

Non-Tuberculous Mycobacterial Pulmonary Disease Prevalence Status in China

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Research

Keywords: NTM, Pulmonary Disease, Prevalence, Drug Resistance

Posted Date: July 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-47871/v1>

Abstract

Introduction: The information on the pulmonary disease caused by non-tuberculosis mycobacterium (NTM) in China is limited, mainly based on local or regional studies.

Methods: Retrospective study involving 72 tuberculosis clusters provided sputa of enrolled presumptive cases for mycobacterial and drug-susceptibility testing in 31 provinces of Chinese mainland. Minimal inhibitory concentrations (MICs) test was used to measure the susceptibility of NTM isolates.

Results: Of 4917 mycobacterial isolates cultured, 317 (6.4%, 95% confidence interval [CI], 5.8 to 7.2) isolates were confirmed as NTM, among 207 (12.1%, 95% CI, 10.6 to 13.8) isolates were detected from the southeastern region with the highest NTM prevalence. Slow growing mycobacteria (SGM) contributed 93.3% (95% CI, 76.4 to 98.8) and 56.1% (95% CI, 50.1 to 61.9) in northern and southern China, respectively. A total of 29 species were detected, the three most frequently isolated NTM belonged to *Mycobacterium abscessus* complex (36.0%, 95% CI, 33.3 to 38.7), *Mycobacterium avium-intracellulare* complex (34.1%, 95% CI, 29.1 to 39.5), *Mycobacterium kansasii* (9.8%, 95% CI, 8.1 to 11.4), respectively. Clarithromycin and amikacin were showed lower resistant rates to NTM in vitro.

Conclusions: The southeastern region should be paid more attention to NTM pulmonary disease. More rapid growing mycobacteria (RGM) were present in southern China than the northern ($P < 0.001$). Considering clear correlations between in vitro activity and the in vivo outcomes of treatment, macrolides and amikacin were recommended in NTM treatment in China.

Introduction

NTM pulmonary disease (NTM-PD) is a severe progressive illness caused by nontuberculous mycobacteria (NTM) and its treatment is more complicated, requiring multiple anti-mycobacterial drugs for more than 12 months [1]. An increasing incidence of pulmonary NTM isolations has been reported in several studies following the development of socioeconomic standard and medical level [2]. The emergence of NTM-PD may also associate with an increase of aging population with chronic lung diseases or an increase of immunocompromised population and advance in radiological diagnostics that have improved the detection rate of pulmonary abnormalities. The mortality of NTM caused pulmonary disease was higher than *Mycobacterium tuberculosis* (MTB) due to inappropriate treatment and high treatment failure [3]. Thus, the information on prevalence of NTM associated disease in China is limited.

The limitations on studies of the epidemiology of pulmonary NTM infection attribute to several ways. First, specimen contamination should be concerned that NTM are widespread in the environment, and diagnosis of NTM disease is complex [4]. Second, the imbalance development of medical and public health communities in different regions leads to discrepant concern about NTM infection in China. Third, NTM disease is not compulsory to report according to the infectious disease control policy in China. Although several studies of NTM epidemiology of China have been reported, these NTM isolates that mostly obtained from medical institutions consist of incomplete samples [3, 5]. Therefore, precise incidence and prevalence data is not available.

The diagnosis of NTM infections need identification of mycobacteria to the species level, as most NTM inherently resistant to the standard anti-tuberculosis drugs and show different resistant phenotype among different species [6]. Drug susceptibility tests (DST) for antimicrobial agents are essential in the treatment of infections, and they play an important role in guiding clinical treatment. In addition, some acid fast bacteria that is not mycobacteria, such as *Gordonia* and *Nocardia*, shows similar colonial morphology on Lowenstein-Jensen (L-J) media. Therefore, an accurate and rapidly method used for NTM identification is particularly important. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), a technology with low time consumption, low cost and high accuracy, has been used for identification of mycobacterium species in some clinical laboratory [7, 8]. In this study, we used MALDI-TOF MS for screening NTM from 5203 cultured clinical strains isolated from sputum of acid-fast. The technique obtained a high NTM detection rate (98.4%, 312/317) and also achieved up to 93.4% (296/317) agreement with 16S rRNA, *hsp65*, ITS and *rpob* genes sequencing. We measured the minimal inhibitory concentrations (MICs) for RGM and SGM of 317 NTM isolates to identify the susceptibility in different antimicrobial drugs recommended by United States Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). MIC50 and MIC90 values were derived from the MIC distribution.

Material And Methods

Study Subjects:

A total of 5487 isolates that isolated from acid-fast bacilli (AFB)-positive tuberculous suspects were collected from 72 stations of nationwide surveillance of drug-resistant tuberculosis in 2013 as shown in Fig.S1. The strains were collected from 72 centers around the country with at least one site in each of the 31 provinces and municipalities of China. The number of centers assigned to each province and municipality was proportional to the number of new smear-positive cases reported by that province relative to the total number of cases nationwide previously [9].

