Tuberculosis.* (2007) 876:565–71. doi: 10.1016/j.tube.2007.08.004

18. Alonso M, Martinez-Lirola M, Jose Palacios J, Menendez A, Herranz M, Martinez S, et al. Evaluation of the potential role of a new mutation in mabA in modifying the response of Mycobacterium tuberculosis to isoniazid. *Tuberculosis.* (2013) 936:664–7. doi: 10.1016/j.tube.2013.07.003

19. Casali N, Nikolayevskyy V, Balabanova Y, Harris SR, Ignatyeva O, Kontsevaya I, et al. Evolution and transmission of drug-resistant tuberculosis in a Russian population. *Nat Genet.* (2014) 463:279–86. doi: 10.1038/ng.2878

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Comparison of clinical characteristics between COVID-19 and H7N9 fatal cases: An observational study

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Objective: The outbreak of COVID-19 in 2020 is reminiscent of the H7N9 outbreak in 2013, which poses a huge threat to human health. We aim to compare clinical features and survival factors in fatal cases of COVID-19 and H7N9.

Methods: Data on confirmed COVID-19 and H7N9 fatal cases identified in mainland China were analyzed to compare demographic characteristics and clinical severity. Survival curves were estimated by the Kaplan–Meier method and compared using log-rank tests and a restricted mean survival time model. A Cox regression model was used to identify survival factors in fatal cases of COVID-19 and H7N9.

Results: Similar demographic characteristics were observed in fatal cases of COVID-19 and H7N9. The proportion of fatal cases of H7N9 receiving antibiotics, antiviral drugs, and oxygen treatment was higher than that of COVID-19. The potential protective factors for fatal COVID-19 cases were receiving antibiotics (HR: 0.37, 95% CI: 0.22–0.61), oxygen treatment (HR: 0.66, 95% CI: 0.44–0.99), and corticosteroids (HR: 0.46, 95% CI: 0.35–0.62). In contrast, antiviral drugs (HR: 0.21, 95% CI: 0.08–0.56) and corticosteroids (HR: 0.45, 95% CI: 0.29–0.69) were the protective factors for H7N9 fatal cases.

Conclusion: The proportion of males, those having one or more underlying medical condition, and older age was high in COVID-19 and H7N9 fatal cases. Offering antibiotics, oxygen treatment, and corticosteroids to COVID-19 cases extended the survival time. Continued global surveillance remains an essential component of pandemic preparedness.

KEYWORDS

COVID-19, H7N9, fatal cases, clinical course, protective factors, survival time

Introduction

The emergence in 2013 of novel avian influenza A H7N9 virus posed a pandemic threat to humans at the time, when human cases of infection from the virus occurred during annual winter–spring epidemics in mainland China (1). Fortunately, in September 2017, the successful development of an H5/H7 bivalent inactivated vaccine for chickens eliminated human infection with H7N9 virus (2, 3), and only three H7N9 cases have been reported since 1 October 2017 (2). Nevertheless, between 2013 and 30 September 2017, 1398 H7N9 cases and 560 H7N9 fatal cases were reported through the national surveillance system for notifiable infectious diseases in mainland China.

In December 2019, a novel coronavirus disease 2019 (COVID-19) outbreak occurred in Wuhan, China (4). Subsequently, outbreaks of human infections with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) occurred in 220 countries around the world (5). On 31 January 2020, the World Health Organization declared the COVID-19 outbreak to be a public health emergency of international concern (6). As of 13 September 2021, more than 21 million cases of COVID-19 had been reported globally, including 4,443,898 fatal cases around the world (7).

Here, we summarize the survival time and causes of clinical course changes in fatal cases of COVID-19 in mainland China compared with confirmed cases of H7N9 virus infections in the same region.

Methods

Study design and participants

This retrospective study includes a total of 290 fatal cases with COVID-19 at Jinyin-tan Hospital between 29 December 2019 and 24 April 2020. All cases were diagnosed based on the diagnosis and treatment protocol of the National Health Commission of the People's Republic of China (7th edition). Of these 290 fatal cases, 239 tested positive for SARS-CoV-2 by RT-PCR, while the remaining 51 were clinically diagnosed with COVID-19. The criterion of clinically diagnosed COVID-19 was confirmed by epidemiological history and clinical manifestations.(8) Epidemiological history: (1) History of travel to or residence in Wuhan and its surrounding areas, or in other communities where cases have been reported within 14 days prior to the onset of the disease; (2) In contact with novel coronavirus infected people (with positive results for the nucleic acid test) within 14 days prior to the onset of the disease; (3) In contact with patients who have fever or respiratory symptoms from Wuhan and its surrounding area, or from communities where confirmed cases have been reported within 14 days before the onset of the disease; (4) Clustered cases (2 or more cases with fever and/or respiratory symptoms in a small area such families, offices, schools etc. within 2 weeks). Clinical manifestations: (1) Fever and/or respiratory symptoms; (2) The imaging characteristics; (3) Normal or decreased white blood cell count, normal or decreased lymphocyte count in the early stage of onset. Clinically diagnosed COVID-19 case has any of the epidemiological history plus any two clinical manifestations or all three clinical manifestations if there is no clear epidemiological history.

In addition, we collected individual records of all 114 laboratory-confirmed H7N9 fatal cases in Zhejiang province from 18 March 2013 to 30 September 2017 from an integrated electronic database managed by Zhejiang CDC. Zhejiang province had the largest number of H7N9 cases. China required every identified H7N9 case to be reported to China CDC within 24 h via a national surveillance system for notifiable infectious diseases. Diagnostic confirmation of H7N9 infection was done either by the isolation of H7N9 virus or a positive real-time reverse-transcription polymerase chain reaction (RT-PCR) assay for H7N9 virus in a respiratory specimen (9).

Data collection

We collected the epidemiological, clinical, laboratory, and clinical management data for 290 fatal COVID-19 cases and 114 fatal H7N9 cases from Jinyin-tan Hospital's information system and Zhejiang province's integrated electronic database, respectively, using standardized forms. We also collected illness onset, diagnosis, and hospital admission times.

Statistical analysis

Descriptive statistical methods were adopted to analyze the continuous variables and categorical variables for H7N9 and COVID-19 cases, respectively. The differences between the two groups were compared by c^2 test. We used the Kaplan–Meier (KM) method to estimate the survival rate and plot the survival curve. Before conducting a log-rank test between the different survival curves, we constructed a test for the proportional hazards (PH) assumption on groups by creating a time-dependent covariate in a PH model. A restricted mean survival time (RMST) model was adopted to plot the mean survival time curves. We were also able to use this model to compare the mean survival time in different groups relative to maximum survival time (τ). We used the Gaussian density estimation method, which is a nonparametric technique, to create a smoothing approximation of time-to-event distributions for illness onset to death and hospital admission to death. The Cox proportional hazards model was used to estimate the effect of covariates on survival time in H7N9 and COVID-19 cases.

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

TABLE 1 Demographics and underlying medical conditions in fatal cases with COVID-19 and H7N9.

Characteristics	COVID-19 ($n = 290$, %)	H7N9 ($n = 114$, %)	c^2	p-value
Gender			0.033	0.855
Male	188 (64.83)	75 (65.80)		
Female	102 (35.17)	39 (34.20)		
Age (years, IQR)	68 (61–75)	65 (57–75)	0.085	0.770
Age group, years			7.213	0.125
<55	43 (14.83)	20 (13.89)		
55–64	69 (23.79)	36 (25)		
65–74	91 (31.38)	29 (25.44)		
75–84	63 (21.72)	26 (22.81)		
≥85	24 (8.28)	3 (2.63)		
Underlying medical conditions				
Any	210/286 (73.43)	83/108 (76.85)	0.244	0.621
Hypertension	126/286 (44.06)	48/108 (44.44)	0.004	0.948
Diabetes	54/286 (18.88)	26/108 (24.07)	1.137	0.286
Cardiovascular disease	28/286 (9.79)	7/108 (6.48)	0.632	0.427
Tumor	20/286 (6.99)	6/108 (5.56)	0.064	0.800
Renal dysfunction	10/286 (3.50)	5/108 (4.63)	0.065	0.799
Chronic obstructive pulmonary disease	6/286 (2.10)	0/108 (0.00)	1.089	0.297
One chronic medical condition	116/286 (40.56)	45/108 (41.67)	0.035	0.852
Two chronic medical conditions	61/286 (21.33)	26/108 (24.07)	0.266	0.606
Three chronic medical conditions	37/286 (12.94)	7/108 (6.48)	2.547	0.111
Hypertension + diabetes	36/286 (12.59)	14/108 (12.96)	0.000	1.000
Hypertension + cardiovascular disease	19/286 (6.64)	5/108 (4.63)	0.230	0.632
Cardiovascular disease + diabetes	8/286 (2.80)	1/108 (0.93)	0.510	0.475

IQR, inter-quartile range.

Ethical approval

The Ethics Review Committee of the Jinyin-tan hospital provided approval for this study (No: KY-2020-62·01). Additionally, patients' personal identifying information was anonymized to ensure privacy.

Results

Demographics and underlying medical conditions

A total of 290 fatal cases with COVID-19 and 114 fatal cases with H7N9 were analyzed in this study. Of those, 188 (64.83%) and 75 (65.80%) were male for COVID-19 and H7N9, respectively; the male-to-female ratios were 1.84:1 and 1.92:1, respectively. The median age was 68.00 years (IQR: 61.00–75.00) for COVID-19 cases and 65.00 years (IQR: 57.00–75.00) for H7N9 cases, and the age group distribution across the two groups was not significantly different ($p = 0.125$; Table 1 and Supplementary Figure 1A). In addition, there was no significant difference in gender and age in the different groups (Supplementary Figures 1B,C).

There was no difference between the prevalence of underlying medical conditions for COVID-19 compared with H7N9 cases. Hypertension (44.06 vs.44.44%), diabetes (18.88 vs.24.07%), and cardiovascular disease (9.79 vs.6.48%) were the most common underlying medical conditions for both COVID-19 and H7N9. In addition, the proportion of fatal COVID-19 cases with three chronic medical conditions was higher than that of H7N9 fatal cases, although the p-value was not significant (Table 1).

Times to events

The median time from illness onset to diagnosis and from illness onset to hospital admission was different between the COVID-19 and H7N9 groups ($p < 0.050$); patients in the COVID-19 group had a longer time from illness onset to both

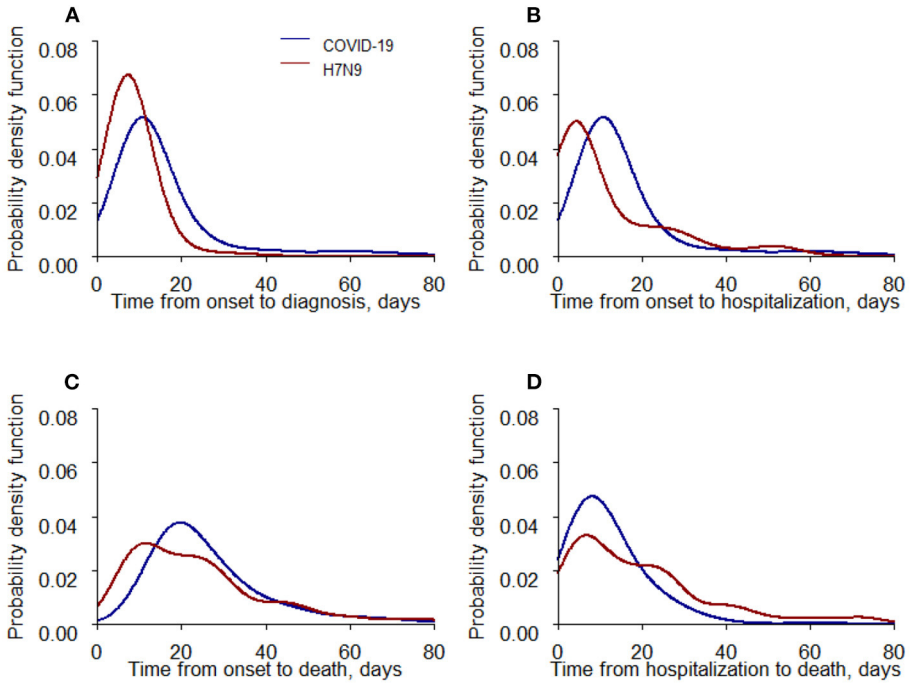

FIGURE 1
Time-to-event distributions of COVID-19 and H7N9 fatal cases. **(A)** Days from illness onset to diagnosis. **(B)** Days from illness onset to hospital admission. **(C)** Days from illness onset to death. **(D)** Days from hospital admission to death.

diagnosis and hospital admission (11.00 and 11.00 days vs.7.00 and 6.00 days; Figures 1A,B). In addition, although the *p*-value was significantly different ($p = 0.03686$), the time from illness onset to death in the COVID-19 group was only 1 day longer than in the H7N9 group (23.00 days vs.22.00 days; Figure 1C), and the time from hospital admission to death was shorter in the COVID-19 group than that in the H7N9 group (10.00 days vs.15.00 days, $p = 0.001$; Figure 1D).

Treatment and complications

From the perspective of treatment, although most COVID-19 and H7N9 fatal cases received antibiotics, antiviral treatment, and oxygen treatment, the proportion of H7N9 cases using the above treatments was significantly higher than that of COVID-19 cases, and the difference between the two groups was statistically significant ($p < 0.05$). However, the proportion of fatal COVID-19 cases (64.48%) was higher than that of H7N9 fatal cases (55.74%). In addition, the proportion of H7N9 fatal cases complicated with acute respiratory distress syndrome was significantly higher than that of fatal COVID-19 cases (25.23 vs.6.55%, $p < 0.001$) (Table 2).

Survival time from hospital admission and illness onset to death

Based on the time from hospital admission to death, a log-rank test showed that the survival time in hospital for H7N9 cases was longer than that for COVID-19 cases ($p < 0.001$; Figure 2A), and this difference was also observed in RMST curves ($p < 0.001$; Figure 2B). However, when using illness onset as the starting point for survival time, the difference between the two KM curves was not statistically significant ($p = 0.413$), and the mean survival time for COVID-19 cases was not statistically higher than that for H7N9 cases within maximum survival time (Figures 2C,D).

Factors associated with survival time interval from hospital admission to death

The effect of covariates on the survival time for COVID-19 cases from hospital admission to death was assessed using a Cox proportional hazards model, and the potential protective factors included receiving corticosteroids, antibiotics, and oxygen treatment, after adjusting for other covariates. The risk of death among COVID-19 cases receiving antibiotics or oxygen

TABLE 2 Treatment and complications in fatal cases with COVID-19 and H7N9.

Characteristics	COVID-19 ($n = 290$, %)	H7N9 ($n = 114$, %)	c^2	p-value
Received antibiotics	272 (93.79)	108/108 (100.00)	5.65	0.017
Received corticosteroid	187 (64.48)	34/61 (55.74)	1.30	0.254
Received antiviral drugs	161 (55.52)	87/105 (82.86)	23.51	<0.001
Oseltamivir	35 (12.07)	75/87 (86.21)	174.44	<0.001
Received ECMO	3 (1.03)	13/69 (18.84)	37.43	<0.001
Received oxygen treatment	258 (88.97)	87/89 (97.75)	5.41	0.020
Received mechanical ventilation	201 (69.31)	71/81 (87.65)	9.97	0.002
Complications				
Respiratory failure	184 (63.45)	71/107 (66.36)	0.175	0.676
ARDS	19 (6.55)	27/107 (25.23)	24.84	<0.001

ARDS, acute respiratory distress syndrome; ECMO, extracorporeal membrane oxygenation; IQR, inter-quartile range.

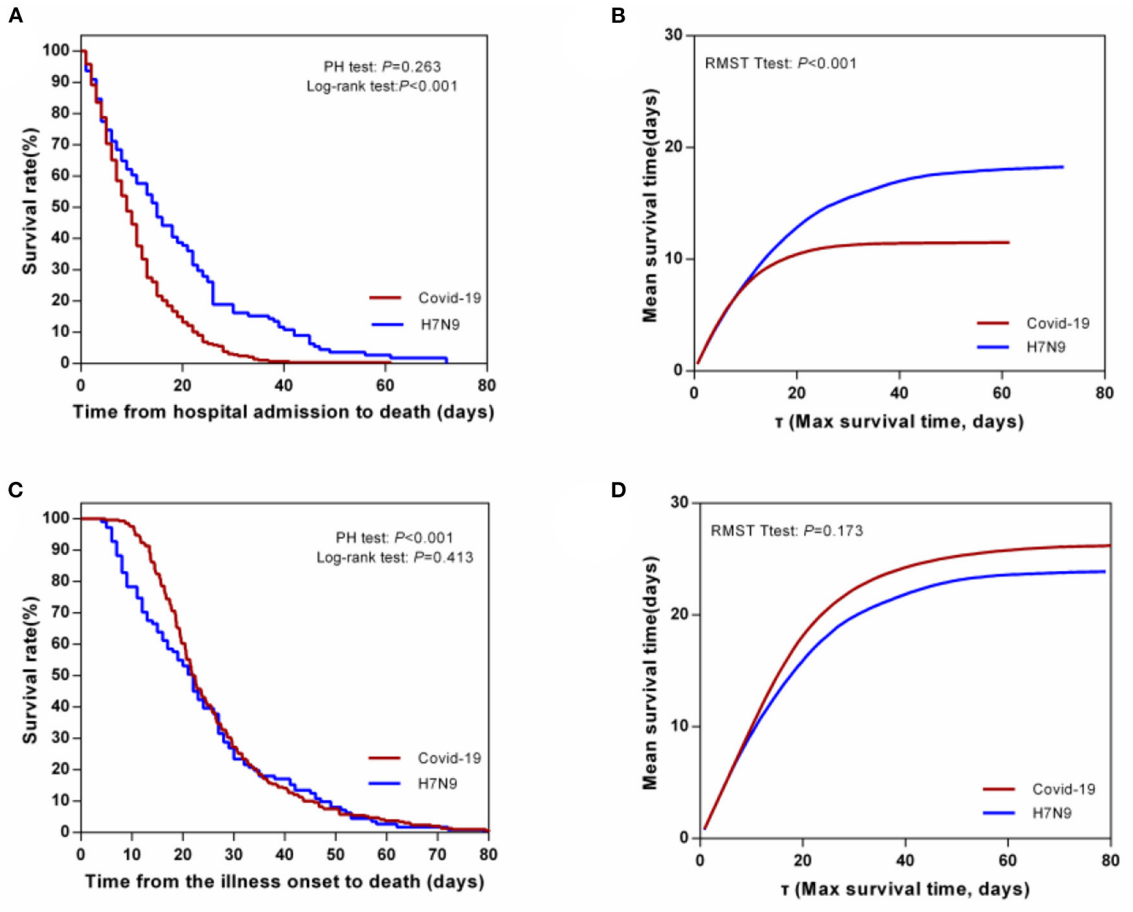

FIGURE 2
Survival time from hospital admission and illness onset to death by log-rank test and RMST curves. **(A)** Survival time from hospital admission to death by log-rank test. **(B)** Survival time from hospital admission to death by RMST curves. **(C)** Survival time from illness onset to death by log-rank test. **(D)** Survival time from illness onset to death by RMST curves.

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TABLE 3 Forest plot of factors associated with survival time interval based on hazard ratio (HR) from hospital admission to death.

Parameter	COVID-19			H7N9		
	Time interval from hospital admission to death (median, IQR, days)	*p*-value	HR (95% CI)	Time interval from hospital admission to death (median, IQR, days)	*p*-value	HR (95% CI)
Age (per 10 years)	-	0.80	1.01 (0.92–1.12)	-	0.3524	1.09 (0.91, 1.3)
Time from onset to admission (days)	-	0.18	1.01 (1.00–1.02)	-	0.1568	1.04 (0.99, 1.09)
Male	10.40 (4.71–14.79)	Ref	Ref	14.0 (5.0, 25.0)	Ref	Ref
Female	9.46 (5.62–15.62)	0.56	0.93 (0.71–1.20)	16.0 (7.0, 30.0)	0.7311	0.93 (0.6, 1.44)
Not received antibiotics	3.66 (2.60–5.63)	Ref	Ref	6.0 (2.5, 11.5)	Ref	Ref
Received antibiotics	10.40 (5.69–15.65)	<0.01	0.37 (0.22–0.61)	15.0 (6.0, 26.0)	0.3442	0.57 (0.18, 1.82)
Not received antivirus	9.63 (5.49–15.48)	Ref	Ref	1.0 (1.0, 2.0)	Ref	Ref
Received antivirus	10.42 (5.57–15.62)	0.56	1.16 (0.71–1.89)	15.0 (6.0, 26.0)	0.002	0.21 (0.08, 0.56)
Not received oxygen treatment	5.55 (2.66–12.58)	Ref	Ref	6.5 (3.0, 9.0)	Ref	Ref
Received oxygen treatment	10.41 (5.75–15.66)	0.04	0.66 (0.44–0.99)	16.0 (6.0, 26.0)	0.2583	0.66 (0.31, 1.37)
No underlying medical conditions	10.08 (5.61–20.46)	Ref	Ref	15.5 (7.5, 26.0)	Ref	Ref
Underlying medical conditions	9.47 (5.43–13.73)	0.15	1.23 (0.93–1.62)	14.0 (5.0, 26.0)	0.3719	0.8 1(0.51, 1.29)
Not received corticosteroid	7.39 (4.42–11.48)	Ref	Ref	6.0 (3.0, 18.0)	Ref	Ref
Received corticosteroid	12.53 (7.78–19.72)	<0.01	0.46 (0.35–0.62)	21.0 (13.0, 30.0)	0.0003	0.45 (0.29, 0.69)

CI, confidence interval.

treatment was significantly lower than among the cases who did not receive these treatments (HR: 0.37, 95% CI: 0.22–0.61 and HR: 0.66, 95% CI: 0.44–0.99, respectively). The risk of death among the COVID-19 cases who received corticosteroids was about half of that among those who did not receive corticosteroids (HR: 0.46, 95% CI: 0.35–0.62). The hazard of death in hospital among the H7N9 cases who receiving antivirus or corticosteroid was less than among the COVID-19 cases (HR: 0.21, 95% CI: 0.08–0.56 and HR: 0.45, 95% CI: 0.29–0.69, respectively). Receiving corticosteroid showed a protective effect both in the COVID-19 and the H7N9 cases. In addition, the time interval from hospital admission to death (median, IQR, days) was generally consistent with the HRs (Table 3).

Discussion

Human infection with H7N9 virus and SARS-CoV-2 were two respiratory infectious disease pandemics in China in recent decades that posed a major threat to public health, since these viruses may acquire mutations that enable efficient and sustained human-to-human transmission and lead to pandemic. Therefore, in this study, we compared the clinical processes of the two infectious diseases and confirmed their significantly different survival times by KM and RMST models. We found that male, having underling medical condition, older age were high-risk groups of COVOD-19 and H7N9 infection. Antibiotics, oxygen treatment, and corticosteroids were protective factors for fatal COVID-19 cases. In contrast, antiviral drugs and corticosteroids were the protective factors for H7N9 fatal cases.

We found that both COVID-19 and H7N9 fatal cases were mostly male, older, and with at least one underlying condition. Vaccine is the most effective method for preventing infectious disease, especially among high-risk populations. The H5/H7 bivalent inactivated vaccine for chickens was first used in September 2017, and the H7N9 virus isolation rate in poultry dropped by 93.3% following vaccination (4). Since most H7N9 cases had avian transmission, and human to human infection was limited, the avian vaccine was also effective in blocking human infections with H7N9 virus, and only three further cases of infection have been reported since September 2019. However, the current pandemic's SARS-CoV-2 pathogen spreads more widely and quickly and is highly transmissible from human to human (10), which leads to it posing a pandemic threat to human beings (11) and having a greater impact on the world. Therefore, effective vaccination of humans is particularly important as a means to prevent and block transmission. In China, as of 26 August 2021, more than two billion doses of COVID-19 vaccine had been distributed, and nearly 890 million people had completed the vaccination program. In the latest round of epidemics, the vaccination of COVID-19 has played a definite role in controlling it.

Early detection, diagnosis, and treatment form the basis for controlling and eliminating infectious diseases and improving survival opportunity. For H7N9 cases, early detection can

control the disease process as much as possible and reduce the proportion of severity and death. However, for COVID-19 cases, early detection and diagnosis are not only to reduce severity and death but also to reduce the transmission of the SARS-CoV-2 virus. In the early stages of the COVID-19 epidemic, this new and emerging infectious disease was not fully understood, chaos, and resource limitations, so the time interval from illness onset to death was shorter, and that from illness onset to diagnosis was longer. However, greater understanding of COVID-19 and the continuous refinement and improvement of diagnostic criteria and improved sensitivity of detection kits not only accelerated the diagnosis of COVID-19 cases but also facilitated timely and accurate prevention and control. Nevertheless, false-negative test results may occur in up to 20–67% of cases (12); therefore, highly clinically suspicious patients should not rely solely on the results of RT-PCR tests, and clinical and epidemiological investigation should be carefully considered (13). In addition, the sensitivity of testing varies with the timing of testing relative to exposure (12). It is estimated that such sensitivity is 62% on the day of symptom onset and 80% on the third day after that, but it falls to only 33% 4 days after exposure (12, 14, 15). Therefore, timely sampling for detection is an important method for reducing false negative tests. Moreover, high-risk groups need to increase the number of their RT-PCR tests and to appropriately extend their isolation period.

At present, although there is no evidence to show that specific drug treatments are effective against suspected or confirmed cases with COVID-19 or H7N9, antiviral therapy and organ support therapy are the cornerstone of the treatment of severe cases with both diseases (16, 17). Our study indicated that more than half of the fatal COVID-19 and H7N9 cases received antibiotics (93.79 and 100.00%), antiviral drugs (55.52 and 82.86%), corticosteroids (64.48 and 55.74%), and oxygen treatment (88.97 and 97.75%) after hospital admission. Our study found that, without specific drug treatment for COVID-19 and H7N9, corticosteroid was a protective factor. This has been proven in research at Oxford University, where the latest study showed the ability of dexamethasone to reduce the risk of death by 54% in cases with COVID-19 requiring ventilation (18). In addition, recent research has indicated that the appropriate use of corticosteroids together with other remedies should be beneficial for severe cases of COVID-19 and H7N9 to prevent ARDS development (19, 20). Separately, severe COVID-19 cases are susceptible to secondary bacterial infection; therefore, a combination of antibiotics can also prolong the survival time of fatal cases and decrease the risk of death of COVID-19 cases requiring oxygen inhalation by 20% (18). For H7N9 cases, early initiation of antiviral therapy in patients is an important method to delay severity and death (17).

Surveillance is the most effective early warning method for emerging infectious diseases, and effective public health emergency management can reduce the adverse impact of emerging infectious diseases (21). In response to the 2003 severe acute respiratory syndrome outbreak, China established the National Notifiable Infectious Disease Surveillance System (NNIDSS) for 39 infectious diseases (22). The establishment of NNIDSS strengthened the construction of China's system for infectious diseases and public health emergencies (23) and played an important role in the outbreak of H7N9 avian influenza and the COVID-19 epidemic (21). However, infectious diseases have emerged one after another in recent years, and the existing surveillance system does not seem to be sufficient for the early warning of emerging infectious diseases. The Chinese government needs to consider further how to improve the existing surveillance system and its early warning role.

This study has some limitations. First, we only collected data on fatal cases, and mild and moderate cases were not included in the study; thus, a comprehensive comparison of survived and deceased cases was not possible. Second, we only collected information on early fatal COVID-19 cases, limiting our ability to characterize the differences between different variants. Third, some potential factors associated with survival time were not collected, which may cause bias. However, the most important factors have been collected.

In conclusion, we have described the clinical characteristics of fatal cases with COVID-19 and H7N9. We found the proportion of males, those having one or more underlying medical condition, and older age were high in these fatal cases. Moreover, from the perspective of individual treatment, offering antibiotics, oxygen treatment, and corticosteroids to COVID-19 cases extended the survival time. However, antiviral therapy and corticosteroids were more effective in H7N9 cases. Last, continued global surveillance of COVID-19 and human infections with avian influenza A viruses remains an essential component of pandemic preparedness.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Review Committee of the Jinyintan hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SL and HJ conceived, designed, and supervised the study. CH, LS, ST, LK, WL, NZ, and DZ collected and cleaned the data. FL, HJ, RH, and JY analyzed the data. HJ and FL wrote

the drafts of the manuscript. SL, HJ, WL, and T-CC interpreted the findings. SL and CH commented on and revised the drafts of the manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1047362/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Age distribution of COVID-19 and H7N9 fatal cases. **(A)** Age distribution of COVID-19 and H7N9 fatal cases by gender. **(B)** Age distribution of all COVID-19 fatal cases by gender. **(C)** Age distribution of all H7N9 fatal cases by gender.

References

1. Wang X, Jiang H, Wu P, Uyeki TM, Feng L, Lai S, et al. Epidemiology of avian influenza A H7N9 virus in human beings across five epidemics in mainland China, 2013-17: an epidemiological study of laboratory-confirmed case series. *Lancet Infect Dis.* (2017) 17:822–32. doi: 10.1016/S1473-3099(17)30323-7

2. Zeng X, Tian G, Shi J, Deng G, Li C, Chen H. Vaccination of poultry successfully eliminated human infection with H7N9 virus in China. *Sci China Life Sci.* (2018) 61:1465–73. doi: 10.1007/s11427-018-9420-1

3. Li C, Chen H. H7N9 influenza virus in China. *Cold Spring Harb Perspect Med.* (2021) 11:a038349. doi: 10.1101/cshperspect.a038349

4. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* (2020) 395:507–13. doi: 10.1016/S0140-6736(20)30211-7

5. Baidu. *Real Time Big Data Report Real Time of Coronavirus Disease (COVID-19) Epidemic*. Beijing: Baidu. (2021). Available online at: https://voice.baidu.com/act/newpneumonia/newpneumonia/?from=osari_aladin_banner#tab4. (accessed on September 13, 2021).

6. World Health Organization (WHO). *Coronavirus Disease (COVID-19) Pandemic.* (2021). Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed on September 13, 2021).

7. Worldometers. *COVID-19 Coronavirus Pandemic.* (2021). Available online at: https://www.worldometers.info/coronavirus/ (accessed on September 13, 2021).

8. The National Health Commission. *Diagnosis and Treatment of Novel Coronavirus Pneumonia.* Available online at: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf (accessed on October 28, 2022).

9. Liao Q, Ip DK, Tsang TK, Cao B, Jiang H, Liu F et al. A clinical prediction rule for diagnosing human infections with avian influenza A (H7N9) in a hospital emergency department setting. *BMC Med.* (2014) 12:127. doi: 10.1186/s12916-014-0127-0

10. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032

11. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci.* (2020) 63:457–60. doi: 10.1007/s11427-020-1637-5

12. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A review. *JAMA.* (2021) 324:782–93. doi: 10.1001/jama.2020.12839

13. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. *Ann Intern Med.* (2020) 173:262–7. doi: 10.7326/M20-1495

14. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA.* (2020) 323:1843–4. doi: 10.1001/jama.2020.3786

15. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *JAMA.* (2020) 323:2249–51. doi: 10.1001/jama.2020.8259

16. Clark A, Jit M, Warren-Gash C, Guthrie B, Wang HHX, Mercer SW, et al. Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study. *Lancet Glob Health.* (2020) 8:e1003–7.

