Antimicrobial Susceptibility in the Mycobacteroides abscessus Complex is Restored by an Imipenem-Clarithromycin Combination

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Research article

Keywords: Clarithromycin, Fractional inhibitory concentration index, Imipenem, Mycobacteroides abscessus

DOI: https://doi.org/10.21203/rs.3.rs-33042/v1

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Abstract

Nontuberculous mycobacteria (NTM) are ubiquitous organisms and the incidence of NTM infections has been increasing in recent years. *Mycobacteroides abscessus* (*M. abscessus*) is one of the most antimicrobial-resistant NTM; however, no reliable antibiotic regimen can be officially advocated. We evaluated the efficacy of clarithromycin in combination with various antimicrobial agents against the *M. abscessus* complex. Twenty-nine clinical strains of *M. abscessus* were isolated from various clinical samples. Of the isolates, 10 (34.5%) were of *M. abscessus* subsp. *abscessus*, 18 (62.1%) of *M. abscessus* subsp. *massiliense*, and 1 (3.4%) of *M. abscessus* subsp. *bolletii*. MICs of three antimicrobial agents (amikacin, imipenem, and moxifloxacin) were measured with or without clarithromycin. The imipenem-clarithromycin combination significantly reduced MICs compared to clarithromycin and imipenem monotherapies, including against resistant strains. The association between susceptibility of the *M. abscessus* complex and each combination of agents was significant (*p* = 0.001). Adjusted residuals indicated that the imipenem-clarithromycin combination had the synergistic effect (adjusted residual = 3.1) and suppressed the antagonistic effect (adjusted residual = -3.1). In subspecies of *M. abscessus* complex, the association with susceptibility of *M. abscessus* subsp. *massiliense* was similarly statistically significant (*p* = 0.036: adjusted residuals of synergistic and antagonistic effect respectively: 2.6 and -2.6). The association with susceptibility of *M. abscessus* subsp. *abscessus* also showed a similar trend but did not reach statistical significance. Our data suggest that the imipenem-clarithromycin combination could be the recommended therapeutic choice for the treatment of *M. abscessus* complex owing to its ability to restore antimicrobial susceptibility.

Background

NTM are ubiquitous organisms that cause diverse types of infectious diseases in humans, including in lungs, the lymphatic system, skin, soft tissue, bone disease, and are disseminated. The morbidity of NTM has been increasing worldwide (1, 2); the 2014 nationwide survey of NTM in Japan revealed that the incidence of pulmonary NTM (14.7 cases/100,000 person/year) has overtaken that of tuberculosis (12.9 cases/100,000 person/year) (3). Above all, the *Mycobacterium avium* complex (88.8%) were the most frequently isolated organisms, followed by *Mycobacterium kansasii* (4.3%) and the *Mycobacteroides abscessus* complex (3.3%). Notably, the incidence of *M. abscessus*-infected pulmonary disease has dramatically increased in Japan, from 0.1 cases/100,000 person/year in 2001 to 0.5 cases/100,000 person/year in 2014. *M. abscessus* is one of the treatment-refractory NTM, characterized by rapid growth and multidrug resistance. It is also frequently isolated from the respiratory tract of patients with cystic fibrosis (CF); *M. abscessus* has been the leading cause of rapid growing mycobacteria in CF since the 2000s (4, 5). The critical feature of *M. abscessus* is its spontaneous resistance to most antibiotics in clinical use, including first line antitubercular drugs (6, 7). The 2007 American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) statement recommended multidrug therapy, including a macrolide and one or more parenteral agents (e.g., amikacin, cefoxitin, or imipenem) (8); however, recommendations for the treatment of *M. abscessus* are known to be of limited efficacy. Recently, three
subspecies of *M. abscessus* have been defined: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, and *M. abscessus* subsp. *massiliense*. *M. abscessus* subsp. *massiliense* specifically lacks the *erm* (41) gene associated with macrolide resistance, and thus, the macrolide susceptibility among *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *abscessus* and *bolletii* is different (9, 10). For this reason, some experts recommend non-macrolide combinations for treatment for macrolide-resistant *M. abscessus* subspecies, based on identified in vitro susceptibilities. Here, we propose new insights into the synergistic effects on *M. abscessus* susceptibility achieved in vitro by clarithromycin in combination with other antimicrobials.

