



Isolation and Antimicrobial Susceptibility of Nontuberculous Mycobacteria in a Tertiary Hospital in Korea, 2016 to 2020

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Abstract

Background: There is a global increase in isolation of nontuberculous mycobacteria (NTM). The aim of the study was to analyze longitudinal trends of NTM identification and pattern of antimicrobial susceptibility testing.

Methods: NTM recovery rates, distribution of NTM species identification, and antimicrobial susceptibility pattern of NTM at Pusan National University Yangsan Hospital between January 2016 and December 2020 were retrospectively analyzed.

Results: A total of 52,456 specimens from 21,264 patients were submitted for mycobacterial culture, of which 2,521 from 1,410 patients were NTM positive over five years (January 2016 to December 2020). NTM isolation showed an increasing trend from 2016 to 2020 ($p < 0.001$, test for trend) mainly caused by *Mycobacterium avium* complex. The vast majority of *M. avium* complex were susceptible to key agents clarithromycin and amikacin. For *Mycobacterium kansasii*, resistance to rifampin and clarithromycin is rare. Amikacin was the most effective drug against *Mycobacterium abscessus* subspecies *abscessus* and *Mycobacterium* subspecies *massiliense*. Most of *M. subspecies massiliense* were susceptible to clarithromycin, while the majority of *M. abscessus* subspecies *abscessus* were resistant to clarithromycin ($p < 0.001$).

Conclusion: There was an increasing trend of NTM isolation in our hospital. Resistance to key drugs was uncommon for most NTM species except for *M. abscessus* subspecies *abscessus* against clarithromycin.

Keywords: Antimicrobial Susceptibility Testing; Nontuberculous Mycobacteria; Mycobacterium avium Complex; Clarithromycin; Amikacin



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Introduction

Nontuberculous mycobacteria (NTM) are defined as a group of *Mycobacterium* species other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*¹. Unlike *M. tuberculosis* that causes tuberculosis (TB) or *M. leprae* that causes leprosy, NTM are opportunistic pathogens that are widespread in the environments such

as soil and water¹. An increase in the number of NTM isolation has been observed globally²⁻⁶. This is partly explained not only by increased awareness of symptoms caused by NTM infection and enhancements in diagnostic techniques, but also by increased numbers of susceptible hosts and the ubiquitous presence of NTM⁷. The situation is expected to become worse because most NTM either inherently possess or have the

ability to acquire resistance against conventional antibiotics⁸.

Currently, macrolide-based antibiotics such as clarithromycin and azithromycin are the first choice of drugs for treating most NTM infections⁹. However, treatment regimens vary by species. The regime also includes ethambutol and rifampin for slowly growing NTM¹⁰, while it adds an aminoglycoside and either cefoxitin, imipenem, or tigecycline for rapidly growing NTM¹¹. Treatment duration is prolonged up to 18 months¹². A complete cure is not always possible^{4,13}. The role of antimicrobial susceptibility testing (AST) in guiding treatment remains a subject of debate¹⁴. Discordance between *in vitro* AST and *in vivo* drug activity has been observed for some drugs and NTM species^{12,15}. For examples, although ethambutol, rifampin, and rifabutin are clinically useful for *Mycobacterium avium* complex (MAC), the relationship between *in vitro* susceptibility results and *in vivo* response to antibiotics is controversial and poorly understood^{16,17}. Two contradictory studies have been conducted in Korea on the relationship between *in vitro* AST results and treatment outcomes of ethambutol and rifampin^{18,19}. AST of the second line drugs moxifloxacin and linezolid might also be taken into consideration for macrolide-resistant MAC isolates and/or isolates from patients who cannot tolerate macrolide therapy using breakpoints in Clinical and Laboratory Standards Institute (CLSI) document M62¹⁶. However, the *in vivo* usefulness of these drugs remains unknown²⁰. In addition, the evidence is limited regarding the use of *in vitro* AST-based treatment with injectable amikacin and beta-lactams for patients with *Mycobacterium abscessus* subspecies *abscessus* infection²¹. For *Mycobacterium kansasii*, there is no correlation between *in vivo* AST and *in vivo* clinical outcomes for any drugs other than rifampin²². These discrepancies partly originate from laboratory technical difficulties associated with AST, standardization of methods, and a lack of clinical validation¹⁴. Nevertheless, laboratory tests for determining AST of NTM can confirm the initial drug treatment choice and any emerging drug resistance²⁰. In addition, AST may provide additional information for different drug treatment choices²⁰. The prevalence of intrinsic and acquired drug resistance can also be estimated based on NTM AST in a community²⁰. The goal of this study was to analyze patterns of AST for NTM isolated in a Korea tertiary university hospital and longitudinal trends of NTM identification.