Methods:

We cultivated all the strains using L-J medium and 284 isolates were recovered failure. All the cultured isolates were identified by MALDI-TOF MS, 286 strains were not mycobacteria. We obtained 317 NTM isolates from 4917 mycobacterium strains according to the MALDI biotype results. Then we confirmed the NTM species by sequencing of partial genes (including 16S rRNA, *hsp65*, *rpoB*, and ITS), 5 strains were identified as *Mycobacterium tuberculosis* complex (MTBC). Another 5 strains that identified as MTBC by MALDI-TOF MS were classified as NTM based on whole gene sequencing in another ongoing research programme. Finally, we identified 317 NTM strains from 31 provinces in China as showed in Fig. S1.

DSTs were performed at the national tuberculosis reference laboratory of China using broth microdilution method. A panel of 13 drugs using the Sensititre® SLOMYCO plates for SGM and a panel of 15 drugs using the Sensititre® RIPMYCOI plates for RGM (TREK Diagnostic Systems, Cleveland, USA) were used to drug resistant test. During the process of experiments, 0.5 McFarland suspensions of bacteria was prepared by Ultrasonic Milling Instrument (TB Healthcare, China) from colonies grown on L-J culture medium. Suspensions were diluted 100-fold with the addition of 100 μ L of the 0.5 Mc suspensions to 10 mL of Mueller-Hinton broth with/without OADC. Aliquots of 100 μ L of standard 1.5×10^5 CFU/mL inoculum were distributed to each well by the semi-automated Sensititre TM Auto-inoculator (Thermo Fisher, Scientific Inc., USA). To prevent evaporation and skipped wells during incubation, plates were sealed with the adhesive membranes accurately and adequately and incubated at recommended temperature[10]. MIC is defined as the lowest concentration that without obvious visible bacteria growth compared to positive controls, which was measured by two readers respectively, aided by VizionTM Digital viewing system. *M. abscessus* (ATCC 19977) and *M. intracellulare* (ATCC 13950) were used as quality control in every batch of drug susceptibility testing.

Statistical Analysis:

The 95% confidence interval for a proportion was calculated according to the Wilson procedure with a correction for continuity described by Robert Newcombe, derived from a procedure outlined by E. B. Wilson [11]. The differences were evaluated by two-tailed Fisher's exact test, and $P < 0.01$ was defined as statistically significant. The breakpoint used to distinguish resistance and susceptibility to antibiotics was suggested by CLSI M24-A3 and M62, as showed in Table 3-7. Additionally, MIC50 and MIC90 values were derived from the MIC distribution.

Results

The Prevalence of Pulmonary NTM diseases

As shown in Table 1, of 317 NTM strains, among 287 (7.7%, 95% CI, 6.9 to 8.7) isolates were detected from the southern region. The NTM infection rate in southern region is higher than in northern region (7.7% vs. 2.5%, $P < 0.01$). In another stratified analysis, the prevalence of NTM infection varied according to geographic areas (Table 1): the highest prevalence was 12.1% (95% CI, 10.6 to 13.8) for southeastern region, 4.4% (95% CI, 3.59 to 5.7) for middle region, 3.1% (95% CI, 2.1 to 4.5) for southwestern region, 2.2% (95% CI, 1.3-3.7) for northeastern region, 1.8% (95% CI, 0.5 to 5.5) for northwest region. The prevalence rate in individual province was showed in Fig. 2 and the details showed in File S1. The composition ratio of rapid growing mycobacteria (RGM) and slow growing mycobacteria (SGM) in northern and southern China is quite different. SGM contributed 93.3% (n/N, 28/30; 95% CI, 76.5 to 98.8) and 56.1% (n/N, 161/287; 95% CI, 50.1 to 61.9) of all the NTM isolates in northern and southern China, respectively. More rapid growing mycobacteria (RGM) were present in southern China than the northern ($P < 0.01$).

Spectrum of different NTM species

A total of 29 species were detected, including 19 SGM and 10 RGM (Table 2), 27 species were detected in southern China and only 7 species were observed in northern China. The five most frequently isolated NTM that accounted for 88.5% of all the NTM species belonged to *M. abscessus* complex (MABC) (36.0%, 95% CI, 30.7 to 41.5), *M. avium-intracellulare* complex (34.1%, 95% CI, 28.9 to 39.6), *M. kansasii* (9.8%, 95% CI, 6.8 to 13.7), *M. paragordoniae* (5.4%, 95% CI, 3.3 to 8.6) and *M. lentiflavum* (3.2%, 95% CI, 1.6 to 5.9) species,

respectively. *M. avium-intracellulare* complex include 7 subspecies: *M. avium* subsp. *hominissuis* (1 isolate), *M. avium* subsp. *vulneris* (2 isolates), *M. avium* (2 isolates), *M. avium* subsp. *marseillense* (16 isolates), *M. intracellulare* (82 isolates), *M. intracellulare* subsp. *yongonense* (4 isolates) and *M. chimera* (1 isolate). *M. abscessus* complex include 3 subspecies: *M. abscessus* (67 isolates), *M. bolletii* (2 isolates), *M. massiliense* (45 isolates). Then we analyzed the geographical distribution of three most frequently NTM species, 114 *M. abscessus* complex isolates were obtained from 6 provinces and 113 strains were distributed in southern China, especially, 93 isolates are located in Guangdong province. 108 *M. avium-intracellulare* complex isolates were observed in 16 provinces (all 318 isolates obtained from 21 provinces) with no obvious regional difference. Although only 31 *M. kansasii* were obtained from 11 provinces, the regional distribution is wide. More detail information of NTM species in different province of China was showed in File S2 and File S3.

