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Mycobacteriological characteristics and treatment outcomes in extrapulmonary *Mycobacterium abscessus* complex infections

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SUMMARY

Objectives: The differentiation between *Mycobacterium abscessus* subspecies *abscessus* (*M. abscessus*) and *Mycobacterium abscessus* subspecies *massiliense* (*M. massiliense*) and determination of the presence of inducible resistance to macrolide antibiotics are important factors in the management of patients with *Mycobacterium abscessus* complex (MABC) infections. Unlike pulmonary MABC infections, little information on extrapulmonary MABC infections is available.
Methods: The molecular identification of clinical isolates was performed, and the clinical characteristics and treatment outcomes of 20 consecutive patients with extrapulmonary MABC infections were assessed.
Results: *M. abscessus* and *M. massiliense* each caused 10 (50%) of the cases. Eight (80%) *M. abscessus* isolates that had inducible resistance to clarithromycin harbored an intact *erm*(41) gene of the T28 variant, whereas two (20%) *M. abscessus* isolates had the C28 *erm*(41) variant and were susceptible to clarithromycin. All *M. massiliense* isolates had a truncated *erm*(41) gene and were susceptible to clarithromycin. The drug susceptibility profiles other than clarithromycin were similar for the *M. abscessus* and *M. massiliense* isolates. Of the 20 patients, 17 (85%) showed a favorable outcome, including all patients with *M. massiliense* infection and 70% (7/10) of patients with *M. abscessus* infection. Favorable outcomes were associated with *M. massiliense* and *M. abscessus* isolates with a non-functional *erm*(41) gene ($p = 0.049$).
Conclusions: Precise species and subspecies identification and the determination of macrolide susceptibility are recommended for the optimal treatment of extrapulmonary MABC infections.

Introduction

Pulmonary and extrapulmonary infections caused by non-tuberculous mycobacteria (NTM) are increasingly reported worldwide (Prevots and Marras, 2015; Stout et al., 2016). The *Mycobacterium abscessus* complex (MABC) is the most important cause of NTM infections by rapidly growing mycobacteria (Griffith et al., 2007; Floto et al., 2016). Moreover, MABC is a highly drug-resistant pathogen and very difficult to treat (Kasperbauer and De Groote, 2015; Lee et al., 2015; Ryu et al., 2016).

The guidelines of the American Thoracic Society and Infectious Diseases Society of America recommend macrolide-based antibiotic therapy combined with intravenous amikacin and cefoxitin or imipenem, based on the results of drug susceptibility testing (DST), for the treatment of MABC infections (Griffith et al., 2007). However, the guidelines recommend different approaches for the treatment of extrapulmonary and pulmonary MABC infections (Griffith et al., 2007). For extrapulmonary MABC infections, such as skin, soft tissue, and bone infections, a total of 4–6 months of antibiotic therapy with at least 2 weeks of an initial combination of parenteral antibiotics is recommended, with a high likelihood of

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cure (Griffith et al., 2007). However, pulmonary MABC disease is regarded as a chronic, incurable infection for most patients, even with the recommended 2–4 months of parenteral antibiotics followed by long-term macrolide-based antibiotic therapy (Griffith et al., 2007). The exact reasons for these different treatment outcomes are largely unknown.

Currently, MABC can be divided into three subspecies: *M. abscessus* subspecies *abscessus* (hereafter *M. abscessus*), *M. abscessus* subspecies *massiliense* (hereafter *M. massiliense*), and *M. abscessus* subspecies *bolletii* (hereafter *M. bolletii*) (Tortoli et al., 2016). *M. abscessus* is the most common pathogen, causing 45–65% of MABC cases, followed by *M. massiliense* (20–55%) and *M. bolletii* (1–18%); the treatment outcomes of patients with pulmonary MABC infections differ according to the etiologic organism (Koh et al., 2014).

The treatment response rates to macrolide-based antibiotic therapy are much higher in patients with pulmonary *M. massiliense* infections than in those with pulmonary *M. abscessus* infections (Koh et al., 2011; Lyu et al., 2014; Koh et al., 2016; Koh et al., 2017). This difference is likely due to the presence of a functional ribosomal methyltransferase gene, *erm*(41), in *M. abscessus* that results in inducible macrolide resistance (susceptible on day 3 but resistant on day 14 of DST). In contrast, the *erm*(41) gene in *M. massiliense* is non-functional due to truncation, so inducible resistance does not occur (Nash et al., 2009; Choi et al., 2012; Brown-Elliott et al., 2015). In addition, previous studies have demonstrated that 7–18% of *M. abscessus* clinical isolates have a T → C polymorphism at nucleotide 28 of the *erm*(41) gene, which inactivates the gene, and as these isolates are susceptible to macrolides (Brown-Elliott et al., 2015; Bastian et al., 2011; Yoshida et al., 2013; Lee et al., 2014; Shallom et al., 2015), macrolides can be useful for treating *M. abscessus* infections caused by the C28 sequevar (Koh et al., 2017). However, information on the responsiveness of extrapulmonary MABC infections to macrolide treatment and the relevance of the different sequevars is very limited.