**Results**

**Clinical features of three subspecies of *M. abscessus* complex**

Twenty-nine clinical strains of *M. abscessus* were isolated from various clinical samples at the Juntendo university hospital from 2011 to 2019. The characteristics of patients from which *M. abscessus* complex were isolated are shown in Table 1. Twenty-two of 29 (75.9%) patients were diagnosed with *M. abscessus* complex from the culturing of sputum or bronchial lavage. Of the isolates, 10 (34.5%) were of *M. abscessus* subsp. *abscessus*, 18 (62.1%) of *M. abscessus* subsp. *massiliense*, and 1 (3.4%) of *M. abscessus* subsp. *bolletii* as determined by multi-locus sequence analysis. The treatment history indicated that 24 of 29 (82.8%) patients had received antibiotics in the last 3 months, including macrolides, and 10 of 29 (34.5%) patients had received immunosuppressive treatment including corticosteroids before the culture.

**Susceptibility to antimicrobial agents in combination with clarithromycin**

The susceptibility to a combination of clarithromycin and antimicrobial agents was compared to that of the antimicrobial agents alone, categorized into each subspecies of *M. abscessus* complex (Figure 1). The MICs of three antimicrobial agents (amikacin, imipenem, and moxifloxacin) were measured with or without clarithromycin. Susceptibility to the imipenem-clarithromycin combination was significantly better than to other clarithromycin combinations. Notably, the use of the imipenem-clarithromycin combination significantly reduced the MIC of clarithromycin, even in clarithromycin-resistant subspecies of *M. abscessus* complex. The effect of restoring susceptibility by the imipenem-clarithromycin combination was stronger than that of the amikacin- and moxifloxacin-clarithromycin combination. In 1 strain of *M. abscessus* subsp. *abscessus* and 3 strains of *M. abscessus* subsp. *massiliense*, susceptibility was not restored by the combined use of clarithromycin and imipenem, and only 1 strain of *M. abscessus* subsp. *bolletii* did not respond to any combination. In subspecies of *M. abscessus* complex, MICs of imipenem and clarithromycin in combination were significantly less than that of either clarithromycin or imipenem alone in both *M. abscessus* subsp. *massiliense* and *abscessus* (*p* < 0.001 for both subspecies) (Table S1).

We next determined the synergistic effect of the imipenem-clarithromycin combination as compared to amikacin- or moxifloxacin-clarithromycin combinations, using the fractional inhibitory concentration (FIC)
index (Figure 2). Susceptibility was divided into two classes, synergy and additive as a synergistic effect and indifference and antagonism as an antagonistic effect. The associations between susceptibility of the *M. abscessus* complex and each combination of antimicrobials were significant (*p* = 0.001) (Table 2). Adjusted residuals indicated that the imipenem-clarithromycin combination had the synergistic effect (adjusted residual = 3.1) and suppressed the antagonistic effect (adjusted residual = -3.1). In the subspecies of *M. abscessus* complex, the association between susceptibility of *M. abscessus* subsp. *massiliense* and each combination of antimicrobials was significant (*p* = 0.036), and imipenem-clarithromycin combination had the synergistic effect (adjusted residual = 2.6) and suppressed the antagonistic effect (adjusted residual = -2.6). The association with susceptibility of *M. abscessus* subsp. *abscessus* also showed a similar trend, but did not reach statistical significance, potentially because of a smaller number of samples.

**Association of clinical features with susceptibilities to the imipenem-clarithromycin combination**

We investigated whether susceptibility to the imipenem-clarithromycin combination might associate with clinical status. The isolates from patients with immunosuppression and/or administered immunosuppressive drugs and/or corticosteroids revealed synergistic effects rather than antagonistic effects (*p* = 0.040) (Table 3). The other clinical parameters such as age, sex, smoking history, bronchiectasis lesion, and a treatment history of antibiotics did not associate with the susceptibility to the imipenem-clarithromycin combination.