Drug susceptibility testing results

Depending on the MIC values obtained, RGM were classified as susceptible, intermediate, or resistant according to the CLSI criteria [12]. The distribution of the corresponding degrees of susceptibility, including MIC50/MIC90 values are shown in Table 3. Graphs of the cumulative distributions of MICs for each drug are shown in Fig.S2; details are provided in File S4. Amikacin was the most active drug for RGM. The resistance rate to amikacin was 4.69% (6/128). Resistance rate to cefoxitin in RGM was 18.90% (24/127). For clarithromycin, 14.96% (19/127) of RGM was resistant at both day 3 and day 14, which corresponded to acquired resistance to clarithromycin, while a total of 38.58% (49/127) of RGM was susceptible at day 3 but resistant at day 14, which corresponded to inducible resistance for clarithromycin. The resistance rate to linezolid was 34.65% for both RGM. Resistance rates to imipenem, tobramycin, doxycycline, cefepime, trimethoprim/sulfamethoxazole (TMP-SMX), minocycline, moxifloxacin, ciprofloxacin, ceftriaxone, and amoxicillin/clavulanic acid were high in both RGM. As showed in Table 4, we compared the drug susceptibility patterns of the major MABC species: *M. abscessus* and *M. massiliense*. A significant difference in clarithromycin inducible resistance rates (83.58% vs 11.11%) were observed between the two species.

We also obtained MIC data for 189 SGM isolates. MIC distributions and MIC50/MIC90 values are shown in Table 5 and Fig.S3. Following in current standard (CLSI M24, 3rd edition) [10] includes recommendations for antimycobacterial susceptibility testing (AST) of SGM, including *M. avium-intracellulare* complex, *M. kansasii* and *M. marinum*. Susceptibility of 108 MAC isolates to 13 antimicrobial agents was presented in Table 6 and Fig.S4. Clarithromycin with 4.67% (5/107) resistant rate was the most active drug for MAC. Resistant rate to amikacin was 10.28% (11/107). Clarithromycin and amikacin which recommended by CLSI as first line antimicrobials for *M. avium-intracellulare* complex showed excellent bacteriostatic effect in vitro. For second line antimicrobials linezolid and moxifloxacin, resistant rate of *M. avium-intracellulare* complex was 66.36% and 47.66%, respectively. Except rifabutin, for other drugs, isoniazid, rifampicin, ethambutol, ethionamide, streptomycin, doxycycline, ciprofloxacin and TMP-SMX showed higher than 50% drug resistant rate. Susceptibility of 31 *M. kansasii* isolates to 13 antimicrobial agents was presented in Table 7 and Fig.S5. Except ethambutol (83.87%), the resistant rate of all antimicrobials tested is lower in *M. kansasii* isolates than *M. avium-intracellulare* complex. No *M. kansasii* isolates were resistant to clarithromycin, amikacin and moxifloxacin. The rate of other first line and second line antimycobacterial drugs resistance is lower than 50%, besides doxycycline (77.42 %, 24/31) and TMP-SMX (51.61%, 16/31). All details of MIC values for each SGM are provided in File S5.

Discussion

It was shown in this study that there was 6.4% NTM pulmonary disease among all myco-bacteriological culture positive cases based on nationwide surveillance of drug-resistant tuberculosis. The pulmonary NTM infection was happened more frequently in southern China, especially, in southeastern regions coast areas with high humidity. The most prevalent SGM was *M. avium-intracellulare* complex which consist of seven subspecies, and the predominant subspecies is *M. intracellulare*. *M. intracellulare* was widely distributed in northern and southern China. The most prevalent RGM was *M. abscessus* complex which consist of three subspecies, and the predominant subspecies is *M. abscessus* that mainly distributed in southern China. The drug susceptibility testing results indicated that drug-resistant spectrum varied greatly in different strains subspecies. Macrolides and amikacin recommended to NTM treatment in China were showed lower resistant rates to NTM in vitro.

Distinguishing NTM from MTBC infection is of great significance in clinic, which can direct accurate and rapid clinical treatment [13–15]. The screening method used for NTM species are based on PNB which can inhibit growth of *M. tuberculosis* complex in most published paper in China [16], a method which is time-consuming and strenuous. The laboratory diagnosis methods used for mycobacterial species identification have evolved though the past decades [17]. With the development of several extraction methods that enhance the amount of bacterial proteins available for MALDI-TOF MS identification and the increasing number of mycobacterial spectra in commercial databases, MALDI-TOF MS technology has been implemented for NTM identification in many laboratories [7, 18]. Several researches

have proved this method achieved more than 95% agreement with the DNA sequencing of variable genomic regions (16S rRNA, hsp65, rpoB and ITS genes) [5, 19]. In our research, we obtained 98.4% NTM detection rate and achieved 93.4% agreement with 16S rRNA, hsp65, ITS and rpoB genes sequencing. Although we cannot identify the NTM strains that were not contained in the Bruker MBT strains database, MALDI-TOF MS is available to identify most clinically NTM in a rapid, reliable, and inexpensive manner.

