

## In Vitro Antimicrobial Susceptibility of *Mycobacterium abscessus* in Korea

*Mycobacterium abscessus* is the second most common etiology of pulmonary disease caused by nontuberculous mycobacteria in Korea. Although antimicrobial susceptibility tests are important for appropriate patient management in *M. abscessus* lung disease, the tests have never been investigated in Korea. Seventy-four isolates of *M. abscessus* recovered from patient respiratory samples were tested against eight antimicrobial agents following the guidelines set forth by the National Committee for Clinical Laboratory Standards. Of the parenteral antibiotics, amikacin (99%, 73/74) and cefoxitin (99%, 73/74) were active against most isolates. Imipenem (55%, 36/66) and tobramycin (36%, 27/74) had activity against moderate number of isolates. Of the oral antibiotics, clarithromycin (91%, 67/74) was active against the majority of isolates. Moxifloxacin (73%, 54/74) and ciprofloxacin (57%, 42/74) had activity against a moderate number of isolates. Doxycycline was the least active, inhibiting only 7% (5/74) of isolates. In conclusion, the variations in susceptibility within *M. abscessus* isolates to currently available antimicrobials suggest that the antimicrobial susceptibilities of any clinically significant *M. abscessus* isolate be needed individually.

**Key Words :** Anti-bacterial Agents; Drug Resistance; Microbial Sensitivity Tests; *Mycobacterium abscessus*; Korea

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## INTRODUCTION

*Mycobacterium abscessus* is the most pathogenic and chemotherapy-resistant rapidly growing mycobacteria (RGM). It accounts for 80% of lung disease caused by RGM and is the second most common RGM species present in extrapulmonary disease (following *M. fortuitum*) (1-4).

*M. abscessus* is resistant to all antituberculosis drugs including isoniazid, rifampin, ethambutol, and pyrazinamide. Thus, routine susceptibility testing to antituberculosis drugs is not recommended for this species. *M. abscessus* is considered to be susceptible to amikacin, cefoxitin, and clarithromycin, and moderately susceptible to imipenem (1-4). However, *M. abscessus* is not an easy organism to treat, and long-term treatment with multiple antimicrobial agents is usually required. Some studies have reported that in vitro susceptibilities to several antimicrobial agents are correlated with clinical responses to treatment in RGM infections (5, 6). Thus, drug susceptibility tests are important for appropriate patient management. In addition, all clinically significant isolates should be tested against selected antimicrobial agents (1, 2).

In Korea, *M. abscessus* is the second most common etiology of lung disease caused by nontuberculous mycobacteria

(NTM) (following the *M. avium-intracellulare* complex). It accounts for 21 to 33% of NTM lung disease in Korea (7-9); however, in vitro antimicrobial susceptibility test results for this organism have never been investigated in Korean population. Therefore, we examined the in vitro susceptibilities of *M. abscessus* isolates from patient respiratory specimens that displayed *M. abscessus* lung disease.

## MATERIALS AND METHODS

From July 2005 to December 2006, 74 nonduplicate isolates of *M. abscessus* (one isolate per patient) recovered from respiratory clinical samples were collected for use in this study. NTM species identification was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method based on the *rpoB* gene (10). All patients met the diagnostic criteria recommended by the American Thoracic Society for *M. abscessus* lung disease (11).

Antimicrobial susceptibility testing was performed at the Korean Institute of Tuberculosis according to the guidelines set forth by the National Committee for Clinical Laboratory Standards (NCCLS) (12, 13). We tested eight antimicrobial

agents against *M. abscessus* isolates: amikacin (Sigma, St. Louis, MO, U.S.A.), cefoxitin (Sigma), imipenem (MSD, Seoul, Korea), tobramycin (Sigma), clarithromycin (Hanmi, Seoul, Korea), ciprofloxacin (Ildong, Seoul, Korea), moxifloxacin (Bayer, Seoul, Korea), and doxycycline (Sigma). Minimal inhibitory concentrations (MICs) of all tested drugs were determined by the broth microdilution method and interpreted according to the guidelines described by the NCCLS document M24-A in 2003 (12). The MIC of moxifloxacin for this RGM has not yet been described by the NCCLS. In its place, we followed the recommendation for aerobic organisms in NCCLS M100-S11 in 2001 (13).