17. Gao HN, Lu HZ, Cao B, Du B, Shang H, Gan JH, et al. Clinical findings in 111 cases of influenza A (H7N9) virus infection. *N Engl J Med.* (2013) 368:2277–85. doi: 10.1056/NEJMoa1305584

18. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol.* (2020) 146:110–8. doi: 10.1016/j.jaci.2020.04.006

19. Banik GR, Alqahtani AS, Booy R, Rashid H. Risk factors for severity and mortality in patients with MERS-CoV: analysis of publicly available data from Saudi Arabia. *Virol Sin.* (2016) 31:81–4. doi: 10.1007/s12250-015-3679-z

20. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet.* (2020) 395:473–5. doi: 10.1016/S0140-6736(20)30317-2

21. Zhang T, Wang Q, Wang Y, Bai G, Dai R, Luo L. Early surveillance and public health emergency responses between novel coronavirus disease 2019 and avian influenza in China: a case-comparison study. *Front Public Health.* (2021) 9:629295. doi: 10.3389/fpubh.2021.629295

22. Yang S, Wu J, Ding C, Cui Y, Zhou Y, Li Y, et al. Epidemiological features of and changes in incidence of infectious diseases in China in the first decade after the SARS outbreak: an observational trend study. *Lancet Infect Dis.* (2017) 17:716–25. doi: 10.1016/S1473-3099(17)30227-X

23. Chinese Center for Disease Control and Prevention. *Public Health Science Data Center.* (2021). Available online at: https://www.phsciencedata.cn/Share/ (accessed on September 13, 2021).

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Novel oxazolidinones harbor potent *in vitro* activity against the clinical isolates of multidrug-resistant *Mycobacterium tuberculosis* in China

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Objective: To investigate the *in vitro* activities of five oxazolidinones in parallel against the reference strains of different mycobacterial species and clinical isolates of *Mycobacterium tuberculosis* (Mtb), and shed light on the differences in the efficacy of these homolog drugs.

Materials and methods: The minimum inhibitory concentrations (MICs) of linezolid, tedizolid, sutezolid, delpazolid, and contezolid against 16 mycobacterial reference strains and 69 *M. tuberculosis* clinical isolates, including 17 drug-susceptible isolates and 52 multidrug-resistant (MDR) isolates, were determined by microplate alamarBlue assay (MABA). The intracellular killing activities of contezolid and linezolid against Mtb H37Rv were compared. In addition, mutations in the linezolid resistance-related genes (*rplC*, *rplD*, and 23S rRNA) of the Mtb clinical isolates were also analyzed.

Results: Tedizolid exhibited the strongest inhibitory activities against the reference strains of both rapidly growing mycobacteria (RGM) and slowly growing mycobacteria (SGM), among the tested oxazolidinones. In contrast, sutezolid only manifested potent activity against reference strains of SGM. Linezolid, delpazolid, and contezolid were less active against the non-tuberculous mycobacterial references. For the Mtb clinical isolates, the antimicrobial action was ranked as: sutezolid > tedizolid > contezolid and linezolid > delpazolid, whereas no difference between drug-sensitive and multiple drug-resistant isolates was observed. Notably, contezolid demonstrated obviously superior intracellular antimicrobial activity than linezolid. Few strains harbored mutations in *rrl* gene or *rplD* genes, although these strains had drug susceptible profiles to linezolid.

Conclusion: Different oxazolidinones can have discrepant antimicrobial activity against different mycobacterial species, or have different manifestations out of cell or in cell. Understanding these differences would be helpful in choosing the appropriate drug in clinical practice.

KEYWORDS

oxazolidinone, *Mycobacterium tuberculosis*, non-tuberculous mycobacteria, drug resistance, minimum inhibitory concentration

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (Mtb), which remains a huge global public health threat for decades. According to World Health Organization (WHO) Global Tuberculosis Report (1), tuberculosis resulted in ∼1.60 million deaths in 2021. In contrast with the drug-susceptible tuberculosis (DS-TB), multidrug-resistant tuberculosis (MDR-TB), and extensively drug-resistant tuberculosis (XDR-TB) incur longer treatment courses, higher costs, and greater drug side-effect. These factors significantly hamper the global tuberculosis control program. Therefore, novel anti-mycobacterial drugs with great safety, tolerability, and efficacy are crucial in curbing tuberculosis, especially drug-resistant tuberculosis.

Oxazolidinones are a relatively new class of synthetic antibiotics that have shown potent activities against drug-resistant Gram-positive bacteria as well as Mtb. Oxazolidinones inhibit bacterial protein synthesis by competitively binding to the 23S rRNA of the bacterial 50S ribosomal subunit and have no cross-resistance to the existing antibacterial agents. Linezolid (LZD) is the first oxazolidinone drug that is used in TB treatment and has been recommended by WHO as the core drug (group A) for the treatment of drug-resistant TB (2). In addition to its very strong efficacy, adverse reactions (such as bone marrow suppression, peripheral neuropathy, and optic nerve damage) associated with LZD raise great concerns. Therefore, LZD must be reconstructed urgently for ensuring its efficacy and overcoming the associated safety problems. A series of oxazolidinones have been developed in recent years, including tedizolid (TZD), sutezolid (SZD), delpazolid (DZD), contezolid (MRX-I), and others. Among these, TZD is the second oxazolidinone that has been clinically approved for MDR-TB treatment, which manifests stronger activities against both DS-TB and MDR-TB strains than LZD (3, 4). Furthermore, fewer hematological toxicity and neuropathy were observed after long-term TZD treatment in contrast with LZD (5). SZD differs from LZD primarily due to the presence of a thiomorpholine substituent, and better anti-tuberculosis activity and a higher safety profile (6). DZD (LCB01-0371) differs structurally from LZD and contains a cyclic amidrazone that replaces the morpholino ring. Studies have shown that the antibacterial activity of DZD is comparable to LZD in *in vitro* susceptibility tests. In a single ascending dose-based clinical trial (7), DZD at a dose of 2400 mg per day had no serious side effects and exhibited bactericidal and bacteriostatic activity comparable to LZD. In 2021, MRX-I was approved by the Chinese Food and Drug Administration (FDA) to treat Gram-positive bacterial infections. The main active structure of MRX-I is the same like LZD, but it demonstrates less mitochondrial protein synthesis inhibition (MPSi) associated with myelosuppression and less monoamine oxidase inhibition (MAOi) associated with drug-drug interactions. In contrast to LZD (MPSi IC50: 7.9 μg/ml; MAOi IC50: 4.1 μg/ml), MRX-I has lower MPSi (IC50: 15.7 μg/ml), and MAOi (IC50: 12.3 μg/ml). Several studies have shown that MRX-I has comparable *in vitro* and *in vivo* anti-tuberculosis activities to LZD (8). To better understand the potential value of these novel oxazolidinones, we evaluated the *in vitro* antimicrobial inhibitory activity of the above-mentioned four new-generation oxazolidinones and compared them to LZD. The reference strains of different mycobacterial species and clinical isolates of *M. tuberculosis* (Mtb) were included to demonstrate the differences in the efficacy of these homolog drugs. Additionally, the mutations in the reported LZD resistance-related genes (*rplC*, *rplD*, and 23S rRNA) were analyzed to better understand the role of these genes in oxazolidinone resistance. As the second approved oxazolidinone drug for treating drug-resistant TB in China, we paid more attention to MRX-I and determined the intracellular bactericidal effects against Mtb H37Rv in contrast to LZD.

Materials and methods

Ethics statement

Because the study involved only laboratory testing with reference strains and clinical isolates, no ethical approval was sought.

Reference strains and clinical isolates

Totally 69 clinical strains were collected from the strain bank of Beijing Chest Hospital, including 17 DS-TB strains and 52 MDR-TB strains. A total of 16 reference strains of different mycobacterial species were also recruited. All of them originated from the American Type Culture Collection (ATCC).

Minimum inhibitory concentration testing

The microplate alamarBlue assay (MABA) was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) to determine the minimum inhibitory concentrations (MICs). The broth microdilution format was set up as a twofold dilution, while the concentrations of all of the tested drugs ranged from 0.0625 to 64 μg/ml. Briefly, fresh culture suspension of 1 McFarland standard was prepared and further diluted (at 1:20) with Middlebrook 7H9 broth containing 10% Middlebrook OADC Enrichment. A total of 100 μl per well of this dilution was added on the 96-well plate. After 7 days of incubation at 37°C, 70 μl of alamarBlue solution was added to each well. Plates were further incubated for 24 h, color changes were then monitored by visual inspection. A change from blue to pink or purple indicated bacterial growth. The MIC was defined as the lowest concentration of antibiotic which prevented a color change from blue to pink. The reference strain Mtb H37Rv was used as a control. The MIC breakpoint concentration was defined as 1.0 μg/ml for LZD, according to the CLSI. All tested oxazolidinones (LZD, MRX-I, DZD, TZD, SZD) came from TargetMOl Chemicals Inc.

Whole genome sequencing

The genomic DNA of the clinical isolates was extracted by using the MasterPure Complete DNA isolation kit (Epicentre, Madison, WI, USA). DNA libraries were constructed and processed using Illumina kit following the manufacturer's instructions. Whole genome sequencing (WGS) was performed on an Illumina HiSeq X-Ten sequencing platform. The sequences of genes known to confer resistance to LZD in *M. tuberculosis* (including 23S rRNA, *rplC* and *rplD* genes) were analyzed specifically.

Intracellular antibacterial assays

The assay was performed according to the method reported previously (9). Bacteria were adjusted to 5×10^6 bacteria per milliliter with RPMI-1640 medium. The monolayers THP-1 cells were infected at a multiplicity of infection of 1:5 (cells:bacilli) and incubated for 4 h at 37°C in a humid atmosphere with 5% CO_2. In order to remove the extracellular bacteria, cultures were washed twice with pre-warmed PBS at 37°C and then 1 ml of gentamicin (20 μg/ml) was added to each well followed by incubation for 2 h. The wells were washed twice with PBS and 1 ml of RPMI-1640 containing the drug in solution to be tested was added, and the plate was incubated for 24, 48, and 72 h. At the end of the incubation, bacterial counts were determined from each well by agar plating. 7H10 plates were incubated at 37°C for 3–4 weeks until colonies were visible. Three replicates were performed for each concentration of the drugs and bacterial counts of each replicate were done in triplicate.

Data analysis

MIC_{50}, MIC_{90} and epidemiological cutoff (ECOFF) values were calculated using ecofinder-xl-2010-v21-webversion software (CLSI). Data were analyzed using SPSS 26.0 software and GraphPad Prism 8.0 software.

Results

MICs of the five oxazolidinones against mycobacterial reference strains

The MICs of the oxazolidinones against the 16 mycobacterial reference strains are shown in **Table 1**. TZD generally presented the best antimicrobial activities against the enrolled strains, 7 out of 8 rapidly growing mycobacteria (RGM) reference strains and 6 out of 8 slowly growing mycobacteria (SGM) strains had MIC $\leq$ 0.5 μg/ml. In contrast, SZD only demonstrated likely activity as TZD against SGM but not RGM. LZD, MRX-I, and DZD were probably less active against either RGM or SGM. Oxazolidinones had weak antibacterial activities against *M. avium* and *M. intracellulare* reference strains. Only TZD had relatively strong activity against *M. avium* reference strain whereas SZD had against *M. intracellulare* reference strain. These had MICs of 0.25 and 0.5 μg/ml, respectively. The activity against *M. abscessus* was generic, only TZD had a MIC of 0.5 μg/ml.

MIC distributions of *Mycobacterium tuberculosis* to the five oxazolidinones

The MIC distributions of the five oxazolidinones against the 69 clinical strains of Mtb are shown in **Table 2** and **Figure 1**.

TABLE 1 MICs of the five oxazolidinones against mycobacterial reference strains.

Reference strain		MIC (μg/ml) of				
		LZD	MRX-I	SZD	DZD	TZD
RGM	*M. abscessus subsp.abscessus*	4	16	4	2	0.5
	M. pulveris	0.5	1	0.5	2	0.5
	M. vaccae	1	1	1	1	0.25
	M. phlei	2	2	2	4	1
	M. smegmatis	1	2	4	2	0.5
	M. senegalense	2	2	1	4	0.5
	M. cosmeticum	2	2	8	1	0.5
	M. flavescens	2	2	1	4	0.5
SGM	*M. tuberculosis* H37Rv	0.5	1	0.25	2	0.25
	M. bovis	0.5	1	0.25	2	0.5
	M. kansasii	0.5	1	≤ 0.0625	1	0.125
	M. africanum	0.5	1	≤ 0.0625	2	0.125
	M. avium subsp.avium	8	8	8	1	0.25
	M. intracellulare	16	32	0.5	32	4
	M. asiaticum	4	8	0.5	4	1
	M. parascrofulaceum	1	2	4	1	0.125

CLSI resistance breakpoint for LZD: *M. tuberculosis* 1 μg/ml; *M. avium* complex, *M. kansasii*, RGM 32 μ g/ml.

The MIC of SZD was 4 to 8 times lower than LZD, whereas the MIC of TZD was 2–4 times lower than LZD. The MIC distribution of MRX-I was similar to LZD, while DZD showed 2–4 times higher MIC than LZD. SZD showed the strongest inhibitory activity among the tested oxazolidinones, with MIC_{50} and MIC_{90} of 0.0625 μg/ml and 0.125 μg/ml, respectively. Notably, the DS- and MDR-TB strains manifested similar MIC distribution profiles. SZD also presented the lowest ECOFFs at 0.125 μg/ml among the five oxazolidinones (Table 2) followed by TZD at 0.25 μg/ml. Notably, the ECOFF for LZD was 1 μg/ml, which is consistent with the CLSI-specified cutoff point for determining LZD resistance. According to the CLSI resistance breakpoint point for LZD, 69 Mtb clinical isolates were sensitive to LZD. For a given isolate, the MICs to different oxazolidinones were correlated (Table 3). With increase of MIC of LZD, the MICs of MRX-I, TZD, and DZD also showed a noticeable upward trend. Such a trend was not observed in the case of SZD.

Mutations conferring linezolid resistance

Although the 69 clinical isolates of Mtb were all susceptible to LZD, WGS identified substitution mutations in the *rrl* gene (C1275T, C2060T, and C2572T) in three strains, whereas 3 other strains harbored a non-synonymous mutation Arg79His (CGT → CAT) in the *rplD* gene. Mutations and corresponding MICs of the five oxazolidinones are shown in Table 4.

TABLE 2 MICs of the 5 oxazolidinones against 69 Mtb clinical isolates.

Agent	MIC_{50} (μ g/ml)	MIC_{90} (μg/ml)	ECOFF (μg/ml)
Linezolid	0.5	1	1
Contezolid	1	2	2
Sutezolid	0.0625	0.125	0.125
Tedizolid	0.125	0.25	0.25
Delpazolid	2	4	4

Intracellular killing activity of contezolid and linezolid against *Mycobacterium tuberculosis* H37Rv

Four hours after H37Rv infection of macrophages, 10% of the bacteria were phagocytosed by macrophages (500,000/5 million) (Figure 2). However, gradually, the intracellular H37Rv also continued to proliferate and proliferated by nearly half after 24 h and more than doubled after 72 h. Both MRX-I and LZD inhibited the intracellular growth of Mtb in contrast with the controls in a dose-dependent manner. MRX-I presented obviously superior intracellular antibacterial effects than LZD. The colony forming units (CFU) counts of MRX-I were lower than LZD at the same drug concentration at each incubation time point. Additionally, only 32 × MIC MRX-I showed bactericidal effects at 24 and 48 h. Furthermore, at 24 h, 1 × MIC MRX-I showed obvious antibacterial activity ($P < 0.05$) while 1 × MIC LZD did not show any antibacterial activity. Even at 4 × MIC, LZD did not demonstrate any obvious bacteriostatic activity.

Discussion

In the present study, we first compared the MICs of five oxazolidinones side by side against the reference strains of mycobacterial species and Mtb clinical isolates. New generation oxazolidinones have intrigued many interests because of their high efficacy but the toxic features (e.g., of LZD) encountered in clinical usage are a major limitation. DZD had excellent pharmacokinetic parameters and a good safety profile (10). In a recent clinical trial, Choi and colleagues demonstrated that LCB01-0371 (DZD) was well tolerated in healthy male subjects after multiple doses of up to 1,200 mg twice daily for 21 days (11). Several studies have shown that SZD

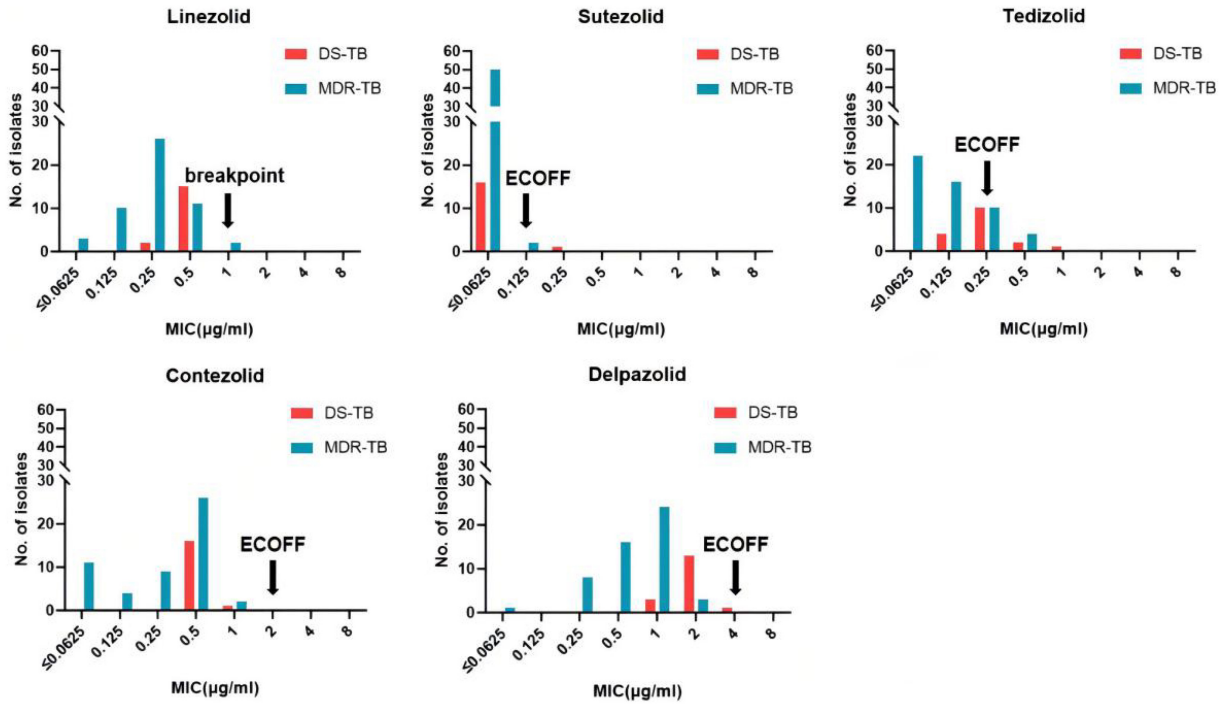

FIGURE 1
MIC distributions of the five oxazolidinones against 69 Mtb clinical isolates.

TABLE 3 MICs of the four novel oxazolidinones for the Mtb clinical isolates with different MICs of linezolid.

Linezolid		No. of isolates with different MICs (μg/ml)			
MIC (μg/ml)	No. of isolates	Contezolid	Tedizolid	Sutezolid	Delpazolid
≤ 0.0625	3	≤ 0.0625 (2) 0.25 (1)	≤ 0.0625 (3)	≤ 0.0625 (3)	≤ 0.0625 (1) 0.25 (1) 1 (1)
0.125	10	≤ 0.0625 (3) 0.125 (2) 0.25 (4) 0.5 (1)	≤ 0.0625 (8) 0.125 (1) 0.5 (1)	≤ 0.0625 (10)	0.25 (5) 0.5 (4) 1 (1)
0.25	28	≤ 0.0625 (6) 0.125 (1) 0.25 (4) 0.5 (17)	≤ 0.0625 (11) 0.125 (6) 0.25 (8) 0.5 (2) 1 (1)	≤ 0.0625 (26) 0.125 (2)	0.25 (2) 0.5 (8) 1 (16) 2 (2)
0.5	26	0.125 (1) 0.5 (22) 1 (3)	0.125 (11) 0.25 (12) 0.5 (3)	≤ 0.0625 (25) 0.25 (1)	0.5 (4) 1 (7) 2 (14) 4 (1)
1	2	0.5 (2)	0.125 (2)	≤ 0.0625 (2)	1 (2)

was more potent than LZD in both *in vitro* and *in vivo* assays (12, 13). In addition, it was also much safer than LZD (14–16) and exhibited superior activity against latent tuberculosis (17). Another study has shown that SZD could shorten the treatment course (18). A 14-day preliminary phase II clinical trial (16) demonstrated that SZD at a daily dose of 1200 mg was safe and well tolerated, with high early bactericidal activity. MRX-I was better tolerated and much safer in healthy Chinese subjects in contrast to LZD (19–21). In a 28-day trial, no serious adverse events were observed at 800 or 1,200 mg every 12 h, and none of the patients discontinued the treatment due to any adverse

TABLE 4 Mutations located in *rrl* and *rplD* in six Mtb clinical strains and MICs of five oxazolidinones.

ID	*rrl*	*rplD*	MIC (μ g/ml)				
			LZD	MRX-I	SZD	TZD	DZD
15104	C2060T	WT	0.5	0.5	≤ 0.0625	0.25	2
6102	C2572T	WT	0.5	0.5	≤ 0.0625	0.125	2
22222	C1275T	WT	≤ 0.0625	≤ 0.0625	≤ 0.0625	≤ 0.0625	1
13204	WT	G236A (Arg79His)	0.5	0.5	0.25	0.25	2
11263	WT	G236A (Arg79His)	0.25	0.5	≤ 0.0625	≤ 0.0625	1
10181	WT	G236A (Arg79His)	0.25	≤ 0.0625	≤ 0.0625	≤ 0.0625	1

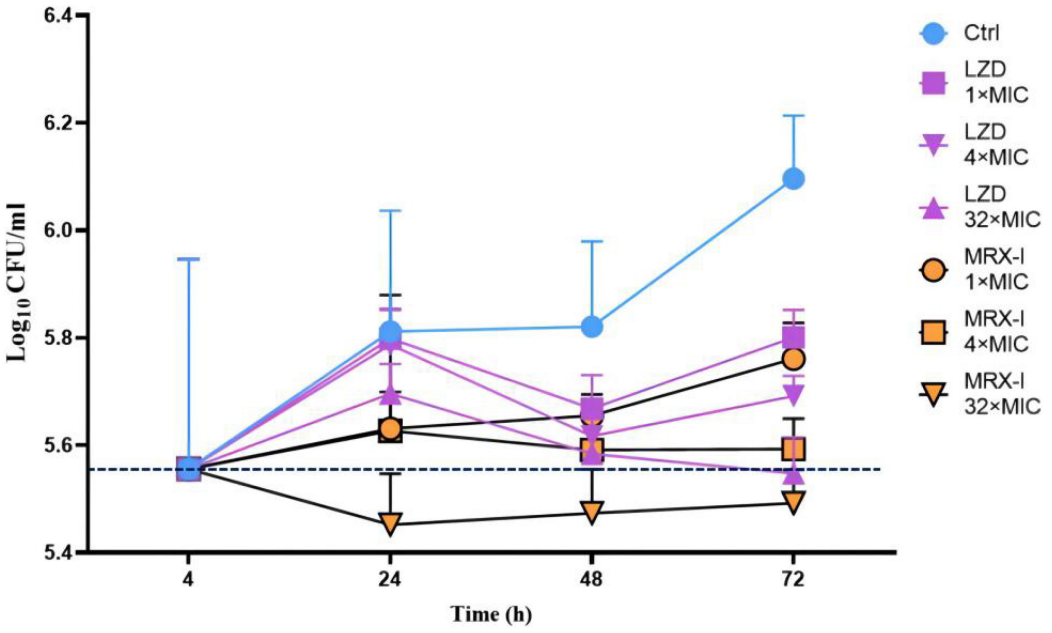

FIGURE 2
Intracellular activities of antibiotics against H37Rv in the THP-1 macrophage model. Ctrl, control; LZD, Linezolid; MRX-I, Contezolid.

events. MRX-I had a lower incidence of myelosuppression than LZD and did not affect the QT interval at the tested therapeutic dose (800 mg/day) (22, 23). Similarly, in a safety and tolerability study, long-term TZD treatment was associated with lesser hematologic toxicity and neuropathy than LZD (5). From the 81 patients treated with TZD (200 mg once daily) for a median duration of 26.5 days, only 6 patients (7.4%) developed myelosuppression-related thrombocytopenia, whereas none developed peripheral or optic neuropathy or allergic reaction.

In this study, TZD exhibited strong inhibitory activities against the RGM and SGM reference strains, while SZD only manifested potent activity against SGM reference strains. However, inconsistent activities were observed against some of the most frequently isolated non-tuberculous mycobacteria (NTM) species. TZD was the only tested drug in this study that had strong activity against *M. avium* reference strain, while only SZD demonstrated strong activity against *M. intracellulare* reference strain, and only TZD demonstrated strong activity against *M. abscessus* reference strain. Similar findings have also been reported by another study (24). The treatment of NTM infection is always challenging due to the shortage of efficacious drugs. Therefore, whether any of the oxazolidinones could be applied for treating a specific NTM infection is worthy of further investigation.

In accordance with the reference strains, SZD also presented the strongest antibacterial effects on clinical isolates of Mtb, followed by TZD. Both drugs harbored better activities than LZD, while MRX-I had comparable activity to LZD. A previous study (8) showed that the anti-TB effects of SZD and MRX-I were comparable to LZD (with MIC_{50} = 0.5 μg/ml), TZD (MIC_{50} = 0.125 μg/ml) had better anti-tuberculosis effects than LZD. In this assay, DZD (MIC_{50} = 2 μg/ml, MIC_{90} = 4 μg/ml)

had the weakest antibacterial effect on Mtb and its MIC was generally 4 times than LZD. Zong Z. et al. (25) showed that the MIC of DZD ($MIC_{50} = 0.5$ μg/ml) against MDR-TB isolates was about 8 times than LZD ($MIC_{50} = 0.064$ μg/ml), whereas the MIC values for both drugs were much lower than the MIC values obtained in this study. The main reason for the above differences may due to the difference between the recruited isolates and the operation process of the MIC test.

According to the CLSI critical concentration of LZD (i.e., 1 μg/ml), the 69 clinical isolates of Mtb recruited in this study were all categorized as LZD-sensitive strains. However, we detected some nucleotide substitutions in *rrl* and *rplD* in a few susceptible strains. Since no LZD-resistant isolate was included in this study, we, therefore, conclude that mutations in these genes are plausibly not always related to LZD resistance in *M. tuberculosis*.

Mycobacterial bacilli preferentially reside in cells. A critical step in anti-tuberculosis drug research is the assessment of its intracellular activity against Mtb. A previous study (9) showed that TZD had good intracellular antibacterial activity, with a 1.3 $\log_{10}$ CFU/ml reduction in the number of intracellular mycobacteria after 72 h exposure to a drug concentration of 16 μg/ml. In another study (26), SZD was bactericidal against Mtb in macrophages. At the drug concentration of 1, 2, and 4 μg/ml, the intracellular survival number of Mtb was reduced by 2 log over the 8th day of action. In this study, MRX-I exhibited obviously stronger intracellular activity than LZD. Even at 1 × MIC after 24 h of incubation, MRX-I showed obvious antibacterial activity, whereas LZD presented a similar effect only in the test with 32 × MIC after 48 h of incubation. Furthermore, MRX-I presented rapid bactericidal activity. At 32 × MIC after 24 h of incubation, about 0.1 log bacilli number reduction was observed compared with the initial infected bacilli number. However, the bacilli number for LZD at 32 × MIC (after 48 h incubation) was equivalent to the initial invading bacilli number, which indicated that LZD had no bactericidal activity against Mtb. Based on this outcome, and also considering the reported better safety profile, MRX-I becomes ideal for treating drug-resistant TB.

There are some limitations in this study. Firstly, the number of Mtb strains in this experiment was small, and the composition ratio of DS-TB and MDR-TB was not in line with the actual situation, which may affect the reliability of ECOFFs defined in this study. Secondly, due to absence of any LZD-resistant isolate among the enrolled cases, the relationship between mutations in the known drug-resistant genes and oxazolidinone resistance could not be evaluated objectively. Thirdly, due to the very low MIC value of SZD against Mtb, the exact MIC distribution of SZD was not elucidated sufficiently. Therefore, whether or not the MIC values of SZD and other oxazolidinones also have consistent trend remains to be studied in the future.

In conclusion, the novel oxazolidinones exhibited potent antibacterial activity against the reference strains of different species and Mtb clinical isolates, including MDR-TB *in vitro*. As the secondly approved oxazolidinone for drug-resistant treatment in China, contezolid manifested much stronger intracellularly bactericidal activity than linezolid against the Mtb bacilli, which encourages its usage in treating drug-resistant tuberculosis.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found in the ENA repository, accession number SRP134826.

Author contributions

CW, GW, and HD participated in the design of the study. CW carried out the experimental studies. CW and HH wrote the manuscript. FH quantitated the drugs. YX, JJ, and LD were responsible for the culture of mycobacteria and THP-1 cells. LZ and FW participated in data analysis. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past collaboration with one of the authors HH at the time of review.