Materials and Methods

1. Data collection

Mycobacterial culture and AST results were obtained from the electric medical records system from the clinical microbiology laboratory of Pusan National University Yangsan Hospital. Data were obtained for all specimens received between January 2016 and December 2020. These specimens were either from patients with presumptive mycobacterial infection or patients with confirmed TB or NTM infection. Various clinical specimens including pulmonary and extrapulmonary samples were included in this study. This study was approved by Pusan National University Yangsan Hospital Institutional Review Board (05-2021-237). Written informed consent by the patients was waived due to a retrospective nature of our study.

2. Mycobacterial culture, identification, and antimicrobial susceptibility testing

Clinical specimens were prepared according to a standard protocol²³. Processed specimens were inoculated into BACTEC 960 mycobacterial growth indicator tubes (MGIT) (Becton Dickinson, Franklin Lakes, NJ, USA) and Ogawa media (Eiken, Tokyo, Japan) for mycobacterial culture. Liquid cultures were kept for 6 weeks. Solid cultures were examined for 8 weeks. Positive acid-fast bacilli (AFB) cultures were confirmed using Ziehl-Neelsen staining. TB/NTM differentiation was performed with an MPT 64 Ag test (SD Bioline Kit, Standard Diagnostics, Yongin, Korea) and an AdvanSure TB/NTM real-time PCR kit (LG Chem, Seoul, Korea).

Positive NTM isolates were sent to Korean Institute of Tuberculosis (KIT) for species identification and AST. NTM species identification was performed using an AdvanSure Mycobacteria GenoBlot assay (LG Chem) at KIT. The assay can identify *M. tuberculosis* complex and 20 different NTM species²⁴. The reverse line probe assay can detect the presence of ≥ 2 NTM species infection in a single specimen. NTM species that could not be differentiated with the assay were confirmed by multigene sequence-based typing. Sequencing results of 16s rRNA, *rpoB*, and *hsp65* were analyzed according to the CLSI guideline MM18-ED2²⁵. Unclassified NTM were given to NTM species that failed to be differentiated at species levels after sequencing. Discrimination between *M. abscessus* subspecies *abscessus* and *M. abscessus* subspecies *massiliense* was performed using an ERM-plus real-time PCR kit (LG Chem; commercially not available). The kit not only can differentiate between *M. abscessus* subspecies *abscessus* and *M. abscessus* subspecies *massiliense*, but also identify

infection of both species in a single specimen. Mixed infection was defined as simultaneous identification of ≥ 2 species in a single specimen.

At KIT, AST was performed by the reference broth microdilution method for slowly and rapidly growing NTM against different drugs according to the CLSI guideline²⁰. Minimum inhibitory concentration (MIC) values were analyzed using Muller-Hinton broth in polystyrene 96-well plates containing drugs in 2-fold increasing concentrations ($\mu\text{g}/\text{mL}$) (Supplementary Table S1). MIC values were categorized as susceptible (S), intermediate (I), or resistant (R) in accordance with the CLSI guideline¹⁶ (Supplementary Table S1). To evaluate inducible resistance to clarithromycin for *M. abscessus* subspecies *abscessus* and *M. abscessus* subspecies *massiliense*, MIC values were established both at days 3 and 14 in accordance with the CLSI guideline²⁰. Clinical specimens that had mixed NTM infections were also subjected to AST at KIT. However, obtaining pure culture isolates of single species was not performed prior to the broth microdilution test. We believed that this AST method for specimens was inappropriate. Hence, AST results from clinical specimens with mixed NTM infection were excluded from the current study.