Because of the high cure rate compared to pulmonary infections, it was hypothesized that a substantial proportion of extrapulmonary MABC infections are caused by *M. massiliense* or the C28 sequevar of *M. abscessus*. The aim of this study was to determine the proportions of M. massiliense and the M. abscessus C28 sequevar among extrapulmonary MABC infections and to examine treatment outcomes of these infections based on causative organisms.

Materials and methods

Study population

The medical records of 22 consecutive patients with extrapulmonary MABC infections treated at Samsung Medical Center (a 1979-bed referral hospital in Seoul, South Korea) from October 2010 to December 2014 were identified using an electronic database. These medical records were reviewed. After the exclusion of two patients who were transferred to another hospital or lost to follow-up during treatment, 20 patients were included in the study. This retrospective study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. 2016-03-050). Informed consent was waived for the use of medical data because patient information was anonymized and de-identified prior to analysis.

Microbiological examination

Acid-fast bacillus (AFB) smears and cultures were performed using standard methods, as described previously (Koh et al., 2016). During the study period, NTM species were identified using a reverse blot hybridization assay of the *rpoB* gene (REBA Myco-ID test; YD Diagnostics, Yongin, South Korea) (Wang et al., 2014). For precise species identification of the isolates, multilocus sequencing analysis of the nearly complete 16S rRNA gene, the 16S–23S rRNA internal transcribed spacer (ITS) sequences, partial *rpoB* sequences, and partial *hsp65* sequences was performed (Ben Salah et al., 2008; Frothingham and Wilson, 1993; Turenne et al., 2001; Adekambi et al., 2003). Detection of *erm*(41) and mutations in the 23S rRNA gene (*rrl*) was performed by PCR sequencing, as described previously (Bastian et al., 2011; Jamal et al., 2000). DST was performed by broth microdilution method, and *Mycobacterium peregrinum* ATCC 700686 was used for quality control, in accordance with the guidelines of the Korean Institute of Tuberculosis (Clinical Laboratory Standards Institute, 2011). The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration capable of inhibiting visible growth of the mycobacteria. The MIC_{50} and MIC_{90} were defined as the concentrations required to inhibit the growth of 50% and 90% of the strains, respectively. The MIC of clarithromycin was determined on days 3 and 14 after incubation; MABC isolates were considered susceptible (MIC $\leq$2 μg/ml on days 3 and 14), resistant (MIC $\geq$8 μg/ml on day 3), or inducibly resistant (susceptible on day 3, but resistant on day 14) to clarithromycin (Clinical Laboratory Standards Institute, 2011).

Antibiotic therapy and treatment outcomes

Treatment regimens and durations for extrapulmonary MABC infections were not standardized but were determined by the attending physician in the institution during the study period. Patients with mild disease received oral or topical antibiotics, such as macrolides (clarithromycin 1000 mg/day or azithromycin 250 mg/day) and fluoroquinolones (moxifloxacin 400 mg/day or ciprofloxacin 500 mg/day), in the outpatient clinic. Patients with severe disease were hospitalized and received intravenous amikacin (10–15 mg/kg/day) combined with cefoxitin (6–12 g/day) or imipenem (2000 mg/day) for several weeks, together with oral antibiotics. Surgical treatment was performed during antibiotic treatment based on discussions among the attending physicians and surgeons. Favorable outcomes were defined as the resolution of clinical symptoms and site lesions after antibiotic treatment and/or initial surgical treatment. Unfavorable treatment outcomes were defined as hospital readmission for the second surgical treatment due to clinical and radiographic deterioration during antibiotic therapy that included at least 2 weeks of combination parenteral antibiotic therapy.

Statistical analysis

All data are presented as the median value and range for continuous variables and as the number and percentage for categorical variables. Data were compared using the Mann–Whitney *U*-test and Kruskal–Wallis test for continuous variables and using the Pearson Chi-square test or Fisher's exact test for categorical variables. A two-sided *p*-value of $<$0.05 was considered statistically significant for all analyses. All analyses were performed using IBM SPSS version 23 statistical software (IBM Corp., Armonk, NY, USA).

