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

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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), cephamycins (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 Σ FIC is ≤0.5, additive when the Σ FIC is >0.5 to ≤1, indifferent when the Σ FIC is >1 to ≤2, and antagonistic when the Σ 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.

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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.

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Reference

1. Bodle EE, Cunningham JA, Della-Latta P, Schluger NW, Saiman L. Epidemiology of nontuberculous mycobacteria in patients without HIV infection, New York city. *Emerg Infect Dis.* 2008;14(3):390-396.
2. Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous Mycobacterial Lung Disease Prevalence at Four Integrated Health Care Delivery Systems. *Am J Resp Crit Care.* 2010;182(7):970-976.
3. Namkoong H, Kurashima A, Morimoto K, et al. Epidemiology of Pulmonary Nontuberculous Mycobacterial Disease, Japan. *Emerg Infect Dis.* 2016;22(6):1116-1117.
4. Roux AL, Catherinot E, Ripoll F, et al. Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in france. *J Clin Microbiol.* 2009;47(12):4124-4128.
5. Olivier KN, Weber DJ, Wallace RJ, Jr., et al. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med.* 2003;167(6):828-834.
6. Swenson JM, Wallace RJ, Jr., Silcox VA, Thornsberry C. Antimicrobial susceptibility of five subgroups of Mycobacterium fortuitum and Mycobacterium chelonae. *Antimicrob Agents Chemother.* 1985;28(6):807-811.
7. Brown BA, Wallace RJ, Jr., Onyi GO, De Rosas V, Wallace RJ, 3rd. Activities of four macrolides, including clarithromycin, against Mycobacterium fortuitum, Mycobacterium chelonae, and M. chelonae-like organisms. *Antimicrob Agents Chemother.* 1992;36(1):180-184.
8. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med.* 2007;175(4):367-416.
9. Bastian S, Veziris N, Roux AL, et al. Assessment of Clarithromycin Susceptibility in Strains Belonging to the Mycobacterium abscessus Group by erm(41) and rrl Sequencing. *Antimicrob Agents Ch.* 2011;55(2):775-781.

10. Kim HY, Kim BJ, Kook Y, et al. Mycobacterium massiliense is differentiated from Mycobacterium abscessus and Mycobacterium bolletii by erythromycin ribosome methyltransferase gene (erm) and clarithromycin susceptibility patterns. *Microbiol Immunol.* 2010;54(6):347-353.
11. Ferro BE, Srivastava S, Deshpande D, et al. Failure of the Amikacin, Cefoxitin, and Clarithromycin Combination Regimen for Treating Pulmonary Mycobacterium abscessus Infection. *Antimicrob Agents Chemother.* 2016;60(10):6374-6376.
12. Lerat I, Cambau E, Roth Dit Bettoni R, et al. In vivo evaluation of antibiotic activity against Mycobacterium abscessus. *J Infect Dis.* 2014;209(6):905-912.
13. Pasipanodya JG, Ogbonna D, Ferro BE, et al. Systematic Review and Meta-analyses of the Effect of Chemotherapy on Pulmonary Mycobacterium abscessus Outcomes and Disease Recurrence. *Antimicrob Agents Chemother.* 2017;61(11).
14. Le Run E, Arthur M, Mainardi JL. In Vitro and Intracellular Activity of Imipenem Combined with Rifabutin and Avibactam against Mycobacterium abscessus. *Antimicrob Agents Chemother.* 2018;62(8).
15. Lefebvre AL, Dubee V, Cortes M, Dorchene D, Arthur M, Mainardi JL. Bactericidal and intracellular activity of beta-lactams against Mycobacterium abscessus. *J Antimicrob Chemother.* 2016;71(6):1556-1563.
16. Miyasaka T, Kunishima H, Komatsu M, et al. In vitro efficacy of imipenem in combination with six antimicrobial agents against Mycobacterium abscessus. *Int J Antimicrob Agents.* 2007;30(3):255-258.
17. Kim BJ, Yi SY, Shim TS, et al. Discovery of a Novel hsp65 Genotype within Mycobacterium massiliense Associated with the Rough Colony Morphology. *Plos One.* 2012;7(6).
18. Yoshida S, Tsuyuguchi K, Suzuki K, et al. Further isolation of Mycobacterium abscessus subsp abscessus and subsp bolletii in different regions of Japan and susceptibility of these isolates to antimicrobial agents. *Int J Antimicrob Ag.* 2013;42(3):226-231.
19. Nakanaga K, Sekizuka T, Fukano H, et al. Discrimination of Mycobacterium abscessus subsp. massiliense from Mycobacterium abscessus subsp. abscessus in clinical isolates by multiplex PCR. *J Clin Microbiol.* 2014;52(1):251-259.
20. Brown-Elliott BA, Vasireddy S, Vasireddy R, et al. Utility of sequencing the erm(41) gene in isolates of Mycobacterium abscessus subsp. abscessus with low and intermediate clarithromycin MICs. *J Clin Microbiol.* 2015;53(4):1211-1215.
21. Woods GL, Brown-Elliott BA, Conville PS, et al. In: nd, ed. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes*. Wayne (PA)2011.