3. Data analysis

The number of specimens positive for AFB culture and the proportion of NTM isolates recovered from positive specimens were calculated for each year. A linear-by-linear association exact test was used to test for significant trends. Chi-squared test and Fischer's exact

test were used to compare differences in antimicrobial susceptibility to each drug between NTM species using SPSS for Windows version 26.0 (IBM, Armonk, NY, USA).

Results

1. Mycobacterial culture results

Data were obtained for 52,456 clinical specimens from 21,264 patients during the study period. The number of specimens received for mycobacterial culture steadily increased from 9,435 in 2016 to 11,396 in 2019, but decreased to 10,309 in 2020 (Figure 1). While the number of specimens positive for culture for *M. tuberculosis* was continuously decreased from 594 (6.3% of total specimens, 60.1% of all positive culture) in 2016 to 381 (3.7% of total specimens, 42.8% of all positive culture) in 2020, the number of NTM isolates was gradually increased from 395 (4.2% of total specimens) in 2016 to 590 (5.2% of total specimens) in 2019 (Figure 1). In total, 5,005 (9.5%) from 2,356 patients were positive for mycobacterial culture. Of these 5,005 culture positive specimens, 2,521 from 1,410 patients were positive for NTM. The proportion of NTM recovered from specimens showed an increasing trend from 39.9% in 2016 to 57.2% in 2020 ($p < 0.001$, test for trend). Interestingly, culture positivity and proportions of NTM recovered from culture isolates were both decreased from 2019 to 2020 (Figures 1, 2).

2. Distribution of NTM species

Of the 2,521 NTM cultures, species identification was obtained for 718 specimens (28.5% of 2,521) from 646

Figure 1. Annual isolation rate of nontuberculous mycobacteria among total clinical specimens compared to *Mycobacterium tuberculosis*.

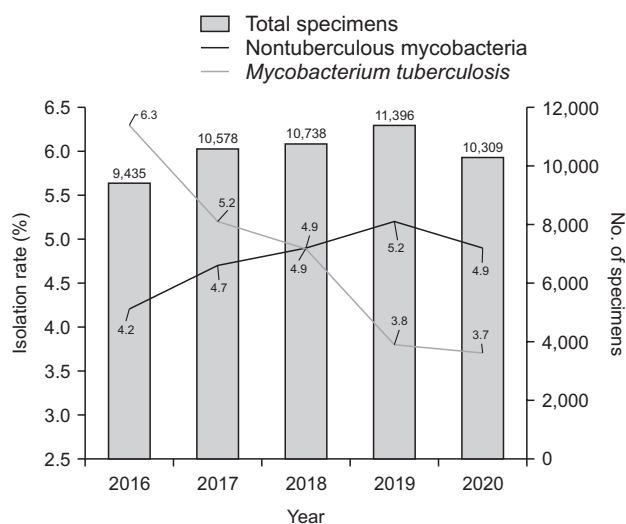
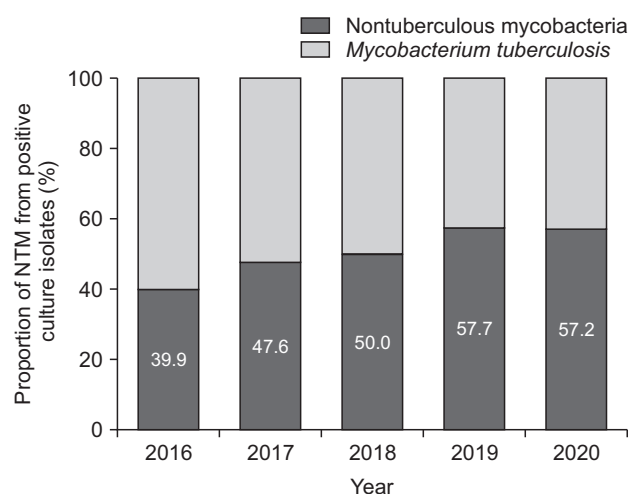