Results

Clinical characteristics of patients

A total of 20 patients with extrapulmonary MABC infections were included in the study. Baseline characteristics of the patients

Table 1
Baseline characteristics of 20 patients with extrapulmonary MABC infections.

Characteristics	Total (N = 20)
Age, years, median (range)	51 (25–76)
Female, *n* (%)	14 (70)
Etiologic organism, *n* (%)	
M. abscessus	10 (50)
M. massiliense	10 (50)
Infection site, *n* (%)	
Skin and soft tissue	7 (35)
Bone and joint	6 (30)
Ocular	7 (35)
Treatment, *n* (%)	
Topical antibiotics alone	4 (20)
Oral antibiotics alone	6 (30)
Oral and intravenous antibiotics	10 (50)
Treatment duration, days, median (range)	
Oral antibiotics	177 (14–840)
Macrolide	177 (14–840)
Quinolone	58 (10–498)
Intravenous antibiotics	27 (4–178)
Amikacin	27 (4–168)
Cefoxitin	18 (13–113)
Imipenem	16 (4–49)

MABC, *Mycobacterium abscessus* complex; *M. abscessus*,*Mycobacterium abscessus* subspecies *abscessus*; *M. massiliense*, *Mycobacterium abscessus* subspecies *massiliense*.

are summarized in Table 1. There were 14 females (70%) and six males (30%), with a median age of 51 years (range 25–76 years). Infection sites were skin and soft tissue (n = 7, 35%), bone and joint (n = 6, 30%), and the ocular system (n = 7, 35%) (Figure 1). There was no patient with a disseminated infection or blood stream infection.

Most patients were previously healthy and did not have any underlying disease or possible risk factors for extrapulmonary MABC infection, such as anti-interferon-gamma autoantibodies. There was one patient who had undergone a liver transplantation and had received immunosuppressive drugs. Among the seven patients with skin and soft tissue infections, five had a documented history of a medical procedure such as acupuncture. All patients with skin and soft tissue infections had skin and subcutaneous involvement. No patient had deep, tendon, or fascia involvement. Among the six patients with bone and joint infections, two had a history of a medical procedure such as intra-articular steroid injection, two patients had recently undergone joint surgery, and two patients had a history of recent trauma. All seven patients with an ocular infection had a history of eye surgery, such as eye implant surgery and lacrimal tube insertion.

Genetic analyses and DST results of the MABC isolates

The genetic analyses of the MABC isolates from the 20 patients are shown in Table 2. The etiologic organisms were *M. abscessus* in 10 (50%) patients and *M. massiliense* in 10 (50%) patients. There were no cases of extrapulmonary infection caused by *M. bolletii*. Eight *M. abscessus* isolates harboring an intact *erm*(41) gene of the T28 sequevar (8/10, 80%) were inducibly resistant to clarithromycin, whereas two *M. abscessus* isolates of the C28 sequevar (2/20, 20%; patients 11 and 13) were susceptible to clarithromycin. All 10 *M. massiliense* isolates harbored a truncated *erm*(41) gene and were susceptible to clarithromycin. All 20 *M. abscessus* and *M. massiliense* isolates were susceptible to clarithromycin on day 3 of DST and had no mutations in the 23S rRNA gene that confer macrolide resistance (Bastian et al., 2011; Shallom et al., 2015; Maurer et al., 2012; Mougari et al., 2016).

The MIC range and the MIC_{50} and MIC_{90} values of each antimicrobial agent for *M. abscessus* and *M. massiliense* isolates are shown in Table 3. Amikacin and cefoxitin showed activity against *M. abscessus*, with MIC_{90} values of 16 μg/ml and 32 μg/ml, respectively. Comparatively, amikacin and cefoxitin showed activity against *M. massiliense*, with MIC_{90} values of 32 μg/ml and 64 μg/ml, respectively. The percentage of resistant isolates in both groups was 10% for amikacin and 0% for cefoxitin. In addition, *M. abscessus* (MIC_{90} 16 μg/ml) appeared more susceptible to imipenem than *M. massiliense* (MIC_{90} 64 μg/ml). In contrast, most MABC isolates showed resistance to ciprofloxacin, moxifloxacin, and doxycycline.