Tables

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

	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 (non HIV)	2 (6.9)
Malignancy	7 (24.1)
Concomitant medications, N (%)	
Corticosteroids	6 (20.7)
Immunosupressant	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
		N (%, Adjusted residual)			
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)	
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		*p* value
		Synergy + Additive N=14 (%)	Indifference + Antagonism 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

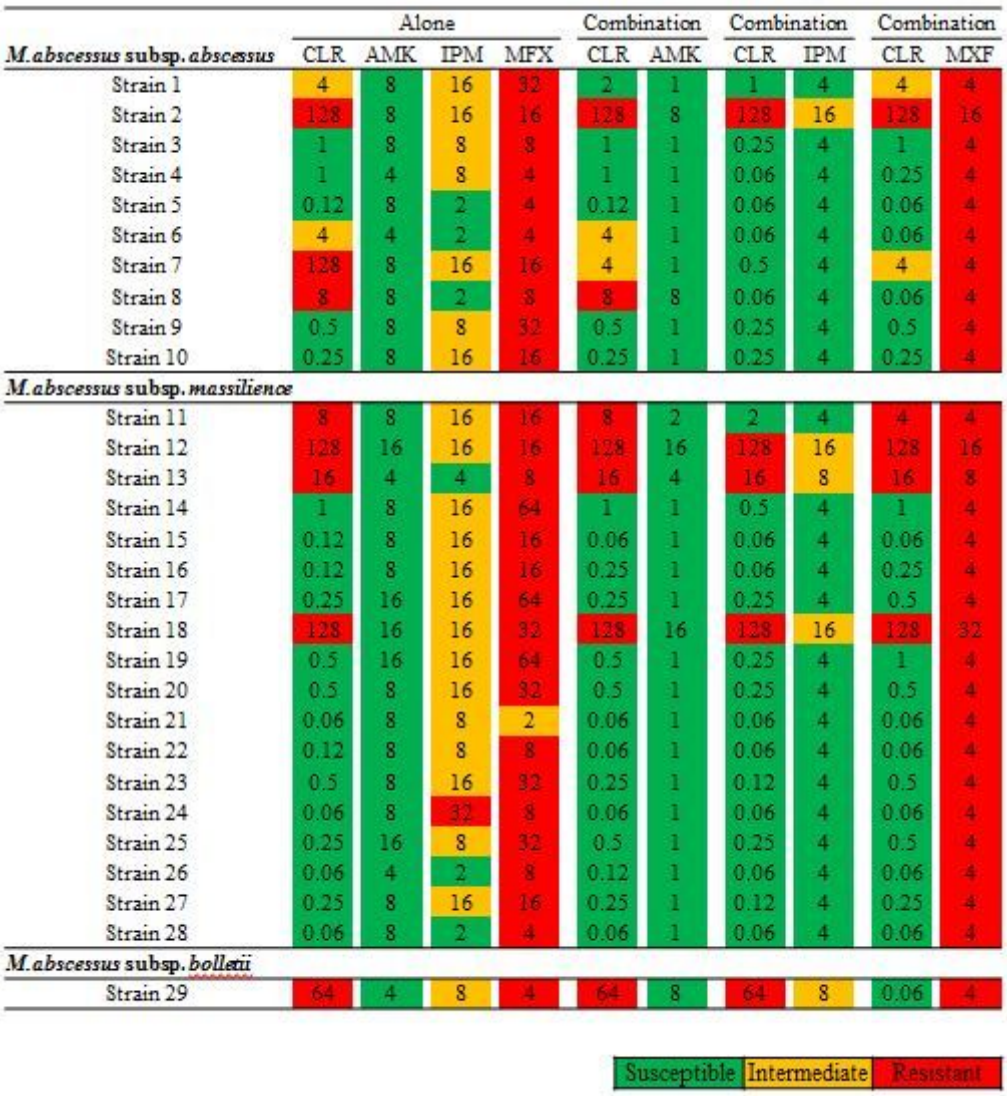
Table 4. Forward and backward primers used for PCR

