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Differences in risk factors and drug susceptibility between *Mycobacterium avium* and *Mycobacterium intracellulare* lung diseases in China

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ABSTRACT

The aim of this study was to investigate the differences in risk factors and drug susceptibility between *Mycobacterium avium* and *Mycobacterium intracellulare* lung diseases in China. In total, 452 nontuberculous mycobacteria (NTM) strains isolated from patients with NTM lung diseases in four specialised TB hospitals were enrolled in this study. The minimum inhibitory concentration (MIC) was used to evaluate the drug susceptibility of *M. avium* and *M. intracellulare* isolates. In addition, demographic and clinical characteristics of patients with NTM lung diseases caused by *M. avium* and *M. intracellulare* were analysed. Of 452 NTM isolates, *M. intracellulare* (188; 41.6%) was the most frequently isolated organism. The percentages of moxifloxacin- and linezolid-resistant strains among the *M. intracellulare* isolates were significantly lower than those among the *M. avium* group ($P = 0.003$ and $P < 0.001$, respectively). In contrast, *M. avium* harboured a lower proportion of rifampicin-resistant strains than *M. intracellulare* ($P = 0.005$). Among patients with *M. intracellulare* lung diseases, the percentages of patients aged >64 years and patients with chronic obstructive pulmonary disease (COPD) were significantly higher than among patients with *M. avium* ($P = 0.008$ for age and $P = 0.001$ for COPD). In conclusion, these data demonstrated that *M. intracellulare* was the most common NTM species in China. This study also revealed that *M. intracellulare* and *M. avium* differed in their drug susceptibility profiles. In addition, clinical cases of *M. intracellulare* lung diseases were more likely to be found in the aged population and among patients with COPD co-morbidity.

1. Introduction

Lung diseases caused by nontuberculous mycobacteria (NTM) have been increasing worldwide [1–3]. In China, a region with a high tuberculosis (TB) burden [4], the rates of NTM can be classified as an epidemic, as demonstrated in the national surveys conducted in 1990 and 2010, which revealed that the proportion of NTM among all mycobacterial isolates had increased from 11.1% to 22.9% [4,5]. Thus, the rising percentage of NTM in China represents a serious public health concern [4].

Among this diverse group of organisms, *Mycobacterium avium* complex (MAC), which predominantly consists of *M. avium* and *Mycobacterium intracellulare*, is the most common disease-causing aetiological NTM species affecting many countries [6,7]. Although *M. avium* and *M. intracellulare* cannot be distinguished by traditional physical and biochemical tests [8,9], these two species differ significantly in their pathogenicity and biology [10]. In contrast to the high incidence of *M. avium* among patients with acquired immune deficiency syndrome (AIDS) [10], *M. intracellulare* appears more likely to infect non-AIDS patients [10]. A previous study by Han et al. demonstrated that *M. intracellulare* infection is more common among older women, regardless of underlying disease [10]. In

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addition, patients with *M. intracellulare* lung disease show a more severe presentation and a worse prognosis than patients with *M. avium* lung disease [9], suggesting a high level of virulence for *M. intracellulare* among MAC. Drug susceptibility of these two neighbouring mycobacteria is an important phenotypic characteristic, which is essential for an appropriate and effective chemotherapy regimen. However, there are limited data available on the differences in drug susceptibility profiles between *M. avium* and *M. intracellulare* [2,11].

A total of 452 NTM strains isolated from patients with NTM lung diseases in four specialised TB hospitals in China were included in this study. Nucleotide sequencing was performed to differentiate *M. avium* and *M. intracellulare*, whilst the minimum inhibitory concentration (MIC) was used to evaluate differences in drug susceptibility. In addition, the demographic and clinical characteristics of patients with NTM lung diseases caused by *M. avium* and *M. intracellulare* were analysed. The aim of this study was to investigate the differences in risk factors and drug susceptibility between *M. avium* and *M. intracellulare* lung diseases in China.

2. Materials and methods

2.1. Ethics statement

The protocols applied in this study were approved by the Ethics Committee of the Chinese Center for Disease Control and Prevention (Beijing, China).

2.2. Patients

Patients enrolled in this study were those who were diagnosed with a NTM lung disease between January 2011 and December 2012 from four specialised TB hospitals that were recruited for this study (Guangzhou Chest Hospital, Lianyungang Forth Hospital, Kaifeng Pulmonary Hospital, and Yongchuan Hospital affiliated to Chongqing Medical University). All patients met the microbiological and clinical criteria established by the American Thoracic Society for NTM lung diseases [1]. The demographic and clinical characteristics of patients were obtained from their medical records. Chest computed tomography and spirometry were performed for all patients, and the diagnosis of all comorbidities met the authorised international criteria. All of the strains isolated from these patients were identified as NTM by the conventional mycobacterial identification method with paranitrobenzoic acid (500 mg/mL) and thiophene-2-carboxylic acid hydrazide (5 mg/mL) [12]. The reagents were purchased from Sigma-Aldrich (St Louis, MO).