Figure 2. Annual proportion of nontuberculous mycobacteria (NTM) from positive culture isolates.



patients. *Mycobacterium intracellulare* (45.8%) was the most commonly isolated NTM, followed by *M. avium* (21.4%) and *M. abscessus* subspecies *abscessus* (7.9%) (Table 1). Interestingly, 8.5% of identified clinical isolates showed mixed NTM infection, of which a combination of *M. intracellulare* and *M. abscessus* subspecies *abscessus* (1.8%) was the most common, followed

by mixed infection of *M. intracellulare* and *M. avium* (1.4%) (Table 1). Annual trends in distribution of NTM species recovered from clinical isolates are shown in Figure 3. The proportion of every NTM species was comparable by year ($p \geq 0.05$).

Table 1. List of identified nontuberculous mycobacteria species recovered from clinical isolates during 2016 to 2020 (n=718)

Identified species	Number of total isolates (%)
1 Species	647 (90.1)
<i>Mycobacterium intracellulare</i>	329 (45.8)
<i>M. avium</i>	154 (21.4)
<i>M. abscessus</i> subspecies <i>abscessus</i>	57 (7.9)
<i>M. kansasii</i>	41 (5.7)
<i>M. abscessus</i> subspecies <i>massiliense</i>	28 (3.9)
<i>M. lentiflavum</i> or <i>M. genavense</i>	13 (1.8)
<i>M. fortuitum</i> complex	10 (1.4)
<i>M. gordonae</i>	9 (1.3)
<i>M. terrae</i> complex	3 (0.4)
<i>M. marinum</i> or <i>M. ulcerans</i>	1 (0.1)
<i>M. xenopi</i>	1 (0.1)
<i>M. mucogenicum</i>	1 (0.1)
≥2 Species	61 (8.5)
<i>M. intracellulare</i> and <i>M. abscessus</i> subspecies <i>abscessus</i>	14 (1.8)
<i>M. avium</i> and <i>M. intracellulare</i>	10 (1.4)
<i>M. avium</i> and <i>M. abscessus</i> subspecies <i>abscessus</i>	5 (0.7)
<i>M. avium</i> and <i>M. abscessus</i> subspecies <i>massiliense</i>	5 (0.7)
<i>M. intracellulare</i> and <i>M. abscessus</i> subspecies <i>massiliense</i>	4 (0.6)
<i>M. avium</i> and <i>M. kansasii</i>	3 (0.4)
<i>M. abscessus</i> subspecies <i>abscessus</i> and <i>M. abscessus</i> subspecies <i>massiliense</i>	3 (0.4)
<i>M. intracellulare</i> and <i>M. fortuitum</i> complex	3 (0.4)
<i>M. intracellulare</i> and <i>M. kansasii</i>	2 (0.3)
<i>M. marinum</i> and <i>M. ulcerans</i>	2 (0.3)
<i>M. intracellulare</i> , <i>M. abscessus</i> subspecies <i>abscessus</i> and <i>M. abscessus</i> subspecies <i>massiliense</i>	2 (0.3)
<i>M. avium</i> , <i>M. intracellulare</i> and <i>M. abscessus</i> subspecies <i>massiliense</i>	2 (0.3)
<i>M. avium</i> and <i>M. fortuitum</i> complex	1 (0.1)
<i>M. avium</i> and <i>M. gordonae</i>	1 (0.1)
<i>M. avium</i> and <i>M. fortuitum</i> complex	1 (0.1)
<i>M. flavescens</i> and <i>M. fortuitum</i> complex	1 (0.1)
<i>M. massiliense</i> and <i>M. fortuitum</i> complex	1 (0.1)
<i>M. intracellulare</i> and <i>M. lentiflavum</i> or <i>M. genavense</i>	1 (0.1)
Unclassified (nontuberculous mycobacteria)	10 (1.4)