Publisher's note

References

1. World Health Organization. *Global Tuberculosis Report*. Geneva: World Health Organization (2022).

2. Mirzayev F, Viney K, Linh NN, Gonzalez-Angulo L, Gegia M, Jaramillo E, et al. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. *Eur Respir J.* (2021) 57:2003300. doi: 10.1183/13993003.03300-2020

3. Ruiz P, Causse M, Vaquero M, Casal M. In vitro activity of tedizolid against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother.* (2019) 63:e1939–1918. doi: 10.1128/AAC.01939-18

4. Vera-Cabrera L, Gonzalez E, Rendon A, Ocampo-Candiani J, Welsh O, Velazquez-Moreno VM, et al. In vitro activities of DA-7157 and DA-7218 against *Mycobacterium tuberculosis* and *Nocardia brasiliensis*. *Antimicrob Agents Chemother.* (2006) 50:3170–2. doi: 10.1128/AAC.00571-06

5. Mensa Vendrell M, Tasias Pitarch M, Salavert Lletí M, Calabuig Muñoz E, Morata Ruiz L, Castells Lao G, et al. Safety and tolerability of more than six days of tedizolid treatment. *Antimicrob Agents Chemother.* (2020) 64:e356–320. doi: 10.1128/AAC.00356-20

6. Barbachyn MR, Hutchinson DK, Brickner SJ, Cynamon MH, Kilburn JO, Klemens SP, et al. Identification of a novel oxazolidinone (U-100480) with potent antimycobacterial activity. *J Med Chem.* (1996) 39:680–5. doi: 10.1021/jm950956y

7. Cho YS, Lim HS, Lee SH, Cho YL, Nam HS, Bae KS. Pharmacokinetics, pharmacodynamics, and tolerability of single-dose oral LCB01-0371, a novel oxazolidinone with broad-spectrum activity, in healthy volunteers. *Antimicrob Agents Chemother.* (2018) 62:e451–418. doi: 10.1128/AAC.00451-18

8. Shoen C, DeStefano M, Hafkin B, Cynamon M. In vitro and in vivo activities of contezolid (MRX-I) against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother.* (2018) 62:e493–418. doi: 10.1128/AAC.00493-18

9. Molina-Torres CA, Barba-Marines A, Valles-Guerra O, Ocampo-Candiani J, Cavazos-Rocha N, Pucci MJ, et al. Intracellular activity of tedizolid phosphate and ACH-702 versus *Mycobacterium tuberculosis* infected macrophages. *Ann Clin Microbiol Antimicrob.* (2014) 13:13. doi: 10.1186/1476-0711-13-13

10. Jeong JW, Jung SJ, Lee HH, Kim YZ, Park TK, Cho YL, et al. In vitro and in vivo activities of LCB01-0371, a new oxazolidinone. *Antimicrob Agents Chemother.* (2010) 54:5359–62. doi: 10.1128/AAC.00723-10

11. Choi Y, Lee SW, Kim A, Jang K, Nam H, Cho YL, et al. Safety, tolerability and pharmacokinetics of 21 day multiple oral administration of a new oxazolidinone antibiotic, LCB01-0371, in healthy male subjects. *J Antimicrob Chemother.* (2018) 73:183–90. doi: 10.1093/jac/dkx367

12. Yip PC, Kam KM, Lam ET, Chan RC, Yew WW. In vitro activities of PNU-100480 and linezolid against drug-susceptible and drug-resistant *Mycobacterium tuberculosis* isolates. *Int J Antimicrob Agents.* (2013) 42:96–7. doi: 10.1016/j.ijantimicag.2013.03.002

13. Williams KN, Stover CK, Zhu T, Tasneen R, Tyagi S, Grosset JH, et al. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. *Antimicrob Agents Chemother.* (2009) 53:1314–9. doi: 10.1128/AAC.01182-08

14. Wallis RS, Jakubiec WM, Kumar V, Silvia AM, Paige D, Dimitrova D, et al. Pharmacokinetics and whole-blood bactericidal activity against *Mycobacterium tuberculosis* of single doses of PNU-100480 in healthy volunteers. *J Infect Dis.* (2010) 202:745–51. doi: 10.1086/655471

15. Wallis RS, Jakubiec W, Kumar V, Bedarida G, Silvia A, Paige D, et al. Biomarker-assisted dose selection for safety and efficacy in early development of PNU-100480 for tuberculosis. *Antimicrob Agents Chemother.* (2011) 55:567–74. doi: 10.1128/AAC.01179-10

16. Wallis RS, Dawson R, Friedrich SO, Venter A, Paige D, Zhu T, et al. Mycobactericidal activity of sutezolid (PNU-100480) in sputum (EBA) and blood (WBA) of patients with pulmonary tuberculosis. *PLoS One.* (2014) 9:e94462. doi: 10.1371/journal.pone.0094462

17. Zhang M, Sala C, Dhar N, Vocat A, Sambandamurthy VK, Sharma S, et al. In vitro and in vivo activities of three oxazolidinones against nonreplicating *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother.* (2014) 58:3217–23. doi: 10.1128/AAC.02410-14

18. Williams KN, Brickner SJ, Stover CK, Zhu T, Ogden A, Tasneen R, et al. Addition of PNU-100480 to first-line drugs shortens the time needed to cure murine tuberculosis. *Am J Respir Crit Care Med.* (2009) 180:371–6. doi: 10.1164/rccm.200904-0611OC

19. Wu X, Li Y, Zhang J, Zhang Y, Yu J, Cao G, et al. Short-term Safety, tolerability, and pharmacokinetics of MRX-I, an oxazolidinone antibacterial agent, in healthy chinese subjects. *Clin Ther.* (2018) 40:322.e–32.e. doi: 10.1016/j.clinthera.2017.12.017

20. Eckburg PB, Ge Y, Hafkin B. Single- and multiple-dose study to determine the safety, tolerability, pharmacokinetics, and food effect of oral MRX-I versus linezolid in healthy adult subjects. *Antimicrob Agents Chemother.* (2017) 61:e2181–2116. doi: 10.1128/AAC.02181-16

21. Wu J, Wu H, Wang Y, Chen Y, Guo B, Cao G, et al. Tolerability and pharmacokinetics of contezolid at therapeutic and supratherapeutic doses in healthy Chinese subjects, and assessment of contezolid dosing regimens based on pharmacokinetic/pharmacodynamic analysis. *Clin Ther.* (2019) 41:1164.e–74.e. doi: 10.1016/j.clinthera.2019.04.025

22. Wu J, Wang K, Chen Y, Yuan H, Li L, Zhang J. Concentration-response modeling of ECG data from early-phase clinical studies to assess QT prolongation risk of contezolid (MRX-I), an oxazolidinone antibacterial agent. *J Pharmacokinet Pharmacodyn.* (2019) 46:531–41. doi: 10.1007/s10928-019-09650-7

23. Wu J, Cao G, Wu H, Chen Y, Guo B, Wu X, et al. Evaluation of the effect of contezolid (MRX-I) on the corrected QT interval in a randomized, double-blind, placebo- and positive-controlled crossover study in healthy Chinese volunteers. *Antimicrob Agents Chemother.* (2020) 64:e2158–2119. doi: 10.1128/AAC.02158-19

24. Guo Q, Xu L, Tan F, Zhang Y, Fan J, Wang X, et al. A novel oxazolidinone, contezolid (MRX-I), expresses anti-*Mycobacterium* abscessus activity in vitro. *Antimicrob Agents Chemother.* (2021) 65:e0088921. doi: 10.1128/AAC.00889-21

25. Zong Z, Jing W, Shi J, Wen S, Zhang T, Huo F, et al. Comparison of in vitro activity and MIC distributions between the novel oxazolidinone delpazolid and linezolid against multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis* in China. *Antimicrob Agents Chemother.* (2018) 62:e165–118. doi: 10.1128/AAC.00165-18

26. Reddy VM, Dubuisson T, Einck L, Wallis RS, Jakubiec W, Ladukto L, et al. SQ109 and PNU-100480 interact to kill *Mycobacterium tuberculosis* in vitro. *J Antimicrob Chemother.* (2012) 67:1163–6. doi: 10.1093/jac/dkr589

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Prevalence and prognostic significance of malnutrition risk in patients with pulmonary tuberculosis: A hospital-based cohort study

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Background: The prevalence and prognostic significance of malnutrition risk remain unclear in Chinese patients with pulmonary tuberculosis. Therefore, we aimed to investigate the malnutrition risk in Chinese patients and explore the relationship between malnutrition risk and follow-up outcomes.

Methods: We conducted a hospital-based cohort study from January 2020 to December 2020. Malnutrition risks were evaluated using nutritional scales, including the Nutritional Risk Screening 2002 (NRS-2002), the controlling nutritional status score (CONUT), the geriatric nutritional risk index (GNRI), and the prognostic nutritional index (PNI). The primary outcome was all-cause mortality at a one-year follow-up. Malnutrition risk was calculated, and the relationship between malnutrition and follow-up outcomes was analyzed. We assessed the performance of malnutrition risks to predict clinical outcomes in prognostic models.

Results: A total of 1,075 patients were included. According to NRS-2002, CONUT, GNRI, and PNI, 818 (76.09%), 954 (88.74%), 682 (63.44%), and 364 (33.86%) patients were at risk of malnutrition, respectively. Before 1-year follow-up, a total of 99 patients (9.2%) had died. After adjustment for risk factors, the association between severe malnutrition in CONUT (HR = 4.78, 95% CI: 1.14–20.11, $P = 0.033$), GNRI (HR = 3.53, 95% CI: 1.70–7.34, $P = 0.001$), or PNI (HR = 2.94, 95% CI: 1.76–4.88, $P < 0.001$) and death before 1-year follow-up remained significant. The addition of the nutritional scales to prognostic models improved death prediction, as validated by the integrated discrimination index (all *P*-values of <0.05).

Conclusion: Malnutrition in patients with pulmonary tuberculosis was associated with an increased risk of all-cause death in the long-term follow-up. Our findings provided evidence for the use of admission nutrition screening in patients with pulmonary tuberculosis.

KEYWORDS

pulmonary tuberculosis, malnutrition, cohort study, prevalence, prognostic

Introduction

Tuberculosis (TB) was the leading cause of mortality worldwide from a single infectious disease, with 10 million new cases and 1.2 million deaths in 2019 (1). Malnutrition is a notable risk factor that leads to higher mortality in patients with TB (2–6). Moreover, malnutrition is an independent predictor for the reactivation of TB (7) and is considered an important, potentially reversible risk factor for treatment failure (8). However, nutritional status is often overlooked in patients with TB. The recently reported prevalence of malnutrition in patients with TB was estimated to be 50%-57% in different countries (2–6), while there are not sufficient data in China.

In 2013, the WHO published the first nutritional guideline for patients with TB. The guideline used the body mass index (BMI) as a tool for assessing nutritional status (9). The BMI, used as the only tool to estimate nutritional status, could not fully assess malnutrition (10). The Chinese Medical Association Parenteral and Enteral Nutrition Branch recommends nutritional risk screening 2002 (NRS-2002) as the preferred tool for assessing nutritional status (11). NRS-2002 has been used to assess malnutrition in patients with TB in a small sample study (11). However, the NRS-2002 could not reflect the severity of malnutrition, and the study is limited by a lack of objective and quantitative evaluation (11).

Recently, several objective scales have been used for assessing malnutrition, including the controlling nutritional status (CONUT) score (12), the geriatric nutritional risk index (GNRI) (13), and the prognostic nutritional index (PNI) (14). Previous studies investigated these nutritional scales in patients with cardiovascular disease (15–17), stroke (18), and cancer (19). These studies demonstrated that nutritional scales could be a good predictor of treatment endpoints (15–19). However, the effectiveness of objective scales on malnutrition screening in patients with TB remains unclear.

In recent years, several predictive models of TB mortality have been developed (20, 21). However, the nutritional scale was ignored in these predictive models. We speculated that adding nutritional scales to statistical models may increase their predictive performance. In the present study, we aimed to investigate malnutrition in Chinese patients and explore the relationship between nutritional status and TB outcome. Moreover, we further compared the predictive performance of malnutrition risk using different nutritional scales.

Materials and methods

Study design

This study was based on single-center, prospective data from January 2020 to December 2020. The inclusion criteria were patients diagnosed with pulmonary tuberculosis and those aged older than 18 years. The diagnosis of pulmonary TB had to be based on the WHO guidelines (22) and was validated by radiographic or etiological examination. We excluded any patient who met the following criteria: a diagnosis of non-tuberculous mycobacterial lung disease; a medical history of retreatment tuberculosis; a medical history of cancer or other end-stage diseases (23); a personal history of alcohol and substance abuse; and missing data on body height, weight, or other blood parameters used to calculate malnutrition risk. This study was approved by the ethics committee at our hospital, and all patients or their relatives signed written informed consent for study participation. The study protocol was reported in accordance with the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) guideline (24).

Data collection

Demographic characteristics, body height or weight, and medical history were collected at baseline. Clinical features and nutrition status were evaluated by an experienced nutritionist from the department of nutrition in our hospital. Laboratory parameters were collected from a hospital-based database. Weight divided by height square [kg/m^2] was used to calculate BMI.

The BMI categories were defined as follows: underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9kg/m^2), and obesity (≥ 30.0 kg/m^2). Baseline sputum samples were collected at admission, and sputum smears and drug-sensitive tests were performed at the laboratory in our hospital. Tuberculosis drug resistance was defined as resistance to at least one first-line anti-TB drug. All the laboratory parameters of the blood samples were obtained from the first-time results at admission.

Nutritional screening scales and follow-up outcomes

The NRS-2002 is a routine scale performed in our hospital to identify patients with TB and malnutrition. The NRS-2002 scale included two main dimensions: impaired nutritional status and disease severity (23). A score between 0 and 3 was given for each dimension. Impaired nutritional status was calculated by three parameters: BMI, recent body mass loss, and food intake during the week before admission. Disease severity was calculated by evaluating the nutritional requirements caused by the medical history and concomitant chronic diseases. For patients over 70 years, an additional score of 1 point was added. The total NRS-2002 score is the sum of an impaired nutritional score, a severity of disease score, and an age score. The total scores range from 0 to 7. Patients with a score of ≥ 3

were considered to be malnutritioned and were recommended nutritional support (23).

We also explored other objective scales in patients with TB to investigate malnutrition, including the CONUT, GNRI, and PNI. To assess malnutrition risk, the CONUT included three parameters (total lymphocyte count, serum albumin, and cholesterol). The total scores ranged from 0 to 12, and malnutrition assessed by CONUT was defined as follows: normal (score 0–1), mild risk (score 2–4), moderate risk (score 5–8), and severe risk (score 9–12) (12).

The GNRI included three parameters (present body weight, ideal body weight, and serum albumin), and the score was calculated as (41.7×present weight [kg]/ideal body weight [kg]+ 1.519×serum albumin [g/L]). We calculated ideal body weight based on the following formula: for women, ideal body weight = height in cm – 100 – ([height in cm – 150]/2); and for men, ideal body weight = height in cm – 100 – ([height in cm−150]/4); malnutrition assessed by GNRI were defined as follows: normal (score >98), mild risk (score 92–98), moderate risk (score 82–91) and severe risk (score<82) (13).

The PNI included two parameters (total lymphocyte count and serum albumin), and the score was calculated using the formula: 0.005×total lymphocyte count (mm^3) + 10×serum albumin (g/dl). Malnutrition assessed by PNI was defined as follows: normal (score>38), moderate risk (score 35–38), and severe risk (score <35) (14).

A face-to-face follow-up or telephone interview was performed 1 year after admission. We obtained follow-up information on all-cause death from relatives of patients or a death certificate from our hospital records.

Statistical analyses

Descriptive characteristics were reported as percentages for categorical variables or as the mean with standard deviation for continuous variables χ^2 test, Fisher exact test, Student's *t*-test, or the Mann–Whitney U-test were performed for statistical analysis whenever deemed appropriate. The malnutrition risk was assessed using the nutritional scales. Cox regression was used to investigate essential factors of mortality at a 1-year follow-up. The following covariates were adjusted in the Cox regression model: age, gender, hypertension, coronary heart disease, chronic obstructive pulmonary disease, meningeal tuberculosis, and tuberculosis drug resistance ($P < 0.1$ by univariate analysis or clinical confounders). We calculated the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to quantify the correct reclassification and sensitivity improvement with the addition of nutritional scales in the predictive model. Sensitivity analyses were performed by different adjusted models. Significant improvement was recognized in the prediction model when NRI >0 or IDI >0 (24). All tests were 2-tailed, and a *P*-value <0.05 was considered statistically significant. All analyses were conducted using R version 4.2.0.

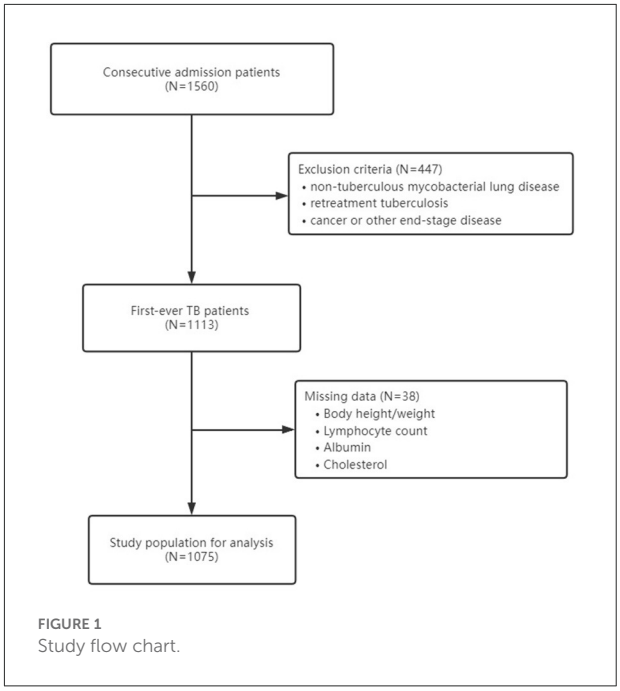

FIGURE 1
Study flow chart.

Results

Baseline characteristics

At baseline, there were 1,560 consecutive in-hospital Chinese patients enrolled in the cohort. After excluding patients with non-tuberculous mycobacterial lung disease ($n = 46$), patients with a medical history of retreatment tuberculosis ($n = 392$), patients with cancer or other end-stage diseases ($n = 9$), and patients with missing data on body height, weight, or other parameters used to calculate malnutrition ($n = 38$), a total of 1,075 patients were analyzed in the study (Figure 1). The mean age was 52.73 (±20.71) years, and 63.16% were men. The baseline information of study participants is shown in Table 1.

Malnutrition risk according to clinical nutrition scales

According to NRS-2002, CONUT, GNRI, and PNI, 818 (76.09%), 954 (88.74%), 682 (63.44%), and 364 (33.86%) patients were at risk of malnutrition (Table 2), whereas 167 (15.53%) patients had malnutrition, as assessed by the underweight BMI. Venn diagram showed malnutrition risk assessed by the 4 nutritional scales (Figure 2).

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TABLE 1 Baseline information of study participants stratified by nutritional status using NRS-2002.

Variables	Overall ($n = 1,075$)	With Malnutrition ($n = 818$)	Without Malnutrition ($n = 257$)	*P*-value
Demographics				
Age, y	52.73 ± 20.71	55.12 ± 21.38	45.14 ± 16.23	<0.001
Men	679 (63.16)	521(63.69)	158(61.48)	0.521
Height, cm	167.80 ± 8.30	167.55 ± 8.29	168.59 ± 8.31	0.078
Weight, kg	60.24 ± 10.45	58.02 ± 9.42	67.30 ± 10.42	<0.001
Body mass index, kg/m^2	21.33 ± 3.01	20.61 ± 2.81	23.60 ± 2.45	<0.001
Medical history				
Hypertension	153(14.23)	119(14.55)	34(13.23)	0.598
Diabetes	272(25.30)	211(25.79)	61(23.74)	0.508
Coronary heart disease	115(10.69)	96(11.74)	19(7.39)	0.049
Stroke	14(1.30)	13(1.59)	1(0.3)	0.208
Chronic obstructive pulmonary disease	19(1.76)	16(1.96)	3(1.67)	0.588
Clinical features				
Military TB	32(2.97)	25(3.06)	7(2.72)	0.784
Meningeal TB	10(0.93)	8(0.98)	2(0.78)	0.971
Positive sputum smear	306(28.46)	261(31.91)	45(17.51)	<0.001
TB drug-resistant	132(12.27)	96(11.74)	36(14.01)	0.333
HIV infection	1(0.10)	1(0.10)	0(0.00)	1.000
Hospital stay	15.99 ± 12.38	16.85 ± 13.10	13.24 ± 9.21	<0.001
Laboratory feature				
Hemoglobin, g/L	120.49 ± 22.30	115.06 ± 21.64	137.75 ± 14.09	<0.001
Lymphocyte, 10^9/L	1.28 ± 0.60	1.18 ± 0.59	1.58 ± 0.53	<0.001
Urea, mmol/l	4.80 ± 2.68	4.97 ± 2.98	4.28 ±1.20	<0.001
Creatinine,μmol/L	64.51 ± 33.29	64.82 ± 37.33	63.53 ± 14.18	0.586
Uric acid, μmol/L	327.86 ± 144.59	311.73 ± 143.85	379.16 ± 134.91	<0.001
Albumin, g/L	35.02 ± 6.94	33.16 ± 6.74	40.98 ± 3.20	<0.001
Cholesterol, mmol/L	4.22 ± 0.86	4.14 ± 0.81	4.52 ± 0.92	<0.001
Triglycerides, mmol/L	1.28 ± 0.58	1.24 ± 0.53	1.40 ± 0.69	<0.001

Continuous variables are expressed as mean±SD, and categorical variables are expressed as frequency (%). TB, tuberculosis; HIV, human immunodeficiency virus.

TABLE 2 The prevalence of the malnutrition risk identified by the nutritional scales.

	Malnutrition risk				
	Normal, %	Any	Mild	Moderate	Severe
BMI	73.02(70.24–75.63)	15.53(13.44–17.87)		…	…
NRS2002	23.91(21.41–26.60)	76.09(73.40–78.59)	…	…	…
CONUT	11.25(9.46–13.34)	88.74(86.66–90.54)	29.48(26.80–32.33)	37.95(35.05–40.94)	21.30(18.91–23.90)
GNRI	36.55(33.69–39.53)	63.44(60.47–66.31)	17.39(15.21–19.83)	24.55(22.04–27.27)	21.49(19.09–24.09)
PNI	66.13(63.21–68.95)	33.86(31.05–36.79)	…	8.93(7.33–10.83)	24.93(22.39–27.65)

BMI, body mass index; NRS, nutritional risk screening; CONUT, controlling nutritional status; GNRI, geriatric nutritional risk index; PNI, prognostic nutritional index.

A more detailed stratified analysis of the severity of malnutrition was performed. Of these patients, 317 (29.48%) and 187 (17.39%) were at mild malnutrition risk, as assessed by CONUT and GNRI; 408 (37.95%), 264 (24.55%), and 96 (8.93%) patients were at moderate malnutrition risk, as assessed by CONUT, GNRI, and PNI; and 229 (21.30%), 231 (21.49%), and 268 (24.93%) patients were at severe malnutrition risk, as assessed by CONUT, GNRI, and PNI, respectively.

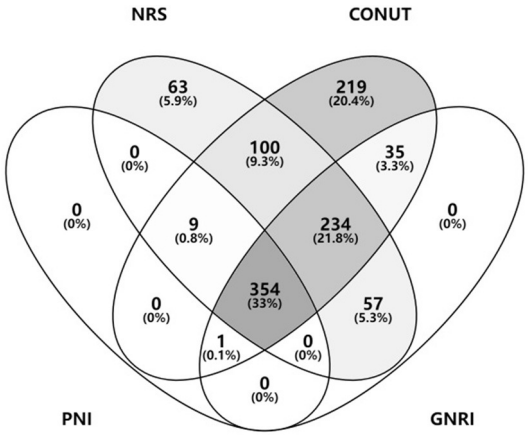

FIGURE 2
Malnutrition risk assessed by the nutritional scales. BMI, body mass index; NRS, nutritional risk screening; CONUT, controlling nutritional status; GNRI, geriatric nutritional risk index; PNI, prognostic nutritional index.

Predictors of follow-up clinical events

Before 1-year follow-up, a total of 99 patients (9.2%) had died. Univariable analyses suggested that malnutrition was significantly associated with follow-up death (Supplementary Table 1). Any malnutrition assessed by the NRS-2002, GNRI, or PNI and moderate to severe malnutrition assessed by CONUT was related to an increased risk of death at 1-year follow-up (Table 3). In multivariable analyses, after adjustment for age, gender, hypertension, coronary heart disease, chronic obstructive pulmonary disease, meningeal tuberculosis, and tuberculosis drug resistance, the association between severe malnutrition in CONUT (HR = 4.78, 95% CI 1.14–20.11, $P = 0.033$), GNRI (HR = 3.53, 95% CI 1.70–7.34, $P = 0.001$), or PNI (HR = 2.94, 95% CI 1.76–4.88, $P < 0.001$) and death before 1-year follow-up remained significant.

Improvement in models upon the addition of adding clinical nutrition scales

We investigated the performance of models to predict clinical outcomes in patients with TB (Table 4). The predictive performance, according to the C-statistic, slightly improved when clinical nutrition scales were added to the different adjusted models. Nutrition scales improved the model's performance, as confirmed by NRI and IDI. The increased NRI ranged from 3.92 to 24.43% in Model 1, from 3.73 to 22.43% in Model 2, and from 3.32 to 13.34% in Model 3, which suggested that the addition of nutrition scales improved the model's performance for predicting death. In the final Model 3, the NRI of CONUT, GNRI, and PNI was 9.40% (−0.02 to 18.82), 10.60% (0.85–20.35), and 13.34% (2.52–24.16), respectively. The improved performance of the model validated by IDI can likewise be interpreted as the explanation of NRI. The addition of BMI and nutrition scales to different adjusted models significantly improved predictive ability (all $P < 0.05$).

Discussion

The present study investigated the nutritional status of Chinese patients with TB and explored the performance of nutritional scales to predict TB outcomes. The findings suggested that severe malnutrition risk ranged from 21.30 to 24.93% in patients with TB. Baseline malnutrition may be a predictor of TB mortality. The addition of nutritional scales to mortality models improved C statistics, NRI, and IDI. The results stress the importance of assessing the nutritional status of patients with TB.

Our data showed that malnutrition risk ranged from 33.86 to 88.74% in patients with TB, the moderate risk was 8.93 to 37.95%, and the severe risk was 21.30 to 24.93%. The 2013 WHO guideline considers BMI as a tool for assessing malnutrition in patients with TB (9). However, in our data, only 15.53 % of individuals who are malnourished used BMI. Malnutrition was underestimated when compared to other nutritional scales. The previous study indicated that patients with catabolic diseases such as TB might be malnourished but still show a BMI between or above the normal range (25). This could be explained by the fact that BMI is a characteristic of chronic malnutrition that involves weight loss (26), whereas disease-associated malnutrition is a subacute or acute condition in which weight loss does not lead to a low BMI (26, 27). These results indicated that patients with TB would not be identified as malnourished when assessing nutritional status only based on BMI. The NRS-2002 is often used to assess for malnutrition in Chinese patients (28). It is an important tool for assessing Chinese patients with malnutrition and whether they need nutritional intervention (29). A recent study confirmed that malnutrition was assessed by the NRS-2002 in Chinese patients with TB with a prevalence of 64.41% (11), which was similar to the results. However, the NRS-2002 is limited by potential subjective bias and cannot quantitatively evaluate the severity of malnutrition.

Therefore, we compared the performance of other objective nutritional scales in our study. The malnutrition risk varied between different objective scales. The malnutrition risk calculated by CONUT was 88.74%, whereas 63.44% was calculated by GNRI and 33.86% by PNI. The GNRI (13) involves weight and serum albumin parameters, and the CONUT (12) includes serum albumin, lymphocyte count, and total cholesterol level. Compared to the CONUT, the PNI (14) only includes the parameters of albumin and lymphocyte count but lacks the parameter of cholesterol level, which may explain the lower prevalence estimated by the PNI. Nevertheless, the prevalence of severe malnutrition risk assessed by objective

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TABLE 3 Multivariable analyses of malnutrition scales to predict 1-year mortality.

Nutritional status	Events, *N* (%)	Unadjusted HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)
BMI					
Underweight	6(6.00)	0.32(0.14–0.73)*	0.51(0.22–1.16)	0.53(0.23–1.21)	0.56(0.24–1.28)
Normal	88(88.80)	Reference	Reference	Reference	Reference
Overweight-Obesity	5(5.00)	0.36(0.15–0.89)*	0.46(0.18–1.16)	0.49(0.19–1.22)	0.50(0.20–1.26)
NRS2002					
Normal	5(5.10)	Reference	Reference	Reference	Reference
Any risk	94(94.90)	2.78(2.16–3.58)*	2.66(1.06–6.70)*	2.61(1.04–6.59)*	2.45(0.97–6.22)
CONUT					
Normal	2(2.00)	Reference	Reference	Reference	Reference
Mild risk	9(9.00)	1.72(0.37–7.95)	1.76(0.38–8.18)	1.76(0.38–8.17)	1.46(0.31–6.86)
Moderate risk	32(32.30)	4.75(1.14–19.80)*	2.64(0.63–11.09)	2.60(0.62–10.98)	2.41(0.57–10.18)
Severe risk	56(56.50)	14.79(3.61–60.63)*	5.29(1.27–22.16)*	4.92(1.17–20.67)*	4.78(1.14–20.11)*
GNRI					
Normal	10(10.10)	Reference	Reference	Reference	Reference
Mild risk	12(12.10)	2.51(1.08–5.81)*	1.76(0.75–4.09)	1.76(0.76–4.13)	1.45(0.60–3.49)
Moderate risk	25(25.20)	3.72(1.78–7.75)*	2.01(0.94–4.25)	1.85(0.86–3.94)	1.98(0.92–4.28)
Severe risk	52(52.50)	8.88(4.52–17.48)*	3.42(1.68–7.01)*	3.31(1.61–6.81)*	3.53(1.70–7.34)*
PNI					
Normal	25(25.20)	Reference	Reference	Reference	Reference
Moderate risk	9(9.00)	2.66(1.25–5.71)*	1.45(0.67–3.15)	1.42(0.65–3.08)	1.48(0.68–3.21)
Severe risk	65(65.60)	6.89(4.35–10.94)*	3.02(1.83–4.99)*	2.85(1.71–4.75)*	2.94(1.76–4.88)*

Model 1: adjust for age, gender; Model 2: adjust for age, gender+hypertension, coronary heart disease, chronic obstructive pulmonary disease; Model 3: adjust for age, gender, hypertension, coronary heart disease, chronic obstructive pulmonary disease+ Meningeal TB, TB drug-resistant. BMI, body mass index; NRS, nutritional risk screening; CONUT, controlling nutritional status; GNRI, geriatric nutritional risk index; PNI, prognostic nutritional index. TB, tuberculosis; HR, hazard ratio.
*$P < 0.05$.

scales ranged from 21.30 to 24.93% in patients with TB, which suggested good internal consistency. Based on the literature review and findings from our study, PNI may be a more suitable scale to screen for malnutrition risk in patients with TB.

Several studies examined the association between nutritional status and TB mortality (2, 4). A prospective cohort of 1,695 adult patients with pulmonary TB was evaluated for malnutrition risk using the BMI, and the results showed that severe undernutrition was associated with a two-fold higher risk of death (2). In another study, nutritional status, also assessed by the BMI, in 1,181 patients with TB revealed that moderate to severe malnutrition is a risk factor associated with early death (4). The correlation between malnutrition and TB mortality was also verified by the Malnutrition Screening Tool (MST) (30), the Mini Nutritional Assessment (MNA) (31), and the Malnutrition Universal Screening Tool (MUST) (32) in small sample size studies (30–34). However, few studies reported the effect of objective scales on nutritional screening for patients with TB. In the present study, we found that severe malnutrition risk evaluated by objective nutritional scales was significantly associated with mortality after adjusting for the potential risk factors. Therefore, physicians should consider on-admission nutritional screening for patients with TB.

Several prognostic models have been developed to predict TB mortality. However, nutritional status was not involved in these studies (20, 21). We added nutritional scales to the statistical models to predict TB outcomes. The predictive ability of nutritional scales for mortality was improved and validated by increased C-statistic, NRI, and IDI. Our study demonstrated that objective nutritional scales might improve malnutrition risk classification for TB mortality.

Our study has its limitations. First, due to the unavailability of necessary variables, we failed to investigate malnutrition according to the European diagnostic criteria (26) and could not perform a comparison with other nutritional screening tools such as MST (30), MNA (31), and MUST (32). However, there is currently no gold standard for nutritional screening in patients with TB. The WHO guidelines only recommend BMI as a tool for assessing nutritional status. Future studies should investigate nutritional status using more evaluation methods. Second, the study only evaluated admission nutritional status—not at follow-up. We were unable to consider dynamic nutritional changes that may affect TB outcomes. Third, the follow-up period in our study was not long enough, and only the death rate 1 year after admission was followed up, which may not be sufficient for evaluating TB outcomes. Finally, we excluded

TABLE 4 Performance of prognostic models with malnutrition scales to predict the 1-year mortality in patients with TB.