**Discussion**

We demonstrated here that the MICs of clarithromycin and imipenem were significantly reduced by the administration of an imipenem-clarithromycin combination. We propose a new therapeutic benefit by which the imipenem-clarithromycin combination could restore the susceptibility of *M. abscessus* isolates, even after acquiring resistance to both clarithromycin and imipenem separately. The isolates included *M. abscessus* subsp. *abscessus*, well known among the three subspecies to easily acquire macrolide-resistance (9, 10). Furthermore, this combination may be suitable for treatment of *M. abscessus* complex in patients with immunosuppression.

In recent years, the incidence of NTM has globally increased. The major issue in treating *M. abscessus* complex centers on its intrinsic resistance against most available antibiotics. The 2007 ATS/IDSA Statement has recommended antimicrobial combination therapy, namely macrolides (clarithromycin), aminoglycosides (amikacin), cephemycins (cefoxitin), and carbapenems (imipenem), to treat *M. abscessus* infections, depending on in vitro susceptibility testing (8). However, there were several problems involved in the recommendation, due to the lack of clinical outcomes, and uncertain interactions present in multidrug combination therapy; thus, there is still limited reliable evidence to promote a global standard treatment regimen for the three subspecies of *M. abscessus* complex. Previous in vitro studies have demonstrated that treatment with the standard regimen therapy (combinations of clarithromycin, amikacin, and cefoxitin) failed to effectively inhibit the growth of *M.*
abscessus due to acquired drug resistance (16). In vivo, the triple-drug regimen was equally or less effective against M. abscessus than cefoxitin alone (17). A systematic review revealed different outcomes of macrolide-containing combination regimens against M. abscessus subsp. abscessus and massiliense. Macrolide-containing combination regimens for M. abscessus subsp. abscessus induced lower rates of negative conversion of sputum culture and higher recurrence rates than that of M. abscessus subsp. massiliense (18). For these reasons, the appropriate drug therapy against M. abscessus remains uncertain. M. abscessus complex spontaneously produce broad-spectrum β-lactamases, resulting in reduced susceptibility to β-lactams, including imipenem. Imipenem in combination with rifabutin or amikacin was more effective than as a monotherapy against M. abscessus complex (19, 20). Miyasaka et al. described that the imipenem-clarithromycin combination had a high rate of synergistic and additive effects, and revealed a decrease in the MIC values inhibiting 50% or 90% of M. abscessus complex (21). Although the exact mechanism for the synergistic effect of clarithromycin combinations was unknown, these data support the results of our present study. Therefore, imipenem may be useful in combination with clarithromycin for the treatment of M. abscessus complex. Limitations of the present study include the lack of clinical outcomes measured in patients with M. abscessus complex treated with imipenem-clarithromycin combination therapy. Thus, a prospective clinical study is required to establish the in vivo efficacy of the combination regimen.

Conclusion

In our in vitro study, we demonstrated the synergistic effect of the imipenem-clarithromycin combination in restoring M. abscessus complex antimicrobial susceptibility. Further, this synergistic effect may occur not only in M. abscessus subsp. massiliense, but also in M. abscessus subsp. abscessus. Thus, our present results suggest that the imipenem-clarithromycin combination could be an effective treatment regimen against both M. abscessus subsp. massiliense and M. abscessus subsp. abscessus.

Methods

Determination of M. abscessus complex

All material samples suspected of mycobacterial contamination in the Juntendo university hospital were cultured in mycobacteria growth indicator tube (MGIT; Becton Dickinson, USA) broth and incubated at 37°C in the BACTEC MGIT 960 (Becton Dickinson, USA) instrument with ambient air. MGIT positive tubes were classified as M. abscessus based on the results of DNA–DNA hybridization (DDH) analysis (DDH Mycobacterium “Kyokuto” kit; Kyokuto Pharmaceutical Industrial, Japan) or matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Detected species were reconfirmed as three subspecies of M. abscessus complex by sequencing the 16S rRNA, rpoB, hsp65, and erm genes (17, 18). All strains of M. abscessus were cultured on BD trypticase soy agar II with 5% sheep blood (Blood agar; Nippon Becton-Dickinson and Company, Japan) at 35°C for approximately 4 to 6 days in an aerobic atmosphere. The study protocol was approved by the Ethics Committee of Juntendo University School of Medicine (no. 18-010 and 19-038).
MALDI-TOF MS analysis

