Prevalence and risk factors of pulmonary nontuberculous mycobacterial infections in the Zhejiang Province of China

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Abstract
Risk factors and prevalence of pulmonary nontuberculous mycobacterial (NTM) diseases were retrospectively evaluated in 1208 suspected pulmonary TB patients seeking care at the Affiliated Hospital of Hangzhou Normal University between July 2018 and December 2018. Further analysis of 390 culture-positive cases demonstrated that 358 (358/390, 91.8%) were infected with Mycobacterium tuberculosis (MTB), 24 (24/390, 6.2%) with NTM and eight (8/390, 2.0%) with both MTB and NTM. M. intracellulare was the most prevalent NTM isolated (16/24, 66.7%), followed by M. abscessus (3/24), M. kansasii (2/24), M. avium (1/24), M. szulgai (1/24) and M. fortuitum (1/24). The difference between NTM and TB case rates for the ≥65-year-old age group significantly exceeded the difference for the reference group (patients aged 25–44 years) (OR (95% CI): 6.30 (1.03–40.90)). Moreover, pulmonary NTM cases were significantly more likely to exhibit underlying bronchiectasis than pulmonary TB patients (OR (95% CI): 18.89 (7.54–47.88)). In conclusion, approximately one-tenth of culture-positive suspected pulmonary TB patients are infected with NTM (most frequently M. intracellulare) in Zhejiang Province, China. The elderly and those with bronchiectasis or a history of TB are at the greatest risk of contracting pulmonary NTM disease.

Introduction
Nontuberculous mycobacteria (NTM) represent a group of opportunistic human pathogens that are normal inhabitants of the environment, especially of the human-engineered environment [1, 2]. A worldwide increase in the prevalence of human NTM infections has been documented as population-based data in recent decades [3, 4]. According to a recent report, the annual incidence of NTM infection increased from 6.0% to 19% in East Asians from 2008 to 2016 [5]. NTM are important causes of pulmonary and extrapulmonary diseases in at-risk populations, such as immunocompromised hosts, the elderly and in patients with cystic fibrosis [6]. Despite the controversy regarding the occurrence of person-to-person spread of NTM-related diseases, recent molecular epidemiological data suggest that pulmonary NTM infections are mainly acquired through transmission of bacteria via fomites and aerosols [7, 8], highlighting the need for public health agencies to dedicate additional resources to the control of pulmonary NTM disease transmission.

Recently, increasing numbers of reports from diverse countries and regions have demonstrated that the relative proportions of NTM species isolated from clinical samples differ significantly by region [9]. For example, the Mycobacterium avium complex (MAC) has been predominantly detected in patient isolates from North America, East Asia and northern Europe [9–11], whereas M. kansasii and M. xenopi have been more frequently isolated within southern Europe [9]. Meanwhile, a recent cross-sectional study has revealed that NTM prevalence and distribution vary greatly across China [12]. M. intracellulare, a member of MAC, is predominately isolated in eastern China, whilst M. abscessus is predominant in southern China [12]. In view of intrinsic differences in pathogenicity and drug susceptibility profiles among various NTM species, understanding this diversity is necessary to guide clinical treatment [13]. Unfortunately, NTM has not been earmarked as an infectious disease priority worldwide, especially in tuberculosis (TB)-endemic settings [14].

The prevalence of NTM infection is rising in China and currently accounts for approximate one-quarter of mycobacterial isolates according to national population-based data [15, 16]. Despite this growing challenge, reporting of NTM disease to public health authorities is not required in China. As a consequence, only limited studies have been conducted to assess temporal changes in the incidence and prevalence of NTM diseases in this county. To address this concern, we carried out a retrospective study in Hangzhou, located in the eastern coastal
region of China. Our objective was to investigate the prevalence of pulmonary NTM diseases among individuals with suspected TB. We also aimed to determine risk factors associated with NTM infections in the population within this region.

Materials and methods

Study design and population

This study was conducted at the Affiliated Hospital of Hangzhou Normal University, a 1500-bed general hospital in Hangzhou, China. We retrospectively reviewed the medical records of individuals with suspected TB seeking care at the hospital between July 2018 and December 2018. Patients presenting with pulmonary TB symptoms who were presumed to have TB disease caused by Mycobacterium tuberculosis (MTB) were each asked to provide two sputum samples for laboratory testing. Routine tests included microscopic examination, mycobacterial culture and/or molecular diagnostic testing. Patients with positive mycobacterial culture test results were recruited for further analysis. This study was approved by the Ethics Committee of the Affiliated Hospital of Hangzhou Normal University.