	C-statistic		Category NRI		IDI	
	Estimate (95% CI)	*P*-value	Estimate (95% CI),%	*P*-value	Estimate (95% CI),%	*P*-value
Model 1	0.8244 (0.7787–0.8700)	Reference	Reference		Reference	
Model+BMI	0.8314 (0.7868–0.8759)	0.183	3.92(−3.28 to 11.12)	0.286	1.01(0.36 to 1.65)	0.002
Model+NRS2002	0.8297 (0.7861–0.8732)	0.290	3.51(−0.01 to 7.94)	0.119	0.46(0.00 to 0.83)	0.016
Model+CONUT	0.8428 (0.7985–0.8871)	0.032	18.77(8.54 to 28.99)	<0.001	3.35(1.99 to 4.72)	<0.001
Model+GNRI	0.8392 (0.7946–0.8835)	0.073	20.89(10.45 to 31.33)	<0.001	2.62(1.31 to 3.92)	<0.001
Model+PNI	0.8434 (0.7985–0.8883)	0.038	24.43(13.29 to 35.58)	<0.001	4.24(2.67 to 5.81)	<0.001
Model 2	0.8334 (0.7877–0.8792)	Reference	Reference		Reference	
Model+BMI	0.8391 (0.7942–0.8840)	0.246	7.48 (−0.13 to 15.09)	0.054	0.91(0.32 to 1.50)	0.002
Model+NRS2002	0.8379 (0.7939–0.8819)	0.365	3.73 (−1.11 to 8.58)	0.131	0.57(0.19 to 0.95)	0.003
Model+CONUT	0.8480 (0.8035–0.8926)	0.056	21.31(11.38 to 31.25)	<0.001	2.83(1.59 to 4.08)	<0.001
Model+GNRI	0.8453 (0.8020–0.8885)	0.079	19.09 (8.99 to 29.18)	<0.001	2.39(1.16 to 3.61)	<0.001
Model+PNI	0.8491 (0.8043–0.8939)	0.066	22.43(11.58 to 33.27)	<0.001	3.71(2.23 to 5.19)	<0.001
Model 3	0.8592 (0.8221–0.8961)	Reference	Reference		Reference	
Model+BMI	0.8592 (0.8226–0.8962)	0.858	7.48(0.99 to 13.97)	0.0239	1.06(0.53 to 1.59)	<0.001
Model+NRS2002	0.8611 (0.8250–0.8971)	0.660	3.32(−1.55 to 8.19)	0.181	0.59(0.11 to 1.08)	0.015
Model+CONUT	0.8713 (0.8357–0.9070)	0.108	9.40(−0.02 to 18.82)	0.051	1.73(0.61 to 2.84)	0.002
Model+GNRI	0.8712 (0.8364–0.9059)	0.119	10.60(0.85 to 20.35)	0.033	1.84(0.67 to 3.01)	0.002
Model+PNI	0.8744 (0.8387–0.9100)	0.070	13.34(2.52 to 24.16)	0.016	2.33(1.01 to 3.66)	0.001

Model 1: adjusted for age, gender; Model 2: adjusted for age, gender+hypertension, coronary heart disease, and chronic obstructive pulmonary disease; Model 3: adjusted for age, gender, hypertension, coronary heart disease, chronic obstructive pulmonary disease+ Meningeal TB, and TB drug-resistance.
NRI, net reclassification improvement; IDI, integrated discrimination Improvement; BMI, body mass index; NRS, nutritional risk screening; CONUT, controlling nutritional status; GNRI, geriatric nutritional risk index; PNI, prognostic nutritional index. TB, tuberculosis.

several patients whose data were incomplete, which may result in potential selection bias. Multicenter studies with large sample sizes are required to generalize these findings.

In conclusion, severe malnutrition risk ranged from 21.30 to 24.93% in Chinese patients with TB. Malnutrition risk was related to an increased risk of death in the long-term follow-up. Nutritional scales may be significant indicators for predicting clinical outcomes in patients with TB.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee at Beijing Chest Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: J-JM and W-ML. Data collection: J-JM, ZL, YC, and HH. Formal analysis: Y-JG. Funding acquisition: W-ML. Methodology: Y-JG and W-ML. Data supervision: J-JM and W-ML. Writing—original draft: J-JM and Y-JG. Writing—review and editing: J-JM, Y-JG, ZL, YC, HH, and W-ML. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1039661/full#supplementary-material

References

1. World Health Organization. *Global Tuberculosis Report*. Geneva: World Health Organization (2020).

2. Bhargava A, Chatterjee M, Jain Y, Chatterjee B, Kataria A, Bhargava M, et al. Nutritional status of adult patients with pulmonary tuberculosis in rural central India and its association with mortality. *PLoS ONE.* (2013) 8:e77979. doi: 10.1371/journal.pone.0077979

3. Wondmieneh A, Gedefaw G, Getie A, Demis A. Prevalence of undernutrition among adult tuberculosis patients in Ethiopia: a systematic review and meta-analysis. *J Clin Tuberc Other Mycobact Dis.* (2021) 22:100211. doi: 10.1016/j.jctube.2020.100211

4. Zachariah R, Spielmann MP, Harries AD, Salaniponi FM. Moderate to severe malnutrition in patients with tuberculosis is a risk factor associated with early death. *Trans R Soc Trop Med Hyg.* (2002) 96:291–4. doi: 10.1016/S0035-9203(02)90103-3

5. Dodor E. Evaluation of nutritional status of new tuberculosis patients at the effia-nkwanta regional hospital. *Ghana Med J.* (2008) 42:22–8.

6. Bhargava A, Benedetti A, Oxlade O, Pai M, Menzies D. Undernutrition and the incidence of tuberculosis in India: national and subnational estimates of the population-attributable fraction related to undernutrition. *Natl Med J India.* (2014) 27:128–33.

7. Dheda K, Barry CE, Maartens G. Tuberculosis. *Lancet.* (2016) 387:1211–26. doi: 10.1016/S0140-6736(15)00151-8

8. Choi R, Jeong BH, Koh WJ, Lee SY. Recommendations for optimizing tuberculosis treatment: therapeutic drug monitoring, pharmacogenetics, and nutritional status considerations. *Ann Lab Med.* (2017) 37:97–107. doi: 10.3343/alm.2017.37.2.97

9. World Health Organization. *Nutritional Care and Support for Patients with Tuberculosis*. Geneva: World Health Organization (2013).

10. Ter Beek L, Bolhuis MS, Jager-Wittenaar H, Brijan RXD, Sturkenboom MGG, Kerstjens HAM, et al. Malnutrition assessment methods in adult patients with tuberculosis: a systematic review. *BMJ Open.* (2021) 11:e049777. doi: 10.1136/bmjopen-2021-049777

11. Li Y, Yang F, Zhou H, Shu L, Wang R, Zhao C. Clinical application of NRS-2002 in nutritional risk screening of tuberculosis inpatients. *Ann Palliat Med.* (2021) 10:5322–8. doi: 10.21037/apm-21-610

12. Ignacio de Ulibarri J, Gonzalez-Madrono A, de Villar NG, Gonzalez P, Gonzalez B, Mancha A, et al. CONUT: a tool for controlling nutritional status. First validation in a hospital population. Nutricion hospitalaria. *Nutr Hosp.* (2005) 20:38–45.

13. Bouillanne O, Morineau G, Dupont C, Coulombel I, Vincent JP, Nicolis I, et al. Geriatric Nutritional Risk Index: a new index for evaluating at-risk elderly medical patients. *Am J Clin Nutr.* (2005) 82:777–83. doi: 10.1093/ajcn/82.4.777

14. Buzby GP, Mullen JL, Matthews DC, Hobbs CL, Rosato EF. Prognostic nutritional index in gastrointestinal surgery. *Am J Surg.* (1980) 139:160–7. doi: 10.1016/0002-9610(80)90246-9

15. Sze S, Pellicori P, Kazmi S, Rigby A, Cleland JGF, Wong K, et al. Prevalence and Prognostic Significance of Malnutrition Using 3 Scoring Systems Among Outpatients With Heart Failure: a Comparison With Body Mass Index. *JACC Heart Fail.* (2018) 6:476–86. doi: 10.1016/j.jchf.2018.02.018

16. Cheng N, Dang A, Lv N, He Y, Wang X. Malnutrition status in patients of very advanced age with nonvalvular atrial fibrillation and its impact on clinical outcomes. *Nutr Metab Cardiovasc Dis.* (2019) 29:1101–9. doi: 10.1016/j.numecd.2019.06.021

17. Raposeiras Roubín S, Abu Assi E, Cespón Fernandez M, Barreiro Pardal C, Lizancos Castro A, Parada JA, et al. Prevalence and prognostic significance of malnutrition in patients with acute coronary syndrome. *J Am Coll Cardiol.* (2020) 76:828–40. doi: 10.1016/j.jacc.2020.06.058

18. Zhang G, Pan Y, Zhang R, Wang M, Meng X, Li Z, et al. Prevalence and prognostic significance of malnutrition risk in patients with acute ischemic stroke: results from the third china national stroke registry. *Stroke.* (2022) 53:111–9. doi: 10.1161/STROKEAHA.121.034366

19. Roshanmehr F, Kagawa M, Taghizadeh-Hesary F, Milani-Bonab A, Haghighian-Roudsari A. Nutritional Status of Cancer Patients: a Unicenteric Report from Iran. *JNFS.* (2022) 7:11–21. doi: 10.18502/jnfs.v7i1.8530

20. Pefura-Yone EW, Balkissou AD, Poka-Mayap V, Fatime-Abaicho HK, Enono-Edende PT, Kengne AP. Development and validation of a prognostic score during tuberculosis treatment. *BMC Infect Dis.* (2017) 17:251. doi: 10.1186/s12879-017-2309-9

21. Nguyen DT, Graviss EA. Development and validation of a prognostic score to predict tuberculosis mortality. *J Infect.* (2018) 77:283–90. doi: 10.1016/j.jinf.2018.02.009

22. World Health Organization. *WHO Consolidated Guidelines on Tuberculosis: Module 3: Diagnosis-Rapid Diagnostics for Tuberculosis Detection*. Geneva: World Health Organization (2020).

23. Ameri A, Rahnama N, Talebi F, Sourati A. An evaluation of Cancer Aging Research Group (CARG) score to predict chemotherapy toxicity in Iranian older cancer patients. *Ann Oncol, Singapore.* (2022) 33:S1276. doi: 10.1016/j.annonc.2022.07.1696

24. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ.* (2007) 335:806–8. doi: 10.1136/bmj.39335.541782.AD

25. Kroc Ł, Fife E, Piechocka-Wochniak E, Sołtysik B, Kostka T. Comparison of nutrition risk screening 2002 and subjective global assessment form as short nutrition assessment tools in older hospitalized adults. *Nutrients.* (2021) 13:225. doi: 10.3390/nu13010225

26. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med.* (2008) 27:157–72. doi: 10.1002/sim.2929

27. Gonzalez MC, Correia MITD, Heymsfield SB, A. requiem for BMI in the clinical setting. *Curr Opin Clin Nutr Metab Care.* (2017) 20:314–21. doi: 10.1097/MCO.0000000000000395

28. Cederholm T, Bosaeus I, Barazzoni R, et al. Diagnostic criteria for malnutrition - An ESPEN Consensus Statement. *Clin Nutr.* (2015) 34:335–40. doi: 10.1016/j.clnu.2015.03.001

29. Correia MI, Waitzberg DL. The impact of malnutrition on morbidity, mortality, length of hospital stay and costs evaluated through a multivariate model analysis. *Clin Nutr.* (2003) 22:235–9. doi: 10.1016/S0261-5614(02)00215-7

30. Miao JP, Quan XQ, Zhang CT, Zhu H, Ye M, Shen LY, et al. Comparison of two malnutrition risk screening tools with nutritional biochemical parameters, BMI and length of stay in Chinese geriatric inpatients: a multicenter, cross-sectional study. *BMJ Open.* (2019) 9:e022993. doi: 10.1136/bmjopen-2018-022993

31. Liu G, Zhang S, Mao Z, Wang W, Hu H. Clinical significance of nutritional risk screening for older adult patients with COVID-19. *Eur J Clin Nutr.* (2020) 74:876–83. doi: 10.1038/s41430-020-0659-7

32. Miyata S, Tanaka M, Ihaku D. Usefulness of the malnutrition screening tool in patients with pulmonary tuberculosis. *Nutrition.* (2012) 28:271–4. doi: 10.1016/j.nut.2011.07.013

33. Miyata S, Tanaka M, Ihaku D. Full mini nutritional assessment and prognosis in elderly patients with pulmonary tuberculosis. *J Am Coll Nutr.* (2013) 32:307–11. doi: 10.1080/07315724.2013.826114

34. Miyata S, Tanaka M, Ihaku D. The prognostic significance of nutritional status using malnutrition universal screening tool in patients with pulmonary tuberculosis. *Nutr J.* (2013) 12:42. doi: 10.1186/1475-2891-12-42

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Effect of a modified regimen on drug-sensitive retreated pulmonary tuberculosis: A multicenter study in China

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Background and objective: Retreatment pulmonary tuberculosis (PTB) still accounts for a large proportion of tuberculosis, and the treatment outcome is unfavorable. The recurrence of retreatment PTB based on long-term follow-up has not been well demonstrated. This study aimed to evaluate effect of a modified regimen on drug-sensitive retreated pulmonary tuberculosis.

Methods: This multicenter cohort study was conducted in 29 hospitals from 23 regions of China from July 1, 2009, to December 31, 2020. Patients were divided into two treatment regimen groups including experimental group [modified regimen (4H-Rt2-E-Z-S(Lfx)/4H-Rt2-E)]and control group [standard regimen (2H-R-E-Z-S/6H-R-E or 3H-R-E-Z/6H-R-E)]. The patients enrolled were followed up of 56 months after successful treatment. We compared the treatment success rate, treatment failure rate, adverse reaction rate, and recurrence rate between

two regimens. Multivariate Cox regression model was used to identify the potential risk factors for recurrence after successful treatment with proportional hazards assumptions tested for all variables.

Results: A total of 381 patients with retreatment PTB were enrolled, including 244 (64.0%) in the experimental group and 137 (36.0%) in the control group. Overall, the treatment success rate was significant higher in the experimental group than control group (84.0 vs. 74.5%, $P = 0.024$); no difference was observed in adverse reactions between the two groups (25.8 vs. 21.2%, $P > 0.05$). A total of 307 patients completed the 56 months of follow-up, including 205 with the modified regimen and 102 with the standard regimen. Among these, 10 cases (3.3%) relapsed, including 3 in the experimental group and 7 in the control group (1.5% vs 6.9%, $P = 0.035$). Reduced risks of recurrence were observed in patients treated with the modified regimen compared with the standard regimen, and the adjusted hazard ratio was 0.19 (0.04–0.77).

Conclusion: The modified retreatment regimen had more favorable treatment effects, including higher treatment success rate and lower recurrence rate in patients with retreated drug-sensitive PTB.

KEYWORDS

pulmonary tuberculosis, retreatment, modified regimen, recurrence, follow-up

Introduction

Tuberculosis (TB) is the second leading infectious killer after Coronavirus disease 2019 globally and also a major contributor to antimicrobial resistance; the incidence of retreatment pulmonary tuberculosis (PTB) cases was about 392,000 in 2019 (1). In China, Ruan et al. (2) reported that 4.9% of 9,828 people in a retrospective study subsequently developed recurrent TB, and 9.6% were infected with *M. tuberculosis* isolates resistant at least to isoniazid and rifampicin. The first national survey of drug-resistant tuberculosis in China showed that 25.6% of patients with retreated TB had multi-drug resistant (MDR) infections (3). Hence, the retreatment PTB needs more attention and should be addressed.

In some developing countries, the treatment success rate of the standard treatment regimen is also unsatisfactory, with a total cure rate of about 70%; also, the rates are different for different patients (4–6). A multicenter, randomized, parallel, controlled, prospective cohort trial showed that the smear conversion was only 77.6% in the patients with retreatment PTB who adopted the standard retreatment regimen (7). Studies on the regimens on retreatment PTB are ongoing. Recently, the efforts on the replacement of ethambutol with moxifloxacin did not improve the treatment outcomes of retreatment PTB significantly (8), suggesting the difficult task for the ideal regimens for retreatment PTB. The World Health Organization (WHO) has recently updated the guidelines for PTB treatment (9) and recommended that the treatment strategies should be adjusted based on drug susceptibility testing. However, most guidelines were published not for drug-sensitive retreatment PTB but for drug-resistant TB. Therefore, it is necessary to improve the treatment outcome of drug-sensitive PTB. Additionally, the recurrence of retreated PTB after successful treatment has always been a global concern. A study from South Africa reported 14% of recurrence rate after successful treatment with a follow-up of 5 years and 6.8% of recurrence rate with a follow-up of 3 years in China. The relapse of retreated PTB during long-term follow-up after treatment success is limited. The recurrence of PTB brings severe challenges to END TB. Therefore, it is important to ensure the long-term follow-up of patients who have been successfully treated, further understand the recurrence, and identify the relevant factors for recurrence to reduce the recurrence rate. This multicenter bidirectional cohort study was conducted to understand the treatment outcome and recurrence of TB and further provide a reference for clinicians to treat patients with retreated PTB.

Materials and methods

Study subjects and settings

Patients with bacteriologically confirmed retreatment PTB having drug susceptibility were retrospectively recruited from 29 TB hospitals and institutions in 23 regions of China from July 1, 2009, to December 31, 2014, with a follow-up by December 31, 2020, who ever attended a bidirectional cohort trial with detailed inclusion and exclusion criteria (Table 1). This study was approved by the ethics committee of the Beijing Chest Hospital affiliated with Capital Medical University (2009–2013). Trial registration: chictr.org Identifier: ChiCTR1800017441 (http://www.chictr.org.cn/historyversionpub.aspx).

Study design

The participants in this bidirectional cohort study adopted the modified and standard regimens. The modified retreatment regimen consisted of a 4-month intensive phase, followed a 4-month continuation phase: 4H-Rt_2-E-Z-S(Lfx)/4H-Rt_2-E (H, isoniazid (INH); Rt, rifapentine; E, ethambutol; Z, pyrazinamide; S, streptomycin; Lfx, levofloxacin). If S was unavailable, it was replaced with Lfx. In China, the standard treatment regimen consisted of a 2-month intensive phase, followed a 6-month continuation phase: 2H-R-E-Z-S/6H-R-E [R, rifampicin (RIF)]. Patients who could not use S had a 3-month intensive phase: 3H-R-E-Z/6H-R-E. The doses of H and Rt in the modified regimen were increased appropriately

TABLE 1 Inclusion and exclusion criteria for patients with retreated TB in the present study.

	Detailed description
Inclusion criteria	(1) Willing to participate in trial treatment and follow-up; signed informed consent. (2) Aged 18–65 years. (3) Retreatment of pulmonary TB for the first time. (4) Sputum culture-positive patients susceptible to first-line antituberculosis drugs confirmed by the DST. (5) Active pulmonary TB. (6) No obvious abnormalities in liver function, renal function, and blood and urine routine testing. (7) Willing to carry out HIV testing.
Exclusion criteria	(1) Resistance to any antituberculosis drug confirmed by DST, including single-drug resistance, poly-drug resistance, and multi-drug resistance. (2) Combined extrapulmonary tuberculosis. (3) HIV antibody positivity. (4) Pregnant or breastfeeding. (5) Severe cardiovascular, liver, kidney, or blood system disease and other serious illnesses. (6) Mental illness. (7) Alcohol abuse. (8) Inability to attend or follow-up treatment. (9) Inability to take oral medications. (10) Allergy or intolerance to any study drug. (11) Participation in another drug clinical trial.

compared with the conventional dose in the standard regimen. Table 2 lists the dose and usage of each drug.

In the present study, patients enrolled were divided into two treatment regimen groups including experimental group [adopted the modified regimen (4H-Rt2-E-Z-S(Lfx)/4H-Rt2-E)] and control group [standard regimen (2H-R-E-Z-S/6H-R-E or 3H-R-E-Z/6H-R-E)].

All the patients with successful treatment were followed up for at least 56 months, and the bacteriological assessment and the evaluation of chest radiological features were performed at least once each year during the follow-up. All patients were told to visit the doctor whenever they felt unwell. Figure 1 shows the flow chart of the study. The primary endpoints included the successful treatment rate at the end of treatment and the recurrence rate after a 56-month follow-up. The secondary endpoints included adverse reactions and treatment failure rate.

Data collection

A work TB system platform (WTSP) was developed for data collection and management. The information on demography, diagnosis, and treatment was entered in a standard data collection form by two researchers and imported to WTSP after verification. The imaging tests were first reviewed by two physicians and then scanned and uploaded to WTSP. The coordinators contacted the site hospitals for any missing key information, and the monthly online quality assessment and irregular on-site training were conducted during project implementation.

Assessments

At baseline, the patients provided three sputum samples for sputum smear fluorescence microscopy and culture of *Mycobacterium tuberculosis* in liquid medium using the MGIT 960 method (Becton Dickinson Diagnostic Systems, MD, USA) (10). The morning sputum specimens were obtained at least once every month in the intensive phase and once every 2 months in the continuation phase during treatment. The specimens were processed using the sodium hydroxide and N-acetyl-L-cysteine (NaOH/NALC) method. Mycobacterial speciation was performed using a GenoType Mycobacterium CM kit (Hain Life Sciences, Germany). Drug susceptibility testing (DST) was performed on positive cultures using the MGIT 960 system following the WHO guidelines (11). All tests were performed at the TB reference laboratory, and quality control was routinely performed (12). Besides bacteriological assessment, each patient underwent a physical examination, routine blood and urine tests, liver and renal function tests, and other examinations at each visit. The liver function, renal function, and routine blood tests were also performed once every month. The chest radiological features were obtained before the treatment initiation, at the end of the intensive treatment, and at the end of treatment. All images were evaluated by two physicians and radiologists. The patients received a baseline evaluation and a series of regular safety assessments. The adverse events were recorded daily, and immediately reportable and clinically significant abnormal laboratory results were evaluated as appropriate or as per demand when necessary.

During the follow-up duration, the sputum specimens and chest radiological features were obtained at least once annually. All patients were told to visit the doctor with the symptoms of cough and expectoration for more than 2 weeks accompanied by ineffective anti-inflammatory therapy.

Definitions and assessments

The treatment outcomes were defined according to the WHO guidelines (13). "Cure" was defined as cases in which the patient completed the treatment according to the program and provided two consecutive negative sputum smear results, including one at the end of the treatment. "Completed treatment" was defined as cases in which the patient had completed the treatment according to the program protocol but did not meet the definition for cure because of the lack of bacteriological results. The "deceased" category included patients who died for any reason during treatment. "Treatment failure" included patients who were sputum smear-positive in the fifth month or later during treatment. "Defaulted" was defined as TB treatment interrupted for two consecutive months or more. "Transfer out" was defined as patients who were transferred to another recording and reporting unit with unknown treatment outcomes. Additionally, the cured and completed treatment categories were classified as "successful treatment," whereas the others were classified as "unfavorable treatment outcome." The term TB recurrence used throughout this study denotes a recorded re-diagnosis of TB (as either sputum smear-positive or sputum smear-negative) after "successful" treatment. "Recurrence" was defined as sputum bacteria (smear or culture) positive again during follow-up after successful retreatment. Meanwhile, chest x-ray (or chest computed tomography scanning) showed new lesions or enlarged original lesions, excluding other lung diseases. The time to recurrence was defined as the time between the documented end date of the treatment and the date of re-diagnosis of active TB.

TABLE 2 Characteristics of the enrolled patients.

Characteristic	Experimental group *N* (%)	Control group *N* (%)	*P* value
Sex			
Male	189 (77.5)	108 (78.8)	0.756
Female	55 (22.5)	29 (21.2)	
Age, year			
18–39	94 (38.5)	49 (35.8)	0.066
40–59	113 (46.3)	77 (56.2)	
≥60	37 (15.2)	11 (8.0)	
BMI, kg/m^2			0.1157
<18.5	93 (38.1)	40 (29.2)	
18.5–23.9	140 (57.4)	86 (62.8)	
≥24	11 (4.5)	11 (8.0)	
Retreatment types			0.236
Relapse	147 (60.3)	90 (65.7)	
Initial treatment failure	32 (13.1)	21 (15.3)	
Unreasonable or irregular antituberculosis treatment for more than 1 month	65 (26.6)	26 (19.0)	
Background regimen			
INH (H)* 0.3 g/day	0	137 (100)	
0.4 g/day	244 (100)	0	
Rft (Rt), 0.6 g × 2/week	244 (100)	0	
RIF (R), 0.45–0.6 g/day	0	137 (100)	
EMB (E), 0.75 g/day	244 (100)	137 (100)	
PZA (Z), 1.5 g/day	244 (100)	137 (100)	
Sm (S), 0.75g/day	118 (48.4)	83 (60.6)	
Lfx (F), 0.6g/day	136 (55.7)	0	

Modified regimen, 4H-L_2-E-Z-S(F)/4H-L_2-E. Standard regimen, 2H-R-E-Z-S/6H-R-E or 3H-R-E-Z/6H-R-E. *Based on the body weight (BW), 0.3 g/day (BW <50 kg), and 0.4 g/day (BW ≥50 kg).

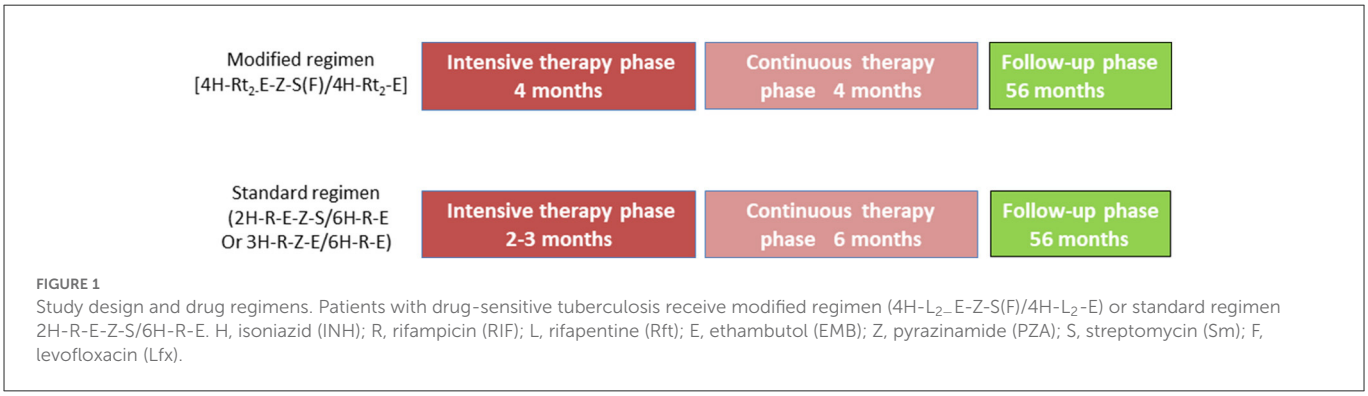

FIGURE 1
Study design and drug regimens. Patients with drug-sensitive tuberculosis receive modified regimen (4H-L_2-E-Z-S(F)/4H-L_2-E) or standard regimen 2H-R-E-Z-S/6H-R-E. H, isoniazid (INH); R, rifampicin (RIF); L, rifapentine (Rft); E, ethambutol (EMB); Z, pyrazinamide (PZA); S, streptomycin (Sm); F, levofloxacin (Lfx).

Statistical analysis

Continuous and categorical variables were presented as medians (interquartile ranges) and percentages (%), respectively. The chi-square test was used for categorical data including demographic and clinical characteristics, and Fisher's exact test was used when the chi-square test was not applicable.

We used the log-rank test to compare the survival curves of time to recurrence after successful treatment in different treatment groups. Further, we used a multivariate Cox regression model to identify the potential risk factors for recurrence after successful treatment with proportional hazards assumptions tested for all variables. Hazard ratios and 95% confidence intervals (CIs) were calculated to demonstrate the risk for recurrence in relation to

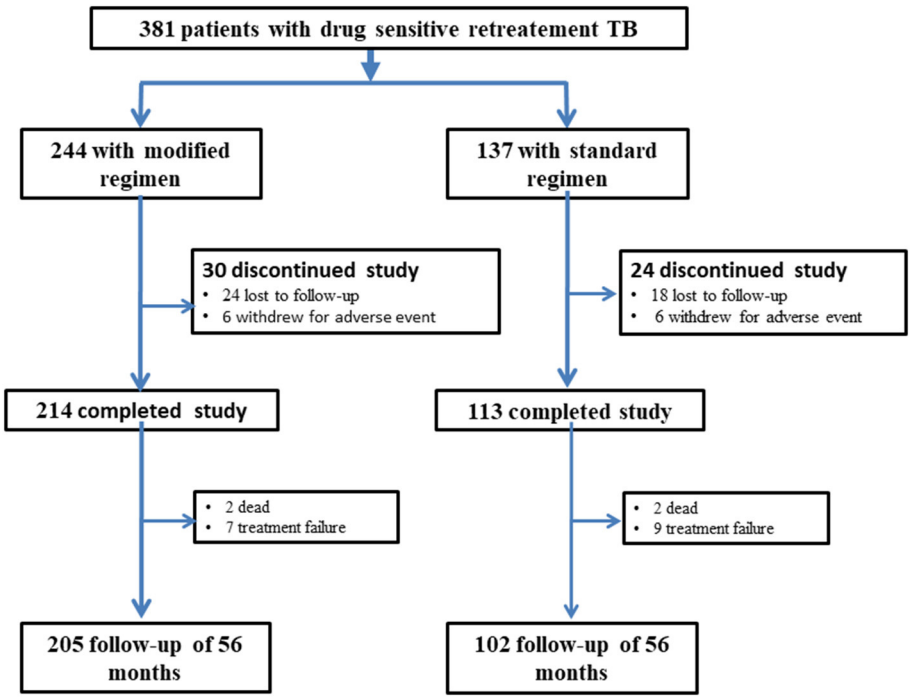

FIGURE 2
Flow chart of TB subjects enrolled in the study.

different treatment regimens. The statistical analysis was conducted using R 3.3.0, and a P value < 0.05 indicated a statistically significant difference.

Results

Study cohort and characteristics of participants

Finally, 381 patients were included with 244 and 137 in the experimental and control regimen groups, respectively (Figure 2). No significant differences were found in the demographic or baseline clinical characteristics between the two groups (Table 2).

Treatment outcomes

The treatment success rate was 84.0% (205/244) in the experimental group, which was significantly higher than control group [74.5% (102/137), $P = 0.024$] (Figure 3). The cure and treatment completion rates were 71.7% (175/244) and 12.3% (30/244) in the experimental group, respectively, however, which were 67.2% (92/137) and 7.3% (10/137) in the control group, respectively. The sputum-negative conversion rate at the end of 2-month after treatment was 81.7% (183/224) in the experimental group and 78.4% (69/88) in the control group ($\chi^2 = 0.44$, $P = 0.507$). There were two deaths because of non-tuberculosis causes in each group. The number of treatment failure cases was seven and nine in the experimental group and control groups, respectively ($\chi^2 = 2.986$, $P = 0.084$) (Figure 3).

Adverse reactions

In the experimental group, 63 patients (25.8%) reported 73 adverse reactions, which mainly manifested as gastrointestinal reactions, joint muscle pain, dizziness, tinnitus, and liver function injury (Table 3). Of these, six patients had serious adverse reactions and withdrew from the group, including one patient with drug-induced liver injury, two patients with drug allergy, one patient with headache and tinnitus, one patient with an influenza-like reaction, and one patient with a gastrointestinal reaction.

In the control group, 29 patients (21.2%) reported 37 adverse reactions, which mainly manifested as gastrointestinal reactions, joint pain, dizziness, tinnitus, and skin reactions. Of these, six patients withdrew from the study because of severe adverse reactions involving severe liver injury. No significant difference was found in adverse reactions between the experimental and control groups ($\chi^2 = 1.037$, $P = 0.309$). Overall, the number of serious adverse reactions, types of reactions, and number of patients with reactions (not including death) were similar between the two study groups during treatment periods.

