Antibiotic Resistance in *Mycobacterium Abscessus* and *Mycobacterium Fortuitum* Isolates from Malaysian Patients

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**Abstract**

**Background:** Rapidly growing mycobacterial species (RGM) are increasingly being recognized as the cause of various superficial and deep infections in humans. Two of the species most frequently isolated from clinical specimens are *Mycobacterium abscessus* and *Mycobacterium fortuitum*. Both species are associated with antibiotic resistances that may complicate therapy. This paper describes the pattern of resistance to five antibiotics commonly prescribed for RGM infections, in *M. abscessus* and *M. fortuitum* isolated from Malaysian patients. **Methods:** The bacterial strains studied were examined with Etest strips to determine their minimum inhibitory concentrations (MICs) toward amikacin, ciprofloxacin, clarithromycin, imipenem, and linezolid. **Results:** Among 51 *M. abscessus* isolates examined by the Etest, the overall MICs of ciprofloxacin, imipenem, amikacin, clarithromycin, and linezolid showed resistance rates of 33.3%, 31.4%, 2.0%, 5.9%, and 21.6%, to the five antibiotics, respectively. *M. abscessus* subspecies *abscessus* was more resistant than *M. abscessus* subs. *massiliense* to ciprofloxacin, imipenem, and linezolid but was more susceptible to clarithromycin and amikacin. *M. fortuitum* isolates were significantly less resistant than *M. abscessus* to ciprofloxacin (3.6%) and imipenem (7.1%) but more resistant to clarithromycin (42.9%) and linezolid (39.3%). **Conclusion:** A suitable combination therapy for Malaysian patients would be amikacin plus clarithromycin and ciprofloxacin, to cover infections by all three *M. abscessus* subspecies and *M. fortuitum*.

**Keywords:** Antibiotic resistance, *Mycobacterium abscessus*, *Mycobacterium fortuitum*

**INTRODUCTION**

Rapid-growing mycobacteria (RGM) are environmental bacteria often found in water, soil, and dust. An increasing number of species have been recognized as opportunistic human pathogens and frequent isolates from clinical specimens. Among them, *Mycobacterium abscessus* is a formidable respiratory pathogen, frequently associated with cystic fibrosis[^1] and causing tuberculosis-like pulmonary disease with substantial mortality. This species complex has been classified into three subspecies, *M. abscessus* subs. *abscessus*, *M. abscessus* subs. *massiliense* and *M. abscessus* subs. *bolletii*, hereafter referred to here as *M. abscessus*, *M. massiliense* and *M. bolletii* for simplicity. The species complex, on the whole, is notorious for their resistance to multiple antibiotics, but the three subspecies differ in their geographical distribution[^2] and susceptibility to antibiotics.[^3] To clarithromycin, a common antibiotic for the treatment of RGMs, *M. massiliense* is mostly susceptible, *M. bolletii* is most often resistant, and both *M. abscessus* and *M. bolletii* show inducible resistance.[^4]

*Mycobacterium fortuitum* is most often associated with skin and superficial infections acquired from community sources, such as contaminated footbaths in beauty parlors[^5] or from contaminated water or medical devices in healthcare settings.[^6] While *M. abscessus* has been reported to be responsible for 90% of the respiratory illness caused by RGMs, *M. fortuitum* is said to be responsible for 60%–80% of postsurgical and catheter-related infections caused by these bacteria.[^7] It has also been associated with serious disseminated infections involving many different organs and tissues in severely immunocompromised individuals.[[^8]-[^10]] Although respiratory infections with *M. fortuitum* are not common, as with *M. abscessus* respiratory infections, *M. fortuitum* lung disease may be difficult to eradicate because of disease chronicity.

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and poor response to antibiotic treatment. The antibiotic of choice for these infections is amikacin, but resistance to aminoglycosides has also been reported.\(^\text{11,12}\) In this study, strains of \textit{M. abscessus} complex and \textit{M. fortuitum} isolated from Malaysian patients are examined for their susceptibility to selected antibiotics to provide guidance for the empirical therapy of RGM infections in the country.

