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Prevalence of Nontuberculous Mycobacteria in a Tertiary Hospital in Beijing, China, January 2013 to December 2018

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Abstract

Background To investigate the species distribution of non-tuberculous mycobacteria (NTM) among tuberculosis (TB) specimens collected from January 2013 to December 2018 at Peking Union Medical Hospital (Beijing), China. NTM species identification was carried out by DNA microarray chip.

Results Mycobacterial species were detected in 1514 specimens from 1508 patients, among which NTM accounted for 37.3% (565/1514), increasing from a prevalence of 15.6% in 2013 to 46.1% in 2018 ($P < 0.001$). Among the 565 NTM positive specimens, the majority (55.2%) were from female patients. Furthermore, patients aged 45-65 years accounted for 49.6% of the total patients tested. Among 223 NTM positive specimens characterized further, the majority (86.2%) were from respiratory tract, whilst 3.6% and 3.1% were from lymph nodes and pus, respectively. Mycobacterium intracellulare (31.8%) and Mycobacterium chelonae / Mycobacterium abscessus (21.5%) were the most frequently detected species, followed by M. avium (13.5%), M. gordonae (11.7%), M. kansasii (7.6%), and others.

Conclusion The proportion of NTM among mycobacterial species detected in a tertiary hospital in Beijing, China, increased rapidly from year 2013 to 2018. Middle-aged patients are more likely to be infected with NTM, especially females. Mycobacterium intracellulare and Mycobacterium chelonae / Mycobacterium abscessus were the most frequently detected NTM pathogens. Accurate and timely identification of NTM is important for diagnosis and treatment.

Background

The substantial increase in the number of patients with immunodeficiency in recent years has contributed to the rise in infectious diseases caused by a variety of rare organisms, including non-tuberculous mycobacteria (NTM)(1, 2). The clinical manifestations of infections caused by NTM are similar to those of *Mycobacterium tuberculosis* (MTB)(3). However, the treatment strategies used for management of infections by these two groups of organisms are quite different(4). Thus accurate identification of *Mycobacteria* strains to species level is crucial for managing infections.

In China, patients diagnosed with TB or suspected of having TB, are referred to a thoracic specialist hospital for further treatment. However, clinical manifestations of NTM diseases and TB are quite similar. A considerable number of patients TB or suspected TB, are lost in tertiary hospitals. Since 2013 to date, the Microbiology lab at Peking Union Medical College hospital has been using PCR-fluorescence probe method to directly detect tuberculous/non-tuberculous *Mycobacterium* in clinical samples. DNA microarray chip method was used to identify the different NTM species. This study retrospectively analyzed the identification data of NTM samples from 2013 to 2018, to provide a general outline of the prevalence of non-tuberculous mycobacteria in a tertiary hospital in China.

Methods

Data collection

This was a retrospective study. Clinical data of patients who tested positive/negative for MTB or NTM using a screening test (Real-time fluorescent PCR detection) from January 2013 to December 2018 in Peking Union Medical College Hospital, Beijing, China, were retrospectively collected. The study was approved by the Human Research Ethics Committee of Peking Union Medical College Hospital (no. S-K890). Data on Mycobacteria species identification, which was carried out by DNA microarray chip, was also collected. To avoid bias in our retrospective analysis, only one specimen per body site of an individual was included in the current study.

Nucleic acid extraction

Mycobacteria Real-time PCR Detection Kit (CapitalBio Corporation, Beijing, China) and Extractor 36 Nucleic Acid Extractor (CapitalBio Corporation, Beijing, China) were used to extract nucleic acid from the specimens directly and thereafter Real-time fluorescent PCR for MTB or NTM screening was performed. Nucleic acid extraction procedures were as outlined below.