3. Antimicrobial susceptibility testing results

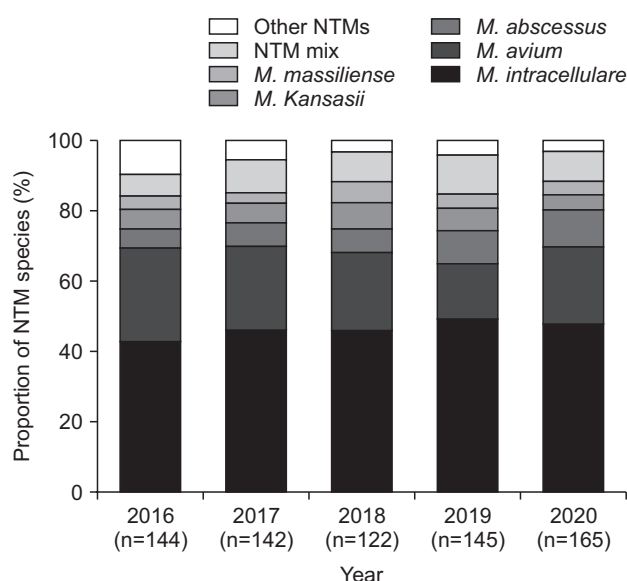
AST was obtained for 387 clinical specimens from 352 patients. Every isolate of *M. intracellulare* was susceptible to clarithromycin. Only one of 91 isolates of *M. avium* was resistant to clarithromycin (Table 2). It was found that 88% (191/217) of *M. intracellulare* isolates and 76% (69/91) ($p=0.01$) of *M. avium* isolates showed amikacin susceptibility. There was a statistically significant difference in the extent of susceptibility to moxifloxacin and linezolid between *M. intracellulare* and *M. avium* (Table 2).

For *M. kansasii*, 96% (22/23) of isolates showed rifampin susceptibility, while 100% (23/23) exhibited susceptibility to clarithromycin (Table 3). In general, *M.*

kansasii showed high rates of susceptibility towards most test drugs except for ciprofloxacin and doxycycline.

Amikacin was the most effective drug against *M. abscessus* subspecies *abscessus* (92%, 35/38) and *M. abscessus* subspecies *massiliense* (100%, 18/18) (Table 4). It was found that 26% (10/38) of *M. abscessus* subspecies *abscessus* were susceptible to clarithromycin at both day 3 and day 14. At day 3, only 3% (1/38) of *M. abscessus* subspecies *abscessus* exhibited clarithromycin resistance, which was indicative of acquired resistance to the drug. It was found that 71% (27/38) of *M. abscessus* subspecies *abscessus* were susceptible or intermediate at day 3 but resistant to clarithromycin at day 14, which correlated with inducible resistance to clarithromycin. Conversely, none of *M. abscessus* subspecies *massiliense* isolates exhibited inducible resistance to clarithromycin. Instead, they all displayed the same susceptibility results at days 3 and 14.

Figure 3. Annual proportion of nontuberculous mycobacteria (NTM) species identification (n=718). *M. massiliense*: *Mycobacterium abscessus* subspecies *massiliense*; *M. abscessus*: *Mycobacterium abscessus* subspecies *abscessus*.