Laboratory examinations

Microscopy analysis of Ziehl-Neelsen (Z-N)-stained direct smears of sputum samples was conducted based on National Tuberculosis Control Programme guidelines developed in China [17]. Then 1 mL of sputum was analysed using the GeneXpert MTB/RIF (Xpert) assay (Cepheid, Sunnyvale, CA, USA) following the manufacturer’s instructions [18]. The remainder of each sputum specimen was decontaminated via the N-acetyl-L-cysteine-NaOH method using the standard recommended final 1% NaOH concentration for 15 min. After decontamination, all specimens were neutralized with phosphate-buffered saline (PBS, pH = 6.8) and centrifuged at 4000 g for 15 min. Pellets were resuspended in 2 mL of PBS then 0.5 mL of each decontaminated specimen was inoculated into a mycobacteria growth indicator tube (MGIT) supplemented with oleic acid, albumin, dextrose and catalase (OADC) and polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin (PANTA) (BD Diagnostic Systems, Sparks, MD, USA) [19]. Cultures were assessed for contamination with other pathogens via observation of colony morphologies and via Z-N staining followed by microscopic examination. An immunochemistry-based MPT64 antigen detection test (Genesis, Hangzhou, China) was performed to identify MTB-positive cultures [20]. MTB-positive cultures were subjected to phenotypic drug susceptibility testing (DST) using the proportional method, as previously reported [21].

Species identification

Crude genomic DNA was extracted from positive cultures grown in MGIT tubes [19]. Briefly, an aliquot (500 µL) of sample was pipetted from the bottom of each positive MGIT culture. Next, each sample was centrifuged at 12 000 × g for 15 min then the bacterial pellet was resuspended in 500 µL of Tris-EDTA (TE) buffer. After incubation at 95 °C for 30 min in a water bath, each supernatant was used as the DNA template for species identification. A DNA fragment of the 16S rRNA gene (containing a region of sequence differences between NTM and MTB) was first amplified to distinguish between MTB and NTM. For NTM isolates, multilocus sequence analysis was performed to classify bacterial isolates into various subspecies [16]. Information on the primers is listed in Table S1. Briefly, the 50 µL polymerase chain reaction (PCR) mixtures contained 25 µL 2 × PCR mixture (TransGen Biotech, Beijing, China), 0.2 µM of each primer set and 5 µL DNA template. The PCR was performed under conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 2 min, and final extension at 72 °C for 10 min. For mixed infections, clones were plated to allow the growth of individual colonies on Middlebrook 7H11 agar medium plates (Difco Laboratories, Detroit, MI, USA) then bacteria lifted from individual colonies were cultured at 37 °C before species identification was conducted via multilocus sequence analysis. Amplicons were sent to Tsingke Company (Beijing, China) for DNA sequencing. Nucleotide sequences were aligned to homologous sequences of reference mycobacteria using the online BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST).

Definitions

The definition of pulmonary NTM disease met criteria established by the American Thoracic Society (ATS) in 2007: (i) clinical symptoms and abnormal chest radiograph suggestive of pulmonary TB; (ii) isolation of the same NTM species from two sputum specimens and (iii) exclusion of other differential diagnoses [22]. The definition of mixed infections with MTB and NTM met the criteria as follows: (i) clinical symptoms and abnormal chest radiograph suggestive of pulmonary TB and (ii) the presence of both MTB and NTM in each sputum sample obtained from each of two separate sputum specimens.

Statistical analysis

Demographic and clinical characteristics were collected from electronic medical records and included gender, age, ethnicity, place of residence, previous TB episode(s) and comorbidities. All collected data were entered into Epi Data version 3.1 (EpiData Association, Odense, Denmark). Careful data entry was carried out and data was cross-checked by two independent researchers to ensure data quality. All variables were tested for association with pulmonary NTM diseases using univariate logistic analysis. Associations between NTM diseases and predictor variables were expressed using odds ratios (ORs) with a 95% confidence interval (95% CI). Differences were considered statistically significant if P < 0.05. All analyses were conducted using SPSS version 20.0 (SPSS Inc, Chicago, USA).