Target	Sequence
16S rRNA	Forward, 5'-AGA GTT TGA TCM TGG 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 CGA TCA GAC CGA TGT T-3'
hsp65	Forward, 5'ACC AAC GAT GGT GTG TCC AT -3' Reverse, 5' CTT GTC GAA CCG CAT ACC CT-3'
erm	Forward, 5'-GAC CGG GCC TTC GTG AT-3' Reverse, 5'-GAC TTC CCC GCA CCG ATT CC-3'

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

	MIC (μg/mL) for category		
Antimicrobial agents	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

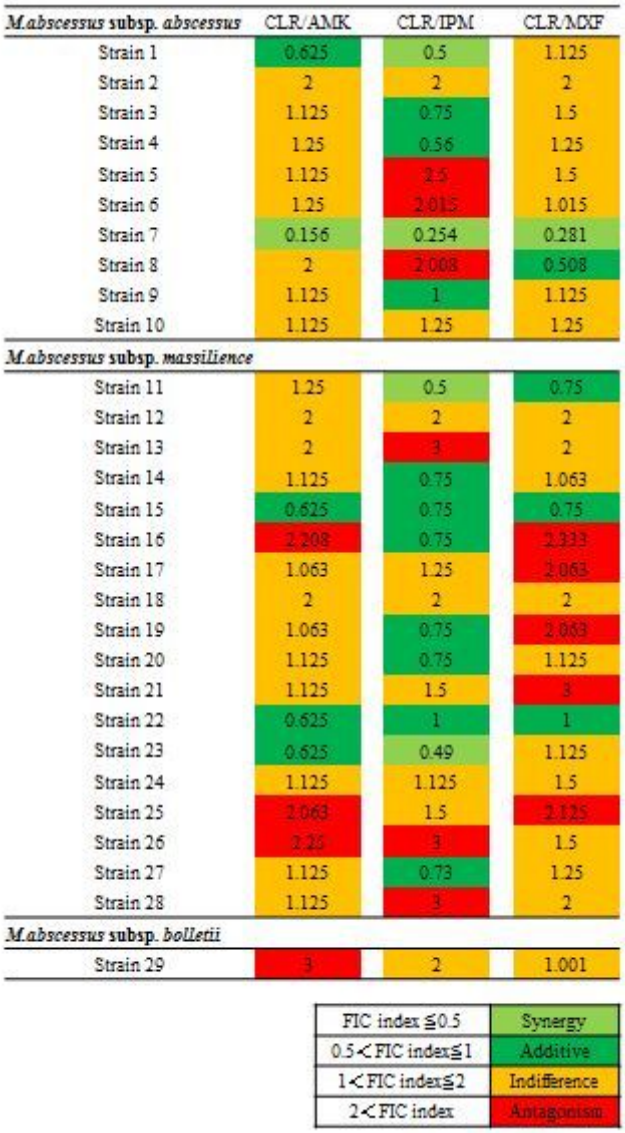


M. abscessus subsp. *abscessus*	Alone				Combination		Combination		Combination	
	CLR	AMK	IPM	MFX	CLR	AMK	CLR	IPM	CLR	MXF
Strain 1	4	8	16	32	2	1	1	4	4	4
Strain 2	128	8	16	16	128	8	128	16	128	16
Strain 3	1	8	8	8	1	1	0.25	4	1	4
Strain 4	1	4	8	4	1	1	0.06	4	0.25	4
Strain 5	0.12	8	2	4	0.12	1	0.06	4	0.06	4
Strain 6	4	4	2	4	4	1	0.06	4	0.06	4
Strain 7	128	8	16	16	4	1	0.5	4	4	4
Strain 8	8	8	2	8	8	8	0.06	4	0.06	4
Strain 9	0.5	8	8	32	0.5	1	0.25	4	0.5	4
Strain 10	0.25	8	16	16	0.25	1	0.25	4	0.25	4
M. abscessus subsp. *massiliense*										
Strain 11	8	8	16	16	8	2	2	4	4	4
Strain 12	128	16	16	16	128	16	128	16	128	16
Strain 13	16	4	4	8	16	4	16	8	16	8
Strain 14	1	8	16	64	1	1	0.5	4	1	4
Strain 15	0.12	8	16	16	0.06	1	0.06	4	0.06	4
Strain 16	0.12	8	16	16	0.25	1	0.06	4	0.25	4
Strain 17	0.25	16	16	64	0.25	1	0.25	4	0.5	4
Strain 18	128	16	16	32	128	16	128	16	128	32
Strain 19	0.5	16	16	64	0.5	1	0.25	4	1	4