The overall NTM pulmonary infection rate is about 6% in our study similar with a systematic review and meta-analysis of NTM infections which demonstrated that the prevalence of NTM infections among tuberculosis suspects was 6.3% in mainland China [20]. The geographic variability both in prevalence of NTM infections and in mycobacterial species composition is distinctly presented in our study. A research in southern-central China demonstrated that the NTM infection rate is 4.0%, and the two most prevalent species are *M. avium-intracellulare* complex and *M. chelonae-abscessus* Complex [6]. An article in Shanghai province proved that the overall rate of NTM isolated from mycobacterial culture-positive patients was 5.9% and the most frequently identified species is *M. kansasii*, with an increasing trend from 3.0% in 2008 to 8.5% in 2012 [21]. In our study, the most frequently species in Shanghai province is also *M. kansasii* (7/11), with an increasing NTM prevalence rate 11% in 2013. In another research from Guangdong and Shanghai province proved that *M. intracellulare* was the most commonly isolated NTM in Shanghai and the most frequently isolated species in Guangzhou is *M. abscessus* [22]. Some researches in eastern and northern region of China demonstrated the NTM prevalence rate is around 2.0%-3.0% and the predominated species is *M. intracellulare*, followed by *M. abscessus* [5, 21–23]. In our study, the NTM infection is more prevalent in southern than northern China and is more frequently in eastern than western China. The most epidemic NTM species are *M. avium-intracellulare* complex that is widely distributed and *M. abscessus* Complex which is mainly distributed in southeastern China. In addition, we isolated 16 *M. marseillense* strains of *M. avium-intracellulare* complex from sputum sample. Pulmonary disease caused by *M. marseillense* should be paid more attention as relevant report is less [24, 25].

Besides Mycobacteria spp., we identified some acid fast staining positive non-mycobacteria. As showed in Fig.S1, we randomly selected 60 non-mycobacteria from 286 polluted or other species for species identification, 15 Gordonia, 2 Nocardia, 2 Streptococcus and 1 Tsukamurella (File S6). The 15 Gordonia (8 G.sputi, 4 G. bronchialis and 3 G. rubripertincta) are distributed in 9 provinces. Interestingly, the occurrence of Gordonia is similar to some report in China [22]. Two Nocardia which often cause chronic lung diseases were isolated, as reported in the several article in China [23]. In addition, Tsukamurella infection case in Jiangxi province has previously been reported in in Southern-central China [6]. Besides NTM and MTBC infection, especially Gordonia and Nocardia species should be identified when using acid-fast staining to diagnosis pulmonary infection.

We evaluated the susceptibility of the RGM and SGM in China by measuring the MIC values of antimicrobials for bacteria using the RAPIDMYCOI and SLOWMYCOI Sensititre™ panel (Thermo Fisher Scientific, Waltham, MA, USA) according to CLSI protocol M24-A2. In China, no such simple MIC measurement commercial kit is available, despite the increase of patients with NTM infections, information on drug susceptibility of NTM isolates is still lacking. We mainly analyzed the susceptibility of the *M. abscessus* complex, which are the most common clinical RGM isolates. Inducible macrolide resistance affected differences of treatment outcome between *M. abscessus* and *M. massiliense*. The same with previous articles [26–28], we found that *M. abscessus* had a higher inducible resistance rate (65.67% vs 2.22%, $p < 0.001$) and acquired resistance rate (17.91% vs 8.89%, $p = 0.2841$) for clarithromycin than *M. massiliense*. These results further emphasized the importance of identification of subspecies for *M. abscessus* and *M. massiliense* to gain an accurate clinical treatment consequent by using different treatment strategies. Amikacin was the most active antimicrobial agent against *M. abscessus* complex species, showing a 94.74% overall susceptibility rate, a similar overall susceptible rate was observed in some researches from China or Australia [29, 30]. However, a higher resistant rate was observed in Japan and South Korea, from 28.2–76.0% [14, 31]. After amikacin, cefoxitin with a 16.67% resistant rate was the second most effective antimicrobial agent against *M. abscessus* complex, which is not the same with results in South Korea [31] where the second most effective antimicrobial agent is linezolid, but same with Japan [14]. The resistance rate to cefoxitin was higher in *M. abscessus* (19.40%) than in *M. massiliense* (11.11%). Linezolid with a resistant rate of 33.33% could be used as an alternative choice against RGM isolates. Considering a high resistant rate with the other drugs tested in our study, they may not be appropriate for *M. abscessus* complex, however, the clinical therapeutic effect need to be observed.