Results

Enrollment of patients

In total, 1208 individuals with suspected pulmonary TB who were retrospectively included in this study sought health care at the Affiliated Hospital of Hangzhou Normal University between July 2018 and December 2018. By reviewing smear, liquid culture and demographic data, 818 (67.7%) were excluded from further analysis, including 15 (1.2%) who failed to produce qualified sputum, 764 (63.2%) with smear-negative/culture-negative results and 11 (0.9%) with contaminated culture results. Ultimately a total of 390 (32.3%) individuals were enrolled in this study. Of these cases, 358 (358/390, 91.8%) were shown to be infected with.
with MTB, 24 (24/390, 6.2%) with NTM and eight (8/390, 2.0%) were coinfected with MTB and NTM (Fig. 1).

**Distribution of NTM species**

A total of 24 NTM isolates were identified by multilocus sequence analysis using online BLAST tools provided by NCBI. As shown in Table 1, 20 isolates (83.3%) were classified as slowly growing mycobacteria, while the remaining 4 were rapidly growing mycobacteria. The most prevalent species was *M. intracellulare* (16 isolates), accounting for 66.7% of all NTM isolates in Hangzhou. *M. abscessus* (three isolates) was the second most frequently isolated species, followed by two *M. kansasii* isolates, one *M. avium* isolate, one *M. szulgai* isolate and one *M. fortuitum* isolate.

**Risk factors associated with NTM infections**

Comparisons of demographic and clinical characteristics between TB and NTM patients are summarized in Table 2. Using the group of patients aged 25–44 years as a reference, the per cent rate of NTM cases within the ≥65 years age group significantly exceeded that of TB cases in that group (OR (95% CI): 4.63 (1.03–20.90), *P* = 0.03), while no significant differences in NTM vs. TB case per cent rates were noted for groups of patients aged <25 years and 45–64 years (*P* > 0.05). We also found that the prevalence of NTM infection was significantly higher in patients with history of TB disease than in those lacking TB history (OR (95% CI): 12.92 (3.24–31.82), *P* < 0.01). In addition, patients with pulmonary NTM diseases were significantly more likely to exhibit underlying bronchiectasis than were those with pulmonary TB diseases (OR (95% CI): 18.89 (7.54–47.88), *P* < 0.01). Meanwhile, no other differences were found based on documented gender, residence or other comorbidities between TB and NTM groups (*P* > 0.05).

**Patients coinfected with NTM and TB**

Here, we further analysed the demographic, clinical and laboratory test results of eight patients coinfected with MTB and NTM. Of these patients, the median age at initial diagnosis was 70 years (range 52–88 years) and 75.0% were male. Five patients (62.5%) were local residents and five patients (62.5%) had previous anti-TB treatment history. In addition, half (4/8) of patients exhibited bronchiectasis. Notably, all patient smears were positive for acid-fast bacilli and all were infected with RIF-susceptible MTB as determined from results of the Xpert assay. Further *in vitro* DST confirmed that seven cases (87.5%) were extensively drug-resistant TB cases. Species identification revealed that six cases were coinfected with MTB and *M. intracellulare*, one case was coinfected with MTB and *M. abscessus* and one case was coinfected with MTB and *M. kansasii* (Table 3).

**Discussion**

The increasing prevalence of pulmonary NTM disease is a neglected public health concern in China [16]. In this study, our data indicate that approximately one-tenth of suspected TB patients with positive mycobacterial culture results were infected with NTM, for an overall NTM prevalence of 6.2% among suspected pulmonary TB patients seeking medical care in Hangzhou. In several previous studies, NTM prevalence rates of various regions had been reported as follows: Shandong (1.6%), Jiangsu (3.4%), Shanghai (5.1%), Europe (6.6%) and Israel (10.8%) [23–27]. Such diverse results obtained in those studies indicate that prevalence of NTM disease may differ from one

![Fig. 1. Enrollment of patients in this study](https://doi.org/10.1017/S0950268819001626)
geographic region to another. In China, initial diagnosis and treatment of pulmonary TB is based on positive sputum smear results obtained using microscopic detection of mycobacteria, especially in resource-limited settings [28]. Given that NTM strains are intrinsically resistant to first-line anti-TB drugs, our findings highlight the urgent need to perform species-level identification of bacilli detected in smear-positive patient samples prior to initiating anti-TB treatment, especially in this coastal region of eastern China, a resource-limited setting. However, conventional biochemical methods require about one to two months to produce reliable results, which would delay treatment and thus would fail to meet clinical requirements for optimal TB care [29]. As molecular diagnostics may provide more rapid identification of MTB and NTM, these techniques should be evaluated for use in resource-limited settings. The ability to achieve bacterial identification in just one day after primary detection of acid-fast bacilli would greatly facilitate the delivery of swift and accurate treatment of MTB and NTM diseases.