Briefly, 4% NaOH solution was added to the specimen in a volume ratio of 1:1. After this, the mixture was thoroughly mixed by vortexing and incubated at room temperature for 30 minutes. A 1mL aliquot of this suspension was added to a 1.5 mL sterile centrifuge tube and centrifuged at 12 000 rpm for 5 minutes. The supernatant was discarded and 50 μL of nucleic acid extract fluid was added to the pellet, vortexed thoroughly, and then transferred into the nucleic acid extracting tube. This was then put into the nucleic acid extractor and centrifuged at maximum rotational speed for 5 minutes. The tube was subsequently put in a 95°C metal bath for 5 minutes, centrifuged at 5 000 rpm for 1 minute, and then the supernatant was used for PCR. The extracted nucleic acid could be preserved at (−20 ± 5) °C for one month.

Real-time fluorescent PCR detection

PCR amplification system was performed by the manufacturer's instructions and was roughly as follows; 18μL PCR amplification reagent and 2μL template DNA were mixed together; amplification conditions: pre-warm at 37 °C for 5 minutes, pre-denaturation at 94°C for 3 minutes, denaturation at 94 °C for 15 seconds, annealing at 60 °Cfor 30 seconds for 40 cycles, and 50 °C for 10 seconds. FAM and HEX channels were selected for fluorescence detection at the same time. 60 °C for 30 seconds was the fluorescence signal acquisition point. A result with a Ct value of < 40 was considered positive. Negative and positive quality control products were included in parallel with the samples. When the positive quality control products were detected as *Mycobacterium tuberculosis* complex group and the negative quality control products as no *Mycobacterium,* the results of the test could be accepted, otherwise the results were invalid and needed to be repeated. Interpretation of real-time fluorescent PCR assay results is shown in Table 1.

NTM species identification

Mycobacteria Identification Array Kit (CapitalBio Corporation, Beijing, China) was used for NTM species identification. DNA microarray chip hybridization system was performed as follows: 9μL hybridization buffer and 6μL PCR products were mixed; reaction conditions: denaturation at 95°C for 5 minutes, ice bath in ice-water mixture for 3 minutes, then blowing and mixing. After this 13.5μL of hybridization reaction mixture was added into the sampling hole, and then the hybridization box was sealed to maintain a 50 °C constant temperature in the water bath pot for 2 hours. Then washing and drying of the chip followed, and then scanning with LuxScan 10K-B Microarray Scanner (CapitalBio Corporation, Beijing, China). The corresponding software was used to read the signals and display the results.

Statistical analyses

Excel was used to establish the database, and SPSS 22.0 was used for statistical analysis. Trend analysis of annual constituent ratio was carried out by trend Chi-square tests and a P value < 0.01 was considered statistically significant.

Results

Changes of MTB/NTM detection rates

From 2013 to 2018, 17287 non-repeat clinical specimens from Peking Union Medical College Hospital were sent to the Microbiology lab for detection of MTB/NTM by Real-time fluorescent PCR method. Of these, 1514 (8.76%) specimens were positive for MTB/NTM. The number of positive specimens per year is shown in Table 2. During the six year study period, there was a significant increase in the number of samples tested for TB and/or NTM each year, with a gradual decrease in MTB detection, and a corresponding significant rise in NTM detection, year by year (^{2} = 21.77，*P*<0.001). Thus altogether there were 1514 positive specimens obtained from 1508 patients, and 6 patients were mixed infection of MTB and NTM. MTB was detected in 949 (62.7%) of the 1514 positive samples, and NTM in 565 (37.3%). From 2013 to 2018, there was a significant rise in the prevalence of NTM, and a corresponding decrease in the prevalence of MTB (^{2} = 58.84，*P*<0.001), as shown in Figure 1.

Demographic data of NTM positive patients

The 565 NTM positive patients comprised of 44.8% (253/565) males and 55.2% (312/565) females. The ages of the patients ranged from 10 to 95years, with an average age of 51.55±16.84 years, and the quartiles were 40, 53 and 63 years, respectively. Only 17 patients (17/565, 3.0%) were under 18 years old. Patients aged 18–44 years accounted for 28.3% (160/565) of the patients, those aged 45–65 years for 49.6% (280/565), and those over 65 years of age accounted for 19.1% (108/565) of the total patients.