Discussion

In accordance with past studies both from Korea and other countries²⁻⁵, our study showed that the number of NTM isolates had increased over time. Currently, NTM constitute the majority of mycobacterial culture isolates in our hospital. Reasons behind this increase of NTM isolation are yet to be fully elucidated. Possible theories include awareness of NTM as pathogens, advancements of laboratory detection methods, increased environmental exposures, and increased numbers of susceptible patients with predisposing factors²⁶. Interestingly, the number of specimens for mycobacterial culture, the number of NTM isolates, and the proportion of NTM were slightly decreased from the previous year to 2020 (Figure 1). A recent study conducted in Korea has shown that environmental exposure such as public bath is a risk factor for NTM dis-

Table 2. Antimicrobial susceptibility testing results for *Mycobacterium avium* complex

Antimicrobial agent	No. (%) of strains for <i>M. intracellulare</i>				No. (%) of strains for <i>M. avium</i>				p-value
	Total	S	I	R	Total	S	I	R	
First line									
Clarithromycin	217	217 (100)	0	0	91	90 (99)	0	1 (1)	0.295
Amikacin	217	191 (88)	26 (12)	0	91	69 (76)	21 (23)	1 (1)	0.01
Second line									
Moxifloxacin	217	18 (8)	95 (44)	104 (48)	91	20 (22)	32 (35)	39 (43)	0.001
Linezolid	217	16 (7)	91 (42)	110 (51)	91	27 (30)	25 (27)	39 (43)	<0.001

S: susceptible; I: intermediate; R: resistant.

Table 3. Antimicrobial susceptibility testing results for *Mycobacterium kansasii*

Antimicrobial agent	No. (%) of strains for <i>M. kansasii</i>			
	Total	S	I	R
First line				
Clarithromycin	23	23 (100)	0	0
Rifampin	23	22 (96)	0	1 (4)
Second line				
Amikacin	23	23 (100)	0	0
Ciprofloxacin	23	11 (48)	0	12 (52)
Doxycycline	23	0	9 (39)	14 (61)
Linezolid	23	23 (100)	0	0
Moxifloxacin	23	23 (100)	0	0
Trimethoprim/sulfamethoxazole	23	19 (83)	0	4 (17)

S: susceptible; I: intermediate; R: resistant.

Table 4. Antimicrobial susceptibility testing results for *Mycobacterium abscessus* complex

Antimicrobial agent	No. (%) of strains for <i>M. abscessus</i> subspecies <i>abscessus</i> (n=38)			No. (%) of strains for <i>M. abscessus</i> subspecies <i>massiliense</i> (n=18)			p-value
	S	I	R	S	I	R	
Amikacin	35 (92)	3 (8)	0	18 (100)	0	0	0.544
Cefoxitin	11 (29)	27 (71)	0	1 (6)	17 (94)	0	0.079
Ciprofloxacin	0	1 (3)	37 (97)	2 (11)	4 (22)	12 (67)	0.099
Clarithromycin (day 3)	31 (82)	6 (15)	1 (3)	17 (94)	0	1 (6)	0.414
Clarithromycin (day 14)	10 (26)	0	28 (74)	17 (94)	0	1 (6)	<0.001
Doxycycline	0	0	38 (100)	0	0	18 (100)	-
Imipenem	23 (61)	15 (39)	0	7 (39)	11 (61)	0	0.159
Linezolid	8 (21)	17 (45)	13 (34)	5 (28)	7 (39)	6 (33)	0.736
Moxifloxacin	0	4 (11)	34 (89)	0	3 (17)	15 (83)	-
Trimethoprim/ sulfamethoxazole	4 (11)	0	34 (89)	0	0	18 (100)	0.294
Tobramycin	0	7 (18)	31 (82)	0	5 (28)	13 (72)	-

S: susceptible; I: intermediate; R: resistant.

ease²⁷. The ongoing pandemic of coronavirus disease 2019 (COVID-19) has drastically changed the hygiene and sanitation procedures in public places in Korea, which might have affected patients in a way that they were less exposed to ubiquitous presence of NTM. In addition, since the number of specimens for mycobacterial culture was decreased from 2019 to 2020 (Figure 1), some patients might be less eager to seek a medical service during the pandemic. COVID-19-related social restrictions intended to control the transmission of severe acute respiratory syndrome coronavirus 2

(SARS-CoV-2) may also have been associated with decreased rates of other infectious diseases such as TB and HIV^{28,29}. Our findings suggest that COVID-19 might affect recovery rates of NTM in a clinical microbiology laboratory of a tertiary hospital.