For SGM, we mainly analyzed the susceptibility of the *M. avium-intracellulare* complex and *M. kansasii*, the most two species in SGM. Consistent with previous studies[32], the macrolides and amikacin have shown excellent in vitro activity against MAC isolates with 90% susceptibility rates, as the first line therapeutic agent for lung diseases caused by *M. avium-intracellulare* complex. As we know, patients with *M. avium-intracellulare* complex pulmonary diseases are frequently administered a combination of clarithromycin, ethambutol, and rifampicin. A study suggests that treatment with clarithromycin and ethambutol is not inferior to treatment with clarithromycin, ethambutol, and rifampicin for *M. avium-intracellulare* complex lung disease [33]. We support the two treatment regimens as the resistant rates of ethambutol and rifampicin are 58.33% and 91.67% in vitro, respectively. Some researchers have reported differential drug

susceptibility patterns of *M. chimaera* and other members of the *M. avium-intracellulare* complex [34]. In our study, we only obtained one *M. chimaera* strain. We compared the drug susceptibility patterns of the most two species of *M. avium-intracellulare* complex, the results showed no significant difference between *M. intracellulare* and *M. marseillense*. As *M. marseillense* infections are rare in humans [24, 25, 35], the drug susceptibility can facilitate our knowledge for the species.

M. kansasii which is the second most species of SGM infection is showed a higher susceptibility rate to majority first and second line antibiotics recommended by CLSI (M24, 3rd Edition). The drug susceptibility patterns presented a huge difference with a previous study which used a total of 78 *M. kansasii* strains from 13 provinces of China for drug susceptibility testing [36]. Except ethambutol (83.87% vs 20.5%), the resistant rate in our study is lower than previous study, clarithromycin (0 vs 20.5%), amikacin (0 vs 5.1%), rifampicin (6.45% vs 56.4%), rifabutin (3.23% vs 34.6%), moxifloxacin (0 vs 16.7%) and linezolid (3.23% vs 32.1%). Our result is similar with a report which used 85 *M. kansasii* obtained from 8 countries in Europe and Asia [37]. All isolates tested in that study were susceptible to rifampicin, amikacin, rifabutin, moxifloxacin, and linezolid. Resistance to ethambutol, ciprofloxacin, and clarithromycin was found in 83 (97.7%), 17 (20%), and 1 (1.2%) isolate, respectively. The drug susceptibility patterns in other studies associated with *M. kansasii* infection presented incompatibility [38–40]. Although all 31 *M. kansasii* in our study were isolated from 13 provinces in China, more isolates should be tested to quality drug susceptibility patterns of *M. kansasii* considering less strain number and regional disparity.

In this study, we obtained the isolates from all the 31 provinces in mainland China; unfortunately, we got recovery failure among strains collected from XinJiang and obtained only one strain from HaiNan province. We also identified no NTM in some provinces from northwestern region considering the small sample size. And we also analyzed the drug susceptibility pattern of all 317 NTM strains, but a limitation in strains number of each species. We have prepared to collect more samples from these regions for completing the NTM infection and drug resistant status in China and planned to analyze the NTM infection using the isolates in following nationwide surveillance of drug-resistant tuberculosis. The acquaintance of NTM pulmonary infection will facilitate the building of TB treatment and control targets.

Declarations

This study was approved by ethics committee of China Center for Disease Control and Prevention and the methods were carried out in accordance with the approved guidelines. All authors are consent for publication, and we declared the availability of data and material and no conflicts of interest.

The work was funded by National Major Science and Technology Projects of China (2017ZX10304402-001-015), and Chinese center for disease control and prevention project (59911905).

YL, YM, DX and CF conceived and designed the experiments; WC, YM, HP, JJ, JL, CQ, CF and DX performed the experiments; CF, WC, XC, ZB, ZY, ZY, YY, XH, SF, YM and DX analyzed the data; CF contributed to the writing of the manuscript.

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Tables

Table 1
Prevalence of NTM infection in different regions based on geographic areas of China

Regions	Isolates	NTM	NTM Rate (95% CI)	p-value
South	3709	287	7.7 (6.9–8.7)	0.01
North	1208	30	2.5 (1.7–3.6)	
Provinces locate in northern region: Hebei, Beijing, Tianjin, Liaoning, Shanxi, Shandong, Henan, Shaanxi, Inner Mongolia, Jilin, Heilongjiang, Tibet, Gansu, Qinghai, Ningxia, Xinjiang;				
Provinces locate in northwestern region: Yunnan, Sichuan, Guizhou, Chongqing, Shanghai, Jiangsu, Zhejiang, Fujian, Anhui, Jiangxi, Hunan, Hubei, Guangxi, Guangdong, Hainan.				
Southeast	1708	207	12.1 (10.6–13.8)	0.01
Southwest	886	27	3.1 (2.1–4.5)	
Northeast	713	16	2.2 (1.3–3.7)	
Northwest	169	3	1.8 (0.5–5.5)	
Middle	1441	64	4.4 (3.5–5.7)	
Provinces locate in northeastern region: Heilongjiang, Jilin, Liaoning, Hebei, Beijing, Tianjin, Inner Mongolia;				
Provinces locate in northwestern region: Xinjiang, Gansu, Qinghai, Ningxia, Shaanxi;				
Provinces locate in middle region: Shanxi, Henan, Anhui, Hubei, Hunan, Jiangxi;				
Provinces locate in southeastern region: Shandong, Jiangsu, Shanghai, Zhejiang, Fujian, Guangdong, Hainan;				
Provinces locate in southwestern region: Guangxi, Tibet, Sichuan, Chongqing, Yunnan, Guizhou.				