2.3. Bacterial strain culture and molecular species identification

NTM strains identified by the conventional biochemical method were subcultured on Löwenstein–Jensen medium (Hangzhou Jiawei Pharmaceutical Co. Ltd., Hangzhou, China). Colonies were scraped and genomic DNA was extracted according to previously reported techniques [13]. Genomic DNA was used for sequencing of multiple genes, including 16S rRNA, *hsp65*, *rpoB* and the 16S–23S rRNA internal transcribed spacer (ITS) sequence, in order to perform molecular species identification [14,15]. The 50 μL PCR mixtures contained 5 μL of 10× PCR buffer, 200 μM of each dNTP, 0.2 μM of each primer set and 1 U of HotStar *Taq* Polymerase (QIAGEN, Hilden, Germany). PCR was performed under the following conditions: initial denaturation at 94 °C for 5 min; 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; followed by a final extension at 72 °C for 10 min. PCR products were sent to Tsingke Company (Beijing, China) for sequencing. DNA sequences were aligned with the homologous sequences of the reference mycobacterial strains using multiple sequence alignments (http://www.ncbi.nlm.nih.gov/BLAST).

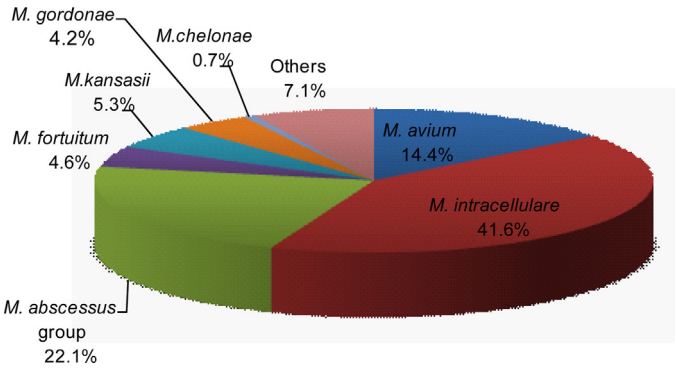

Fig. 1. Distribution of different nontuberculous mycobacteria species.

2.4. Minimum inhibitory concentration determination

To determine the drug susceptibility of *M. avium* and *M. intracellulare* strains, a microplate alamarBlue® assay (MABA) was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [16]. Cation-adjusted Mueller–Hinton broth (Becton Dickinson, Cockeysville, MD) supplemented with 5% oleic acid–albumin–dextrose–catalase (OADC) (Becton Dickinson, Sparks, MD) was used for broth microdilution testing. A total of 12 agents were selected for MIC assessment, including clarithromycin, moxifloxacin, linezolid, rifampicin, rifabutin, ethambutol, streptomycin, gatifloxacin, levofloxacin, amikacin, azithromycin and capreomycin. The concentrations of the drugs ranged from 0.0625 μg/mL to 128 μg/mL.

The antimicrobial agents were classified into three groups according to the breakpoint value of each drug. The breakpoint values of Group I drugs were taken from the CLSI reference, including clarithromycin, moxifloxacin and linezolid [16], whilst the breakpoint values of Group II drugs were referenced from previous literature, including rifampicin [17], ethambutol [17] and amikacin [18]. In addition, the drugs with no available breakpoint were classified as Group III, including azithromycin, capreomycin, levofloxacin, gatifloxacin, rifabutin and streptomycin.

2.5. Statistical analysis

SPSS v.14.0 (SPSS Inc., Chicago, IL) was used to perform χ^2 analysis. Differences were considered to be statistically significant at a *P*-value of <0.05.

3. Results

3.1. Identification of nontuberculous mycobacteria species

A total of 452 NTM isolates identified by the conventional biochemical method were further confirmed at the species level through the use of multilocus sequence analysis. As shown in Fig. 1, *M. intracellulare* (188; 41.6%) was found to be the most abundant organism, followed by *Mycobacterium abscessus* group (100; 22.1%), *M. avium* (65; 14.4%), *Mycobacterium kansasii* (24; 5.3%), *Mycobacterium fortuitum* (21; 4.6%), *Mycobacterium gordonae* (19; 4.2%), *Mycobacterium chelonae* (3; 0.7%) and other rare mycobacterial species (32; 7.1%). In total, 253 MAC isolates, including 188 *M. intracellulare* and 65 *M. avium* isolates, were used for further analysis.

3.2. Drug susceptibility profiles

The range of MICs of each antimicrobial agent for *M. intracellulare* and *M. avium* is shown in Table 1. Clarithromycin was highly