Colonies of *M. abscessus* complex on blood agar were scratched with a needle, and particles on the needle surface were diluted in 50 μL 80% trifluoroacetic acid. After incubation for 15 minutes at room temperature, the solution was added to 150 μL distilled water and 200 μL 100% acetonitrile, followed by a centrifugation step (16,200 × g, 2 min). One microliter of the cleared supernatant containing the bacterial extract was transferred onto a MALDI target plate (Bruker Daltonik, Germany). Dried spots were overlaid with MALDI matrix (10 mg/mL α-cyano-4-hydroxy-cinnamic acid [α-HCCA] in 50% acetonitrile:2.5% trifluoroacetic acid) (Bruker Daltonik, Germany). After drying of the matrix, MALDI-TOF MS measurements were performed with a Microflex LT/SH benchtop mass spectrometer (Bruker Daltonik, Germany) equipped with a 60-Hz nitrogen laser. Parameter settings (ion source 1 [IS1], 20 kV; IS2, 18.2 kV; lens, 6.85 kV; detector gain, 2854 V; gating, none) had been optimized for the mass range between 2,000 and 20,000 Da. Spectra were recorded in the positive linear mode with the maximum laser frequency. An external standard (bacterial test standard [BTS]) (Bruker Daltonik, Germany) was used for instrument calibration. Data evaluation was performed by visually comparing spectra to search for peak shifts using flexAnalysis 3.4 (Bruker Daltonik, Germany).

PCR amplification and DNA sequencing

DNA was extracted from cultured colonies using the DNeasy UltraClean Microbial Kit (QIAGEN, Germany), and PCR was conducted using Ex Taq DNA polymerase, hot-start version (Takara, Japan) according to the manufacturer’s instructions. The gene-specific primer pairs used for PCR analysis are listed in Table 4; these primers were used in previous studies (19, 20). The sequencing PCR products were purified with the BigDye XTerminator purification kit (Life Technologies, USA) and samples were loaded on the ABI Prism 3130 Genetic Analyzer (Thermo Fisher Scientific, USA). The DNA sequencing results were analyzed using a BLAST search to identify sequence similarity between samples and the three species of *M. abscessus* complex.

Antimicrobial susceptibility testing

Susceptibility testing was performed according to Clinical and Laboratory Standard Institute (CLSI) guideline M24-A2 (21), using frozen broth microdilution panels. The ranges of antibiotic concentrations tested were as follows: amikacin (AMK) 0.25 to 64 μg/mL, clarithromycin (CLR) 0.06 to 64 μg/mL, imipenem (IPM) 4 to 32 μg/mL, and moxifloxacin (MXF) 1 to 32 μg/mL. MICs of each antimicrobial agent were determined by broth microdilution methods, as recommended by the CLSI, using frozen broth microdilution plates (Eiken Chemical Co., Ltd., Japan). The MICs were determined after 7 days of incubation at 35°C. The MIC breakpoints, indicating susceptible, intermediate, and resistant strains, were interpreted according to the CLSI criteria for amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, moxifloxacin, trimethoprim/sulfamethoxazole, and tobramycin (Table 5) (21). The effect of each agent combined with clarithromycin was evaluated using FIC index analysis. FIC index was calculated as follows: Σ (FIC A + FIC B), where FIC A is the MIC of compound A in combination / MIC of compound A alone, and FIC B is the MIC of compound B in combination / MIC of compound B alone. The
combination is considered synergistic when the $\Sigma$ FIC is $\leq 0.5$, additive when the $\Sigma$ FIC is $>0.5$ to $\leq 1$, indifferent when the $\Sigma$ FIC is $>1$ to $\leq 2$, and antagonistic when the $\Sigma$ FIC is $>2$.