Table 2
Species distribution among the NTM isolates form China.

Species distribution among the NTM isolates from China.				
Category	Number	NTM species	Number	Rate
Slow growing mycobacteria	189	M. avium-intracellulare complex	108	34.1%
		M. kansasii	31	9.8%
		M. paragordoniae	17	5.4%
		M. lentiflavum	10	3.2%
		M. gordonae	4	1.3%
		M. colombiense	3	0.9%
		M. nonchromogenicum	2	0.6%
		M. paraense	2	0.6%
		M. arupense	2	0.6%
		M. europaeum	1	0.3%
		M. nebraskense	1	0.3%
		M. parascrofulaceum	1	0.3%
		M. senuense	1	0.3%
		M. seoulense	1	0.3%
		M. sinense	1	0.3%
		M. szulgai	1	0.3%
		M. terrae	1	0.3%
		M. virginiense	1	0.3%
		M. xenopi	1	0.3%
		Rapid growing mycobacteria	128	M. abscessus complex
M. chelonae	3			0.9%
M. fortuitum	2			0.6%
M. peregrinum	3			0.9%
M. chitae	1			0.3%
M. engbaekii	1			0.3%
M. porcinum	1			0.3%
M. setense	1			0.3%
M. vanbaalenii	1			0.3%
M. senegalense	1			0.3%
M. avium-intracellulare complex including M. avium subsp. hominissuis (1 isolate), M. avium subsp. vulneris (2 isolates), M. avium (2 isolates), M. avium subsp. marseillense (16 isolates), M. intracellulare (82 isolates), M. intracellulare subsp. yongonense (4 isolates) and M. chimaera (1 isolate);				
M. abscessus complex including M. abscessus (67 isolates), M. bolletii (2 isolates), M. massiliense (45 isolates).				

Table 3
Susceptibility of RGM to 15 antimicrobial agents determined by the broth dilution method

Grouping/species	RGM								
Antimicrobial agent	Broth dilution ranges (µg/mL)	MIC50	MIC90	Susceptible	Intermediate	Resistant	S (%)	I (%)	R (%)
Antimicrobial agents and susceptibility breakpoints (MICs) for testing rapidly growing mycobacteria ^a									
Amikacin	1–64	8	16	≤ 16	32	≥ 64	93.75%	1.56%	4.69%
Clarithromycin (3D)	0.06-16	0.12	8	≤ 2	4	≥ 8	85.04%	0	14.96%
Clarithromycin (14D)		8	16				44.09%	2.36%	53.54%
Imipenem	2–64	64	64	≤ 4	8–16	≥ 32	0	3.15%	96.85%
linezolid	1–32	16	32	≤ 8	16	≥ 32	33.07%	32.28%	34.65%
Cefoxitin	4-128	64	128	≤ 16	32–64	≥ 128	6.30%	74.80%	18.90%
Tobramycin	1–16	8	16	≤ 2	4	≥ 8	13.39%	12.60%	74.02%
Doxycycline	0.12-16	16	16	≤ 1	2–4	≥ 8	4.72%	0	95.28%
Moxifloxacin	0.25-8	8	8	≤ 1	2	≥ 4	7.81%	1.56%	90.63%
Ciprofloxacin	0.12-4	4	4	≤ 1	2	≥ 4	11.02%	0.79%	88.19%
Tigecycline	0.015-4	1	2	-	-	-	-	-	-
TMP-SMX	0.25/4.75-8/152	8/152	8/152	≤ 2/38	-	≥ 4/76	4.72%	-	95.28%
Others									
Cefepime	1–32	32	32	≤ 8	16	≥ 32	0.79%	1.57%	97.64%
Minocycline	1–8	8	8	≤ 1	2–4	≥ 8	3.94%	0.79%	95.28%
Ceftriaxone	4–64	64	64	≤ 8	16–32	≥ 64	0	2.36%	97.64%
Amoxicillin/clavulanic acid	2/1–64/32	64/32	64/32	≤ 8/4	16/8	≥ 32/16	1.57%	0	98.43%
^a CLSI M24, 3rd ed., 2018 (2).									