Another interesting finding of this study is the occurrence of mixed infections with MTB and NTM among patients with presumptive TB, although coinfections were observed in only a small proportion of cases. This result aligns with the reported results obtained from surveys of TB clinics in Japan conducted by

| Classification | Species | Number of isolates (%) |
|----------------|---------|------------------------|
| Slowly growing mycobacteria (n = 20) | *M. intracellulare* | 16 (66.7) |
| | *M. avium* | 1 (4.2) |
| | *M. kansasii* | 2 (8.3) |
| | *M. szulgai* | 1 (4.2) |
| Rapidly growing mycobacteria (n = 4) | *M. abscessus* | 3 (12.5) |
| | *M. fortuitum* | 1 (4.2) |

Table 1. Demographic and clinical characteristics of mycobacteria infections in this study

| Characteristics | No. of pulmonary mycobacteria cases (%) | OR (95% CI) | P value |
|----------------|-----------------------------------------|-------------|---------|
| Gender         |                                         |             |         |
| Female         | 116 (32.4) | 9 (37.5) | 1.00 | – |
| Male           | 242 (67.6) | 15 (62.5) | 0.80 (0.34–1.88) | 0.61 |
| Age group (years) |                                     |             |         |
| <25            | 71 (19.8) | 0 (0.0) | 1.02 (0.99–1.06) | 0.50 |
| 25–44          | 84 (23.5) | 2 (8.3) | 1.00 | – |
| 45–64          | 76 (21.2) | 8 (33.3) | 4.42 (0.91–21.47) | 0.06 |
| ≥65            | 127 (35.5) | 14 (58.4) | 4.63 (1.03–20.90) | 0.03 |
| TB history     |                                         |             |         |
| No             | 310 (86.6) | 8 (33.3) | 1.00 | – |
| Yes            | 48 (13.4) | 16 (66.7) | 12.92 (3.24–31.82) | <0.01 |
| Population     |                                         |             |         |
| Residence      | 204 (57.0) | 13 (54.2) | 1.00 | – |
| Migration      | 154 (43.0) | 11 (45.8) | 1.12 (0.49–2.57) | 0.79 |
| Diabetes       |                                         |             |         |
| No             | 316 (88.3) | 21 (87.5) | 1.00 | – |
| Yes            | 42 (11.7) | 3 (12.5) | 1.08 (0.31–3.76) | 0.75 |
| Bronchiectasis |                                         |             |         |
| No             | 340 (95.0) | 12 (50.0) | 1.00 | – |
| Yes            | 18 (5.0) | 12 (50.0) | 18.89 (7.54–47.88) | <0.01 |
| COPD           |                                         |             |         |
| No             | 343 (95.8) | 21 (87.5) | 1.00 | – |
| Yes            | 15 (4.2) | 3 (12.5) | 3.27 (0.88–12.17) | 0.10 |
| Tumor          |                                         |             |         |
| No             | 346 (96.6) | 23 (95.8) | 1.00 | – |
| Yes            | 12 (3.4) | 1 (4.2) | 1.25 (0.16–10.07) | 0.58 |

COPD, chronic obstructive pulmonary disease; OR: odds ratio; 95% CI: 95% confidence interval.
Shigeto and colleagues demonstrating that NTM was a complication in only 1.2% of new registered TB cases there [30]. In contrast, a population-based study in the United States demonstrated that one in seven confirmed pulmonary TB patients were coinfected with NTM in 2006 [30]. Notably, in the latter study NTM were often isolated soon after initial TB diagnosis [30]. As it appears to be difficult to distinguish between coinfection at initial diagnosis and subsequent NTM infection during the follow-up period, the methodology used in that study may potentially explain the extremely high rate of NTM coinfection among active TB cases in the United States. Consequently, our findings also have important clinical implications, especially due to the fact that among our study population, coinfection with MTB and NTM is a major contributor of suspected extensively drug-resistant TB cases. However, current methods in widespread clinical use are unable to distinguish among multiple species of mycobacteria present within a single collected specimen. Fortunately, recent advances in next-generation sequencing (NGS) have ushered in new opportunities for identifying all bacterial pathogens within a sample [31]. Nevertheless, further studies are required to evaluate NGS performance in improving the resolution of bacterial species identification in specimens collected from patients coinfected with TB and NTM.