In our study, *M. intracellulare* and *M. avium* were the most common organisms recovered from clinical specimens followed by *M. abscessus* subspecies *abscessus*, consistent with previous reports from Korea^{3,30}. Geographic distribution of NTM species recovered from clinical isolates can vary. For example, MAC was

the most common NTM species isolated in the United States³¹, whereas *M. kansasii* and *M. abscessus* complex (MABC) were the most frequent species isolated in Singapore³² and Shanghai China³³, respectively. Such differences in the distribution of NTM species around the world can be in part due to variations in sample sources, climate changes, and population density³⁴. In this study, 8.5% of all clinical specimens contained mixed infection. This proportion was similar to 7.5% in a study conducted in southwest China³⁵, but different from 30.1% in a study performed in Singapore³⁶. Studies on isolation rates of mixed NTM infection in Korea are lacking. These results indicate that distribution of NTM multispecies infection could vary geographically as single NTM species. Although the clinical significance of mixed NTM species infection from the same patient is not fully understood yet, it is a frequent finding of NTM lung infection³⁵. Disease status and clinical outcomes of patients with mixed NTM infection might be different from those with a single NTM infection³⁷. Unfortunately, the correlation between mixed NTM infection and patient outcomes could not be investigated in our study due to the lack of data pertaining to clinical treatment. Misidentification of mixed NTM infection is not an uncommon finding, which can cause a critical problem in patient management³⁷. Therefore, future studies should elucidate clinical outcomes of mixed NTM infection with accurate diagnosis of single or mixed NTM infection.

It has been reported that a vast majority of NTM show resistance against anti-mycobacterial drugs³⁸. Although the association between *in vitro* AST patterns of NTM and *in vivo* patient outcomes was poor in previous studies^{39,40}, the evidence of clinical impacts of AST was observed for rifampin in *M. kansasii* infection⁴¹, macrolides and amikacin in MAC infection^{42,43}, and macrolides in MABC⁴⁴. CLSI's recommendations for selecting antimicrobial agents are based on published studies about appropriate therapy²⁰. Although the exact role of AST for NTM remains to be elucidated by larger clinical studies, clinical benefits of AST for NTM are manifold²⁰. First, AST results can guide treatment choices for NTM infection. Second, AST profiles might lead to better prediction of treatment outcomes. Third, detailed studies of NTM AST can be accessed by a community to estimate the prevalence of drug resistance.

MAC primary consists of *M. intracellulare* and *M. avium*. Clarithromycin and amikacin are the two first line drugs against MAC²⁰. In our study, most of MAC isolates showed susceptibility to clarithromycin and amikacin, consistent with their roles in most NTM treat-

ment regimens and correlation between *in vitro* susceptibility and *in vivo* clinical outcomes^{4,42,43}. Although moxifloxacin is recommended by the CLSI as the second line drug of AST for MAC, clinical evidence is limited for clinical efficacy and safety of moxifloxacin for MAC pulmonary disease⁴⁴. In addition, no correlation was observed between *in vitro* and *in vivo* response to moxifloxacin⁴⁵. Clinical efficacy of linezolid, another second drug for MAC, is also uncertain⁴⁶. According to our study, both drugs had limited activity against MAC isolates, as described in other studies⁴⁷⁻⁵⁰.