**Statistical analysis**

Categorical variables were compared using the chi-square test or Fisher's exact test. The evaluation of changes in MIC was performed using the Wilcoxon signed-rank test. Differences were considered significant at $p < 0.05$. When the chi-square test results were statistically significant, adjusted residuals were calculated to determine which particular associations were significant. Adjusted residuals were significant at $p < 0.05$ level if they were less than $-1.96$ or more than $1.96$, and were significant at $p < 0.01$ level if they were less than $-2.58$ or more than $2.58$. All statistical analyses were performed using the SPSS software program (version 20, IBM Japan, Japan).

**Abbreviations**

NTM: nontuberculous mycobacteria; *M. abscessus*: *Mycobacteroides abscessus*; CF: cystic fibrosis; ATS/IDSA: American Thoracic Society/Infectious Diseases Society of America; FIC: fractional inhibitory concentration; MGIT: mycobacteria growth indicator tube; DDH: DNA–DNA hybridization; MALDI-TOF MS: matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; BTS: bacterial test standard; CLSI: Clinical and Laboratory Standard Institute; AMK: amikacin; CLR: clarithromycin; IPM: imipenem; MXF: moxifloxacin; HIV: human immunodeficiency virus

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Independent Ethics Committee at Juntendo University Hospital (approval no. 18-010 and 19-038) and adhered to the tenets of the Declaration of Helsinki.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets during the current study available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**
This work was supported by the Grant for Cross-disciplinary Collaboration, Juntendo University (grant no. 2019-46 to T. Okabe).

**Authors' contributions**

Conceived and designed the experiments: ST, HI, ST, and AN. Performed the experiments: ST. Analyzed the data: ST, HI, ST, and AN. Collected the data and/or samples: ST, YH, JW, KK, SK, IS, YO, MI, MC, and SM. Contributed reagents/materials/analysis tools: ST, TO, MC, and SM. Reviewed the initial and final drafts of the manuscript: AO and KT. Wrote the paper: ST, HI, ST, and AN. All authors read and approved the final manuscript.

**Acknowledgements**

The authors would like to thank the staff of Juntendo University Hospital for their contribution in collecting data.

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**Tables**
Table 1. The characteristics of patients from which *M. abscessus* complex were isolated

| Characteristic                                      | N=29   |
|-----------------------------------------------------|--------|
| Sex (Male/Female)                                   | 12/17  |
| Median age (range)                                  | 65 (38-83) |
| Smoking history, N (%)                              | 9 (31.0) |
| *M. abscessus* complex subtype, N (%)               |        |
| *M. abscessus* subsp. *abscessus*                   | 10 (34.5) |
| *M. abscessus* subsp. *masiliense*                  | 18 (62.1) |
| *M. abscessus* subsp. *bolletii*                    | 1 (3.4) |
| *M. abscessus* complex detected from, N (%)         |        |
| Sputum or bronchial lavage                          | 22 (75.9) |
| Others                                              | 7 (24.1) |
| Pretreatment of antibiotics within 3 months, N (%)  |        |
| Macrolides                                          | 5 (17.2) |
| Fluoroquinolones                                    | 5 (17.2) |
| Tetracyclines                                       | 2 (6.9) |
| Others                                              | 12 (41.4) |
| Comorbidity, N (%)                                  |        |
| Bronchiectasis                                      | 10 (34.5) |
| Diabetes mellitus                                   | 4 (13.8) |
| Immunodeficiency (nonHIV)                           | 2 (6.9) |
| Malignancy                                          | 7 (24.1) |
| Concomitant medications, N (%)                      |        |
| Corticosteroids                                     | 6 (20.7) |
| Immunosuppressant                                   | 4 (13.8) |