**Methods**

The bacterial strains studied were isolated between 2012 and 2014, from the sputum and bronchoalveolar lavage fluids of patients with clinical signs of lower respiratory tract infections. Acid-fast bacilli grown on Lowenstein-Jensen slopes within 7 days of inoculation were kept at 80°C until required for further testing. \textit{M. abscessus} ATCC 19977 was used as the reference strain for the determination of minimum inhibitory concentrations (MIC).

**Identification of rapidly growing mycobacterial species**

The identification of \textit{M. abscessus} complex and \textit{M. fortuitum} was based on the DNA sequencing of the \textit{hsp} 65 gene. For gene amplification, DNA was extracted from each isolate by heating a suspension of the isolate at 100°C for 15 min, followed by centrifugation at 1500 rpm for 10 min. Of the supernatant obtained, 2.5 µl was used as the DNA template in a 25 µl reaction mixture containing 6 µl of ddH\(_2\)O, 12.5 µl of Master mix (Promega), and 2 µl each of forward and reverse primers. The primers used were Tb11 (5′-ACCAACGATGGTGTGTCCAT-3′) and Tb12 (5′-CTGGTGAACCGCATACCT-3′), described by Telenti \textit{et al.}, 1993.\(^\text{13}\) The thermal cycling profile, also by Telenti,\(^\text{13}\) consisted of 45 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, followed by extension at 72°C for 10 min. The expected product size was 439 base pairs.

Since the differentiation of \textit{M. abscessus} subspecies with the \textit{hsp} 65 PCR is not entirely reliable,\(^\text{14,15}\) an additional PCR assay based on the \textit{erm} (41) gene was performed to distinguish \textit{M. massiliense} from the other two \textit{M. abscessus} subspecies. The \textit{erm} (41) gene in almost all \textit{M. massiliense} is characterized by a 2 bp deletion at nucleotides 64–65 and a 274 bp deletion of nucleotides 159-432,\(^\text{16}\) and hence, is 276 bp shorter compared to the other two subspecies. The primers for the amplification of the \textit{erm} (41) gene were \textit{erm} F (5′-TGGTATCCGCTCAGTGATGA-3′) and \textit{erm} R (5′-GCGGTGGATGTAAGGAAG-3′). The thermal cycling profile consisted of an initial denaturation at 95°C for 5 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, and ending with a final extension at 72°C for 10 min.\(^\text{16}\) The expected product size was 451 bp. All amplicons obtained were purified using QIAquick PCR purification kit and were evaluated for their purity using the Thermo Scientific NanoDrop Spectrophotometer. They were then sent out for Sanger sequencing with the same primers as those used for the PCR.

\textit{Hsp} 65 gene sequences were analyzed using NCBI BLASTN and hsp65BLAST (http://hsp65blast.phsa.ca/) while \textit{erm} (41) gene sequences were aligned and analyzed using MEGA6 software, an integrated tool which conducts automatic and manual sequence alignments.

**Determination of minimum inhibitory concentrations**

All strains were examined with Etest strips (ABBiodisk, bioMe´rieux) for susceptibility to amikacin, ciprofloxacin, clarithromycin, imipenem, and linezolid, as per manufacturer’s instructions. Inocula from a suspension prepared in broth to a 1 McFarland standard were plated on Mueller Hinton Blood agar. Etest strips were placed on the air-dried inoculated plates which were then incubated in an ambient air incubator at 36°C. The MICs were read after 72 h of incubation, except for clarithromycin MICs which were read on the 3\textsuperscript{rd}, 7\textsuperscript{th}, and 14\textsuperscript{th} day of incubation, for the detection of inducible resistance. The MICs were interpreted according to the Clinical and Laboratory Standards Institute breakpoints.\(^\text{17}\)

**Results**

**Subspecies identification of \textit{Mycobacterium abscessus} strains**

Based on the \textit{hsp} 65 and \textit{erm} (41) PCR results, the 51 strains of \textit{M. abscessus} complex were identified as 12 strains of \textit{M. abscessus}, 38 of \textit{M. massiliense}, and only 1 \textit{M. bolletii}. The 28 \textit{M. fortuitum} strains were identified as \textit{M. fortuitum} subspecies \textit{fortuitum} [Table 1].