A multidrug antibiotic regimen comprising of rifampin, ethambutol, and isoniazid is currently the recommended course of treatment for *M. kansasii*⁴⁶. MIC values and clinical outcomes for ethambutol and isoniazid do not correlate well. Therefore, the current CLSI guideline does not recommend reporting *in vitro* AST for the drugs¹⁶. In addition, a short-course therapy that substitutes a macrolide for isoniazid has been proposed. However, its effectiveness has only been examined in limited studies⁵¹. In our study, most of *M. kansasii* isolates were susceptible to tested drugs except for ciprofloxacin and doxycycline, in agreement with another study⁵².

MABC includes three subspecies, *M. abscessus* subspecies *abscessus*, *M. abscessus* subspecies *bolletti*, and *M. abscessus* subspecies *massiliense*⁵³. Since some drugs' susceptibility testing outcomes can only be applied to particular species, the CLSI and the majority of mycobacterial experts strongly suggest species-level identification or, in the case of the MABC, subspecies-level identification⁴⁶. This is also required for the appropriate selection of treatment choices⁴⁶. Our study showed that the vast majority of MABC isolates were susceptible to amikacin. For clarithromycin, we demonstrated that *M. abscessus* subspecies *abscessus* had a 71% inducible resistance rate and a 3% acquired resistance rate, which were comparable to results of a previous study⁵³. However, there was a significant difference in susceptibility to clarithromycin between the two MABC species: 94% of *M. abscessus* subspecies *massiliense* were susceptible to clarithromycin, in contrast to a low susceptibility to the drug for *M. abscessus* subspecies *abscessus* ($p < 0.001$), as observed in other studies^{49,53}. The status of *erm*(41) gene functionality can lead to this difference in macrolide susceptibility^{53,54}. These results highlight that correct identification of NTM infection is of utmost importance to guide an appropriate treatment using species-specific strategies.

Our study has several limitations. Firstly, we did not investigate the correlation of positive culture isolate

with clinical or radiologic findings mainly due to the retrospective nature of this study. Given the copious number of clinical specimens, manual analysis of clinical features and radiologic findings was not possible. Thus, caution should be exercised when performing data interpretation because NTM isolation rate does not necessarily reflect the rate of NTM clinical infections. In addition, more than one specimen per patient were included in this study. Therefore, the recovery rate of NTM could be biased by this type of sampling method. Secondly, out of positive cultures for NTM, only a small portion (around 30%) were tested for species identification. In this regard, results should be interpreted with caution. Thirdly, as a tertiary hospital, most of our patients and samples came from a single province of the country. Thus, findings of this study might not be generalized to other regions or countries. In addition, the correlation between *in vitro* AST and *in vivo* clinical features was not studied, as stated previously. Future studies using patient outcomes are needed to shed light on the clinical significance of *in vitro* NTM AST in our hospital. Lastly, proper AST results for specimens with multispecies infection were not accomplished. Since multispecies NTM infection in a patient is not an uncommon finding, accurate AST technique using single culture isolate is necessary in the case of multispecies infection.

In summary, the recovery rate of NTM showed an increasing trend in our hospital, although the trend was not observed in 2020. It would be interesting to determine whether the COVID-19 pandemic and crisis-related regulations play a role in the change of epidemiology of NTM infection. *MAC*, *M. abscessus* subspecies *abscessus*, and *M. kansasii* were the most common NTM species in our center. Little less than 9% of total isolates were shown to be mixed NTM infection. Most of isolated NTM species were susceptible to key drugs such as clarithromycin, amikacin, and rifampin. However, most of *M. abscessus* subspecies *abscessus* isolates showed inducible resistance to clarithromycin.

Authors' Contributions

Conceptualization: Kim KJ, Jeon D, Chang CL. Methodology: Kim KJ, Oh SH. Formal analysis: Kim KJ, Oh SH, Jeon D. Writing - original draft preparation: Kim KJ. Writing - review and editing: Kim KJ, Chang CL. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article

was reported.

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Supplementary Material

Supplementary material can be found in the journal homepage (<http://www.e-trd.org>).

Supplementary Table S1. Antimycobacterial agents and breakpoints for testing nontuberculous mycobacteria.

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