Table 2
Clarithromycin susceptibility and *erm*(41) and *rrl* genotypes of clinical isolates from 20 patients with extrapulmonary MABC infections.

Patient No.	Isolate	DST result[a]	*erm*(41) sequevar[b]	*erm*(41) length[c]	*rrl* positions 2058–2059[d]
01	*M. massiliense*	S		Truncated	WT
02	*M. massiliense*	S		Truncated	WT
03	*M. massiliense*	S		Truncated	WT
04	*M. massiliense*	S		Truncated	WT
05	*M. abscessus*	IR	T28	Complete	WT
06	*M. abscessus*	IR	T28	Complete	WT
07	*M. abscessus*	IR	T28	Complete	WT
08	*M. massiliense*	S		Truncated	WT
09	*M. massiliense*	S		Truncated	WT
10	*M. massiliense*	S		Truncated	WT
11	*M. abscessus*	S	C28	Complete	WT
12	*M. massiliense*	S		Truncated	WT
13	*M. abscessus*	S	C28	Complete	WT
14	*M. abscessus*	IR	T28	Complete	WT
15	*M. abscessus*	IR	T28	Complete	WT
16	*M. abscessus*	IR	T28	Complete	WT
17	*M. massiliense*	S		Truncated	WT
18	*M. massiliense*	S		Truncated	WT
19	*M. abscessus*	IR	T28	Complete	WT
20	*M. abscessus*	IR	T28	Complete	WT

MABC, *Mycobacterium abscessus* complex; *M. abscessus*, *Mycobacterium abscessus* subspecies *abscessus*; *M. massiliense*, *Mycobacterium abscessus* subspecies *massiliense*.
[a] Results of drug susceptibility testing to clarithromycin using the microdilution method. S, susceptible; IR, inducibly resistant.
[b] A thymine (T) or cytosine (C) was present at nucleotide position 28 of *erm*(41).
[c] 'Truncated' indicates that *erm*(41) was truncated and non-functional due to deletions of nucleotides 64, 65, and 159–432; 'complete' indicates that the full-length gene was present (Bastian et al., 2011).
[d] Wild-type (WT) sequence of AA at nucleotides 2058–2059 in *rrl* (*Escherichia coli* numbering system).

Table 3
Results of drug susceptibility testing against *M. abscessus* and *M. massiliense*.

	M. abscessus ($n = 10$)				*M. massiliense* ($n = 10$)			
	MIC[a] range	MIC_{50}[a]	MIC_{90}[a]	% resistant[b]	MIC[a] range	MIC_{50}[a]	MIC_{90}[a]	% resistant[b]
Clarithromycin, day 3	0.5–4	0.5	2	–	0.5	0.5	0.5	–
Clarithromycin, day 14	0.5–64	32	64	80	0.5	0.5	0.5	–
Amikacin	8–64	16	16	10	8–128	16	32	10
Cefoxitin	16–32	32	32	–	16–64	32	64	–
Imipenem	2–32	4	16	10	8–64	8	64	30
Ciprofloxacin	4–16	16	16	100	4–16	16	16	100
Moxifloxacin	2–16	8	16	90	2–16	4	8	80
Doxycycline	32	32	32	100	0.25–32	32	32	90

M. abscessus, *Mycobacterium abscessus* subspecies *abscessus*; *M. massiliense*, *Mycobacterium abscessus* subspecies *massiliense*.
[a] MIC = minimum inhibitory concentration (μg/ml); $MIC_{50/90}$ = MIC required to inhibit the growth of 50% and 90% of the strains, respectively.
[b] Breakpoint values are referenced from the Clinical and Laboratory Standards Institute (CLSI) recommendations (clarithromycin, 8 μg/ml; amikacin, 64 μg/ml; cefoxitin, 128 μg/ml; imipenem, 32 μg/ml; ciprofloxacin, 4 μg/ml; moxifloxacin, 4 μg/ml; doxycycline, 8 μg/ml) (Clinical Laboratory Standards Institute, 2011).

Comparison of clinical characteristics and treatment modalities according to etiologic organism

Among the 20 study patients, four (20%) with ocular infections received topical antibiotics alone and the other 16 (80%) received oral antibiotic treatment for a median duration of 177 days (range 14–840 days); 10 patients (50%) received a combination of oral and intravenous antibiotic treatment for a median duration of 27 days (range 4–178 days)(Table 1).