Abbreviations: HIV, human immunodeficiency virus
Table 2. The number of synergistic and antagonistic combination with clarithromycin and each antimicrobial

| Species                     | Categories of FIC index       | CLR/AMK     | CLR/IPM    | CLR/MXF    | p value   |
|-----------------------------|-------------------------------|-------------|------------|------------|-----------|
| M. abscessus complex        | Synergy + Additive            | 5 (17.2, -1.5) | 14 (48.3, 3.1***) | 5 (17.2, -1.5) | 0.001**   |
| N=29†                       | Indifference + Antagonism     | 24 (82.8, 1.5) | 15 (51.7, -3.1***) | 24 (82.8, 1.5) |           |
| M. abscessus subsp. massilience | Synergy + Additive        | 3 (16.7, -1.3) | 9 (50.0, 2.6***) | 3 (16.7, -1.3) | 0.036*    |
| N=18                        | Indifference + Antagonism     | 15 (83.3, 1.3) | 9 (50.0, -2.6***) | 15 (83.3, 1.3) | 0.24      |
| M. abscessus subsp. abscessus | Synergy + Additive           | 2 (20.0, -0.8) | 5 (50.0, 1.7) | 2 (20.0, -0.8) | 0.24      |
| N=10                        | Indifference + Antagonism     | 8 (80.0, 0.8) | 5 (50.0, -1.7) | 8 (80.0, 0.8) |           |

† including M. abscessus subsp. boletii (n=1)
* p value <0.05, ** p value <0.01
* adjusted residuals > |1.96|, ** adjusted residuals > |2.58|
Abbreviations: FIC index, fractional inhibitory concentration index; CLR, clarithromycin; AMK, amikacin; IPM, imipenem; MXF, moxifloxacin

Table 3. The number of synergistic and antagonistic combination with clarithromycin and imipenem in each clinical status

| FIC index                      | Synergy + Additive | Indifference + Antagonism | p value |
|--------------------------------|---------------------|---------------------------|---------|
|                                | N=14 (%)            | N=15 (%)                  |         |
| Age                            | <65 years           | 7 (24.1)                  | 7 (24.1) | 0.86     |
|                                | ≥65 years           | 7 (24.1)                  | 8 (27.6) |           |
| Sex                            | Male                | 6 (20.7)                  | 6 (20.7) | 0.88     |
|                                | Female              | 8 (27.6)                  | 9 (31.0) |           |
| Smoking history                | Yes                 | 3 (10.3)                  | 6 (20.7) | 0.43     |
|                                | No                  | 11 (37.9)                 | 9 (31.0) |           |
| With bronchiectasis            | Yes                 | 4 (13.8)                  | 6 (20.7) | 0.70     |
|                                | No                  | 10 (34.5)                 | 9 (31.0) |           |
| With immunosuppression         | Yes                 | 10 (34.5)                 | 5 (17.2) | 0.040*   |
|                                | No                  | 4 (13.8)                  | 10 (34.5)|           |
| Pretreatment of antibiotics    | Yes                 | 6 (20.7)                  | 8 (27.6) | 0.57     |
|                                | No                  | 8 (27.6)                  | 7 (24.1) |           |

Antibiotics including clarithromycin (n=3)
* p value <0.05, ** p value <0.01
Abbreviations: FIC index, fractional inhibitory concentration index
### Table 4. Forward and backward primers used for PCR

| Target  | Sequence                                           |
|---------|----------------------------------------------------|
| 16S rRNA| Forward, 5'-AGA GTT TGA TCMTGG CTC AG-3'          |
|         | Reverse, 5'-TAC GGT TAC CTT GTT ACG AC-3'         |
| rpoB    | Forward, 5'-GAG GGT CAG ACC ACG ATG AC-3'         |
|         | Reverse, 5'-AGC C6A TCA GAC CGA TGT T-3'          |
| hsp65   | Forward, 5' ACC AAC GAT GGT GTG TCC AT -3'        |
|         | Reverse, 5' CTT GTG GAA CCG CAT ACC CT-3'         |
| erm     | Forward, 5'-GAC CSG GCC TTC GTG AT-3'             |
|         | Reverse, 5'-GAC TTC CCC GCA CCG ATTC C-3'         |