The colonies present on the culture medium were transferred to 15-mL tubes of 7H9 broth (Becton Dickinson, Piscataway, NJ, U.S.A.) with 0.02% Tween 80 (Junsei Chemical, Tokyo, Japan). The suspension was mixed vigorously on a vortex mixer for 15 to 20 sec until the turbidity matched the density of a 0.5 McFarland standard. The suspension was then diluted a few times following consideration of the final inoculum concentrations in the well. Serial 2-fold dilutions of antimicrobial agents were prepared and added across the 96-well plates (Becton Dickinson). The concentrations of antimicrobial agents in the wells were determined based on the NCCLS recommendation (12, 13). The final inoculum suspension (150  $\mu$ L) was transferred to the wells of a microtiter tray containing 150  $\mu$ L of each antimicrobial type and dilution. The final inoculum concentration was  $5 \times 10^4$  CFU/mL per well. The inoculated tray was covered and incubated at 30°C for over 3 days in room air. Endpoint MICs of all the antimicrobial drugs were read as the first well in which no growth occurred. *Staphylococcus aureus* ATCC 29213 and *Mycobacterium peregrinum* ATCC 700686 were also used for quality control purposes. The susceptibility categories of all antimicrobial agents were determined according to the breakpoints recommended by the NCCLS and are presented in Table 1 (12, 13).

**Table 1.** Antimicrobial drugs and MIC breakpoints

| Drug           | Suggested breakpoints |       |            |
|----------------|-----------------------|-------|------------|
|                | S                     | I     | R          |
| Amikacin       | $\leq 16$             | 32    | $\geq 64$  |
| Cefoxitin      | $\leq 16$             | 32-64 | $\geq 128$ |
| Imipenem       | $\leq 4$              | 8     | $\geq 16$  |
| Tobramycin     | $\leq 4$              | 8     | $\geq 16$  |
| Clarithromycin | $\leq 2$              | 4     | $\geq 8$   |
| Ciprofloxacin  | $\leq 1$              | 2     | $\geq 4$   |
| Moxifloxacin*  | $\leq 1$              | 2     | $\geq 4$   |
| Doxycycline    | $\leq 1$              | 2-8   | $\geq 16$  |

MIC, minimal inhibitory concentration.

Drugs and breakpoints are listed according to the recommendations set forth by the NCCLS document M24-A (12).

\*The breakpoints of this antimicrobial agent for the RGM have not yet been addressed by the NCCLS. The values are those recommended for aerobic organisms in NCCLS M100-S11 (13).

## RESULTS

In total, 74 isolates of *M. abscessus* were tested for antimicrobial susceptibility. The results of antimicrobial susceptibility tests are presented in Table 2. Of the parenteral antibiotics, amikacin (99%, 73/74) and cefoxitin (99%, 73/74) were active against most *M. abscessus* isolates. Amikacin demonstrated better in vitro activity against *M. abscessus* than tobramycin (36%, 27/74). Imipenem (55%, 36/74) had in vitro activity against a moderate number of isolates. Of the oral antibiotics, clarithromycin (91%, 67/74) was active against the majority of isolates. The fluoroquinolones showed moderate in vitro activities against the *M. abscessus* isolates. Moxifloxacin (73%, 54/74) had better in vitro activity than ciprofloxacin (57%, 42/74). Doxycycline was the least active, inhibiting only 7% (5/74) of the *M. abscessus* isolates.

## DISCUSSION

*M. abscessus* isolates have been determined to be uniformly resistant to standard antituberculous agents. For pulmonary diseases caused by *M. abscessus*, it is recommended that susceptible oral antibiotics (including clarithromycin or azithromycin) be administered in combination with parenteral medications (amikacin, cefoxitin, or imipenem) (1, 2). Due to its variable in vitro susceptibilities to some drugs, antibiotic susceptibility testing of all clinically significant isolates is recommended, although the correlation between in vitro susceptibility results for *M. abscessus* and clinical response for specific antimicrobial agents has not been established (1, 2). Our results in this study demonstrated that the resistance rates of *M. abscessus* isolates to various antibiotics are high. However, amikacin and cefoxitin were active against nearly all *M. abscessus* isolates. Clarithromycin was generally active against the majority of the *M. abscessus* isolates. In addition, ciprofloxacin, moxifloxacin, and imipenem exhibited moderate activity (>50% susceptibility), but the susceptibility of *M. abscessus* to doxycycline was poor.

Aminoglycosides are important parenteral antibiotics used in the treatment of *M. abscessus* infection. Traditionally, amikacin was the most active agent against RGM species.