There were no differences in age, sex, or infection site between patients with *M. abscessus* and *M. massiliense* infections (Table 4), and no significant differences were found between the two groups in the proportion of patients who received oral antibiotics and/or intravenous antibiotics. The median duration of treatment with oral antibiotics did not differ between patients with *M. abscessus* infections (177 days, range 14–840 days) and patients with *M. massiliense* infections (176 days, range 30–348 days, $p = 0.837$). The median duration of intravenous antibiotic treatment also did not differ between patients with *M. abscessus* infections (76 days, range 4–178 days) and those with *M. massiliense* infections (23 days, range 15–27 days, $p = 0.476$).

Comparison of clinical characteristics and treatment modalities according to infection site

The patients with bone and joint infections were older than those with skin and soft tissue or ocular infections ($p = 0.037$) (Table 5). However, there were no differences in sex or etiologic organism between patients with skin and soft tissue infections, bone and joint infections, and ocular infections. All patients with skin and soft tissue infections or bone and joint infections received oral antibiotic treatment with or without intravenous antibiotics. Patients with bone and joint infections were more likely to receive intravenous antibiotics ($n = 6$, 100%) than were those with skin and soft tissue infections ($n = 3$, 43%) or ocular infections ($n = 1$, 14%, $p = 0.011$). The median duration of oral antibiotic treatment was longer in patients with bone and joint infections (343 days, range 14–840 days) than in those with skin and soft tissue infections (176 days, range 62–188 days) or ocular infections (38 days, range 30–174), although this difference was not statistically significant ($p = 0.053$).

Treatment outcomes

Of the 20 patients, 17 (85%) showed a favorable treatment response (Table 6). The duration of treatment with intravenous antibiotics was shorter in patients with favorable outcomes than in those with unfavorable outcomes (median 20 days vs. 173 days, $p = 0.017$). The duration of treatment with oral antibiotics was also shorter in patients with favorable outcomes than in those with unfavorable outcomes (median 174 days vs. 558 days), although this was not statistically significant ($p = 0.057$). All patients with *M. massiliense* infection and 70% (7/10) of patients with *M. abscessus* infection showed a favorable treatment response. All three patients with an unfavorable response were infected with *M. abscessus* isolates that had an intact *erm*(41) gene of the T28 sequevar, whereas a favorable outcome was associated with *M. massiliense* and *M. abscessus* isolates with a non-functional *erm* (41) gene ($p = 0.049$).

Discussion

Extrapulmonary MABC infections are uncommon disorders (Piersimoni and Scarparo, 2009; Kasperbauer and Huitt, 2013).

Table 4
Comparison of 20 patients with extrapulmonary MABC infection according to etiologic organism.

	M. abscessus ($n = 10$)	*M. massiliense* ($n = 10$)	*p*-Value
Age, years, median (range)	53 (25–76)	50 (37–74)	0.853
Female, *n* (%)	7 (70)	7 (70)	1.000
Infection site, *n* (%)			0.297
Skin and soft tissue	2 (20)	5 (50)	
Bone and joint	3 (30)	3 (30)	
Ocular	5 (50)	2 (20)	
Treatment, *n* (%)			
Oral antibiotics alone	1 (10)	5 (50)	0.141
Oral and intravenous antibiotics	6 (60)	4 (40)	0.371
Surgical treatment	9 (90)	9 (90)	1.000
Treatment duration, days, median (range)			
Oral antibiotics	177 (14–840)	176 (30–348)	0.837
Intravenous antibiotics	76 (4–178)	23 (15–27)	0.476

MABC, *Mycobacterium abscessus* complex; *M. abscessus*, *Mycobacterium abscessus* subspecies *abscessus*; *M. massiliense*, *Mycobacterium abscessus* subspecies *massiliense*.

Table 5
Comparison of 20 patients with extrapulmonary MABC infections according to infection site.

	Skin and soft tissue (n = 7)	Bone and joint (n = 6)	Ocular (n = 7)	p-Value
Age, years, median (range)	45 (30–67)	72 (41–76)	50 (25–68)	0.037
Female, n (%)	6 (86)	5 (83)	3 (43)	0.260
Causative organism, n (%)				0.297
M. abscessus	2 (29)	3 (50)	5 (71)	
T28 sequevar	2	3	3	
C28 sequevar	0	0	2	
M. massiliense	5 (71)	3 (50)	2 (29)	
Treatment, n (%)				
Oral antibiotics alone	4 (57)	0 (0)	2 (29)	0.153
Oral and intravenous antibiotics	3 (43)	6 (100)	1 (14)	0.011
Surgical treatment	7 (100)	6 (100)	5 (71)	0.300
Treatment duration, days, median (range)				
Oral antibiotics	176 (62–188)	343 (14–840)	38 (30–174)	0.053
Intravenous antibiotics	37 (27–114)	23 (14–178)	4[a]	0.229

MABC, *Mycobacterium abscessus* complex; *M. abscessus*, *Mycobacterium abscessus* subspecies *abscessus*; *M. massiliense*, *Mycobacterium abscessus* subspecies *massiliense*.
[a] Only one patient with an ocular infection received intravenous antibiotics.