**Antibiotic susceptibility testing**

The antibiotic susceptibilities of the strains examined are summarized in Table 1. For all \textit{M. abscessus} complex strains

| Table 1: Summary of resistance rates in \textit{Mycobacterium abscessus} complex and \textit{Mycobacterium fortuitum} |
|-----------------------------------------------|
| **Bacterium (n)** | **Ciprofloxacin** | **Imipenem** | **Amikacin** | **Clarithromycin** | **Linezolid** |
| **S** | **I** | **R** | **S** | **I** | **R** | **S** | **I** | **R** | **S** | **I** | **R** |
| \textit{Mycobacterium abscessus} (12) | 5 | 2 | 5 | 5 | 0 | 7 | 12 | 0 | 0 | 0 | 1 | 0 | 8 | 1 | 3 |
| Percentage resistance | 41.7 | 58.3 | 0 | 0 | 0 | 25.0 |
| \textit{Mycobacterium massiliense} (38) | 17 | 9 | 12 | 27 | 3 | 8 | 31 | 6 | 1 | 36 | 0 | 2 | 29 | 2 | 7 |
| Percentage resistance | 31.6 | 21.1 | 2.6 | 5.3 | 18.4 |
| \textit{Mycobacterium bolletii} (1) | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Percentage resistance | - | - | - | - | - | - |
| \textit{Mycobacterium fortuitum} (28) | 27 | 0 | 1 | 25 | 1 | 12 | 26 | 1 | 1 | 14 | 2 | 12 | 15 | 2 | 11 |
| Percentage resistance | 3.6 | 7.1 | 3.6 | 42.9 | 39.3 |

\((n): number of strains, S: Susceptible, I: Intermediate, R: Resistant\)
and *M. fortuitum*, amikacin seems to be the most effective antibiotic *in vitro*, with <4% resistance within the respective subspecies/species. Clarithromycin, likewise, showed good antimicrobial activity on the *M. abscessus* complex but had a rather high rate of resistance (42.9%) among *M. fortuitum*, with three (10.8%) of the isolates showing inducible resistance. Conversely, imipenem, which had a low rate of resistance among *M. fortuitum*, showed resistance rates of 21.1% to 58.3% among the *M. abscessus* complex. Similarly, ciprofloxacin resistance was low (3.6%) among *M. fortuitum* but high among *M. massiliense* (31.6%) and *M. abscessus* (41.7%). Linezolid resistance increased from 18.4% in *M. massiliense* to 25% in *M. abscessus* and 39.3% in *M. fortuitum*. Overall, 33.3% of *M. abscessus*, 60.1% of *M. massiliense*, and 42.9% of *M. fortuitum* showed *in vitro* susceptibility to all five of the antibiotics. Of the resistant strains, none were resistant to all five antibiotics, two (5.1%; a *M. massiliense* and a *M. fortuitum*) were resistant to four antibiotics, and only seven (8.9%) were resistant to three antibiotics each. On the other hand, no antibiotic showed consistent activity on all strains. While ciprofloxacin, amikacin, and imipenem had good activity for *M. fortuitum*, amikacin and clarithromycin had the best activity for the *M. abscessus* complex.

**DISCUSSION**

The goal of antimicrobial susceptibility testing is to predict whether patients treated with antibiotics are likely to be cured of their infections. For most bacteria, antibiotic susceptibilities differ substantially among strains in different geographical locations and clinical settings. Hence, for empirical therapy, local antibiotic susceptibility data are immensely helpful as a guide to the choice of antibiotics for the treatment of infections.