**Table 2.** In vitro susceptibility of *M. abscessus* isolates

| Drug           | No. | No. (%) of isolates |              |           |
|----------------|-----|---------------------|--------------|-----------|
|                |     | Susceptible         | Intermediate | Resistant |
| Amikacin       | 74  | 73 (99)             | 1 (1)        | 0 (0)     |
| Cefoxitin      | 74  | 73 (99)             | 1 (1)        | 0 (0)     |
| Imipenem       | 66  | 36 (55)             | 22 (33)      | 8 (12)    |
| Tobramycin     | 74  | 27 (36)             | 25 (34)      | 22 (30)   |
| Clarithromycin | 74  | 67 (91)             | 5 (7)        | 2 (3)     |
| Ciprofloxacin  | 74  | 42 (57)             | 19 (26)      | 13 (18)   |
| Moxifloxacin   | 74  | 54 (73)             | 15 (20)      | 5 (7)     |
| Doxycycline    | 74  | 5 (7)               | 9 (12)       | 60 (81)   |

According to a study by Swenson et al., 95% of *M. abscessus*, 99% of *M. fortuitum*, and 88% of *M. chelonae* isolates were inhibited by this agent with a MIC of  $\leq 16$   $\mu\text{g}/\text{mL}$  (5). Intravenous amikacin is administered at a dose of 10 to 15 mg/kg daily to adult patients with normal renal function. This dose elicits peak serum levels in the low 20 mg/mL range. In this study, 99% of *M. abscessus* isolates were susceptible to amikacin and 36% were susceptible to tobramycin.

Cefoxitin is another important parenteral antibiotic used in the treatment of *M. abscessus* infection. This agent is administered at a dose of up to 12 g/day intravenously in divided doses. In this study, cefoxitin inhibited the growth of 99% of *M. abscessus* isolates. Another parenteral antibiotic that has been used in *M. abscessus* infection is imipenem. This agent has been reported to be moderately susceptible against *M. abscessus* (14). This study demonstrated that imipenem inhibits 55% of *M. abscessus* isolates at the breakpoint of  $<8$   $\mu\text{g}/\text{mL}$  and 88% of isolates at the intermediate breakpoint of  $<16$   $\mu\text{g}/\text{mL}$ . Therefore, imipenem may be useful in clinical treatment regimens for *M. abscessus*, especially in situations when cefoxitin cannot be used due to adverse effects.

Of the variable oral antibiotics, the most effective agents are the newer macrolides. Clarithromycin is representative of this class and has been considered to be the most effective (12). In this study, 91% of *M. abscessus* isolates were susceptible and 3% were resistant to clarithromycin. Brown et al. reported that the newer macrolides generally show better activity against RGM species, and among them, clarithromycin is the strongest (15).

Oral fluoroquinolones have also been used in the treatment of RGM diseases. Ciprofloxacin is the class representative of the older fluoroquinolones (i.e., ciprofloxacin, ofloxacin, and levofloxacin). In the current study, we tested ciprofloxacin and moxifloxacin (newer 8-methoxyfluoroquinolone), and both antimicrobials showed high activities, with 57 and 73%, respectively, of *M. abscessus* isolates susceptible. Even though fluoroquinolone cannot be used as a single-drug therapy due to the risk of developing mutational resistance (16), both ciprofloxacin and moxifloxacin could be used as alternative oral agents during combination treatment for *M. abscessus* lung disease. Doxycycline is a member of the tetracycline antibiotics and has shown poor activity against *M. abscessus* and *M. chelonae* in previous studies. In the current study, only 21% of *M. abscessus* isolates were susceptible or intermediate and approximately 80% were resistant to this agent. Doxycycline was the least active agent among all those tested in this study.

In this study, NTM species identification was performed using the PCR-RFLP method based on the *rpoB* gene. RGM are difficult to speciate by conventional methods. Several genes such as 16S rRNA, *hsp65*, and *rpoB* gene have been used to identify NTM to the species level. However, some gene target is often limited by the lack of sequence divergence among closely related *Mycobacterium* species. For exam-

ple, 16S rRNA internal transcribed spacer assay cannot differentiate *M. abscessus* from *M. massiliense* or *M. bolletii*, although this assay has proven to be valuable to distinguish *M. abscessus* and *M. chelonae* (17). Accurate identification of isolated NTM species is important since distinguishing these species is clinically relevant.

In conclusion, the variations in susceptibility within *M. abscessus* isolates to currently available antimicrobials suggest that the antimicrobial susceptibility testing of all clinically significant *M. abscessus* isolates be needed. In addition, our results offer clinicians choices for empirical treatment when *M. abscessus* lung disease is diagnosed and the in vitro susceptibilities are not available.

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