Types of NTM positive specimens

The 565 NTM positive samples were collected from different clinical departments. Most of them (348/565, 61.6%) were from the respiratory department, followed by infection department (55/565, 9.7%), and other departments were relatively less represented (no one was more than 4.4%). According to sample type category, the majority was sputum (243/565, 43.0%), followed by broncho-alveolar lavage fluid (101/565, 17.9%) and tracheobronchial aspiration (74/565, 13.1%), cerebrospinal fluid (69/565, 12.2%), lymph nodes (19/565, 3.4%), pus and urine (both 14/565, 2.5%). Other types of specimens were less than 1.0%. The total number of specimens from the respiratory tract was 418 (74.0%).

NTM species distribution

Based on the requesting physician's requirements for further testing, 223 NTM positive samples were characterized for NTM species, with repetitive isolates from the same body part of the same patient eliminated. These 223 samples were from 220 patients. Among them, two cases were multiple location infection, and one case was mixed infection of *M. intracellulare* and other *Mycobacterium spp.* isolate which was beyond the detection rangeof our kit and thus could not be identified to species level accurately. The NTM species identified by DNA microarray chip in the 223 samples included *M. intracellulare(*71), *M. chelonae/M. abscessus(*48), *M. avium(*30), *M. gordonae(*26), *M. kansasii(*17), *M. fortuitum(*15), *M. xenopi(*2), *M. gilvum(*2), *M. smegmati(*1), *M. marinum/M. ulcerans(*1), *M. terrae(*1), *M. phlei(*1) and other *Mycobacterium* spp.(8). In addition, 7 specimens initially identified as other *Mycobacterium spp.* as they could not be identified to species level by Mycobacteria Identification Array Kit (CapitalBio Corporation, Beijing, China), were further identified as *M. simiae* (2), *M. iranicum, M. chimaera, M. marswillense, M. holsaticum* and *M. colombiense* by 16S rDNA sequencing. Distribution of NTM species identified by DNA microarray chip during the period 2013—2018, is shown in Table 3.

NTM species distribution from different specimen types

As shown in Table 4, there are several differences in sample sources for different NTM species. Besides the respiratory tract which was a major collection site for all NTM species, 45.5% (5/11) of *M. chelonae/M. abscessus* and 50.0% (2/4) of *M. kansasii* were detected from lymph node specimens. Also, there were 33.3% (2/6) and 27.3% (3/11) of *M. intracellulare* and *M. chelonae/M. abscessus* detected from pus, respectively.

Discussion

Non-tuberculous mycobacteria (NTM) do not cause tuberculosis or leprosy but can cause pulmonary disease resembling tuberculosis, lymphadenitis, skin disease, or disseminated disease. The pulmonary infection caused by NTM is difficult to clinically differentiate from that caused by MTB. Most of the NTM strains are not susceptible to anti-tuberculosis drugs. The treatment plan is related to the species, infection site and the severity of infection(5). Therefore, rapid and accurate identification of *Mycobacterium* to species level is very important for managing these infections. Real-time fluorescent PCR detection

(Mycobacteria Real-time PCR Detection Kit, CapitalBio Corporation, Beijing, China) could be used to detect both MTB or NTM in the specimen directly in three hours, and there is no significant difference in the detection performance of this method compared to traditional methods (culture and microscopy)(6). In addition, the DNA microarray chip method (Mycobacteria Identification Array Kit, CapitalBio Corporation, Beijing, China)can detect and identify 17 species or groups of clinically common *Mycobacteria,* including *M. tuberculosis* complex, *M. intracellular, M. avium, M. gordonae, M. kansasii, M. fortuitum, M. scrofulaceum, M. gilvum, M. terrae, M. chelonei /M. abscessus, M. phlei, M. nonchromogenicum, M. marinum / M. ulcerans, M. aureus, M. senegalense / M. malmoense, M. xenopi* and *M. smegmati*. Using this method, results are available within 6 hours, which is helpful for early diagnosis and treatment of tuberculosis and NTM disease.