*M. abscessus* complex and *M. fortuitum* are not infrequently isolated from the respiratory secretions of Malaysian patients, many of whom require antibiotic treatment for their infections. We compared the antibiotic susceptibilities of our local isolates with those reported in medical literature. Although most of these isolates are single isolates and many of the infections do not satisfy the American Thoracic Society’s diagnostic criteria for lung disease, the majority of culture-positive patients had a productive cough or abnormal chest X-rays and was treated with antibiotics for suspected respiratory infections.

Overall, our AST results are consistent with those reported by others[18,19] in that the greatest *in vitro* activity against both groups of RGM is seen in amikacin. This aminoglycoside has been the preferred treatment for *M. fortuitum* infections and is often used in combination with imipenem for the treatment of serious lung disease.[20] In our series, however, the rates of imipenem resistance suggest that this combination therapy is not likely to be effective in many *M. abscessus* subspecies infections (21%–58% resistance) but should still be adequate for *M. fortuitum* infections (7.1% resistance). High rates of imipenem resistance have also been reported by others, with rates ranging from 55% to 95%.[18,21]

Fluoroquinolones have been used successfully for the treatment of RGM infections.[22] Our results showed very good ciprofloxacin activity for *M. fortuitum* but relatively high rates of resistance for *M. abscessus* and *M. massiliense*. Thus, where NTM species identification is not available, fluoroquinolones might not be suitable for empirical therapy. An additional concern with the use of fluoroquinolones for respiratory infections is that these antibacterials have been associated with delayed anti-TB treatment and resistance in tuberculosis, and hence, should not be used indiscriminately in regions where the incidence of tuberculosis is high.[23]

The macrolide clarithromycin is one of the most widely prescribed antibiotics for NTM infections, and our *in vitro* test results indicate that it would be a suitable antibiotic of choice for *M. abscessus* and *M. massiliense* infections. Against *M. fortuitum*, however, the 39% resistance we observed predicts substantial treatment failures with clarithromycin therapy. One drawback pertinent to both NTM species is the occurrence of inducible resistance in apparently clarithromycin-susceptible isolates. This is caused by the derepression of erythromycin resistance methylase (erm) genes, the *erm* (41) gene in *M. abscessus* and *M. bolletii*,[41] and the *erm* (39) gene in *M. fortuitum*.[24] These inducible resistances are not detected in routine antibiotic susceptibility tests, and hence, will not be reported to referring physicians. They are deduced when resistance emerges after the initiation of therapy. In most *M. massiliense* strains, the *erm* (41) gene is truncated and inactive. Hence, this subspecies is not affected by inducible resistance, and clarithromycin can be safely prescribed for susceptible strains.

When linezolid was first introduced for clinical use in the early 2000s, it was reported to be active against most gram-positive bacteria, including many species of RGM.[25] However, reports of resistance among RGM soon appeared. Yang et al.[19] found 42% resistance among clinical isolates of *M. abscessus* and 25% among *M. fortuitum*. We found similar high resistance rates of 18.4% among *M. massiliense*, 25% among *M. abscessus*, and 39.3% among *M. fortuitum*. The reason for this rapid emergence of resistance is still unknown, but the high prevalence of resistance indicates the need for linezolid to be used in combination with a more reliable antibiotic, in serious RGM infections.

**CONCLUSION**

In summary, our 2-year collection of RGM from respiratory secretions showed *M. massiliense* to be the most frequently isolated *M. abscessus* subspecies and *M. bolletii*, the least often encountered. The rarity of *M. bolletii* isolation from clinical specimens is also reported from our neighboring countries, Korea[26] and Australia.[3] In view of varying antibiotic susceptibilities among different RGM species and subspecies, our results reinforce the need for species and
subspecies identification of RGM to ensure the choice of appropriate antibiotics for therapy. When this is not possible, combination therapy is recommended for most patients and is, especially, important in serious infections, since monotherapy may lead to the further emergence of resistance. Based on our results, a suitable combination therapy for Malaysian patients would be amikacin plus clarithromycin and ciprofloxacin, to cover infections by all three *M. abscessus* subspecies and *M. fortuitum*.

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Conflicts of interest
There are no conflicts of interest.

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