This study demonstrates that the predominant NTM species isolated from prospective TB patients in Zhejiang is *M. intracellulare*, which aligns with results of recent studies from Shanghai [12]. In contrast, *M. abscessus* was the most frequently isolated NTM from prospective TB patients in Guangdong [12]. We therefore hypothesize that geographical variations in the prevalence and specific characteristics of causative organisms of NTM infections may reflect environmental NTM diversity across China. In addition, here we identified several risk factors associated with NTM diseases. First, in line with findings of previous studies [32, 33], pulmonary NTM disease is more common among elderly people, which may result from decreased immune function in older patients [34]. Second, patients previously diagnosed with TB are at greater risk of pulmonary NTM disease. Although the exact reasons for this phenomenon remain unclear, we speculate that lung cavitations due to previous TB activity may provide an infection niche for NTM. Third, NTM infections occur more frequently in patients with bronchiectasis. Numerous previous reports have shown an association between this structural lung disease and pulmonary NTM infections [35, 36], with the appearance of bronchiectasis cited as an important cause of impaired mucociliary clearance of pathogens from the bronchial tree [36]. The lack of pathogen clearance subsequently increases the risk of NTM colonization within the respiratory tract, thereby contributing to the high prevalence of pulmonary NTM diseases in populations with underlying bronchiectasis.

Numerous weaknesses in this report warrant discussion here. First, this study only analysed mycobacterial isolates from one pilot study site in Hangzhou. Despite being the sole TB designated hospital in that region, the small sample size in this study likely weakens the statistical strength of our conclusions. Second, as HIV status is an important risk factor for NTM infections [34], all patients enrolled in this study were tested for HIV and were HIV-negative, probably due to low-HIV prevalence in Hangzhou. Thus, we could not evaluate the association between HIV status and NTM infection in this population. Third, although we isolated both MTB and NTM from coinfected patients, evaluation of the clinical relevance of these pulmonary NTM isolates is difficult without conducting long-term follow-up of coinfection cases. Future studies focusing on long-term outcomes will provide new insight into the clinical significance of concurrent isolation of NTM and MTB from patient respiratory tract specimens.

In conclusion, our data demonstrate that in the Zhejiang Province of China approximately one-tenth of patients with positive mycobacterial cultures are infected with NTM; of these cases, one-fourth are coinfected with MTB. *M. intracellulare* is the most frequently isolated NTM from suspected pulmonary TB patients seeking medical care in Zhejiang Province. Notably, elderly people and patients with previous TB and bronchiectasis are at greater risk of pulmonary NTM disease than other patient groups in this population. Ultimately, more attention should be paid to the clinical importance of these NTM isolates. This is particularly important in developing countries like China where TB and NTM diseases frequently coexist.
achieve more timely identification of MTB and NTM after primary detection of acid-fast bacilli in patient samples in order to improve patient care in NTM-endemic areas.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0950268819001626

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**Conflict of interest.** The authors declare that there is no conflict of interest regarding the publication of this paper.