An analysis of the current study showed that the absolute numbers of both NTM positive and MTB positive samples increased by yearly from 2013 to 2018. Notably, the proportion of NTM positive samples among the total positive samples for MTB or NTM as per the Real-time fluorescent PCR detection method, increased from 15.6% in 2013 to 46.1% in 2018. This is in agreement with the results of the national tuberculosis epidemiological sampling surveys carried out in China in 1990 (4.9%), 2000 (11.1%) and 2010 (22.9%)(7, 8).This finding is consistent with multiple studies from diverse countries, demonstrating an increasing prevalence of NTM infections in recent years (9, 10). Many factors may contribute to the observed rise in NTM detection, including improved clinical awareness of NTM infection, use of better and more sensitive NTM detection techniques, increased number of immunocompromised patients, aging of the population and so on(11, 12). Among the most common NTM pathogens, the incidence of MAC from 2013 to 2018, grew faster than that of *M. chelonei /M. abscessus* (Table 3), suggesting a more prominent role for MAC in NTM infections. Furthermore, the fast increase in the prevalence of *M. gordona* and M. *fortuitum* in NTM infections should be noted.

The major clinical manifestation of NTM is pulmonary disease, with *M. avium-intracellulare* complex (MAC or MAIC) the most common species involved in infection(13, 14). In this retrospective study, *M. intracellulare* (65/193, 33.7%) was the most commonly detected species in respiratory tract samples, followed by *M. chelonei / M. abscessus* (37/193, 19.2%), *M.avium (*27/193, 14.0%)*, M.gordonae (*24/193, 12.4%). So MAC (92/193, 47.7%) was still the most common NTM pathogen in respiratory tract, which is consistent with previous findings in US, Canada, Australia, Japan, Korea and Southern China(11–16). Although MAC is the most common pathogen in NTM pulmonary disease, the relative frequency of MAC varies widely among different geographical regions. For example, MAC represented 31% of isolates from South America, 52% from North America and 42.1% from China in this study. Many factors such as the climate type and population density can affect the distribution of NTM species (2).

In the present study, NTM infection was much more common in women(55.2%) than men(44.8%). Furthermore, the age range of infected people was relatively wide, being most common in the 45–65 year age group (49.6%), probably due to some issues related to the function of the immune system(17). This finding is in agreement with those of multiple studies in US(18), Japan(19, 20) and South Korea(16), which all indicated that older women were more susceptible to NTM infection. According to a previous study, abnormal expression of adipokines, sex hormones, and/or TGF-β may predispose slender, older women to NTM infection(21). However, contrasting findings have been reported in Europe, where patients with NTM pulmonary disease were more likely to be male, possibly owing to smoking history and chronic obstructive pulmonary disease (COPD)(9, 20).

DNA microarray chip method (Mycobacteria Identification Array Kit, CapitalBio Corporation, Beijing, China) can accurately distinguish between *M. avium* and *M. intracellulae,* which have quite similar phenotypes. We found out that the prevalence of *M. intracellulae(*31.8%) was always higher than *M. avium(*13.5%) from 2013 to 2018. It is important to distinguish between *M. avium* and *M. intracellulae* because theyshow different pathogenic characteristics. *M. intracellulae* is more virulent compared to *M. avium,* indicating a more intensive therapeutic strategy (22). However, the DNA microarray chip method could not distinguish between *M. chelonei* and *M. abscessus,* and between *M. marinum* and *M. ulcerans*. This is because *M. chelonei* and *M. abscessus* have the same 16S rRNA gene sequences, and so are *M. marinum* and *M. ulcerans.* However, *M. chelonei* tends to cause disseminated infections (5), and *M. ulcerans* produces a cytotoxin (mycolactone) with immune-modulating properties that causes necrosis(23). Therefore, accurate identification of these strains to species level still has an important clinical significance. Microbiology laboratories could further identify these strains by 16S–23S Internal Transcribed Spacer Region sequencing and Sequencer-Based Capillary Gel Electrophoresis (24). Moreover, Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR) markers can be used for typing *M. intracellulae* clinical isolates for molecular epidemiological studies (25). Other than pulmonary infectious diseases, NTM can also cause lymph node and skin and soft tissue infections. In this study, lymph node and pus were the second most common specimen types, only less than respiratory tract infections.