Follow-up

A total of 307 patients completed the 56-month follow-up, including 205 with the modified regimen and 102 with the standard regimen. Among these, 10 (3.3%) relapsed, including 3 in the experimental group and 7 in the control group (1.5% vs. 6.9%; $P = 0.035$), as shown in Table 4 and Figure 4. Table 4 showed the results of the Cox proportional hazards model for the factors associated with recurrent retreated PTB. The multivariable analysis showed that

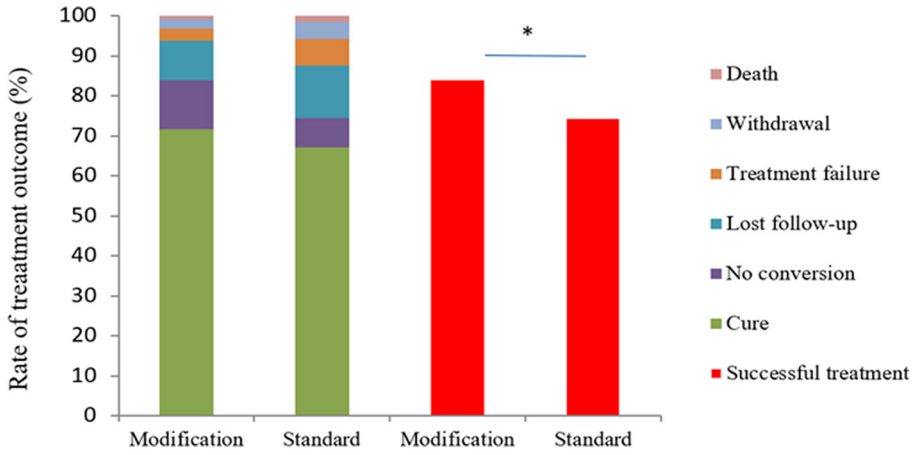

FIGURE 3
Study outcomes after 8 months according to the protocol-defined analysis and analysis based on the WHO definition. The figure shows study outcomes in the modified intention-to-treat population based on the WHO definitions with respect to study data after 8 months. Patients in the modified regimen group had a higher rate of successful treatment (cure plus no conversion) compared with those in the standard regimen group ($\chi^2 = 5.128$, $P = 0.024$). *The value of $P < 0.05$ indicate statistical significance.

TABLE 3 Adverse reactions in the studied population*.

Group	Abnormal liver function	Headache and tinnitus	Joint pain	Gastrointestinal reactions	Skin reaction and others	Total
Experimental group	9 (3.7)	16 (6.6)	17 (7.0)	15 (6.1)	16 (6.6)	73 (29.9)
Control group	8 (5.8)	6 (4.4)	5 (3.6)	8 (5.8)	10 (7.3)	37 (27.0)
P	0.329	0.382	0.183	0.904	0.783	0.547

*No significant differences were found between the two groups in any category, as calculated using Fisher's exact test in a *post hoc* analysis.

TABLE 4 Factors associated with recurrence after successful treatment.

Characteristic	HR (95% CI)	*p*
Regimen = Modified regimen	0.19(0.04–0.77)	**0.02**
Age group = 40–59 years[a]	1.5 (0.29–7.81)	0.6
Age group ≥60 years[a]	5.09 (0.74–34.9)	0.1
BMI < 18.5[b]	1.85 (0.37–9.21)	0.5
BMI≥24 [b]	3.38 (0.29–39.8)	0.3
With any comorbidity	2.15 (0.40–11.5)	0.4
Sex = Female	10.221 (0.62–13.6)	>0.9

P-value ≤ 0.05 indicated a statistically significant difference and is presented in boldface in the table. BMI, Body mass index; HR, hazard ratio. [a]HRs in comparison with the 18–39 age group. [b]HRs in comparison with the 18.5–23.9 BMI group.

the modified regimen was associated with a lower risk of recurrence (hazard ratios, 0.19; 95% CI, 0.04–0.77) compared with the standard treatment regimen, after adjusting for age, sex, body mass index (BMI), and comorbidity.

Discussion

The present study showed that the treatment success rate was higher in the experimental group than control group. Moreover, the overall recurrence rate was 3.3% at 56 months after successful treatment, and 1.5 and 6.9% in the experimental and control groups, respectively. We also found that the use of the modified regimen was associated with a lower recurrence rate. The results might provide a reference for clinicians in treating previous PTB patients.

The retreatment PTB is one of the key reasons for the origin of MDR/rifampicin-resistant TB and has imposed great challenges for the TB epidemic, and need to be more attention. Jones-López et al. (14) found that the recommended regimen (category II) yielded unacceptably low treatment response rates in previously treated patients with TB, particularly in subgroups with multidrug-resistant TB and human immunodeficiency virus and was associated with poor long-term outcomes in Kampala, Uganda. In China, the smear conversion of the patients with retreatment PTB who adopted the standard retreatment regimen was only 71.11% as reported 10 years ago (7). In this study, the treatment success rate of the modified regimen (84.0%) increased by nearly 10% and the recurrence rate after the 56-month follow-up decreased by 5.4% compared with the control group.

In this study, the dose of INH was modified in the experimental group based on the patient's body mass instead of the fixed dose of INH (0.3 g/day) in the standard regimen group because the mean body weight of inpatients with PTB increased in decades (15). Appropriately increasing the dose of INH benefits the treatment effect, which has many explanations. First, exposure to lower therapeutic levels of anti-TB drugs is likely to increase drug tolerance and cause the proliferation of resistant strains of *M. tuberculosis* and treatment failure (16, 17), which might be attributed to the increase in the thickness of the cell wall, efflux pump activity, and gene mutations (18). Moreover, the clinical isolates with special genotypes, such as MANU2, has the minimum inhibitory concentration (MIC) to INH between sensitivity and resistance (19). Furthermore, some patients

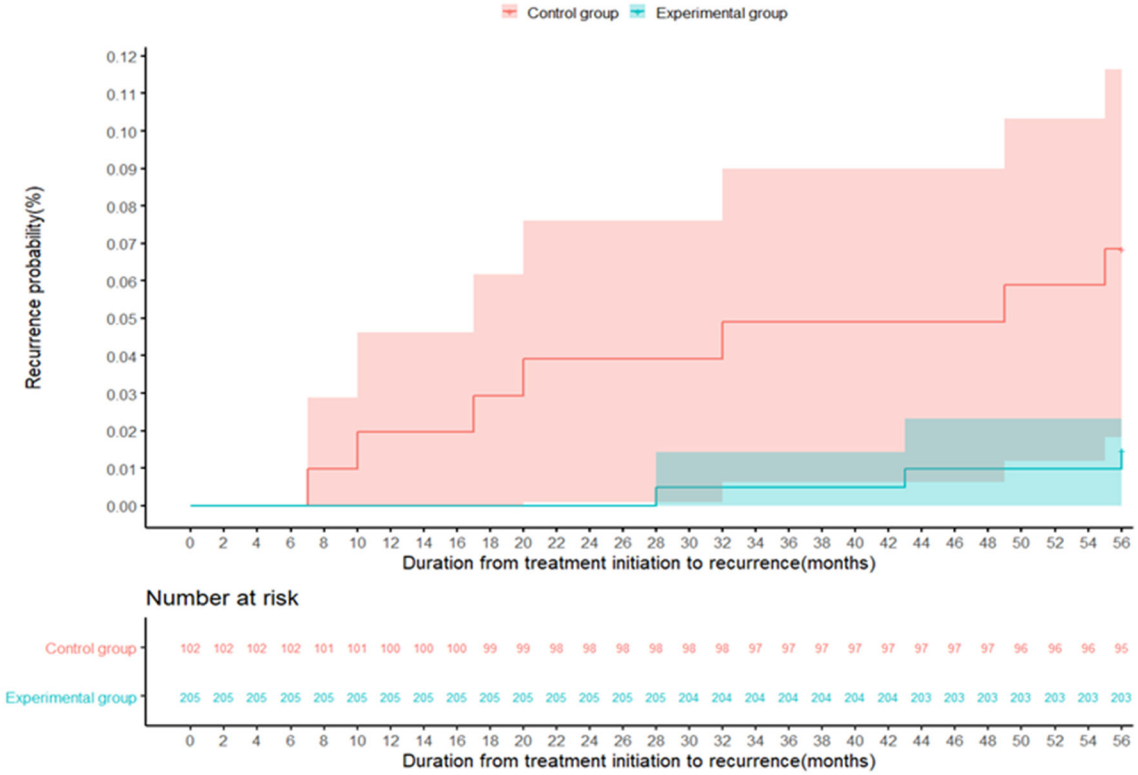

FIGURE 4
The recurrence probability in the control group (red) and the experimental group (green) during the follow-up period of 56 months. The recurrence of tuberculosis is defined as either a positive M. tb culture or a positive sputum smear fluorescence microscopy during the follow-up.

taking INH 0.3 g/day had MIC lower than the target concentration range (20).

Compared with RIF, Rft has a longer half-life and lower effective bacteriostatic concentration (21). Previous studies showed that the Rft doses of 0.6 g/day (22) and 20 mg/(kg · day) (23, 24) showed a trend toward greater efficacy based on the occurrence of culture conversion and did not increase the incidence of adverse events. In this study, we chose Rft at a dose of 0.6 g/day twice a week instead of RIF. Additionally, S had ototoxicity, nephrotoxicity, inconvenient administration, and a high drug resistance rate, while Lfx was much easier to be administered orally with less toxicity to the liver and kidney.

Moreover, a previous study demonstrated that smear positivity after 2 months of treatment for the intensive phase was independently associated with recurrent PTB (2). Nearly 30% of patients were still smear positive at the end of the intensive phase (7). Therefore, in this study, the intensive treatment period was extended from 2–3 months to 4 months to maximize the elimination of rapidly proliferating and most slowly proliferating bacteria.

Recurrent PTB remains a problem in successfully treated patients with sputum smear-positive PTB (2). This study reported a recurrence rate of 1.5% with the modified regimen after the 56-month follow-up, which was much lower than that with the standard regimen. It was also lower than the values reported by studies from India (25) and 12% after a 5-year follow-up in Brazil (26). Notably, the use of the modified regimen reduced the risk of recurrence compared with the standard regimen, which was in line with previous findings (27). However, we also observed that sex, age, BMI, and comorbidity were not associated with the reduced risk of occurrence of recurrent PTB among successfully treated patients with retreated PTB. It suggested that we should pay more attention to the follow-up of patients with retreated PTB receiving different regimens after successful treatment.

Data availability statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Chinese Clinical Trial Registry (ChiCTR1800017441) and the Ethics Committee of Beijing Chest Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

QG: conceptualization, investigation, visualization, and writing—original draft. YM and LZ: investigation, visualization, and writing—original draft. LM: investigation. CZ, YC, XHu, SC, FeW, BL, XHa, LS, XWa, YL, SY, WC, QL, LC, CW, BO, FuW, PL, XWu, XX, XL,

HZ, HL, JL, CY, PZ, HC, and CK: patient inclusion, follow-up, and data entry. WS and YL: methodology, software, and formal analysis. ZS, JD, and WG: review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. World Health Organization. Global tuberculosis report 2020. Geneva: World Health Organization (2020).

2. Ruan Q, Yang Q, Sun F, Liu W, Shen Y, Wu J, et al. Recurrent pulmonary tuberculosis after treatment success: a population-based retrospective study in China. *Clin Microbiol Infect.* (2022) 28:684–89. doi: 10.1016/j.cmi.2021.09.022

3. Zhao Y, Xu S, Wang L, Chin DP, Wang S, Jiang G, et al. National survey of drug-resistant tuberculosis in China. *N Engl J Med.* (2012) 366:2161–70. doi: 10.1056/NEJMoa1108789

4. Yoshiyama T, Shrestha B, Maharjan B. Risk of relapse and failure after retreatment with the Category II regimen in Nepal. *Int J Tuberc Lung Dis.* (2010) 14:1418–23.

5. Ige OM, Akindele MO. Five year review of treatment outcome of directly observed therapy (DOT) for re-treatment pulmonary tuberculosis patients in UCH,Ibadan,Nigeria. *Afr J Med Med Sci.* (2011) 40:15–21. doi: 10.13748/j.cnki.issn1007-7693.2018.10.020

6. Ottmani SE, Zignol M, Bencheikh N, Laâsri L, Chaouki N, Mahjour J. Results of cohort analysis by category of tuberculosis retreatment cases in Morocco from 1996 to 2003. *Int J Tuberc Lung Dis.* (2006) 10:1367–72.

7. Zhang L, Shao W, Fang Q, Zhu Y. Curative effect of increasing dose and prolonging the intensive phase in the retreated pulmonary .*Chin J Mod Appl Pharm.* (2018) 35:1521–25.

8. Perumal R, Padayatchi N, Yende-Zuma N, Naidoo A, Govender D, Naidoo K, et al. Moxifloxacin-based regimen for the treatment of recurrent, drug-sensitive pulmonary tuberculosis: an open-label, randomized, controlled trial. *Clin Infect Dis.* (2020) 70:90–8. doi: 10.1093/cid/ciz152

9. World Health Organization. *Guidelines for Treatment of Drug Susceptible Tuberculosis and Patient Care*. Geneva: World Health Organization (2017).

10. Pang Y, Su B, Zheng H, Zhang Z, Ma A, Wang Y, et al. Factors associated with missed detection of mycobacterium tuberculosis by automated BACTEC MGIT 960 system. *Biomed Res Int.* (2016) 2016:5972021. doi: 10.1155/2016/5972021

11. World Health Organization. *Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*. Geneva: World Health Organization (2014).

12. World Health Organization. *Guidelines for Surveillance of Drug Resistance in Tuberculosis*. Geneva: World Health Organization, (2009).

13. World Health Organization. *Treatment of Tuberculosis: Guidelines for National Programs*. Geneva: World Health Organization (2005).

14. Jones-López EC, Ayakaka I, Levin J, Reilly N, Mumbowa F, Dryden-Peterson S, et al. Effectiveness of the standard WHO recommended retreatment regimen (category II) for tuberculosis in Kampala, Uganda: a prospective cohort study. *PLoS Med.* (2011) 8:e1000427. doi: 10.1371/journal.pmed.1000427

15. Chen YQ, Du J, Liu YH, Sun ZG, Ma Y, Shu W, et al. Relationship between the weight of hospitalized patients with pulmonary tuberculosis and the oral dose of isoniazid. *Zhonghua Jie He He Hu Xi Za Zhi.* (2018) 41:529–33. doi: 10.3760/cma.j.issn.1001-0939.2018.07.005

16. Sekaggya-Wiltshire C, von Braun A, Lamorde M, Ledergerber B, Buzibye A, Henning L, et al. Delayed sputum culture conversion in tuberculosis-human immunodeficiency virus-coinfected patients with low isoniazid and rifampicin concentrations. *Clin Infect Dis.* (2018) 67:708–16. doi: 10.1093/cid/ciy179

17. Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. *Clin Infect Dis.* (2012) 55:169–77. doi: 10.1093/cid/cis353

18. Ley SD, de Vos M, Van Rie A, Warren RM. Deciphering within-host microevolution of Mycobacterium tuberculosis through whole-genome sequencing: the phenotypic impact and way forward. *Microbiol Mol Biol Rev.* (2019) 83:e00062–18. doi: 10.1128/MMBR.00062-18

19. Xu Y, Zhang Z, Sun Z. Drug resistance to Mycobacterium tuberculosis: from the traditional Chinese view to modern systems biology. *Crit Rev Microb.* (2015) 41:399–410. doi: 10.3109/1040841X.2013.860948

20. Guo S, Zhu H, Guo X, Wang B, Liu Z, Xu J, et al. Analysis of plasma concentrations of first-line anti-tuberculosis drugs in 909 tuberculosis patients. *Chin J Antituber.* (2018) 40:744–49. doi: 10.3969/j.issn.1000-6621.2018.07.014

21. Keung AC, Eller MG, McKenzie KA, Weir SJ. Single and multiple dose pharmacokinetics of rifapentine in man: part II. *Int J Tuberc Lung Dis.* (1999) 3:437–44.

22. Dawson R, Narunsky K, Carman D, Gupte N, Whitelaw A, Efron A, et al. Two-stage activity-safety study of daily rifapentine during intensive phase treatment of pulmonary tuberculosis. *Int J Tuberc Lung Dis.* (2015) 19:780–86. doi: 10.5588/ijtld.14.0868

23. Dorman SE, Savic RM. Goldberg S, Stout JE, Schluger N, Muzanyi G, et al. Daily rifapentine for treatment of pulmonary tuberculosis A randomized, dose-ranging trial. *Am J Respir Crit Care Med.* (2015) 191:333–43. doi: 10.1164/rccm.201410-1843OC

24. Dooley KE, Bliven-Sizemore EE, Weiner M, Lu Y, Nuermberger EL, Hubbard WC, et al. Safety and phamacokinetics of escalating daily doses of the antituberculosis drug rifapentine in healthy volunteers. *Clin Phamacol Ther.* (2012) 91:881–88. doi: 10.1038/clpt.2011.323

25. Narayanan S, Swaminathan S, Supply P. Impact of HIV infection on the recurrence of tuberculosis in South India. *J Infect Dis.* (2010) 201:691–703 doi: 10.1086/650528

26. Picon PD, Rizzon CF, Bassanesi SL, Silva LC, Della Giustina L (2011). Retreatment of tuberculosis patients in the city of Porto Alegre, Brazil: outcomes. *J Bras Pneumol.* 37:504–11 doi: 10.1590/S1806-37132011000400013

27. Tabarsi P, Chitsaz E, Tabatabaei V. Revised category II regimen as an alternative strategy for retreatment of category I regimen failure and irregular treatment cases. *Am J Ther.* (2011) 18:343. doi: 10.1097/MJT.0b013e3181dd60ec

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Effects of smoking on the severity and transmission of pulmonary tuberculosis: A hospital-based case control study

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Introduction: There is a high incidence of both smoking and tuberculosis (TB) in China. This study examined the risk factors for severe pulmonary TB (PTB) and positive tubercle bacilli in sputum.

Methods: We conducted a retrospective case-control study in a tertiary hospital from January 2017 to December 2018 ($n = 917$). The clinical and biological characteristics of patients were collected, and univariable and multivariable logistic regression analyses were performed to assess the factors associated with smoking in terms of the severity and transmission of PTB.

Results: Positive tubercle bacilli in sputum and severe PTB were much higher in smoking patients. Together with nutrition status, heavy smoking exhibited a 284% greater risk in severe PTB. Positive tubercle bacilli in sputum was significantly associated with hypoproteinemia and smoking regardless of the status, duration, and degree.

Conclusion: Because cigarette smoking was strongly and inversely associated with hypoproteinemia, we conclude that smoking plays a critical role in the severity and transmission of PTB. Smoking cessation interventions should be employed to prevent severe PTB and decrease the transmission of PTB.

KEYWORDS

pulmonary tuberculosis, smoking, severity of the disease, positive tubercle bacilli, transmission

Background

Although efforts are underway worldwide to end the global tuberculosis (TB) epidemic, TB remains a leading cause of infectious disease, creating a significant public health burden worldwide. As reported by WHO, in 2021, an estimated 10.6 million people were infected with TB, and 1.6 million people died from it (1). Currently, evidence of the impact of smoking on TB is growing (2–4). China, which has a high TB burden, also ranks among the top countries in the world in the number of smokers, particularly among adolescents (5). It has been estimated that if 80% coverage by the WHO-recommended strategy for TB control (directly observed treatment, short-course chemotherapy, or DOTS) is sustained, complete smoking cessation and complete elimination of solid fuel use by 2033 would reduce the projected annual TB incidence by 14–52% (6). Therefore, to achieve the goal of ending TB by 2035, it is important to understand the characteristics of smoking-related TB.

Previous studies of smoking-related TB have largely focused on the risk of developing TB, treatment outcomes, and prognoses (2, 7). Amere et al. (2) estimated the incidence and mortality of TB among smoking patients in 32 high-TB-burden countries, reporting 17.6% (95%CI = 8.4–21.4) TB cases and 15.2% (95%CI = 15.9–37.6) TB mortality attributable to smoking. Burusie et al. (8) systematically reviewed 22 studies and found that smoking significantly increased the likelihood of poor TB outcomes by 51% (OR = 1.51, 95%CI = 1.3–1.70, I-square = 75.1%). These findings are notable, but to the best of our knowledge, there is little information available about the severity and transmission of the disease in smoking patients with PTB, particularly in China.

This study investigated the severity of the disease and level of disease transmission for PTB in smoking patients. Furthermore, to explore the risk factors for severe PTB and PTB transmission, we analyzed smoking and associated factors in terms of the degree, duration, and status of smoking.

Methods

Study design and populations

A case-control study was conducted among patients with PTB at a tertiary hospital between January 2017 and December 2018 in southwest China. All patients over the age of 18 who visited our hospital with a diagnosis of PTB were invited to participate. Those who experienced multiple organ dysfunction syndrome (MODS), septic shock, or respiratory failure not caused by PTB were excluded; in addition, those who were unable to obtain intact information data were also excluded (Figure 1). Data on all of the enrolled patients were collected using the hospital's medical record systems and telephone interviews. The survey was designed to investigate smoking among patients with PTB as well as the risk factors for the severity and transmission of PTB. No personally identifying details were collected. Consent was obtained during the telephone interviews, and only participants who provided consent entered the survey.

Data collection, definitions, and criteria

Demographic information, clinical data, and self-reported cigarette smoking status were collected using the medical records system and telephone interviews. Smokers were defined as individuals who smoked regularly or had done so for at least 6 months during their lives. Non-smokers were defined as patients who neither smoked more than 100 cigarettes in their lifetime nor were currently smoking. Smokers were divided into current smokers and ex-smokers (past smokers). To prevent misclassification of patients who temporarily quit smoking at the onset of symptoms, the definition of current smoker was expanded to include past smokers who had stopped smoking after the onset of PTB-associated symptoms. To further clarify the influence of smoking on PTB, the extent of smoking was evaluated. Heavy smoking and light smoking

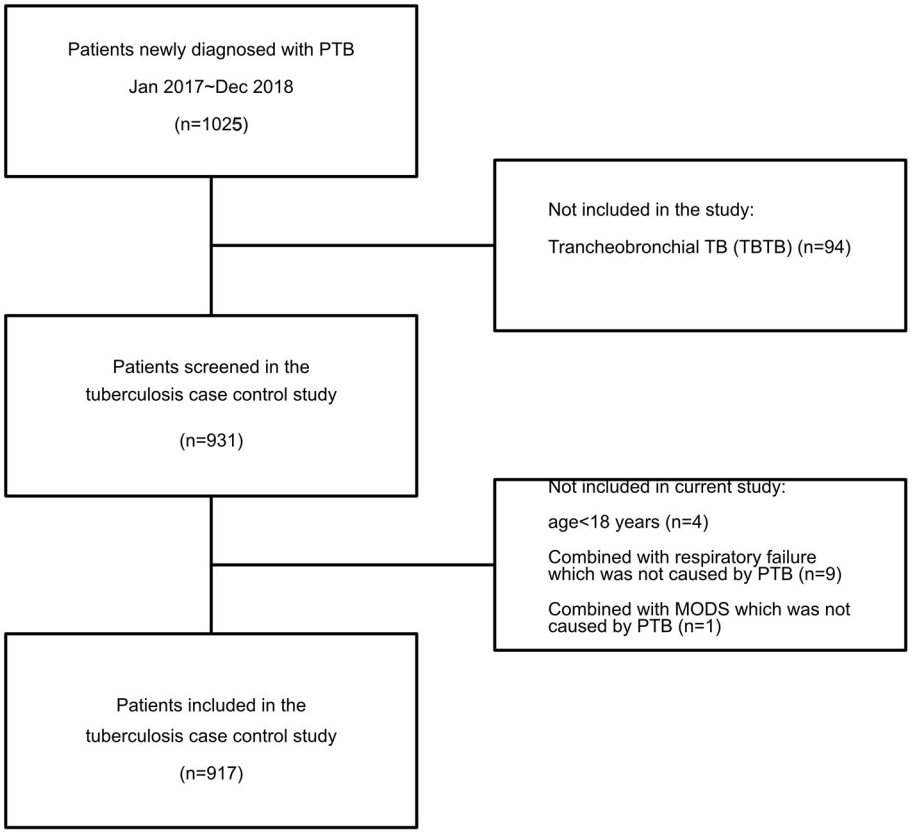

FIGURE 1
Flow diagram.

TABLE 1 TBscore and adjusted TBscore.

Variables	TBscore	Adjusted TBscore
Symptoms		
Cough	1	1
Dyspnea	1	1
Chest pain	1	1
Night sweats	1	1
Signs		
Anemia	1	1
Pulse >90 beats/min	1	1
Positive finding at lung auscultation	1	1
Temperature >37°C	1	1
BMI <18	1	1
BMI <16	1	1
MUAC <220 mm	1	–
MUAC <200 mm	1	–
Hypoproteinemia	–	1
Bilateral lung involvement	–	1
Total	13	13

BMI, body mass index; MUAC, mid upper arm circumference.

were defined following a previous study (9) such that heavy smoking was 20 or more cigarettes per day, or more than 20 pack-years, and light smoking was <20 cigarettes per day, or <20 pack-years.

The Bandim TBscore is an easily implemented self-rated instrument for evaluating severity in patients with PTB (10). It assesses five symptoms (cough, hemoptysis, dyspnea, chest pain, and night sweats) and six signs [pale inferior conjunctivae, pulse >90 per minute, positive findings at lung auscultation, temperature >37°C (axillary), body mass index (BMI) <18/<16, and mid-upper-arm circumference (MUAC) <220 mm/<200 mm]. Each variable contributes one point. BMI and MUAC contribute an extra point each, if BMI <16 and MUAC <200 mm; hence, the maximum score is 13 (Table 1). A score of 8 or greater is defined as severe TB, which is associated with a strong prognostic capacity of mortality. As MUAC data could not be obtained, we adjusted the self-rated health score to assess disease severity. As in a previous study (11), because hypoproteinemia and bilateral lung involvement have been shown to be associated with increased mortality in PTB (12, 13), we adjusted the Bandim TBscore system for hypoproteinemia, with bilateral lung involvement in CT or X-ray instead of MUAC (Table 1).

Statistical analysis

All data were analyzed using R software version 3.6.2 (R Foundation for Statistical Computing). Quantitative variables were expressed as means/medians and standard deviations. Qualitative variables were summarized as counts and proportions for each category. Smokers' and non-smokers' characteristics were compared using Pearson's χ^2 test. To further evaluate the factors (mainly focused on smoking) that influence the severity of PTB and positive tubercle bacilli in sputum, univariable and multi-variable logistic regression analyses were conducted. The variables for inclusion were carefully chosen, using statistically significant associations in univariate analysis. The results are presented as *P*-values, odds ratios (ORs), and 95% confidence interval (CIs). A *P*-value of <0.05 was considered statistically significant.

TABLE 2 Characteristics of patients and factors related to smoking at the time of TB diagnosis.

	Non-smoking ($N = 470$)	Smoking ($N = 447$)	*P*-value
Sex			
Female	319 (67.9%)	8 (1.8%)	<0.001
Male	151 (32.1%)	439 (98.2%)	
Age (years)			
Mean (SD)	45.2 (19.9)	58.1 (15.4)	<0.001
BMI (kg/m^2)			
Mean (SD)	20.6 (3.05)	20.7 (15.4)	0.658
Hypoproteinemia			
No	321 (68.3%)	231 (51.7%)	<0.001
Yes	149 (31.7%)	216 (48.5%)	
BTS			
Mean (SD)	4.26 (1.91)	4.91 (1.96)	<0.001
Multi-system TB			
No	437 (93%)	418 (93.5%)	0.849
Yes	33 (7%)	29 (6.5%)	
Tubercle bacilli			
Negative	290 (61.7%)	203 (45.4%)	<0.001
Positive	180 (38.3%)	244 (54.6%)	
BTS level			
<8	446 (94.9%)	403 (90.2%)	0.009.4
≥8	24 (5.6%)	44 (9.8%)	
CCI			
0	338 (71.9%)	229 (51.2%)	<0.001
1	94 (20%)	157 (35.1%)	
2	30 (6.4%)	39 (8.7%)	
≥3	8 (1.7%)	22 (4.9%)	

BMI, body mass index; TB, tuberculosis; BTS, Bandim TBscore; CCI, Charlson comorbidity index.

Ethical considerations

This study adhered to the tenets of the Declaration of Helsinki, and the ethics committee of the First Affiliated Hospital of Chongqing Medical University approved the study (No. 2020-140). All patient records were anonymized before analysis. All subjects received electronic information before inclusion and provided oral consent in the telephone interview.

Results

Characteristics of participants

In all, 931 inpatients at the First Affiliated Hospital of Chongqing Medical University suffering from PTB (between January 2017 and December 2018) were screened, of which 14 were excluded because of age younger than 18 years or having either MODS or dysfunction respiratory failure not caused by PTB. As shown in Table 2, there were 447 smokers among the enrolled patients, of whom 98.2% were male. The mean age of smokers was much higher than that of the non-smokers (58.1 ± 15.4 vs. 45.2 ± 19.9, $P < 0.001$). In 64.2% of the smokers, smoking duration was more than 20 years, and 62.2% of smokers had not given up smoking (Supplementary Table 1). Although the BMI was similar between smokers and non-smokers (20.6 ± 3.05 vs. 20.7 ± 3.0), the proportion of hypoproteinemia was

TABLE 3 Odds ratios (ORs) for severe PTB in smoking and associated factors.

Variables	Univariable		Multivariable	
	OR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Age (years)				
18–39	Reference			
40–59	0.78 (0.4–1.53)	0.467	0.62 (0.28–1.42)	0.261
60–69	1.67 (0.88–3.18)	0.118	0.93 (0.42–2.07)	0.862
>70	0.88 (0.4–1.95)	0.761	0.31 (0.12–0.79)	0.014
Sex				
Female	Reference			
Male	1.73 (0.98–3.04)	0.059		
BMI				
Normal	Reference			
Underweight	6.32 (3.63–11)	<0.001	5.81 (3.17–10.63)	<0.001
Overweight	0.81 (0.24–2.77)	0.736	1.13 (0.31–4.07)	0.856
Obese	0.99 (0.29–3.41)	0.99	1.24 (0.34–4.52)	0.744
Hypoproteinemia				
No	Reference			
Yes	18.62 (7.96–43.55)	<0.001	17.27 (7.17–41.62)	<0.001
CCI				
0	Reference			
1	0.94 (0.53–1.67)	0.836		
2	0.75 (0.26–2.16)	0.593		
≥3	1.35 (0.39–4.64)	0.63		
Smoking status				
Ex-smoker	Reference			
Current smoker	2.03 (1.21–3.40)	0.007	2.4 (1–5.8)	0.051
Smoking degree				
Non-smoker	Reference			
Light smoker	1.41 (0.75–2.64)	0.29	1.66 (0.63–4.36)	0.306
Heavy smoker	2.93 (1.63–5.24)	<0.001	3.84 (1.45–10.2)	0.007
Smoking duration				
Non-smoker	Reference			
<10 years	2.44 (1.05–5.67)	0.039	2.86 (0.92–8.9)	0.071
10–20 years	1.08 (0.40–2.91)	0.878	1.49 (0.42–5.28)	0.536
>20 years	2.25 (1.29–3.92)	0.004	2.6 (0.99–6.84)	0.052

BMI, body mass index; PTB, pulmonary tuberculosis; OR, odds ratios; AOR, adjusted odds ratios; CCI, Charlson comorbidity index. Except for independent variables, the AOR for multivariable analyses was adjusted for factors that did not enter the model.

much higher in the smoking PTB patients ($P < 0.001$). In addition to the higher positive tubercle bacilli in smokers, the smoking patients also had a higher disease severity (TBscore >8) and a higher Charlson comorbidity index (CCI). However, we did not find a significant difference in multi-system TB between smokers and non-smokers.