**References**

1. Falkingham III JO (2009) Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *Journal of applied microbiology* 107, 356–367.
2. Falkingham III JO (2015) Environmental sources of nontuberculous mycobacteria. *Clinics in Chest Medicine* 36, 35–41.
3. Hoefsloot W et al. (2013) The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. *European Respiratory Journal* 42, 1604–1613.
4. Marras TK et al. (2007) Isolation prevalence of pulmonary nontuberculous mycobacteria in Ontario, 1997 2003. *Thorax* 62, 661–666.
5. Lee H et al. (2019) Epidemiology of nontuberculous mycobacterial infection, South Korea, 2007–2016. *Emerging Infectious Diseases* 25, 569–572.
6. Falkingham III JO (2011) Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. *Emerging Infectious Diseases* 17, 419–424.
7. Bryant JM et al. (2016) Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. *Science* 354, 751–757.
8. Nishiuchi Y, Iwamoto T and Maruyama F (2017) Infection sources of a common non-tuberculous mycobacterial pathogen, Mycobacterium avium complex. *Frontiers of Medicine* 4, 27.
9. Stout JE, Koh WJ and Yew WW (2016) Update on pulmonary disease due to non-tuberculous mycobacteria. *International Journal of Infectious Diseases* 45, 123–134.
10. Adjemian J et al. (2012) Prevalence of nontuberculous mycobacterial lung disease in U.S. Medicare beneficiaries. *American Journal of Respiratory and Critical Care Medicine* 185, 881–886.
11. Simons S et al. (2011) Nontuberculous mycobacteria in respiratory tract infections, Eastern Asia. *Emerging Infectious Diseases* 17, 343–349.
12. Pang Yet al. (2017) Diversity of nontuberculous mycobacteria in eastern and southern China: a cross-sectional study. *European Respiratory Journal* 49, 1601429.
13. Aksamit TR, Philley JV and Griffith DE (2014) Nontuberculous mycobacterial (NTM) lung disease: the top ten essentials. *Respiratory Medicine* 108, 417–425.
14. Rau R et al. (2016) Leveraging advances in tuberculosis diagnosis and treatment to address nontuberculous mycobacterial disease. *Emerging Infectious Diseases* 22, 365–369.
15. Wang L et al. (2014) Tuberculosis prevalence in China, 1990–2010; a longitudinal analysis of national survey data. *Lancet* 383, 2057–2064.
16. Zhang Z et al. (2015) Differences in risk factors and drug susceptibility between Mycobacterium avium and Mycobacterium intracellulare lung diseases in China. *International Journal of Antimicrobial Agents* 45, 491–495.
17. Xia H et al. (2013) Multicentre evaluation of Ziehl-Neelsen and light-emitting diode fluorescence microscopy in China. *International Journal of Tuberculosis and Lung Disease* 17, 107–112.
18. Tang P et al. (2019) Additional benefits of GeneXpert MTB/RIF assay for the detection of pulmonary tuberculosis patients with prior exposure to fluoroquinolones. *Infection and Drug Resistance* 12, 87–93.
19. Pang Y et al. (2014) Evaluation of the Xpert MTB/RIF assay in gastric lavage aspirates for diagnosis of smear-negative childhood pulmonary tuberculosis. *Pediatric Infectious Disease Journal* 33, 1047–1051.
20. Pang Y et al. (2016) Rapid diagnosis of MDR and XDR tuberculosis with the MeltPro TB assay in China. *Scientific Reports* 6, 25330.
21. Pang Y et al. (2012) Spoligotyping and drug resistance analysis of Mycobacterium tuberculosis strains from national survey in China. *PLoS One* 7, e32976.
22. Griffith DE et al. (2007) An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *American Journal of Respiratory and Critical Care Medicine* 175, 367–416.
23. Jing H et al. (2012) Prevalence of nontuberculous mycobacteria infection, China, 2004–2009. *Emerging Infectious Diseases* 18, 527–528.
24. Shao Y et al. (2015) The epidemiology and geographic distribution of nontuberculous mycobacteria clinical isolates from sputum samples in the eastern region of China. *PLoS Neglected Tropical Diseases* 9, e0003623.
25. Roux AL et al. (2009) Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. *Journal of Clinical Microbiology* 47, 4124–4128.
26. Wu J et al. (2014) Increase in nontuberculous mycobacteria isolated in Shanghai, China: results from a population-based study. *PLoS One* 9, e107363.
27. Levy I et al. (2008) Multicenter cross-sectional study of nontuberculous mycobacterial infections among cystic fibrosis patients, Israel. *Emerging Infectious Diseases* 14, 378–384.
28. Pang Y et al. (2016) An overview on tuberculosis-specific hospitals in China in 2009: results of a national survey. *Emerging Infectious Diseases* 47, 1584–1587.
29. Chimara E et al. (2008) Reliable identification of mycobacterial species by PCR-restriction enzyme analysis (PRA)-hsp65 in a reference laboratory and elaboration of a sequence-based extended algorithm of PRA-hsp65 patterns. *BMC Microbiology* 8, 48.
30. Kendall BA et al. (2010) Isolation of non-tuberculous mycobacteria from the sputum of patients with active tuberculosis. *International Journal of Tuberculosis and Lung Disease* 14, 654–656.
31. Shokralla S et al. (2012) Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology* 21, 1794–1805.
32. Huang HL et al. (2017) Epidemiology and predictors of NTM pulmonary infection in Taiwan – a retrospective, five-year multicenter study. *Scientific Reports* 7, 16300.
33. Dailloux M et al. (2006) Respiratory infections associated with nontuberculous mycobacteria in non-HIV patients. *European Respiratory Journal* 28, 1211–1215.
34. Tan Y et al. (2018) Epidemiology of pulmonary disease due to nontuberculous mycobacteria in Southern China, 2013–2016. *BMC Pulmonary Medicine* 18, 168.
35. Winthrop KL et al. (2010) Pulmonary nontuberculous mycobacterial disease prevalence and clinical features: an emerging public health disease. *American Journal of Respiratory and Critical Care Medicine* 182, 977–982.
36. Chu H et al. (2014) Prevalence of nontuberculous mycobacteria in patients with bronchiectasis: a meta-analysis. *Archives of Medical Science* 10, 661–668.