This study has several limitations. First, most NTM strains are widely distributed in the environment, being found in the soil, and water, including even treated water. Therefore caution must be exercised in interpreting positive results from specimens as this doesn’t necessarily mean that the patient is infected by the bacteria. Positive results may be due to bacterial colonization, or transient infection which is quickly cleared by the immune system, or contamination in sample collection and transportation(14). Second, among the common NTM species, MAC, *M. abscessus* and *M. kansasii* are highly pathogenic, and can cause lung diseases and lymph node, skin and soft tissue infections. However, *M. gordonae* and *M. fortuitum* rarely cause any infections, and only cause diseases when the patient’s general condition is particularly poor. During the clinical diagnosis and treatment, the clinical significance of the strain should be considered by combining with the patient’s symptoms, signs and imaging findings.

Conclusions

The proportion of NTM among mycobacterial species detected in a tertiary hospital in Beijing, China, increased rapidly from year 2013 to 2018. Middle-aged and elderly patients are more likely to be infected with NTM, especially females. The most frequently detected NTM pathogens were *M. intracellulare* and *M. chelonae / M. abscessus.* Accurate and timely identification of NTM is crucial for diagnosis and treatment.

Abbreviations

MTB：*Mycobacterium tuberculosis*

NTM：non-tuberculous mycobacteria

Declarations

Ethics approval and consent to participate

The retrospective study was approved by the Human Research Ethics Committee of Peking Union Medical College Hospital (no. S-K890).

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are not publicly available due to the privacy of patients but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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The funding bodies had no role in study design, data collection and analysis, interpretation of data, decision to publish, or preparation of the manuscript.

Authors' contributions

JJH analyzed and interpreted the patient data. WHY collected the data from two systems of laboratory. MX and YCX guided the idea of analysis. YZ, YXL and TK were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Interpretation of real-time fluorescent PCR assay results

Detection results	Interpretation
FAM (+) HEX(+)	MTB nucleic acid detection positive
FAM (+) HEX(-)	MTB nucleic acid detection positive
FAM (-) HEX(+)	NTM nucleic acid detection positive
FAM (-) HEX(-)	*Mycobacterium spp.* nucleic acid detection negative

Table 2. The number of positive specimens from *Myobacterium* detection during 2013- 2018 in Beijing, China.

Year	Total	Positive specimens	
		MTB (%)	NTM (%)
2013	859	76 (8.85)	14 (1.63)
2014	1857	141 (7.59)	57 (3.07)
2015	2271	156 (6.87)	66 (2.91)
2016	3259	184 (5.65)	88 (2.70)
2017	4130	193 (4.67)	170 (4.12)
2018	4911	199 (4.05)	170 (3.46)
Total	17287	949(5.49)	565 (3.27)

Table 3. Non-tuberculous species identified from 2013 to 2018 in a tertiary hospital in Beijing, China.