Smoking and associated factors in severe PTB

Table 3 presents the ORs for severe PTB. The risk factors were age, sex, BMI, hypoproteinemia, smoking, and CCI. To further explore the influence of smoking on severe PTB, the smokers were classified into different types based on smoking status, degree, and duration. As the *P*-value of those variables was more than 0.05 in univariable analysis, the analyses were adjusted for the factors that did not enter the model. Age more than 70 years, BMI in the underweight group, and hypoproteinemia were associated with severe PTB. Although smoking status and smoking duration did not exhibit a relationship with severe PTB, heavy smokers exhibited an increased rate of severe PTB, 284% of that for other patients (AOR = 3.84; 95% CI = 1.45–10.2, $P = 0.007$).

Smoking and associated factors in positive tubercle bacilli

The transmission of PTB was evaluated using positive tubercle bacilli. According to multivariable analysis (Table 4), the following factors were independently associated with PTB transmission: BMI in the underweight group (AOR = 1.58; 95% CI = 1.12–2.22), hypoproteinemia (AOR = 1.67; 95%CI = 1.25–2.22), CCI with 1 score (AOR = 1.79; 95% CI = 1.3–2.47) and more than 3 scores (AOR = 4.28, 95%CI = 1.77–10.35), smoking status in current smokers (AOR = 1.64; 95% CI = 1.11–2.42), smoking degree in heavy smokers (AOR = 2.11; 95% CI = 1.33–3.33), and smoking duration of more than 20 years (AOR = 1.82; 95% CI = 1.18–2.82) or <10 years (AOR = 1.85; 95% CI = 1.03–3.3).

Discussion

PTB remains the respiratory infectious disease with the highest mortality rate worldwide. Countermeasures are needed to control the source of infection and reduce mortality. It is very important to know the risk factors and intervene in advance. In this study, we found that smoking and nutrition status were independent risk factors for severe PTB and bacterial-positive TB.

In line with a previous study (13), more than 95% (439/447) of smokers were male in our study, whereas only 2% (8/325) of females were smokers. The sex difference in smoking is consistent with smoking prevalence in China (5, 14): men must stop using tobacco.

Hypoproteinemia is an independent risk factor for various diseases (15–17). Although evidence already exists for its effect on treatment outcomes (18), we further evaluated the relationship between hypoproteinemia and PTB in terms of severity and positive tubercle bacilli. Hypoproteinemia was not only associated with positive tubercle bacilli but could also be seen as an independent risk factor for severe PTB. Previous studies have shown that cigarette smoking is strongly and inversely associated with serum concentrations of albumin (19). We observed the same phenomenon in our study, with a higher proportion of hypoproteinemia in patients with a smoking history. Conducting tobacco cessation education should be considered equally as important as nutritional support.

Severe PTB is associated with higher mortality. It is essential to explore risk factors for critically ill patients. However, no diagnostic criteria have been provided for severe PTB. The Bandim TBscore system, first developed by Wejse in 2009, is an effective and convenient scoring instrument for the assessment of PTB disease severity. Thus, we evaluated the severity of PTB with an adjusted Bandim TBscore and found that the proportion of severe PTB was higher in smokers. By further evaluating the influence of smoking status, degree, and duration in regard to PTB severity, we found that heavy smoking was an independent factor for severe PTB. This result is in concordance with a previous study (2), in particular, that smoking contributes to PTB incidence and mortality in high-TB-burden countries. This provided a hint that to decrease the incidence of severe PTB, smoking, particularly heavy smoking, should be avoided.

TB is an infectious disease that claims many human lives. Preventing person-to-person spread is central to halting the TB epidemic. The transmission of TB requires the expulsion of viable tubercle bacilli from an active source case (20), so it is critical to explore the risk factors for positive tubercle bacilli in sputum. It has been reported that higher sputum mycobacterial loads are found in smoking patients (21). This was the cased in our study. Among other risk factors, we found that hypoproteinemia, BMI at underweight level, CCI, and smoking were strongly associated with positive tubercle bacilli in sputum. This connection varies with status, degree, and duration of smoking. As smoking is a risk factor for comorbidites (e.g., chronic pulmonary disease, heart disease, diabetes et al.) and abnormal nutrition status (e.g., hypoproteinemia) (22–25), we conclude that smoking plays a key role in positive tubercle bacilli in sputum.

Previous studies have also reported the negative influence of comorbidities on clinical outcomes in PTB (26). The CCI was developed as a comorbidity-based indicator for estimating the risk of death and classifying the severity of a patient's clinical condition (27). A previous study reported that a CCI score >3 was an independent risk factor for all-cause death in PTB patients (28). Min et al. (29) also found that LIBT patients with a CCI score of 3 or higher were less likely to complete its treatment, indicating a poorer prognosis. However, the role of CCI in the severity and spread of TB has not been established. In this study, we investigated the use of this scoring system in evaluating the severity and transmission of PTB, with a particular focus on smoking populations. The results revealed that the CCI score was significantly higher in the smoking group than in the non-smoking group ($P < 0.001$). According to further evaluation with multivariable logistic analysis, CCI was not associated with the severity of PTB, while there was a relationship between CCI and positive TB in sputum. Because the sample size with a CCI score of more than 2 was small in this study, more evidence is needed to confirm the link between CCI and the disease severity/transmission of PTB.

This study had some limitations, such as the retrospective data collection. Due to the properties of the case-control study, we were unable to identify the timepoint for mycobacteria

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TABLE 4 Odds ratios (ORs) for transmission of PTB in smoking and associated factors.

Variables	Univariable		Multivariable	
	OR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Age (years)				
18–39	Reference			
40–59	1.24 (0.89–1.73)	0.212	1.01 (0.7–1.45)	0.964
60–69	1.96 (1.34–2.85)	<0.001	1.39 (0.92–2.11)	0.115
>70	1.9 (1.27–2.83)	0.002	1.22 (0.78–1.9)	0.384
Sex				
Female	Reference			
Male	1.63 (1.24–2.14)	<0.001	1.34 (1–1.8)	0.05
BMI				
Normal	Reference			
Underweight	1.68 (1.21–2.32)	0.002	1.58 (1.12–2.22)	0.009
Overweight	0.96 (0.62–1.49)	0.865	0.97 (0.61–1.54)	0.909
Obese	0.75 (0.46–1.22)	0.25	0.68 (0.41–1.14)	0.144
Hypoproteinemia				
No	Reference			
Yes	2.1 (1.6–2.75)	<0.001	1.67 (1.25–2.22)	<0.001
CCI				
0	Reference			
1	1.98 (1.47–2.68)	<0.001	1.79 (1.3–2.47)	<0.001
2	1.22 (0.74–2.02)	0.435	1.06 (0.62–1.68)	0.838
≥3	4.92 (2.08–11.66)	<0.001	4.28 (1.8–10.35)	0.001
Smoking status				
Ex-smoker	Reference			
Current smoker	1.94 (1.49–2.52)	<0.001	1.64 (1.11–2.42)	0.013
Smoking degree				
Non-smoker	Reference			
Light smoker	1.64 (1.2–2.23)	0.002	1.39 (0.92–2.12)	0.122
Heavy smoker	2.44 (1.73–3.44)	<0.001	2.11 (1.33–3.33)	0.001
Smoking duration				
Non-smoker	Reference			
<10 years	1.86 (1.12–3.1)	0.016	1.85 (1.03–3.3)	0.039
10–20 years	1.21 (0.77–1.9)	0.415	1.11 (0.64–1.92)	0.715
> 20 years	2.27 (1.69–3.07)	<0.001	1.82 (1.18–2.82)	0.007

BMI, body mass index; PTB, pulmonary tuberculosis; OR, odds ratios; AOR, adjusted odds ratios; CCI, Charlson comorbidity index; adjusted for age and sex. Except for the independent variable, the AOR or multivariable analyses was adjusted for factors that did not enter the model.

in sputum conversion to negative and the prognosis of PTB smokers. In addition, the sample size, particularly for smoking subgroups such as status, duration, and degree, was insufficient. To further clarify the important role that smoking plays in PTB, the sample size should be enlarged. Moreover, we confined our study to a single center. The results may be affected by the nature and policies of our hospital. To understand the role of various states of smoking in PTB development, the logical next step would be to perform a study of this type in Chongqing or the southwestern region of China in a multicenter prospective study.

Conclusion

Our findings indicate risk factors for severe PTB and PTB transmission. Smoking plays a critical role in the severity and transmission of PTB. Smoking cessation interventions should be

offered to PTB patients. Further trials are needed to investigate the possible mechanisms and possible benefits of early smoking cessation in the improvement of the clinical prognosis of PTB patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by committee of the First Affiliated Hospital of Chongqing Medical University (No. 2020-140). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YF analyzed all of the data and wrote the manuscript. YF, HC, and YX reviewed the literature. HS, YX, and GY collected the raw data. YY assisted in the statistical analysis. RG, JJ, and PW edited and supervised the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1017967/full#supplementary-material

References

1. Chakaya J, Petersen E, Nantanda R, Mungai BN, Migliori GB, Amanullah F, et al. The WHO global tuberculosis 2021 report–not so good news and turning the tide back to End TB. *Int J Infect Dis.* (2022) 22:149–57. doi: 10.1016/j.ijid.2022.03.011

2. Amere GA, Nayak P, Salindri AD, Narayan KMV, Magee MJ. Contribution of smoking to tuberculosis incidence and mortality in high-tuberculosis-burden countries. *Am J Epidemiol.* (2018) 187:1846–55. doi: 10.1093/aje/kwy081

3. Wang EY, Arrazola RA, Mathema B, Ahluwalia IB, Mase SR. The impact of smoking on tuberculosis treatment outcomes: a meta-analysis. *Int J Tuberc Lung Dis.* (2020) 24:170–5. doi: 10.5588/ijtld.19.0002

4. Wang MG, Huang WW, Wang Y, Zhang YX, Zhang MM, Wu SQ, et al. Association between tobacco smoking and drug-resistant tuberculosis. *Infect Drug Resist.* (2018) 11:873–87. doi: 10.2147/IDR.S164596

5. Zhao Y, Di X, Li S, Zeng X, Wang X, Nan Y, et al. Prevalence, frequency, intensity, and location of cigarette use among adolescents in China from 2013–14 to 2019: findings from two repeated cross-sectional studies. *Lancet Reg Health West Pac.* (2022) 27:100549. doi: 10.1016/j.lanwpc.2022.100549

6. Senanayake MGB, Wickramasinghe SI, Samaraweera S, De Silva P, Edirippulige S. Examining the social status, risk factors and lifestyle changes of tuberculosis patients in Sri Lanka during the treatment period: a cross-sectional study. *Multidiscip Respir Med.* (2018) 13:9. doi: 10.1186/s40248-018-0121-z

7. Thomas BE, Thiruvengadam K, Rani S, Kadam D, Ovung S, Sivakumar S, et al. Smoking, alcohol use disorder and tuberculosis treatment outcomes: a dual co-morbidity burden that cannot be ignored. *PLoS ONE.* (2019) 14:e0220507. doi: 10.1371/journal.pone.0220507

8. Burusie A, Enquesilassie F, Addissie A, Dessalegn B, Lamaro T. Effect of smoking on tuberculosis treatment outcomes: a systematic review and meta-analysis. *PLoS ONE.* (2020) 15:e0239333. doi: 10.1371/journal.pone.0239333

9. Neumann T, Rasmussen M, Heitmann BL, Tonnesen H. Gold standard program for heavy smokers in a real-life setting. *Int J Environ Res Public Health.* (2013) 10:4186–99. doi: 10.3390/ijerph10094186

10. Bohlbro AS, Patsche CB, Mendes AM, Sifna A, Gomes V, Wejse C, et al. A self-rated health score predicts severe disease and high mortality in patients with pulmonary TB. *Int J Tuberc Lung Dis.* (2022) 26:158–65. doi: 10.5588/ijtld.21.0545

11. McHenry ML, Bartlett J, Igo RP, Wampande E, Benchek P, Mayanja-Kizza H, et al. Interaction between host genes and M. tuberculosis lineage can affect tuberculosis severity: evidence for coevolution. *PLoS Genet.* (2020) 4:e1008728. doi: 10.1371/journal.pgen.1008728

12. Singla R, Raghu B, Gupta A, Caminero J, Sethi P, Tayal D, et al. Risk factors for early mortality in patients with pulmonary tuberculosis admitted to the emergency room. *Pulmonology.* (2021) 1:35–42. doi: 10.1016/j.pulmoe.2020.02.002

13. Haque G, Kumar A, Saifuddin F, Ismail S, Rizvi N, Ghazal S, et al. Prognostic factors in tuberculosis related mortalities in hospitalized patients. *Tuberc Res Treat.* (2014) 2014:624671. doi: 10.1155/2014/624671

14. Zhang M, Yang L, Wang L, Jiang Y, Huang Z, Zhao Z, et al. Trends in smoking prevalence in urban and rural China, 2007 to 2018: findings from 5 consecutive nationally representative cross-sectional surveys. *PLoS Med.* (2022) 19:e1004064. doi: 10.1371/journal.pmed.1004064

15. Ali AM, Kunugi H. Hypoproteinemia predicts disease severity and mortality in COVID-19: a call for action. *Diagn Pathol.* (2021) 16:31. doi: 10.1186/s13000-021-01092-5

16. Hu J, Lv C, Hu X, Liu J. Effect of hypoproteinemia on the mortality of sepsis patients in the ICU: a retrospective cohort study. *Sci Rep*. (2021) 11:24379. doi: 10.1038/s41598-021-03865-w

17. Rudasill SE, Morales RR, Sanaiha Y, Sareh S, Antonios JW, Khoury H, et al. Predicting morbidity and mortality in laparoscopic cholecystectomy: preoperative serum albumin still matters. *Am J Surg*. (2020) 220:432–7. doi: 10.1016/j.amjsurg.2019.12.005

18. Liu R, Shu W, Song Y, Liu Y, Ma L, Gao M. Use of serum albumin level as a predictive marker of clinical outcomes for active tuberculosis. *Ann Clin Lab Sci*. (2020) 50:681–6.

19. Rahmatallah FA, Alkhair AA, Osman EA, Mohamed GZ, Ebrahim HG, Abdu Samia A, et al. Effect of cigarette smoking on aminotransferase enzymes activities and albumin level among sudanese male smokers. *JNMS1*. (2019) 1–6.

20. Peters JS, Andrews JR, Hatherill M, Hermans S, Martinez L, Schurr E, et al. Advances in the understanding of Mycobacterium tuberculosis transmission in HIV-endemic settings. *Lancet Infect Dis*. (2019) 19:e65–76. doi: 10.1016/S1473-3099(18)30477-8

21. Adegbite BR, Edoa JR, Achimi Agbo P, Dejon-Agobe JC, Ne P, Lotola-Mougeni F, et al. Epidemiological, mycobacteriological, and clinical characteristics of smoking pulmonary tuberculosis patients, in lambarene, gabon: a cross-sectional study. *Am J Trop Med Hyg*. (2020) 103:2501–5. doi: 10.4269/ajtmh.20-0424

22. Campagna D, Alamo A, Di Pino A, Russo C, Calogero AE, Purrello F, et al. Smoking and diabetes: dangerous liaisons and confusing relationships. *Diabetol Metab Syndr*. (2019) 11:85. doi: 10.1186/s13098-019-0482-2

23. Wheaton AG, Liu Y, Croft JB, VanFrank B, Croxton TL, Punturieri A, et al. Chronic obstructive pulmonary disease and smoking status - United States, 2017. *MMWR Morb Mortal Wkly Rep*. (2019) 68:533–8. doi: 10.15585/mmwr.mm6824a1

24. Gallucci G, Tartarone A, Lerose R, Lalinga AV, Capobianco AM. Cardiovascular risk of smoking and benefits of smoking cessation. *J Thorac Dis*. (2020) 12:3866–76. doi: 10.21037/jtd.2020.02.47

25. Boiko O, Rodionova V. Does smoking affect nutritional status and renal function in patients with COPD? *Eur Respir J*. (2020) 56:1003. doi: 10.1183/13993003.congress-2020.1003

26. Jakimova MA, Karpina N, Gordeeva O, Asanov R. Comorbidity: pulmonary tuberculosis and chronic obstructive pulmonary disease. *Eur Respir J*. (2019) 54:PA2969. doi: 10.1183/13993003.congress-2019.PA2969

27. Charlson ME, Carrozzino D, Guidi J, Patierno C. Charlson comorbidity index: a critical review of clinimetric properties. *Psychother Psychosom*. (2022) 91:8–35. doi: 10.1159/000521288

28. Zhang S, Tong X, Wang L, Zhang T, Huang J, Wang D, et al. Clinical characteristics and prognostic analysis of patients with pulmonary tuberculosis and type 2 diabetes comorbidity in china: a retrospective analysis. *Front Public Health*. (2021) 9:710981. doi: 10.3389/fpubh.2021.710981

29. Min J, Kim HW, Stagg HR, Rangaka MX, Lipman M, Abubakar I, et al. The cascade of care for latent tuberculosis infection in congregate settings: a national cohort analysis, Korea, 2017–2018. *Front Med*. (2022) 9:927579. doi: 10.3389/fmed.2022.927579

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A study of risk factors for tuberculous meningitis among patients with tuberculosis in China: An analysis of data between 2012 and 2019

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Purpose: The present study aimed to explore the risk factors for tuberculous meningitis (TBM) among patients with tuberculosis (TB).

Methods: This retrospective study was conducted on patients with TB who were hospitalized in Beijing Chest Hospital between January 2012 and December 2019. Demographic and clinical data of patients with TB were extracted from electronic medical records using a standardized data collection system. Logistic regression was used to analyze the risk factors associated with TBM.

Results: Of the total number of 22,988 cases enrolled, 3.1% were cases of TBM, which included 127 definite and 581 probable TBM, respectively. Multivariate analysis showed that definite TBM was significantly associated with patients aged < 30 years [adjusted odds ratio (aOR) = 3.015, 95% confidence interval (CI): (1.451–6.266)], who were farmers [aOR = 1.490, 95%CI: (1.020–2.177)], with miliary pulmonary TB [aOR = 105.842, 95%CI: (71.704–156.235)], and with malnutrition [aOR = 2.466, 95%CI: (1.110–5.479)]. Additionally, probable TBM was significantly associated with patients aged < 30 years [aOR = 2.174, 95% CI: (1.450–3.261)], aged 30–59 years [aOR = 1.670, 95% CI: (1.222–2.282)], who were farmers [aOR = 1.482, 95%CI: (1.203–1.825)], with miliary pulmonary TB [aOR = 108.696, 95%CI: (87.122–135.613)], and with a digestive system TB [aOR = 2.906, 95%CI: (1.762–4.793)].

Conclusion: An age of <30 years, being a farmer, and having miliary pulmonary TB were risk factors for TBM among patients with TB. Further screening of patients with TB with aforementioned characteristics could facilitate clinicians to identify patients with TBM at an early stage.

KEYWORDS

tuberculosis, tuberculous meningitis, miliary pulmonary tuberculosis, risk factors, malnutrition

Introduction

Tuberculosis (TB) is ranked as the second leading cause of death from a single infectious agent after coronavirus 2019 (COVID-19) (1), and tuberculous meningitis (TBM) is the most severe form of TB (2). Despite 1-5% of TBM among cases of TB, it is universally fatal if left untreated (3, 4). Previous studies demonstrated that TBM had high mortality and morbidity, and the death rate reached up to 20-30% in TBM without HIV (human immunodeficiency virus) infection, but up to 50-60% in TBM with HIV infection (5–9). Moreover, nearly half of the survivors would have neurological sequelae, even if they are receiving treatment.

An early diagnosis of TBM can facilitate timely treatment and improve prognosis ultimately. However, it is difficult to diagnose TBM. Its symptoms and signs are variable and nonspecific. Existing diagnostic technologies still have some limitations. Elevated protein, lymphocytic pleocytosis, and low glucose with cerebrospinal fluid (CSF) cannot reliably distinguish TBM from other subacute meningitis types (cryptococcal meningitis, etc.) (10). Neuroimaging, especially cerebral magnetic resonance imaging (MRI), which is sensitive to the detection of the lesion, exhibits low specificity (11). Microbiological tests have good specificity but unfavorable sensitivity. Adenosine deaminase and T-SPOT.TB testing have different sensitivities and specificities and are expensive (12, 13). Notably, most of the aforementioned tests are unavailable in a resource-limited area. Risk factors for TBM should be identified, which could help clinicians to facilitate an early detection of TBM, especially in medical resource-limited areas.

Literature published demonstrated that being a farmer (14), having diabetes mellitus (15), having kidney failure (15–17), having HIV infection (1), having malnutrition (1), and having a rheumatic disease (18) are the risk factors for TB. Patients with the aforementioned characteristics are more likely to have TB, which can help clinicians identify TB early. Previous studies reported that TBM was the second stage of TB (19, 20), which was accompanied by the occurrence of any other TB. Up to 80% of miliary pulmonary TB is accompanied by TBM, and TBM was especially common in young children and people with untreated HIV infection (21, 22).

Early detection of TBM and targeted interventions are crucial for reducing the risk of TBM.

Thus, this study aimed to determine different factors associated with TBM in China.

Methods

Study population and area

Patients with TB who were hospitalized at Beijing Chest Hospital from 1 January 2012 to 31 December 2019 were enrolled in this retrospective study. Beijing Chest Hospital is a Class-3A level (the top level of hospital ranking in China) specialized hospital for treating TB infectious diseases in Beijing (north of China), equipped with 1,100 beds, and designated as a municipal-level hospital for treating patients with TB.

The participants were excluded, if they belonged to any of the following patient categories: (1) HIV-infected patients with TB; (2) possible cases of TBM according to the uniform case definition; and (3) suspected cases of TBM without results of cerebral imaging and lumbar puncture.

Definitions

In the present study, patients were categorized as definite, probable, possible TBM, and non-TBM cases, based on a uniform case definition for use in clinical research on TBM (23). Briefly, three types of TBM, including definite, probable, and possible TBM of diagnostic criteria, are defined as follows: (1) definite TBM: microbiological identification or evidence from commercial nucleic acid amplification tests of the central nervous system (CNS) mycobacterium tuberculosis infection; (2) probable TBM: when imaging is available, a diagnostic score of 12 or above is required, and when imaging is not available, a diagnostic score of 10 or above is required; and (3) possible TBM: when imaging is available, a diagnostic score of 6–11 is required, and when imaging is not available, a diagnostic score of 6–9 is required.

The definition of miliary pulmonary TB included the presence of a miliary pattern on chest radiograph or computed tomography (CT), along with one or more of the following features: (1) clinical features compatible with pulmonary TB, including cough for a duration of 3 weeks or more, fever, weight loss, night sweats, loss of appetite, or hemoptysis; (2) microbiological and/or histopathological evidence of TB; and (3) response to antituberculosis treatment (24, 25).

Secondary pulmonary tuberculosis, also known as reactivated or adult pulmonary tuberculosis, refers to the reactivation of dormant tuberculosis lesions or re-exogenous infection.

Malnutrition was defined as the body mass index of patients (BMI) of<18.5 kg/m^2 according to the consensus statement of the European Society for Clinical Nutrition and Metabolism (ESPEN) (26). The BMI was calculated as weight in kilograms (kg) divided by height in meter square (kg/m^2).

In this study, patients with TB included secondary pulmonary TB, miliary pulmonary TB, tuberculous pleuritis, tuberculous peritonitis, tuberculous pericarditis, lymph node TB, cutaneous and soft tissue TB, TB of the head and the neck, digestive system TB, genitourinary TB, vertebral TB, and osteoarticular TB, as seen in Table 1.

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TABLE 1 Demographic and clinical characteristics of 22,988 cases from 2012 to 2019.

Characteristics	Total (n = 22,988)	Non-TBM (n = 22,280)	Definite TBM (n = 127)	Probable TBM (n = 581)	P-value
Age group, years					
<30	6,358 (27.7)	6,024 (27.0)	68 (53.6)	266 (45.8)	**<0.001**
30–59	9,694 (42.1)	9,441 (42.4)	38 (29.9)	215 (37.0)	
≥60	6,936 (30.2)	6,815 (30.6)	21 (16.5)	100 (17.2)	
Sex					**<0.001**
Male	14,714 (64.0)	14,322 (64.3)	73 (57.5)	319 (54.9)	
Female	8,274 (36.0)	7,958 (35.7)	54 (42.5)	262 (45.1)	
Marital status					**<0.001**
Married	15,288 (66.5)	14,916 (66.9)	64 (50.4)	308 (53.0)	
Unmarried	6,083 (26.5)	5,784 (26.0)	57 (44.9)	242 (41.7)	
Others	1,617 (7.0)	1,580 (7.1)	6 (4.7)	31 (5.3)	
Occupation					**<0.001**
Farmer	7,552 (32.9)	7,242 (32.5)	55 (43.3)	255 (43.9)	
Other	15,436 (67.1)	15,038 (67.5)	72 (56.7)	326 (56.1)	
Comorbidity					
Diabetes mellitus	4,124 (17.9)	4,059 (18.2)	12 (9.4)	53 (9.1)	**<0.001**
Hypertension	3,362 (14.6)	3,289 (14.8)	9 (7.1)	64 (11.0)	**0.002**
Coronary heart disease	1,392 (6.1)	1,376 (6.2)	2 (1.6)	14 (2.4)	**<0.001**
Liver cirrosis	195 (0.8)	193 (0.9)	1 (0.8)	1 (0.2)	0.098
Kidney failure	525 (2.3)	506 (2.3)	6 (4.7)	13 (2.2)	0.182
Rheumatic disease	537 (2.3)	507 (2.3)	3 (2.4)	27 (4.6)	**0.001**
Carcinoma	1,188 (5.2)	1,183 (5.3)	2 (1.6)	3 (0.5)	**<0.001**
Malnutrition	563 (2.4)	532 (2.4)	8 (6.3)	23 (4.0)	**0.001**
Concurrent TB					
Secondary pulmonary TB	15,847 (68.9)	15,349 (68.9)	100 (78.7)	398 (68.5)	0.056
Miliary pulmonary TB	650 (2.8)	249 (1.1)	68 (53.5)	333 (57.3)	**<0.001**
Tuberculous pleuritis	4,993 (21.7)	4,872 (21.9)	21 (16.5)	100 (17.2)	**0.01**
Tuberculous peritonitis	324 (1.4)	308 (1.4)	2 (1.6)	14 (2.4)	0.115
Tuberculous precarditis	183 (0.8)	180 (0.8)	1 (0.8)	2 (0.3)	0.516
Lymph node TB	1,189 (5.2)	1,121 (5.0)	8 (6.3)	60 (10.3)	**<0.001**
Cutaneous and soft tissue TB	1,170 (5.1)	1,142 (5.1)	5 (3.9)	23 (4.0)	0.378
TB of the head and neck	112 (0.5)	104 (0.5)	2 (1.6)	6 (1.0)	**0.023**
Digestive system TB	341 (1.5)	298 (1.3)	3 (2.4)	40 (6.9)	**<0.001**
Genitourinary TB	289 (1.3)	259 (1.2)	4 (3.1)	26 (4.5)	**<0.001**
Vertebral TB	2,032 (8.8)	1,927 (8.6)	11 (8.7)	94 (16.2)	**<0.001**
Osteoarticular TB	1,336 (5.8)	1,299 (5.8)	4 (3.1)	33 (5.7)	0.432

Data are presented as n (%). TBM, tuberculous meningitis; TB, tuberculosis.
P values are compared from chi-square (χ^2) test, Fisher's exact test, and Kruskal–Wallis test, as appropriate.
The value of $P < 0.05$ indicates statistical significance.

In the present study, TBM included definite TBM and probable TBM. Non-TBM cases included all other cases of TB without TBM.

Data collection

The present study collected demographic (including age, sex, marital status, and occupation) and clinical (comorbidities and concurrent TB) data for all hospitalized patients with TB from electronic medical records. In the hospital, all diseases are coded using the International Classification of Diseases, 10th Revision with Clinical Modification (ICD-10-CM). The information about patients was collected only during the first hospitalization, but not if the patient was hospitalized repeatedly. Moreover, to reclassify patients with TBM into definite, probable, and possible TBM according to the uniform case definition, the present study also collected symptoms and symptom duration, signs, history of recent (within the past 1 year) close contact with an individual with pulmonary TB, routine biochemistry and biochemical analysis of the cerebrospinal fluid (CSF), imaging (computed tomography (CT), magnetic resonance imaging (MRI), or ultrasound (US)) of the cereba and other organs, mycobacterial evidence by any of the smear microscopy, culture, polymerase chain reaction (PCR), Xpert from sputum, urine, and stool, pleural effusion, pericardial effusion, peritoneal effusion, pus, and bronchoalveolar lavage fluid from patients with TBM.

Statistical analysis

Categorical variables were described as frequency and percentages (%) and continuous variables as mean and standard deviation (SD) or median and interquartile range (IQR), as appropriate. The means for continuous variables were compared using the independent group variance analysis when the data were normally distributed; otherwise, the Kruskal–Wallis test was used. A comparison of categorical variables was done using the chi-square (χ^2) test or the Fisher exact test if the cell counts were small. To investigate and identify the risk factors of TBM further, the present study generated multivariate logistic regression analyses. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to demonstrate the risk for TBM. Statistical analysis was conducted using SPSS software (version 19.0, IBM, Armonk, NY, USA), and a value of P of < 0.05 indicated statistical significance.

Ethics statement

The study was approved by the Ethics Committee of Beijing Chest Hospital (20210113YJS-2021-007). In the present study, the retrospective collection and analysis of cases were patients' demographic characteristics and diagnostic information. All data were supplied and analyzed, without access to personal identifiable information (PII). No informed consent was required.

Results

Demographics and clinical characteristics of patients

A total of 23,121 inpatient cases with TB were enrolled in this study between 2012 and 2019. Of these, 133 cases were excluded, including 102 possible cases of TBM diagnosed by the uniform case definition, 8 suspected cases of TBM who did not undergo lumbar puncture and cerebral imaging, and 23 HIV-positive cases. A total of 22,988 patients with TB were thus included in the present study (Figure 1).

The median age of the patient was 47 years, and 64.0% of patients were men. Of these, 17.9% and 14.6% had diabetes mellitus and hypertension, respectively. Of 22,988 patients, 708 (3.1%) cases were patients with TBM, including 127 definite and 581 probable cases, and 22,280 (96.9%) cases were patients without TBM. Patients with definite and probable TBM were more likely to be younger, and nearly two-thirds of patients without TBM were women. Patients with definite and probable TBM were more likely to be male, to be less than aged 30 years, and to have rheumatic disease and malnutrition than patients without TBM. They were also more likely to have miliary pulmonary TB. The percentage of definite patients with TBM categorized as having miliary pulmonary TB (53.5%) was more than 48-fold greater than the percentage of patients without TBM (1.1%). Moreover, the percentage of probable TBM patients having miliary pulmonary TB (57.3%) was more than 52-fold greater than the percentage of patients without TBM (Table 1).

Among the 127 definite TBM cases, 4 (3.1%) of them were positive with smear microscopy, 23 (18.1%) for Mycobacteria Growth Indicator Tube (MGIT) culture, 24 (18.9%) for Lowenstein-Jenson culture, 42 (33.1%) for PCR, and 74 (58.3%) for Xpert, and two were pathologically diagnosed by biopsy from the brain (Figure 1). Additionally, although the hospitalized cases of TB increased gradually from 2,066 in 2012 to 3,475 in 2019, the proportion of TBM showed a decreasing trend from 4.4 to 2.4% during the same period (Figure 2). Miliary pulmonary TB (61.7%) with TBM was the highest, followed by digestive system TB (12.6%) and genitourinary TB (10.4%) (Figure 3).