Table 4
Susceptibility of *M. abscessus* and *M. massiliense* to 15 antimicrobial agents determined by the broth dilution method

Grouping/species	<i>M. abscessus</i> complex (114)			<i>M. abscessus</i> (67)			<i>M. massiliense</i> (45)		
Antimicrobial agent	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Amikacin	94.74%	1.75%	3.51%	92.54%	1.49%	5.97%	100.00%	0.00%	0.00%
Clarithromycin (3D)	85.09%	0.00%	14.91%	82.09%	0.00%	17.91%	91.11%	0.00%	8.89%
Clarithromycin (14D)	42.98%	2.63%	54.39%	13.43%	2.99%	83.58% a	86.67%	2.22%	11.11% a
Imipenem	0.00%	1.75%	98.25%	0.00%	0.00%	100.00%	0.00%	2.22%	97.78%
linezolid	32.46%	34.21%	33.33%	29.85%	34.33%	35.82%	35.56%	35.56%	28.89%
Cefoxitin	4.39%	78.95%	16.67%	4.48%	76.12%	19.40%	4.44%	84.44%	11.11%
Tobramycin	11.40%	12.28%	76.32%	8.96%	10.45%	80.60%	13.33%	15.56%	71.11%
Doxycycline	1.75%	0.00%	98.25%	1.49%	0.00%	98.51%	2.22%	0.00%	97.78%
Moxifloxacin	2.63%	1.75%	95.61%	1.49%	1.49%	97.01%	2.22%	0.00%	97.78%
Ciprofloxacin	5.26%	0.88%	93.86%	5.97%	0.00%	94.03%	4.44%	0.00%	95.56%
Tigecycline	-	-	-	-	-	-	-	-	-
TMP-SMX	2.63%	-	97.37%	2.99%	-	97.01%	2.22%	0.00%	97.78%
Cefepime	0.00%	0.88%	99.12%	0.00%	0.00%	100.00%	0.00%	0.00%	100.00%
Minocycline	0.88%	0.88%	98.25%	0.00%	1.49%	98.51%	2.22%	0.00%	97.78%
Ceftriaxone	0.00%	0.88%	99.12%	0.00%	0.00%	100.00%	0.00%	0.00%	100.00%
Amoxicillin/clavulanic acid	0.00%	0.00%	100.00%	0.00%	0.00%	100.00%	0.00%	0.00%	100.00%
^a Significant difference between <i>M. abscessus</i> and <i>M. massiliense</i> was observed in resistance rate to clarithromycin (Fisher's exact tests $P < 0.001$).									

Table 5
MIC50/MIC 90 and antimicrobial concentration ranges for ($\mu\text{g/mL}$) DSTs of SGM

Grouping/species	Antimicrobial agent	Broth dilution range ($\mu\text{g/mL}$)	MIC50	MIC90
SGM	Linezolid	1–64	16	64
	Moxifloxacin	0.12-8	2	8
	Clarithromycin	0.06-64	2	4
	Isoniazid	1–8	8	8
	Rifampicin	0.12-8	8	8
	Rifabutin	0.25-8	0.5	2
	Ethambutol	0.5–16	8	16
	Ethionamide	0.3–20	20	20
	Amikacin	1–64	8	32
	Streptomycin	0.5–64	16	64
	Doxycycline	0.12-16	16	16
	Ciprofloxacin	0.12-16	8	16
	TMP-SMX	0.12/2.38-8/152	4/76	8/152

Table 6
Susceptibility of *M. avium-intracellulare* complex to 13 antimicrobial agents determined by the broth dilution method

Grouping/species	<i>M. avium-intracellulare</i> complex								
Antimicrobial agent	Broth dilution range (µg/mL)	MIC50	MIC90	Susceptible	Intermediate	Resistant	S (%)	I (%)	R (%)
First line									
Clarithromycin	0.06-64	2	4	≤ 8	16	≥ 32	95.37%	0	4.63%
Amikacin	1-64	16	64	≤ 16	32	≥ 64	71.30%	18.52%	10.19%
Second line									
Linezolid	1-64	32	64	≤ 8	16	≥ 32	14.81%	18.52%	66.67%
Moxifloxacin	0.12-8	2	8	≤ 1	2	≥ 4	15.74%	37.04%	47.22%
Others									
Isoniazid	1-8	8	8	-	-	2	-	-	97.22%
Rifampicin	0.12-8	8	8	-	-	1	-	-	91.67%
Rifabutin	0.25-8	1	2	-	-	2	-	-	6.48%
Ethambutol	0.5-16	8	8	-	-	4	-	-	58.33%
Ethionamide	0.3-20	20	20	-	-	5	-	-	93.52%
Streptomycin	0.5-64	32	64	-	-	2	-	-	96.30%
Doxycycline	0.12-16	16	16	≤ 1	2-4	≥ 8	0.93%	0	99.07%
Ciprofloxacin	0.12-16	16	16	≤ 1	2	≥ 4	6.48%	1.85%	91.67%
TMP-SMX	0.12/2.38-8/152	4/76	8/152	≤ 2/38	-	≥ 4/76	47.22%	-	52.78%

Table 7
Susceptibility of *Mycobacterium kansasii* to 13 antimicrobial agents determined by the broth dilution method