Strain 20	0.5	8	16	32	0.5	1	0.25	4	0.5	4
Strain 21	0.06	8	8	2	0.06	1	0.06	4	0.06	4
Strain 22	0.12	8	8	8	0.06	1	0.06	4	0.06	4
Strain 23	0.5	8	16	32	0.25	1	0.12	4	0.5	4
Strain 24	0.06	8	32	8	0.06	1	0.06	4	0.06	4
Strain 25	0.25	16	8	32	0.5	1	0.25	4	0.5	4
Strain 26	0.06	4	2	8	0.12	1	0.06	4	0.06	4
Strain 27	0.25	8	16	16	0.25	1	0.12	4	0.25	4
Strain 28	0.06	8	2	4	0.06	1	0.06	4	0.06	4
M. abscessus subsp. *bolletii*										
Strain 29	64	4	8	4	64	8	64	8	0.06	4

Susceptible | Intermediate | Resistant

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	0.56	1.25
Strain 5	1.125	2.5	1.5
Strain 6	1.25	2.015	1.015
Strain 7	0.156	0.254	0.281
Strain 8	2	2.008	0.508
Strain 9	1.125	1	1.125
Strain 10	1.125	1.25	1.25
M.abscessus subsp. *massilience*			
Strain 11	1.25	0.5	0.75
Strain 12	2	2	2
Strain 13	2	3	2
Strain 14	1.125	0.75	1.063
Strain 15	0.625	0.75	0.75
Strain 16	2.208	0.75	2.333
Strain 17	1.063	1.25	2.063
Strain 18	2	2	2
Strain 19	1.063	0.75	2.063
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	2.063	1.5	2.125
Strain 26	2.25	3	1.5
Strain 27	1.125	0.73	1.25
Strain 28	1.125	3	2
M.abscessus subsp. *bolletii*			
Strain 29	3	2	1.001

FIC index ≦0.5	Synergy
0.5＜FIC index≦1	Additive
1＜FIC index≦2	Indifference
2＜FIC index	Antagonism

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

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- Supplementarytable20200127.pptx