When comparing patients with TB to patients without TBM, the univariate analysis showed that age, sex, marriage, occupation, comorbidities (including diabetes mellitus, hypertension, coronary heart disease, rheumatic disease,

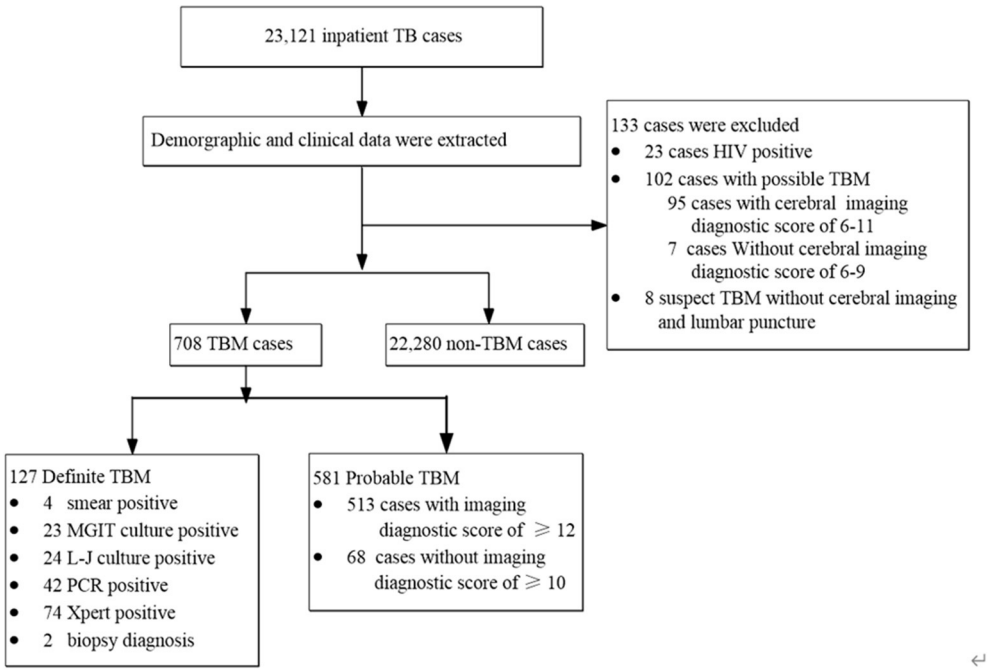

FIGURE 1
Flowchart of subjects enrolled in the study.

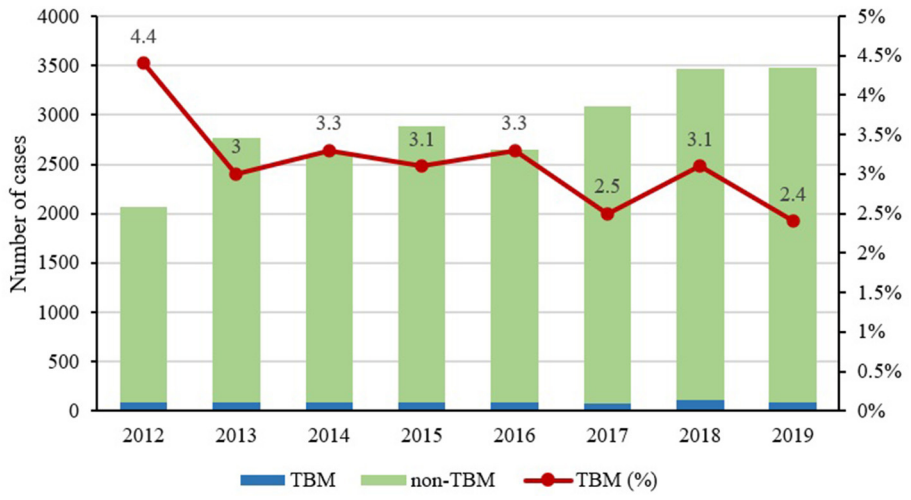

FIGURE 2
Proportion of tuberculosis (TB) and tuberculous meningitis (TBM) from 2012 to 2019.

carcinoma, and malnutrition), and concurrent TB (miliary pulmonary TB, tuberculous pleuritis, lymph node TB, TB of the head and neck, digestive system TB, genitourinary TB, and vertebral TB) were significantly different among the three groups ($p < 0.05$), as reported in Table 1.

Risk factors for definite TBM and probable TBM

Table 2 shows the results of the logistic regression model for factors related to definite TBM and probable TBM. In the

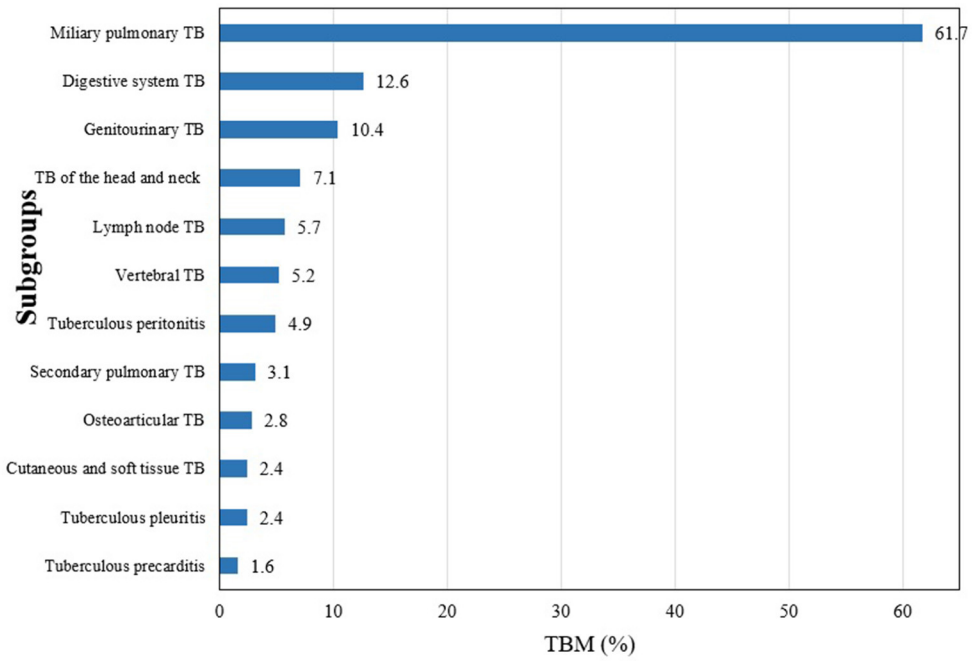

FIGURE 3
The proportion of TBM in different types of tuberculosis.

present multivariable logistic regression model, compared with patients without TBM, patients aged <30 years were associated with a greater likelihood of definite TBM compared with patients aged >59 years [aOR = 3.015, 95% CI: (1.451–6.266)]. Farmers were more likely to have definite TBM [aOR = 1.490, 95%CI: (1.020–2.177)]. Compared with patients without miliary pulmonary TB, patients with miliary pulmonary TB increased the risk of definite TBM [aOR = 105.842, 95%CI: (71.704–156.235)]. Malnutrition also increased the risk of definite TBM [aOR = 2.466, 95%CI: (1.110–5.479)]. Additionally, probable TBM was significantly associated with patients aged <30 years [aOR = 2.174, 95% CI: (1.450–3.261)], aged 30–59 years [aOR = 1.670, 95%CI: (1.222–2.282)], who are farmers [aOR = 1.482, 95%CI: (1.203–1.825)], with miliary pulmonary TB [aOR = 108.696, 95%CI: (87.122–135.613)], and with digestive system TB [aOR = 2.906, 95%CI: (1.762–4.793)] (Table 2).

Discussion

The present study found that 3.1% of TBM was prevalent among patients with TB, which was consistent with those given in previous studies (2, 3, 27). The present study also found that patients aged < 30 years, being farmers, and having miliary pulmonary TB were associated with a higher risk of definite and probable TBM. Additionally, malnutrition increased the probability of definite TBM, while digestive system TB increased the probability of probable TBM. Meanwhile, tuberculous pleuritis was associated with decreased probability.

Our study reported patients with TB who were < 30 years of age were two to three times more likely to have TBM, and compared with patients aged > 59 years, younger patients were more susceptible to TBM. Previous studies observed that TBM affected young children most commonly, and the peak was 2–4 years old, while the progression from latent tuberculosis infection (LTBI) to more severe forms of TB disease was faster in younger children (~1–4 months for TBM) (28–30). However, there is no report about patients aged < 30 years who are more prone to suffer from TBM. A retrospective cohort study from China found that patients aged > 20 years had a significantly lower risk for treatment delay (31). The possible reason was that younger patients are more likely to come to the hospital to seek health care, once they have suspected symptoms of TB. Additionally, the present study also observed that farmer was associated with an increased probability of TBM, while the results on farmers had not been reported in previous studies. Wang et al. reported that the farmer increased the risk of TB (14). Possible reasons were that farmers have a relatively low income, live in a relatively crowded poor environment, and are malnourished, thus making them vulnerable to TB. Second, farmers have low education level and poor knowledge of TB, which are the other reasons why they do not seek medical care when they are suspected symptoms of TB (32–34). However, TB progresses without treatment, which increases the probability

TABLE 2 Associated factors for definite TBM and probable TBM.

Variables	Definite TBM (vs. non-TBM)		Probable TBM (vs. non TBM)	
	aOR (95% CI)	P value	aOR (95% CI)	P value
Age group, years				
≥60	Ref		Ref	
<30	3.015 (1.451–6.266)	**0.003**	2.174 (1.450–3.261)	**<0.001**
30–59	1.348 (0.736–2.468)	0.333	1.670 (1.222–2.282)	**0.001**
Occupation				
Other	Ref		Ref	
Farmer	1.490 (1.020–2.177)	**0.039**	1.482 (1.203–1.825)	**<0.001**
Malnutrition	2.466 (1.110–5.479)	**0.027**	1.423 (0.820–2.470)	0.210
Miliary pulmonary TB	105.842 (71.704–156.235)	**<0.001**	108.696 (87.122–135.613)	**<0.001**
Tuberculous pleuritis	0.563 (0.340–0.930)	**0.025**	0.672 (0.514–0.879)	**0.004**
Digestive system TB	0.945 (0.280–3.186)	0.927	2.906 (1.762–4.793)	**<0.001**

TBM, tuberculous meningitis; TB, tuberculosis; aOR, adjusted odds ratio.
The value of $P < 0.05$ indicates statistical significance.

of TBM. Therefore, increasing income and improving living conditions are helpful to prevent TBM from affecting farmers. Moreover, an appropriate way of promoting health education is to propagate the knowledge of TB among farmers, especially among illiterate farmers, which in turn promotes them to seek timely healthcare, which plays a more important role in reducing the incidence of TBM.

Miliary pulmonary TB results from the hematogenous spread of mycobacterium tuberculosis (Mtb) in the pulmonary. The present study found that 61.7% of patients with miliary pulmonary TB had TBM, which was similar to those mentioned in previous studies (11, 21). On the other hand, more than one-half (56.6%) of the patients with TBM had miliary pulmonary TB and only 1.1% of patients without TBM had miliary pulmonary TB. Miliary pulmonary TB was the most common hematogenous TB, followed by digestive system TB (30). In the present study, patients with miliary pulmonary TB had more than 100 times increased risk of both definite and probable TBM compared with TB patients without miliary pulmonary TB, and digestive system TB was also associated with increased risk for probable TBM. Lymph node TB, vertebral TB, and TB of the head and the neck were not associated with the risk for TBM, and the results indicated from the TBM pathogenesis mentioned previously that TBM was secondary to hematogenous spread, other than lymphangitic or contiguous spread. The results also suggested that, when physicians encounter such patients they consider performing cerebral imaging and lumbar puncture to exclude TBM for TB patients with miliary pulmonary TB in clinical practice.

The results of the present study observed that malnutrition increased the risk of definite TBM. The cellular immunity plays a critical role in immune responses to Mtb infection. Malnutrition could impair T-cell function, particularly the production of T-helper-1 cytokines and functions of the macrophage antimycobacterial effector (35). A previous study demonstrated that malnutrition increased the incidence and exacerbated clinical manifestations of TB (36). Patients with TB have an increased metabolism and a decreased appetite that compounds the already present malnutrition. Thus, a merciless vicious circle between TB and malnutrition continues (37). Previous studies revealed that malnutrition increased the risk of death for TB (38) and TBM (30). Improving the living conditions and reducing malnutrition can not only reduce the occurrence of TB and TBM but also improve the prognosis.

Our results found that tuberculous pleuritis was related to decreased risk for definite and probable TBM. The reason can be given that most patients with tuberculous pleuritis have fever, chest pain, shortness of breath, etc. Urgently, these types of discomfort promote patients to seek healthcare, while the diagnosis and treatment of tuberculous pleuritis reduce its progression to TBM. In addition, tuberculous pleuritis decreases the probability of TBM that may relate to their pathogenesis, but the pathogenesis of these two diseases is not so clear; hence, further studies need to be conducted in the future.

There are some limitations to our study. First, the study included inpatients only from a single center in China. Second, patients with HIV infection had not been analyzed further based on a small sample with 23 cases. In addition, the present study

did not collect information about smoking and drinking, which is likely to correlate with TBM.

In summary, the present data reported on the proportion of TBM, including definite and probable TBM among patients with TB from a large sample study, and revealed risk factors including patients aged < 30 years, being farmers, and having miliary pulmonary TB affecting patients with TBM. The results of the present study suggested screening patients with TB having the aforementioned characteristics that could facilitate clinicians to identify patients with TBM at an early stage and improve the prognosis of patients with TBM further. Further studies should be conducted to confirm the findings of the present study.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Ethics statement

The study was approved by the Ethics Committee of Beijing Chest Hospital. In our study, the retrospective collection and analysis of case were patients' demographic characteristics and diagnosis information. All data were supplied and analyzed, without access to personal identifying information. No informed consent was required.

Author contributions

MH, YM, QL, and NC were involved in the conception and design of the project. MH and YM carried out the analysis and wrote the first draft of the manuscript. MH, YM, XJ, HJ, and FL conducted the literature search, data acquisition, and input data. QL had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors read and contributed to the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. World Health Organization. *Global tuberculosis report 2022*. Geneva. Available online at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022

2. Torok ME. Tuberculous meningitis: advances in diagnosis and treatment. *Brit Med Bull.* (2015) 113:117–31. doi: 10.1093/bmb/ldv003

3. Lincoln EM. Tuberculous meningitis in children; with special reference to serous meningitis; tuberculous meningitis. *Am Rev Tuberc.* (1947) 56:75–94. doi: 10.1164/art.1947.56.2.75

4. Seddon JA, Wilkinson R, van Crevel R, Figaji A, Thwaites GE, Tuberculous MIRC. Knowledge gaps and research priorities in tuberculous meningitis. *Wellcome Open Res.* (2019) 4:188. doi: 10.12688/wellcomeopenres.15573.1

5. Thao LTP, Heemskerk AD, Geskus RB, Mai NTH, Ha DTM, Chau TTH, et al. Prognostic models for 9-month mortality in tuberculous meningitis. *Clin Infect Dis.* (2018) 66:523–32. doi: 10.1093/cid/cix849

6. Wen L, Li M, Xu T, Yu X, Wang L, Li K. Clinical features, outcomes and prognostic factors of tuberculous meningitis in adults worldwide: systematic review and meta-analysis. *J Neurol.* (2019) 266:3009–21. doi: 10.1007/s00415-019-09523-6

7. Wang M, Luo L, Zhang Y, Liu X, Liu L, He J. Treatment outcomes of tuberculous meningitis in adults: a systematic review and meta-analysis. *BMC Pulm Med.* (2019) 19:1. doi: 10.1186/s12890-019-0966-8

8. Thwaites GE, Duc Bang N, Huy Dung N, Thi Quy H, Thi Tuong Oanh D, Thi Cam Thoa N, et al. The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with tuberculous meningitis. (2005) 192:2134–41. doi: 10.1086/498220

9. Heemskerk AD, Bang ND, Mai NTH, Chau TTH, Chau NVV, Phu NH, et al. Intensified antituberculosis therapy in adults with tuberculous meningitis. *N Engl J Med.* (2016) 374:124–34. doi: 10.1056/NEJMoa1507062

10. Bahr NC, Boulware DR. Methods of rapid diagnosis for the etiology of meningitis in adults. *Biomark Med.* (2014) 8:1085–103. doi: 10.2217/bmm.14.67

11. Venkatraman N, King T, Bell D, Woltmann G, Wiselka M, Abubakar I, et al. High levels of neurological involvement but low mortality in miliary tuberculosis: a 6-year case-series from the UK. *Eur Respir J.* (2016) 47:1578–81. doi: 10.1183/13993003.01958-2015

12. Li XL, Xie N, Wang SW, Wu QH, Ma Y, Shu W, et al. Diagnostic value of cerebrospinal fluid T-SPOTTB for tuberculousis meningitis in China. *Biomed Environ Sci.* (2017) 30:681–4. doi: 10.3967/bes2017.091

13. Gupta BK, Bharat A, Debapriya B, Baruah H. Adenosine deaminase levels in CSF of tuberculous meningitis patients. *J Clin Med Res.* (2010) 2:220–4. doi: 10.4021/jocmr429w

14. Wang X, Yin S, Li Y, Wang W, Du M, Guo W, et al. Spatiotemporal epidemiology of, and factors associated with, the tuberculosis prevalence in northern China, 2010-2014. *BMC Infect Dis.* (2019) 19:365. doi: 10.1186/s12879-019-3910-x

15. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. *Lancet Infect Dis.* (2009) 9:737–46. doi: 10.1016/S1473-3099(09)70282-8

16. Shu C, Wei Y, Yeh Y, Lin H, Chen C, Wang P, et al. The impact on incident tuberculosis by kidney function impairment status: analysis of severity relationship. *Resp Res.* (2020) 21:51. doi: 10.1186/s12931-020-1294-5

17. Cho PJ, Wu C, Johnston J, Wu M, Shu C, Lin H. Progression of chronic kidney disease and the risk of tuberculosis: an observational cohort study. *Int J Tuberc Lung Dis.* (2019) 23:555–62. doi: 10.5588/ijtld.18.0225

18. Vuorela M, Mars NJ, Salonen J, Kauppi MJ. Tuberculosis in people with rheumatic disease in Finland 1995-2007: a nationwide retrospective register study. *Rheumatol Adv Pract.* (2019) 3:z20. doi: 10.1093/rap/rkz020

19. Davis AG, Rohlwink UK, Proust A, Figaji AA, Wilkinson RJ. The pathogenesis of tuberculous meningitis. *J Leukocyte Biol.* (2019) 105:267–80. doi: 10.1002/JLB.MR0318-102R

20. Donald P, Schaaf H, Schoeman J. Tuberculous meningitis and miliary tuberculosis: the Rich focus revisited. *J Infection.* (2005) 50:193–5. doi: 10.1016/j.jinf.2004.02.010

21. Garg RK, Sharma R, Kar AM, Kushwaha RAS, Singh MK, Shukla R, et al. Neurological complications of miliary tuberculosis. *Clin Neurol Neurosur.* (2010) 112:188–92. doi: 10.1016/j.clineuro.2009.11.013

22. Thwaites GED, van Toorn RM, Schoeman JP. Tuberculous meningitis: more questions, still too few answers. *Lancet Neurology.* (2013) 12:999–1010. doi: 10.1016/S1474-4422(13)70168-6

23. Marais S, Thwaites G, Schoeman JF, Torok ME, Misra UK, Prasad K, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis.* (2010) 10:803–12. doi: 10.1016/S1473-3099(10)70138-9

24. Hussain SF, Irfan M, Abbasi M, Anwer SS, Davidson S, Haqqee R, et al. Clinical characteristics of 110 miliary tuberculosis patients from a low HIV prevalence country. *Int J Tuberc Lung Dis.* (2004) 8:493

25. Sharma SK, Mohan A, Sharma A, Mitra DK. Miliary tuberculosis: new insights into an old disease. *Lancet Infect Dis.* (2005) 5:415–30. doi: 10.1016/S1473-3099(05)70163-8

26. Cederholm T, Bosaeus I, Barazzoni R, Bauer J, Van Gossum A, Klek S, et al. Diagnostic criteria for malnutrition - an ESPEN consensus statement. *Clini Nutr. (Edinburgh, Scotland).* (2015) 34:335–40. doi: 10.1016/j.clnu.2015.03.001

27. Seddon JA, Tugume L, Solomons R, Prasad K, Bahr NC. Tuberculous MIRC. The current global situation for tuberculous meningitis: epidemiology, diagnostics, treatment and outcomes. *Wellcome Open Res.* (2019) 4:167. doi: 10.12688/wellcomeopenres.15535.1

28. Duque-Silva A, Robsky K, Flood J, Barry PM. Risk factors for central nervous system tuberculosis. *Pediatrics.* (2015) 136:e1276–84. doi: 10.1542/peds.2014-3958

29. Donovan J, Thwaites GE, Huynh J. Tuberculous meningitis: where to from here? *Curr Opin Infect Dis.* (2020) 33:259–66. doi: 10.1097/QCO.0000000000000648

30. Starke JR. Tuberculosis in children. *Semin Resp Crit Care.* (2004) 25:353–64. doi: 10.1055/s-2004-829507

31. He Y, Han C, Chang K, Wang M, Huang T. Total delay in treatment among tuberculous meningitis patients in China: a retrospective cohort study. *BMC Infect Dis.* (2017) 17:341. doi: 10.1186/s12879-017-2447-0

32. Asres M, Gedefaw M, Kahsay A, Weldu Y. Patients' delay in seeking health care for tuberculosis diagnosis in East Gojjam zone, Northwest Ethiopia. *Am J Trop Med Hyg.* (2017) 96:1071–5. doi: 10.4269/ajtmh.16-0892

33. Luba TR, Tang S, Liu Q, Gebremedhin SA, Kisasi MD, Feng Z. Knowledge, attitude and associated factors towards tuberculosis in Lesotho: a population based study. *BMC Infect Dis.* (2019) 19:96. doi: 10.1186/s12879-019-3688-x

34. Ali Warsi SM, Danish SH, Ahmad F, Khan AI, Khan MP, Bano S, et al. Tuberculosis knowledge and health seeking behaviour: a tale of two districts of Sindh, Pakistan. *JPMA.* (2016) 66:1120–6

35. Boelaert JR, Gordeuk VR. Protein energy malnutrition and risk of tuberculosis infection. *Lancet.* (2002) 360:1102. doi: 10.1016/S0140-6736(02)11169-X

36. Macallan DC. Malnutrition in tuberculosis. *Diagn Micr Infec Dis.* (1999) 34:153–7. doi: 10.1016/s0732-8893(99)00007-3

37. Martin SJ, Sabina EP. Malnutrition and associated disorders in tuberculosis and its therapy. *J Diet Suppl.* (2019) 16:602–10. doi: 10.1080/19390211.2018.1472165

38. Zachariah R, Spielmann MP, Harries AD, Salaniponi FML. Moderate to severe malnutrition in patients with tuberculosis is a risk factor associated with early death. *T Roy Soc Trop Med H.* (2002) 96:291–4. doi: 10.1016/s0035-9203(02)90103-3

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Digitizing tuberculosis treatment monitoring in Wuhan city, China, 2020–2021: Impact on medication adherence

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Introduction: Digital technologies can improve adherence to tuberculosis (TB) treatment. We studied the impact of digitizing TB treatment monitoring on adherence among TB patients in Wuhan, China, during 2020-2021.

Methods: We compared an electronic system introduced to monitor TB medication adherence (e-Patient Service System; e-PSS) with the p paper-based standard of care (TB Control Information System; TCIS) in terms of prescribed TB treatment doses taken by patients and patient outcome after six months of follow up. We designed a cross sectional study using retrospective data for all drug susceptible pulmonary TB patients recorded on both systems. The main indicators were: compliant first follow up visit (within 3 days of start of treatment); medication adherence (80% or more of monthly doses taken); and end of treatment success ratio.

Results: A total of 1,576 TB patients were recorded in TCIS in July September, 2020 and 1,145 TB cases were included in e-PSS in January March, 2021. The distribution of patient demographic and clinical features was similar between the two groups. A larger proportion from the e-PSS group visited the community doctor in the first three days compared with the TCIS group (48.91 versus 29. 76 % respectively). Medication adherence was also higher in the e-PSS group during the 6 months of treatment than in the TCIS group (84. 28 versus 80.3 3 % respectively). Treatment success was 92.52% in the e-PSS group and 92.07% in the TCIS group. Multivariate logistic regress ion analysis demonstrated that adjusted odds ratios for compliant first follow up visit, medication adherence and favorable treatment outcome in the e-PSS versus TCIS groups were 2.94 (95% 2.47 3.50), 1.33 (95% 1.08 1.63), and 1. 12 (95% CL: 0.79 1.57) respectively.

Discussion: This study revealed improvements in TB care following an intervention to monitor treatment digitally in patients in Wuhan, China.

KEYWORDS

tuberculosis, China, medication adherence and treatment outcome, digital adherence technology, operational research

Introduction

Tuberculosis (TB) is a communicable disease that remains a major global public health threat (1). In 2021, 6.4 million people were newly diagnosed with TB and an estimated 1.6 million deaths were reported, making TB the second most important infectious killer worldwide after COVID-19 that year (2). China is one of

the 30 high-TB burden countries in the world, and the incidence rate as of 2021 is currently 55/100,000, with 780,000 new reported TB cases (2). Wuhan is the biggest city in China's central region, with 5,596 new reported TB cases in 2021.

Most TB treatment regimens in use globally require multiple drugs to be taken daily for 6 months in order to achieve high efficacy. This long treatment is one of the most significant obstacles to TB control and interruption can lead to the emergence of drug-resistant TB (DR-TB) and continued spread. In a patient-level pooled analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis, non-adherence was the most significant risk factor for an unfavorable outcome: Missing 10% of doses or more was associated with an adjusted hazard ratio of 5.7 [95% confidence interval (CI): 3.3–9.9] (3). Therefore, many programs support patients with TB to improve adherence and completion of treatment.

Different strategies are recommended by the World Health Organization (WHO) to help people with TB take their treatment using person-centered approaches (4). These include in-person assistance and the use of different digital adherence technologies. A systematic review found that ∼52% of patients with TB adopted self-administered therapy (SAT) in China, attesting to the difficulties in implementing in-person support in large high-TB burden countries (5). Compared with directly observed therapy (DOT) alone, SAT has been associated with lower rates of treatment success, adherence, and sputum smear conversion as well as higher rates of development of drug resistance (6, 7). China's National TB Programme (NTP) offers different forms of treatment adherence support, such as home visits or phone calls from community or village doctors (village-level licensed general practitioners), family members, or volunteers. The village doctors are expected to visit each patient every 10 days during the intensive phase followed by once a month in the continuation phase. Since village supporters may be busy with their own routine business and may find it difficult to remind and follow patients for such a long period, digital technologies are being increasingly used for improving the adherence of patients with TB.

Several digital technologies are being applied to help TB treatment completion globally (7), such as short message services (SMSs) *via* mobile phones, phone calls, and video-supported therapy (VOT) (4, 8). In general, these digital technologies may not have a clear impact on treatment success compared with in-person observation, while they help save resources and improve adherence (9, 10). Evidence in Northwest Ethiopia reported that mobile phone-based weekly refilling with a daily medication reminder system improved adherence to patient-centered TB treatment (79 vs. 66.4%; RR = 1.63, 95% lower CI 1.16, one-tailed, $p = 0.02$); however, there was no significant effect on treatment success (89.5 vs. 85.1%, $p = 0.12$) (11). Similar evidence from a systematic review showed that clinic attendance and TB treatment completion were higher in people receiving pre-appointment reminder phone calls [clinic attendance: 66 vs. 50%, relative risk (RR) = 1.32, 95% CI 1.10–1.59; TB treatment completion: 100 vs. 88%, RR = 1.14, 95% CI 1.02–1.27] (12). Studies show that VOT achieved adherence to anti-TB treatment and significantly reduced the time and money patients spent on their treatment (13, 14). In China, studies showed that implementing an electronic medication monitor improved the treatment success rate (15) and medication adherence (16). For example, a pill box could remind the patient to take medicine on time and record the patient's medication each time the patient opens the device (16). Innovative approaches that can aid healthcare workers in tracing patients and supporting patients in adhering to TB treatment are needed in China.

Very few studies have evaluated the impact of data management on TB treatment outcomes. In this study, we looked at the impact of electronic monitoring of TB medication adherence (e-Patient Service System; e-PSS) on prescribed care and TB treatment completion 6 months after the start in Wuhan, China, in 2021. We compared this with a paper-based standard of care (TB Control Information System; TCIS) in late 2020. We used the findings to discuss the implications for programmatic enhancements.

Methods

Study design and setting

This was a comparative cross-sectional study using retrospective data to compare between TCIS and e-PSS in Wuhan, China. With a population of more than 11 million people, Wuhan is the capital city of Hubei province in central China. The city has an area of approximately 8,569.15 square kilometers and consists of 17 districts with TB programs (17).

In China, the Tuberculosis Control and Prevention Strategy provides patient-centered "prevention, diagnosis, treatment, management, and education" as part of comprehensive care services, aimed at reducing TB incidence and death and the economic burden on patients. Alongside the strategy, TB surveillance has been strengthened in China in recent years through an Infectious Disease Reporting System and Tuberculosis Information Management System (18). Through these two systems, both TB notification and TB treatment outcomes can be monitored in a timely manner (19). These measures help optimize TB service provision systems and improve the quality of case detection and treatment management. However, there is no national system to manage and monitor medication adherence and follow-up on an individual patient level. China is looking to establish a technical support system to assist in TB patient treatment management. At this time, e-PSS, developed by Beijing SINOVO Power Technology Company Limited, has been recently introduced in Chinese primary health institutions, in Beijing (20) and Tianjin (21). It is targeted mainly for use by hospitals, community clinics, and patients with TB based on the Technical Guide for Tuberculosis Control in China. Early results show that these tools help to combine patient data from hospitals and communities and lead to improvements in management and services for both patients and medical staff. To maintain the operation of this system, the Institution for Tuberculosis Control and Prevention will regularly supervise and inspect the hospital and community clinics.

In Wuhan, both TCIS and e-PSS have been introduced to improve adherence and treatment outcomes among patients with TB. TCIS was the paper-based standard of care, used by community doctors. The Wuhan Institute for Tuberculosis Control established TCIS with a paper-based standard in 2008. The functions of TCIS

are recording diagnosis and treatment status, collecting medication adherence records, and recording routine community home visits. E-PSS (Figure 1) was introduced by the Wuhan Institute for Tuberculosis Control in 2021, as a new TB patient treatment support system administered through a website, mobile phone application, or WeChat (an instant messaging service). The e-PSS has four modules: patient referral to the TB hospital, tracing of patients in the community, patient treatment management, and health education. The patient management module can (1) assist community clinic doctors to manage and follow up patients with TB *via* mobile phone, (2) send reminder alerts of medication and follow-up visits to community doctors and patients, and (3) allow hospital doctors to get feedback from patients and monitor patients' medication status in real time. In our study, we considered TCIS to manage patients with TB diagnosed before 2021 and e-PSS to manage patients with TB diagnosed after 2020.

Ethics approval

Permission for the study was sought from Hubei Provincial Center for Disease Control and Prevention and Wuhan Pulmonary Hospital, Wuhan, China. As this study involved analysis of routinely collected anonymous secondary data, the ethics committee waived the need for informed consent and formal ethics approval.