### Table 5. Antimicrobial agents and MIC breakpoints for rapidly growing mycobacteria

| Antimicrobial agents | MIC (µg/mL) for category |
|----------------------|--------------------------|
|                      | Susceptible | Intermediate | Resistant |
| Amikacin             | ≤16         | 32           | ≥64       |
| Cefoxitin            | ≤16         | 32-64        | ≥128      |
| Ciprofloxacin        | ≤1          | 2            | ≥4        |
| Clarithromycin       | ≤2          | 4            | ≥8        |
| Doxycycline          | ≤1          | 2 - 4        | ≥8        |
| Imipenem             | ≤4          | 8 - 16       | ≥32       |
| Linezolid            | ≤8          | 16           | ≥32       |
| Moxifloxacin         | ≤1          | 2            | ≥4        |
| Trimethoprim-sulfamethoxazole | ≤ 2/38 | – | ≥ 4/76 |
| Tobramycin           | ≤2          | 4            | ≥8        |

### Figures
Figure 1

MIC distributions for amikacin, imipenem, and moxifloxacin combined with clarithromycin, categorized into three subspecies of M. abscessus complex. Green color indicates susceptibility, yellow color indicates intermediate, and red color indicates resistance to M. abscessus. Abbreviations: CLR, clarithromycin; AMK, amikacin; IPM, imipenem; MXF, moxifloxacin.
| M. abscessus subsp. abscessus | CLR/AMK | CLR/IPM | CLR/MXF |
|-------------------------------|---------|---------|---------|
| Strain 1                      | 0.625   | 0.5     | 1.125   |
| Strain 2                      | 2       | 2       | 2       |
| Strain 3                      | 1.125   | 0.75    | 1.5     |
| Strain 4                      | 1.25    | 0.5     | 1.25    |
| Strain 5                      | 1.125   | 0.75    | 1.5     |
| Strain 6                      | 1.25    | 0.5     | 1.25    |
| Strain 7                      | 0.156   | 0.254   | 0.281   |
| Strain 8                      | 2       | 0.008   | 0.559   |
| Strain 9                      | 1.125   | 1.125   | 1.125   |
| Strain 10                     | 1.125   | 1.25    | 1.25    |
| M. abscessus subsp. massillae |         |         |         |
| Strain 11                     | 1.25    | 0.5     | 0.75    |
| Strain 12                     | 2       | 2       | 2       |
| Strain 13                     | 2       | 2       | 2       |
| Strain 14                     | 1.125   | 0.75    | 1.063   |
| Strain 15                     | 0.625   | 0.75    | 0.75    |
| Strain 16                     | 2.098   | 0.75    | 2.317   |
| Strain 17                     | 1.069   | 1.25    | 2.008   |
| Strain 18                     | 2       | 2       | 2       |
| Strain 19                     | 1.069   | 0.75    | 2.008   |
| Strain 20                     | 1.125   | 0.75    | 1.125   |
| Strain 21                     | 1.125   | 1.5     | 3       |
| Strain 22                     | 0.625   | 1       | 1       |
| Strain 23                     | 0.625   | 0.49    | 1.125   |
| Strain 24                     | 1.125   | 1.125   | 1.5     |
| Strain 25                     | 2.009   | 1.5     | 2.512   |
| Strain 26                     | 2.009   | 1.5     | 2.512   |
| Strain 27                     | 1.125   | 0.75    | 1.25    |
| Strain 28                     | 1.125   | 1.125   | 1.125   |

| M. abscessus subsp. bolletii |         |         |         |
|-------------------------------|---------|---------|---------|
| Strain 29                     | 4       | 2       | 1.001   |

### Figure 2

FIC index of amikacin, imipenem, and moxifloxacin combined with clarithromycin, categorized into three subspecies of M. abscessus. Light green color indicates synergy, green color indicates additive, yellow color indicates indifference, and red color indicates antagonism in each combination. Abbreviations: CLR, clarithromycin; AMK, amikacin; IPM, imipenem; MXF, moxifloxacin; FIC index, fractional inhibitory concentration index.

### Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarytable20200127.pptx](#)