Grouping/species	<i>Mycobacterium kansasii</i>								
Antimicrobial agent	Broth dilution ranges (µg/mL)	MIC50	MIC90	Susceptible	Intermediate	Resistant	S (%)	I (%)	R (%)
First line	0.06-64	0.25	0.5	≤ 8	16	≥ 32	100%	0	0
Clarithromycin									
Rifampicin	0.12-8	0.5	0.5	≤ 1	-	≥ 2	93.55%	-	6.45%
Second line									
Amikacin	1-64	2	8	≤ 16	32	≥ 64	96.77%	3.23%	0
Ciprofloxacin	0.12-16	2	4	≤ 1	2	≥ 4	32.26%	41.93%	25.81%
Doxycycline	0.12-16	8	16	≤ 1	2-4	≥ 8	9.68%	12.90%	77.42%
Linezolid	1-64	2	4	≤ 8	16	≥ 32	96.77%	0	3.23%
Moxifloxacin	0.12-8	≤ 0.12	0.25	≤ 1	2	≥ 4	96.77%	3.23%	0
Rifabutin	0.25-8	≤ 0.25	0.5	≤ 2	-	≥ 4	96.77%	0	3.23%
TMP-SMX	0.12/2.38-8/152	4/76	8/152	≤ 2/38	-	≥ 4/76	48.39%	-	51.61%
Others									
Ethambutol	0.5-16	8	16	-	-	≥ 4	-	-	83.87%
Isoniazid	1-8	2	8	-	-	-	-	-	-
Streptomycin	0.5-64	4	16	-	-	-	-	-	-
Ethionamide	0.3-20	0.6	20	-	-	≥ 5.0	-	-	12.90%

Figures

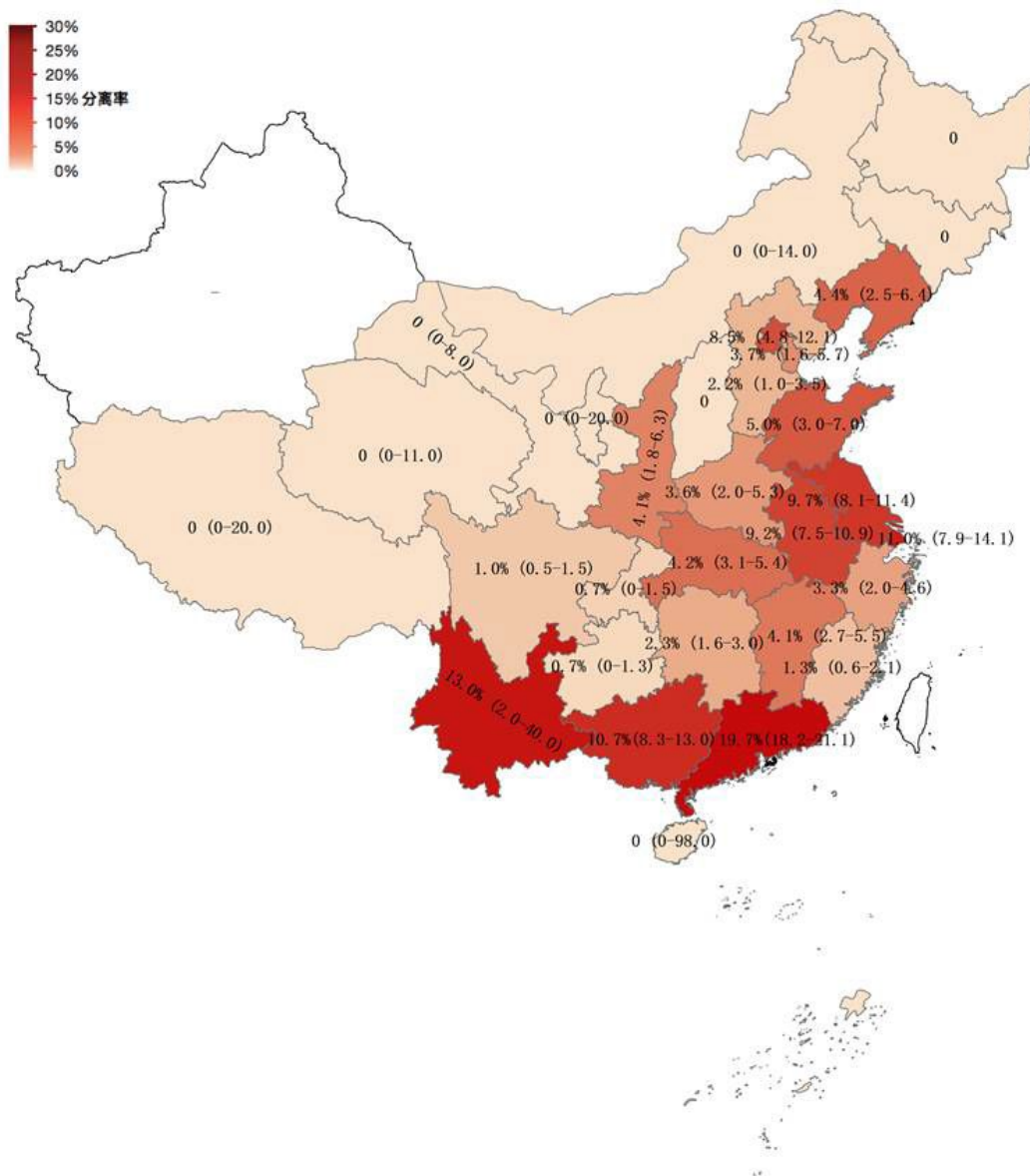


Figure 1

Prevalence of NTM in different province of China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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