Study population

The study population included all patients with drug-sensitive pulmonary TB registered with TCIS and e-PSS residing in Wuhan, China. The study included all 17 districts in Wuhan with a TB program.

Indicators, sources of data, and data collection

The three indicators used to compare the performance of TCIS and e-PSS were the following: the proportion of patients in whom there was a first follow-up visit to the community doctor within 3 days of the start of treatment; medication adherence defined as 80% or more of prescribed monthly doses taken; and the percentage of end-of-treatment success in patients under e-PSS and TCIS.

All data were extracted from TCIS and e-PSS. The data for the e-PSS group were collected from January to March 2021, and the data for the TCIS group were retrospectively collected from July to September 2020. All records were consolidated into a single database in Excel with standardized fields.

Analysis plan

Descriptive statistics were used to describe the characteristics of the study population, using means to summarize continuous variables and percentages for categorical variables. The chi-square

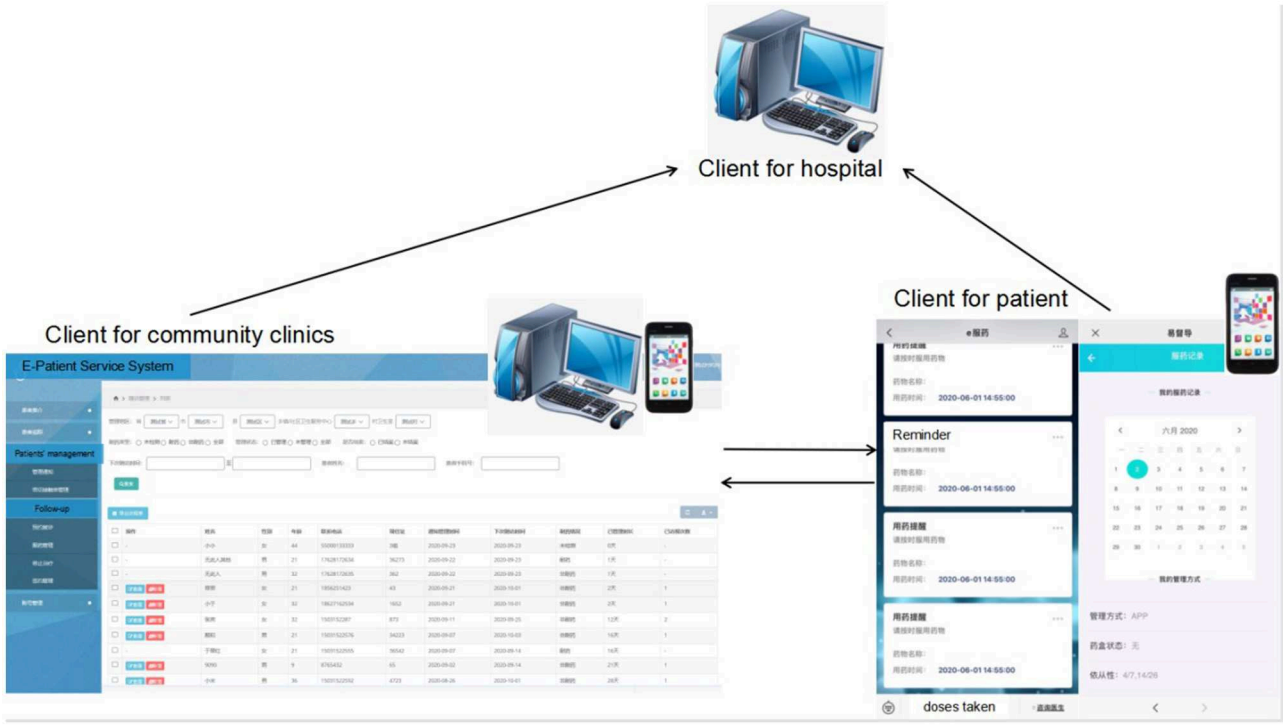

FIGURE 1
E-Patient Service System.

test was used to analyze categorical variables. Multivariate logistic regression analysis was also carried out to adjust for confounding factors. A p-value < 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 25.0. The forest plots were created using the forest plot package for R software version 4.1.1.

Results

A total of 1,576 patients with TB were recorded in TCIS between July and September 2020 in Wuhan, China, and 1,145 TB cases were included in e-PSS during the period from January to March 2021. Of these, the age group of 45–64 years was the largest in both systems, 32.49 and 33.36%, respectively, and the majority of cases were men (62.56 and 66.90%, respectively). The proportion of unemployed was higher in TCIS (37.82%) than in e-PSS (24.54%), whereas employed (22.14%) was lower than in e-PSS (26.64%). The majority of the participants were newly diagnosed patients with TB (95.24% in TCIS and 94.85% in e-PSS), and more than 49% were laboratory bacteriological positive. There were nearly 85% of cases without previous history and ∼7% with diabetes (Table 1).

During 6 months of follow-up of 1,576 patients in TCIS and 1,145 patients in e-PSS, there was a 19.15% increase in community doctors who visited patients within the first 3 days (from 29.76% in TCIS to 48.91% in e-PSS) (Table 2). There was a slightly lower percentage of patient months with good medication adherence (i.e., >80% of prescribed treatment doses taken) in the TCIS group compared with the e-PSS group (80.33 vs. 84.28%, respectively). Good medication adherence was higher in the e-PSS group than in the TCIS group for each of the 6 months of treatment (Figure 2).

After 6-month follow-up, 555 cases (35.22%) in TCIS and 169 cases (14.76%) in e-PSS were not evaluated. Using cases with known outcome status as the denominator, the percentages of favorable treatment outcomes (including cured and treatment completed) were 92.52% in the e-PSS group and 92.07% in the TCIS group ($P = 0.704$). A total of 41.92% were cured whereas 50.15% were categorized as treatment completed in TCIS as compared to e-PSS where 38.42% were cured and 54.10% were treatment completed. The proportions of unfavorable outcomes (including failure, death, lost to follow-up, transferred to RR/MDR-TB, and others) were 7.93 and 7.48% in TCIS and e-PSS, respectively (Table 3).

We retrospectively analyzed the association between independent variables (e-PSS/TCIS) and dependent variables (compliant first follow-up visit, good medication adherence, and favorable treatment outcome), after adjusting for covariates (age, sex, occupation, category of TB report, and type of TB). When comparing the e-PSS group with the TCIS group, a statistically significant association was observed with compliant first follow-up visit [adjusted OR: 2.94, (95% CI: 2.47–3.50); Figure 3] and good medication adherence [adjusted OR: 1.33, (1.08–1.63); Figure 4], while the association with favorable treatment outcome was not statistically significant [adjusted OR: 1.12 (0.79–1.57); Figure 5]. Patients with TB who had been previously treated were less likely to attend the first follow-up visit [adjusted OR: 0.64, (0.42–0.97)] and to have a favorable outcome (adjusted OR: 0.40, 95% CI: 0.23–0.72) than previously untreated patients. There was a 4% decrease in the odds of a favorable outcome (OR: 0.96, 95% CI: 0.94–0.97) for every 1-year increment in age.

TABLE 1 Patient demographics and clinical characteristics recording in TCIS and e-PSS during 2020–2021 in Wuhan.

Variables	TCIS ($n = 1,576$) N (%)	E-PSS ($N = 1,145$) N (%)	P-value
Age (yr)			0.324
0–14	14 (0.89)	6 (0.52)	
15–24	184 (11.68)	109 (9.52)	
25–44	501 (31.79)	369 (32.23)	
45–64	512 (32.49)	382 (33.36)	
>65	365 (23.16)	279 (24.37)	
Mean (SD)	47.89 (19.27)	48.92 (18.66)	
Gender			0.017
Male	986 (62.56)	766 (66.90)	
Female	590 (37.44)	379 (33.10)	
Occupation			<0.001
Farmer	166 (10.53)	144 (12.58)	
Employee	349 (22.14)	305 (26.64)	
Unemployed	596 (37.82)	281 (24.54)	
Retiree	234 (14.85)	239 (20.87)	
Others	231 (14.66)	176 (15.37)	
Type of TB			0.639
New case	1,501 (95.24)	1,086 (94.85)	
Previously treated	75 (4.76)	59 (5.15)	
Category of TB report			0.004
Positive	781 (49.56)	647 (56.51)	
Negative	742 (47.08)	461 (40.26)	
TB pleurisy	44 (2.79)	31 (2.71)	
Unknown	9 (0.57)	6 (0.52)	
Previous history			0.381
None	1,329 (84.33)	978 (85.41)	
Diabetes	114 (7.23)	91 (7.95)	
Silicosis	1 (0.06)	0 (0.00)	
Mental disease	2 (0.13)	2 (0.17)	
Other	130(8.25)	74 (6.46)	

Discussion

Our study showed that the use of e-PSS among patients with TB could support compliant first follow-up visits and enhance medication adherence levels compared with TCIS during the project period (2020–2021) in Wuhan, China.

TABLE 2 Status of community visit in TCIS and e-PSS during 2020–2021 in Wuhan, China.

Variables	TCIS ($n = 1,576$)	E-PSS ($n = 1,145$)	Percentage difference (%)	*P*-value
Compliant first follow-up visit (based on days)				<0.001
<4	469 (29.76)	560 (48.91)	19.15	
4–14	380 (24.11)	286 (24.98)	0.87	
>14	706 (44.80)	200 (17.47)	−27.33	
Not evaluated	21 (1.33)	99 (8.65)	7.31	

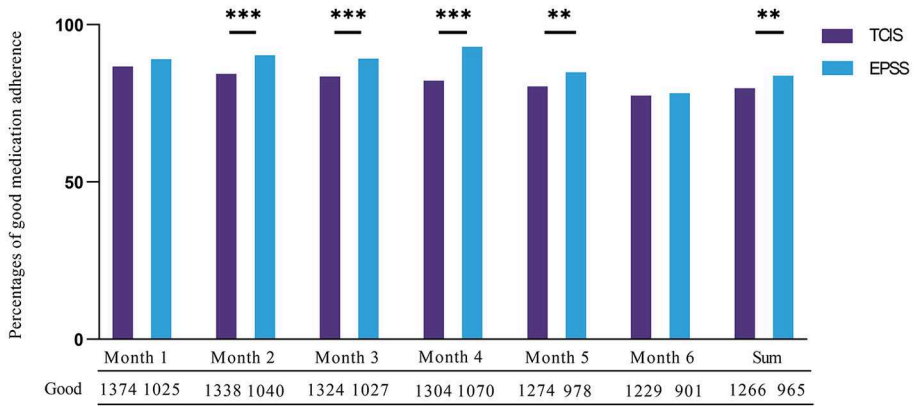

FIGURE 2
Medication adherence recorded in TCIS and e-PSS for 6 months. Good medication adherence defined as more than 80% of prescribed monthly doses taken. $^{**}P < 0.01$. $^{***}P < 0.001$. All the bar graphs were grouped by month; the bar graph on the left in each group depicted the data distribution of good medication adherence in TCIS, and the number of good medication adherence was denoted by the digit under each column. Conversely, the bar graph on the right depicted that in e-PSS.

TABLE 3 Treatment outcomes among patients with TB recorded in TCIS and e-PSS during 2020–2021 in Wuhan, China.

Treatment outcome*	TCIS ($n = 1,021$)	E-PSS ($n = 976$)	*P*-value
	N (%)	*N* (%)	
Favorable outcome	940 (92.07)	903 (92.52)	0.704
Cured	428 (41.92)	375 (38.42)	
Treatment completed	512 (50.15)	528 (54.10)	
Unfavorable outcome	81 (7.93)	73 (7.48)	
Failure	1 (0.10)	2 (0.20)	
Died	19 (1.86)	27 (2.77)	
Lost to follow up	9 (0.88)	4 (0.41)	
Transferred to RR/MDR-TB	8 (0.78)	2 (0.20)	
Other	44 (4.31)	38 (3.89)	

*There were 555 cases in TCIS and 169 cases in e-PSS without evaluation recorded.

The e-PSS played an important role in tracing and managing patients with TB in the community. A compliant first follow-up visit was more frequent in e-PSS compared to TCIS. In e-PSS, community doctors can receive patients' information in a timely fashion *via* short message service (SMS) and complete all the work of follow-up visits with a smartphone and computer. However, community doctors only used paper files to record follow-up information in TCIS and lack of reminders.

The results demonstrated that e-PSS (84.28%) improved medication adherence among patients with TB as compared with TCIS (80.33%) during the 6 months of TB treatment. For the medication adherence data in TCIS, the community doctors asked the patients about the status of missed or delayed medication for the past 10 days or 1 month through face-to-face or telephone interviews. Through the e-PSS, patients with TB could record their medication adherence daily by mobile phone, and the community doctors could check the medication data of all patients instantly. There is an intervention of daily medication reminders by sending SMS in real time, which can greatly improve patient medication adherence. A study available from Northwest Ethiopia reported that mobile phone-based weekly refilling with a daily medication reminder system improved adherence to patient-centered TB treatment and provider–patient relationship (11). Thus, good adherence is significantly associated with increased use of adherence strategies and digital interventions. Meanwhile, e-PSS is convenient for patients with TB to view or search for authoritative and practical knowledge, such as medical guidance on the diagnosis. It also supports bi-directional communication between patients with TB and doctors when adverse drug reactions and concerns arose during the treatment course by using mobile phone applications directly. Similar evidence from Thailand, the CARE-call system, a mobile-based system, was able to

Variable	Univariable analysis OR (95%CI)	Multivariable analysis OR (95%CI)	P
System			
TCIS	Ref	Ref	
EPSS	2.67(2.27~3.14)	2.94(2.47~3.50)	<0.001
Age (Year)			
0~	Ref	Ref	0.843
15~	1.2(0.43~3.35)	1.34(0.46~3.89)	0.592
25~	1.17(0.43~3.21)	1.45(0.50~4.18)	0.497
45~	1.26(0.46~3.43)	1.33(0.46~3.86)	0.603
65~	1.17(0.43~3.19)	1.25(0.43~3.67)	0.683
Gender			
Female	Ref	Ref	
Male	1.3(1.1~1.53)	1.18(0.99~1.42)	0.067
Occupation			
Farmer	Ref	Ref	0.000
Employee	0.21(0.15~0.28)	0.17(0.12~0.25)	<0.001
Unemployed	0.23(0.17~0.3)	0.24(0.18~0.33)	<0.001
Retiree	0.16(0.12~0.23)	0.14(0.10~0.20)	<0.001
Others	0.34(0.25~0.47)	0.33(0.23~0.47)	<0.001
Category of TB report			
Positive	Ref	Ref	0.601
Negative	0.9(0.76~1.05)	0.96(0.80~1.14)	0.604
TB pleurisy	1.05(0.65~1.68)	1.12(0.68~1.85)	0.659
Unknown	0.32(0.07~1.5)	0.39(0.08~1.90)	0.243
Type of TB			
New case	Ref	Ref	
Previously treated	0.67(0.46~0.98)	0.64(0.42~0.97)	0.036

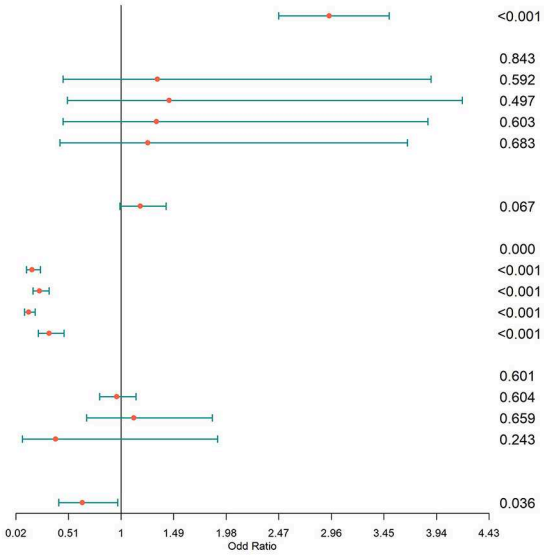

FIGURE 3
Multivariate logistic regression analysis on the factors associated with a compliant first follow-up visit. A compliant first follow-up visit, the first visit to the community doctor within 3 days of the start of treatment; OR, odds ratio; CI, confidence interval; model was adjusted for age, gender, occupation, category of TB report, and type of TB.

Variable	Univariable analysis OR (95%CI)	Multivariable analysis OR (95%CI)	P
System			
TCIS	Ref	Ref	
EPSS	1.32(1.08~1.61)	1.33(1.08~1.63)	0.006
Age (Year)			
0~	Ref	Ref	
15~	1.06(0.3~3.76)	0.94(0.26~3.40)	0.929
25~	0.89(0.26~3.08)	0.76(0.21~2.70)	0.670
45~	0.72(0.21~2.47)	0.51(0.14~1.81)	0.296
65~	0.72(0.21~2.49)	0.46(0.13~1.64)	0.232
Gender			
Female	Ref	Ref	
Male	0.9(0.73~1.11)	0.92(0.74~1.13)	0.419
Occupation			
Farmer	Ref	Ref	
Employee	0.89(0.62~1.27)	0.64(0.43~0.94)	0.025
Unemployed	0.88(0.63~1.24)	0.80(0.57~1.13)	0.212
Retiree	1.11(0.76~1.64)	1.18(0.80~1.75)	0.401
Others	0.88(0.6~1.3)	0.64(0.42~0.96)	0.034
Category of TB report			
Positive	Ref	Ref	
Negative	1.1(0.9~1.34)	1.11(0.91~1.36)	0.305
TB pleurisy	0.85(0.48~1.5)	0.85(0.48~1.51)	0.581
Unknown	1.5(0.34~6.66)	1.51(0.34~6.79)	0.593
Type of TB			
New case	Ref	Ref	
Previously treated	0.87(0.56~1.34)	0.97(0.62~1.51)	0.887

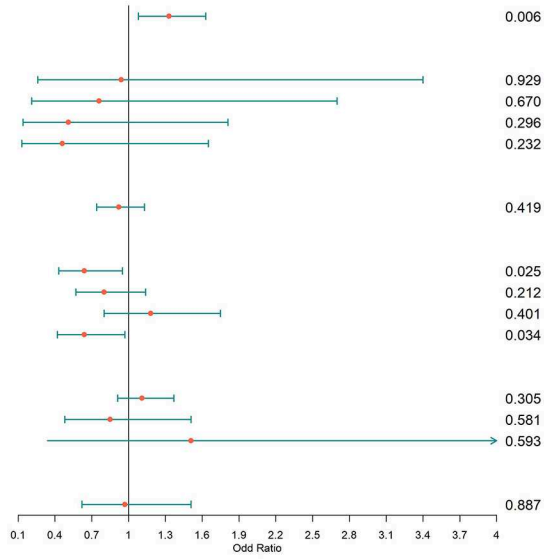

FIGURE 4
Multivariate logistic regression analysis on the factors associated with good medication adherence. Good medication adherence, more than 80% doses taken during 6 months; OR, odds ratio; CI, confidence interval; model was adjusted for age, gender, occupation, category of TB report, and type of TB.

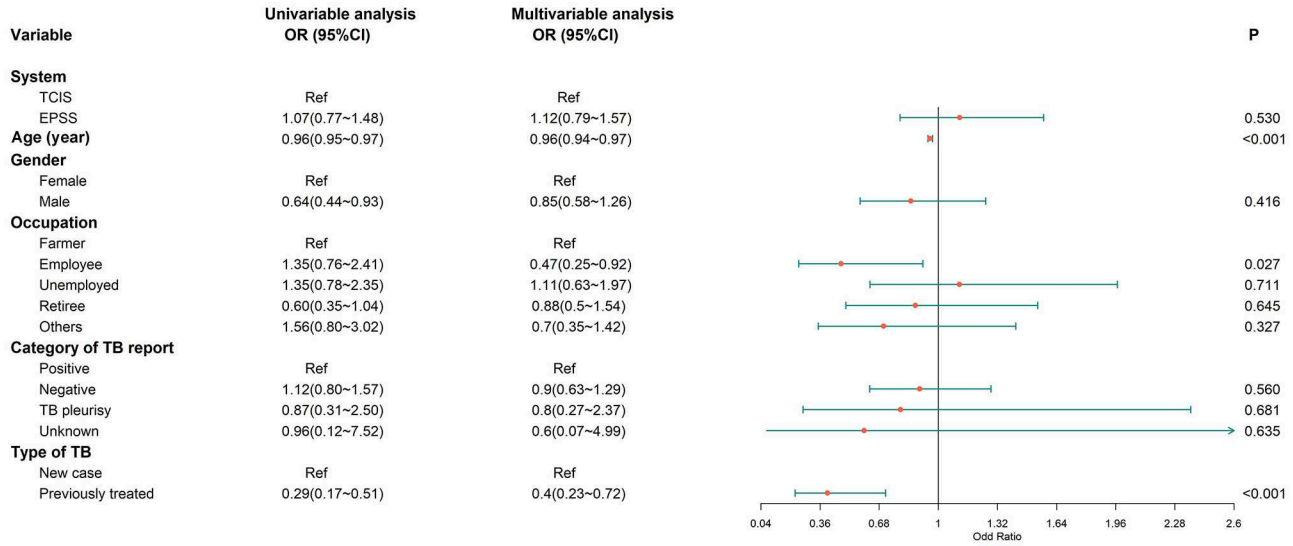

FIGURE 5
Multivariate logistic regression analysis on the factors associated with favorable treatment outcomes. Favorable treatment outcome, cured or treatment completed; OR, odds ratio; CI, confidence interval; model was adjusted for age, gender, occupation, category of TB report, and type of TB.

prevent non-adherence in this rural setting and was satisfied by participants (22).

A systematic review of digital interventions, including short message service (SMS), video-observed therapy (VOT), and medication monitors (MMs), to support treatment for active TB showed that the effect of digital technologies to improve TB care remains limited (10). Another study in Karnataka, India, showed that using daily treatment regimens with an innovative adherence support tool among HIV-infected TB did not improve successful treatment outcomes (23). We could not find a statistically significant association with favorable treatment outcomes, which might be due to incomplete data on treatment outcomes, and such an association should be further explored in similar future studies of this type.

These findings are important not only for better medication adherence and successful treatment outcome among patients with TB but also for preventing TB to spread in public. There were several limitations of this study. First, when the study could not assess all patients' treatment outcomes, both systems mainly facilitate patient management, and there are no strict requirements for the entry of treatment outcomes; however, the result shows that the data of treatment outcomes in e-PSS are more complete than in TCIS. Second, our study design cannot compare two system effects at the same time, since TCIS stopped running in 2021. Third, as of now, e-PSS has only been running for more than a year, and the doctors are not skilled in operation, thus, the functions of the new system cannot be fully demonstrated. But what is certain is that the efficacy of the new system will become more and more high quality over time. Finally, because hospital follow-up function in e-PSS was not yet available in Wuhan, the data of hospital follow-up have not been involved in this study, and further operational research will be needed.

To the best of our knowledge, this is the first study that evaluates how digitizing data management influences TB medication adherence and patient outcomes. The digital approach we describe from Wuhan, China, in 2020–2021 improved follow-up visit attendance and medication adherence. We showed that the monitoring of TB treatment in the community using mobiles and cloud-based computing is feasible, and, in contrast to the current paper-based system, allows for access to real-time information and more timely action. We propose that such a system be more widely deployed and its effect on TB treatment efficiency and effectiveness be studied further.

Data availability statement

The datasets presented in this article are not readily available because we need to ensure privacy of Chinese citizens' information is under protection. Requests to access the datasets should be directed to zhangmengxian1990@163.com.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

MZ and GW designed and implementation of the research, completed the analysis, and drafted the manuscript. HN and AY provided expertise with the data and

edited the manuscript. TL and YiX provided guidance with regards to theory. YeX provided raw data from the system. JY, SH, LZ, and YL supervised the project, critically reviewed, and approved the final version of the manuscript.

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Conflict of interest

YeX was employed by the company Beijing SINOVO Power Technology LTD., Beijing, China.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Chakaya J, Petersen E, Nantanda R, Mungai BN, Migliori GB, Amanullah F, et al. The WHO global tuberculosis 2021 REport–not so good news and turning the tide back to end TB. *Int J Infect Dis.* (2022) 124:S26–9. doi: 10.1016/j.ijid.2022.03.011

2. *World Health Organization*. (2022). Available online eat: https://www.who.int/publications/m/item/global-tuberculosis-report-2022-factsheet (accessed October 27, 2022).

3. Imperial MZ, Nahid P, Phillips PP, Davies GR, Fielding K, Hanna D, et al. A patient-level pooled analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis. *Nat Med.* (2018) 24:1708–15. doi: 10.1038/s41591-018-0224-2

4. Organization WJGWH. *Guidelines for Treatment of Drug-Susceptible Tuberculosis and Patient Care* (2017).

5. Hou W, Song F, Zhang N, Dong X, Cao S, Yin X, et al. Implementation and community involvement in DOTS strategy: a systematic review of studies in China. *Int J Tuberc Lung Dis.* (2012) 16:1433–40. doi: 10.5588/ijtld.12.0080

6. Wang X, Liu J, Wang J, Wu T, Zhan S-y. Systematic review of directly observed therapy on tuberculosis control in China. (2006) 27:63–7.

7. Alipanah N, Jarlsberg L, Miller C, Linh NN, Falzon D, Jaramillo E, et al. Adherence interventions and outcomes of tuberculosis treatment: a systematic review and meta-analysis of trials and observational studies. *PLoS Med.* (2018) 15:e1002595. doi: 10.1371/journal.pmed.1002595

8. Tola HH, Shojaeizadeh D, Tol A, Garmaroudi G, Yekaninejad MS, Kebede A, et al. Psychological and educational intervention to improve tuberculosis treatment adherence in Ethiopia based on health belief model: a cluster randomized control trial. *PLoS ONE.* (2016) 11:e0155147. doi: 10.1371/journal.pone.0155147

9. Nsengiyumva NP, Mappin-Kasirer B, Oxlade O, Bastos M, Trajman A, Falzon D, et al. Evaluating the potential costs and impact of digital health technologies for tuberculosis treatment support. *Eur Respir J.* (2018) 52:1801363. doi: 10.1183/13993003.01363-2018

10. Ngwatu BK, Nsengiyumva NP, Oxlade O, Mappin-Kasirer B, Nguyen NL, Jaramillo E, et al. The impact of digital health technologies on tuberculosis treatment: a systematic review. *Eur Respir J.* (2018) 51:1701596. doi: 10.1183/13993003.01596-2017

11. Gashu KD, Gelaye KA, Lester R, Tilahun BJBH, Informatics C. Effect of a phone reminder system on patient-centered tuberculosis treatment adherence among adults in Northwest Ethiopia: a randomised controlled trial. *BMJ Health Care Inform.* (2021) 28:e100268. doi: 10.1136/bmjhci-2020-100268

12. Liu Q, Abba K, Alejandria MM, Sinclair D, Balanag VM, Lansang MAD. Reminder systems to improve patient adherence to tuberculosis clinic appointments for diagnosis and treatment. *Cochrane Database Syst Rev.* (2014) 2014:CD006594. doi: 10.1002/14651858.CD006594.pub3

13. Garfein RS. Doshi RP. Synchronous and asynchronous video observed therapy (VOT) for tuberculosis treatment adherence monitoring and support. *J Clin Tuberc Other Mycobact Dis.* (2019) 17:100098. doi: 10.1016/j.jctube.2019.100098

14. Ravenscroft L, Kettle S, Persian R, Ruda S, Severin L, Doltu S, et al. Video-observed therapy and medication adherence for tuberculosis patients: randomised controlled trial in Moldova. (2020) 56: 00493–00493. doi: 10.1183/13993003.00493-2020

15. Wang N, Guo L, Shewade HD, Thekkur P, Zhang H, Yuan Y-L, et al. Effect of using electronic medication monitors on tuberculosis treatment outcomes in China: a longitudinal ecological study. *Infect Dis Poverty.* (2021) 10:29. doi: 10.1186/s40249-021-00818-3

16. Liu X, Lewis JJ, Zhang H, Lu W, Zhang S, Zheng G, et al. Effectiveness of electronic reminders to improve medication adherence in tuberculosis patients: a cluster-randomised trial. *PLoS Med.* (2015) 12:e1001876. doi: 10.1371/journal.pmed.1001876

17. Wuhan Municipal Statistics Bureau, Team SSWI. *Wuhan Statistical Yearbook 2020.* China Statistics Press (2020). p. 9.

18. Huang F, Cheng S, Du X, Chen W, Scano F, Falzon D, et al. Electronic recording and reporting system for tuberculosis in China: experience and opportunities. *J Am Med Inform Assoc.* (2014) 21:938–41. doi: 10.1136/amiajnl-2013-002001

19. Li T, Chen W, Zhao Y, Wang L, Chen M, Du X, et al. Underreporting of notifiable pulmonary tuberculosis cases to the national tuberculosis information management system—China. (2015) *China CDC Wkly.* (2020) 2:185. doi: 10.46234/ccdcw2020.049

20. Xie Y, Du J, Luo P, Wu Y, Zhang K, Yang CJ. CJA. Preliminary report of a mobile supervision application used for treatment management in patients with tuberculosis in Tongzhou District, Beijing in 2016. *Chin Jour Of Antituberculosis.* (2017) 39:708–12.

21. Jian D, Yu-hong L, Shu-yu MJ. Strengthen the supervision and management for tuberculosis patients with mobile internet technology. (2016) 5:23.

22. Ratchakit-Nedsuwan R, Nedsuwan S, Sawadna V, Chaiyasirinroje B, Bupachat S, Ngamwithayapong-Yanai J, et al. Ensuring tuberculosis treatment adherence with a mobile-based CARE-call system in Thailand: a pilot study. *Infect Dis.* (2020) 52:121–9. doi: 10.1080/23744235.2019.1688862

23. Thekkur P, Kumar AM, Chinnakali P, Selvaraju S, Bairy R, Singh AR, et al. Outcomes and implementation challenges of using daily treatment regimens with an innovative adherence support tool among HIV-infected tuberculosis patients in Karnataka, India: a mixed-methods study. *Glob Health Action.* (2019) 12:1568826. doi: 10.1080/16549716.2019.1568826

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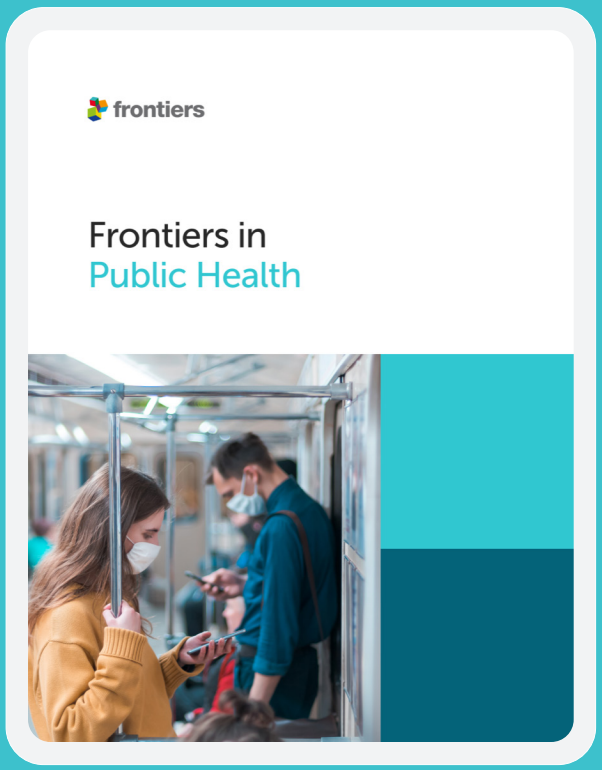

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