Gene chip identified *Mycobacterial* Species	2013	2014	2015	2016	2017	2018	Total (%)
M. intracellulare	2	2	8	8	20	31	71 (31.8)
M. chelonae/M. abscessus	2	3	11	13	10	9	48 (21.5)
M. avium	0	1	5	2	6	16	30(13.5)
M. gordonae	0	1	2	3	8	12	26 (11.7)
M. kansasii	0	0	2	6	4	5	17 (7.6)
M. fortuitum	0	0	1	2	4	8	15 (6.7)
M. gilvum	0	0	0	0	1	1	2 (0.9)
M. xenopi	0	0	0	0	1	1	2 (0.9)
M. marinum/M. ulcerans	0	1	0	0	0	0	1 (0.4)
M. smegmati	0	0	1	0	0	0	1 (0.4)
M. terrae	0	0	0	0	0	1	1 (0.4)
M. phlei	0	0	0	0	0	1	1 (0.4)
Other *Mycobacterium*	0	0	0	2	1	5	8* (3.6)
Total	4	8	30	36	55	90	223 (100)

*Seven of the isolates were further identified as *M. simiae* (2), *M. iranicum*, *M. chimaera*, *M. marswillense*, *M. holsaticum* and *M. colombiense* by 16S rDNA sequencing respectively.

Table 4. Specimen types among which NTM were detected in this study.

rial	Sputum	Bronchoalveolar lavage fluid	Tracheobronchial aspiration	Lymph node	Pus	Hydrothorax	Urine	Lung tissue	Ascitic fluid	Marrow	Joint fluid	Subcutaneous nodule	Vertebrae puncture tissue	Others	Tota
re	46	12	7	0	2	1	0	0	0	1	1	1	0	0	71
I.	35	1	1	5	3	1	0	0	0	0	0	0	0	2	48
	18	8	1	1	0	1	0	1	0	0	0	0	0	0	30
ae	20	4	0	0	0	1	1	0	0	0	0	0	0	0	26
i	9	4	0	2	1	0	0	0	0	0	0	0	1	0	17
m	13	1	0	0	0	0	1	0	0	0	0	0	0	0	15
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
I.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
ati	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
rium	7	0	0	0	0	0	0	0	0	0	0	0	0	1	8
	154	30	9	8	7	4	2	1	1	1	1	1	1	3	223

Figures

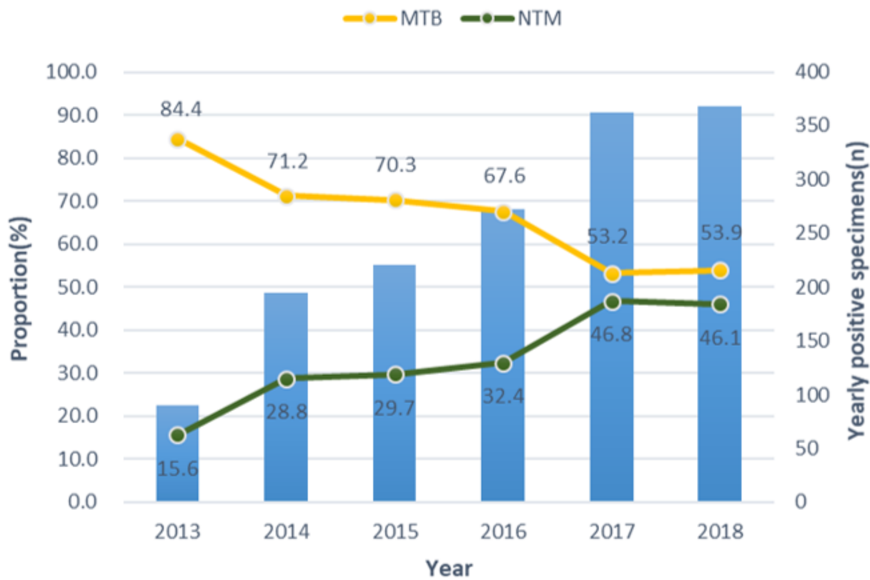

Figure 1

Distribution of Positive specimens in Mycobacterium nucleic acid detection during 2013 - 2018.