Original Article

Prevalence and Antimicrobial Susceptibility of *Mycobacterium abscessus* in a General Hospital, China*

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Abstract

Objective To gain greater insight into the prevalence drug resistant profiles of *M. abscessus* from a general hospital in Beijing, China.

Methods Partial gene sequencing of *16S*, *hsp65*, and *rpoB* were used to distinguish the species of NTM isolates. All strains identified as *M. abscessus* were further enrolled in the drug susceptibility testing by using broth microdilution method.

Results We found that *M. avium* complex was the most frequent NTM organism, accounting for 54.1% (33/61) of all isolates. Behind MAC, the second most common organisms were *M. abscessus* (22 out of 61, 36.1%). Average rates of resistance were 4.5% for AMK, 9.1% for LZD, and 13.6% for CLA, respectively. In contrast, resistance to LEV (17/22, 77.3%), IMI (9/22, 40.9%), and SMX (10/22, 45.5%) was noted in more than 40% of *M. abscessus* isolates. DNA sequencing revealed that all the CLA-resistant isolates harbored nucleotide substitutions in position 2058 (1/3, 33.3%) or 2059 (2/3, 66.7%) of 23S rRNA.

Conclusion In conclusion, our data demonstrated that *M. intracellulare* and *M. abscessus* were the most common NTM species in the general hospital of Beijing. CLA, AMK, LZD showed promising activity, where as LEV, IMI, and SMX exhibited poor activity against *M. abscessus in vitro*.

Key words: *Nontuberculous mycobacteria*; *Mycobacterium abscessus*; China

Biomed Environ Sci, 2016; 29(2): 85-90 *doi: 10.3967/bes2016.009* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

INTRODUCTION

Although *Mycobacterium tuberculosis* is one of the most important mycobacterial species threatening the public health all over the world, other species, known as *nontuberculous mycobacteria* (NTM), are being responsible for increasing emergency of NTM disease in human[1-3]. NTM are commonly isolated from different environmental sources, including soil, treated and untreated water, animals and food, divided into slow growing mycobacteria (SGM) and rapid growing mycobacteria (RGM) according to their behavior in the culture[2].

*This work was supported by the grant 2012BAI05B02 from National Key Technology Research and Development Program; China and grant 81400037 from the National Natural Science Foundation of China.

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Of the rapid growing mycobacteria relevant to human disease (including *M. fortuitum*, *M. abscessus*, and *M. chelonae*), *M. abscessus* is the most common type to cause lung disease, and is also the most difficult to treat in the clinical practice[4-5], the mobility rate of which have be nearly 20% in susceptible individuals in the past decades[5]. The major problem during treatment is that *M. abscessus* is not responsive to 'standard' antituberculosis agents, but susceptible to other common antibiotics, such as macrolides, beta-lactams or tetracyclines[6]. The drug susceptibility of the isolates is variable, which make it essential to obtain *in vitro* drug susceptibility profile of individual *M. abscessus* strains to generate an effective therapeutic regimen for the patients[7].

In China, *M. abscessus* is one of the most common nontuberculous mycobacetria causing lung disease[8]. To gain greater insight into the prevalence drug resistant profiles of *M. abscessus*, we firstly identified the *in vitro* susceptibility of *M. abscessus* isolates from patient's respiratory specimens from a general hospital in Beijing, China. Thirteen antibiotics, which were extensively used in the clinical practice for the treatment of NTM infections, were selected to perform the minimum inhibitory concentration (MIC) of *M. abscessus*.

MATERIALS AND METHODS

Bacterial Strains

Data were obtained from patients at Beijing Hospital during the 36-month period between April 1, 2012, and March 31, 2015. During this period, sputum samples from all TB suspect patients were collected, and the sputum was digested with NALC-NaOH (4%) for 15 min, and inoculated onto Löwenstein-Jensen (L-J) medium according to the previous report[9]. The NTM isolates were firstly distinguished from *M. tuberculosis* isolates with PNB and TCH modified L-J medium. Partial gene sequencing of *16S*, *hsp65*, and *rpoB* were used to distinguish the species of NTM isolates[8]. All strains identified as *M. abscessus* were further enrolled in the drug susceptibility testing. In addition to clinical isolates, one reference strain of *M. abscessus*, ATCC35761 were obtained from National Tuberculosis Reference Laboratory of China. The protocols applied in this study were approved by the Ethics Committee of Beijing Hospital, and informed consent was obtained from all patients whose sputum specimens were used in scientific studies.

Species Identification

All the clones growing on the L-J medium were scraped and genomic DNA was extracted by a rapid-boiling method[10]. The genomic DNA was used for the sequencing of multiple genes, including 16S rRNA, *hsp65*, and 16S-23S rRNA internal transcribed spacer (ITS) sequence, to perform molecular species identification[8]. The 50 μL PCR mixtures were prepared as follows: 5 μL 10×PCR buffer, 200 μmol/L of each dNTP, 0.2 μmol/L of each primer set, 5 μL crude genomic DNA, 1 U HotStar Taq polymerase (Qiagen). The amplification was performed in athemocycler (Bioer, Hangzhou, China) as follows: 5 min at 94 °C for initial denaturation, and then 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. The PCR products were sent to Sango Company (Beijing, China) for sequencing. All the sequencing results were aligned to the GenBank database over the Internet by using the NCBI BLAST server (www.ncbi.nlm.nih.gov).

Minimal Inhibition Concentration (MIC)

Antimicrobial susceptibility testing was performed using broth microdilution method according to the guidelines by the National Committee for Clinical Laboratory Standards (NCCLS)[11]. Briefly, organisms scraped from the culture media was transferred to saline with 0.02% Tween 80. The suspension was mixed vigorously on a votex for 1 min until the bacterial colonies were dispersed homogeneously. Then the suspension was diluted to the density of a 0.5 McFarland standard. Followed by further dilution two hundred times with cation-adjusted Mueller-Hinton broth media (CAMHB), the diluted bacterial suspension was inoculated at a final concentration of 3.75×10^5 CFU/mL. MICs were determined for 13 antimicrobial agents, which were used in the treatment regimen against *M. abscessus* infection, were selected for drug susceptibility profile analysis, including clarithromycin (CLA), azithromycin (AZM), amikacin (AMK), cefoxitin (CFX), imipenem (IMI), linezolid (LZD), moxifloxacin (MOX), levofloxacin (LEV), tigecycline (TGC), capreomycin (CAP), tobramycin (TOB), sulfamethoxazole (SMX), and clofazimine (CLO). All agents mentioned above were purchased from Sigma-Aldrich. The breakpoints used to