Table 6
Treatment outcomes of 20 patients with extrapulmonary MABC infection.

	Favorable outcome (n = 17)	Unfavorable outcome (n = 3)	p-Value
Age, years, median (range)	49 (25–76)	71 (51–72)	0.146
Female, n (%)	11 (65)	3 (100)	0.521
Functioning *erm*(41) gene, n (%)	5 (29)	3 (100)	0.049
Etiologic organism, n (%)			0.211
M. abscessus	7 (41)	3 (100)	
T28 sequevar	5 (71)	3 (100)	
C28 sequevar	2 (29)	0	
M. massiliense	10 (59)	0	
Infection site, n (%)			0.263
Skin soft tissue	6 (35)	1 (33)	
Bone and joint	4 (24)	2 (67)	
Ocular	7 (41)	0	
Treatment, n (%)			
Oral antibiotics alone	6 (35)	0 (0)	0.521
Oral and intravenous antibiotics	7 (41)	3 (100)	0.211
Surgical treatment	15 (88)	3 (100)	1.000
Treatment duration, days, median (range)			
Oral antibiotics	174 (14–348)	558 (177–840)	0.057
Intravenous antibiotics	20 (4–37)	173 (114–178)	0.017

MABC, *Mycobacterium abscessus* complex; *M. abscessus*, *Mycobacterium abscessus* subspecies *abscessus*; *M. massiliense*, *Mycobacterium abscessus* subspecies *massiliense*.

Many previous studies have not differentiated the subspecies of MABC and have included a wide range of treatment strategies (Bodle et al., 2008; Novosad et al., 2016). This is the first study to comprehensively analyze the clinical characteristics and treatment outcomes of patients with extrapulmonary MABC infections according to mycobacterial characteristics, including subspecies differentiation. In this study, half of the extrapulmonary MABC infections were caused by *M. abscessus* and the other half were caused by *M. massiliense*. All *M. massiliense* isolates were susceptible to clarithromycin, and of the *M. abscessus* isolates, 20% (2/10) had the C28 variant of *erm*(41) and were susceptible to clarithromycin and 80% (8/10) had the T28 variant and were inducibly resistant to clarithromycin. Favorable treatment outcome rates were higher in patients infected with either *M. massiliense* or the *M. abscessus* C28 sequevar than in those infected with the *M. abscessus* T28 sequevar. These results suggest that both precise subspecies differentiation and the determination of the presence of inducible resistance using *erm*(41) gene sequencing are very important for predicting the treatment outcome in patients with extrapulmonary MABC infections.

Among extrapulmonary NTM infections in adults, skin and soft tissue infections are the most common (Piersimoni and Scarparo, 2009; Kasperbauer and Huitt, 2013) and can occur after medical procedures or traumatic injuries. Rapidly growing mycobacteria, including MABC, are the most important NTM pathogens of skin and soft tissue infections (Kothavade et al., 2013; Lin et al., 2014); however, many previous studies regarding MABC skin and soft tissue infections have not differentiated between *M. abscessus* and *M. massiliense* (Chen et al., 2011; Mudedla et al., 2015). Recently, postsurgical and post-procedure outbreaks of skin and soft tissue infection caused by *M. abscessus* or *M. massiliense* have occurred in several countries (Cheng et al., 2013; Leão et al., 2010; Kim et al., 2007; Song et al., 2012; Koh et al., 2010); patients with such MABC infections usually recover well after several months of oral antibiotics, including a macrolide and fluoroquinolone, with or without initial intravenous antibiotics (Song et al., 2012; Choi et al., 2011; Jung et al., 2014).

Several outbreaks of septic arthritis caused by MABC after medical procedures have also been reported (Jung et al., 2015; Lee et al., 2016). The treatment response in patients with septic arthritis due to *M. massiliense* was good after about 12 months of antibiotic treatment with or without surgery (Jung et al., 2015; Lee et al., 2016). Vertebral osteomyelitis due to NTM is usually caused by slowly growing mycobacteria such as *Mycobacterium avium*

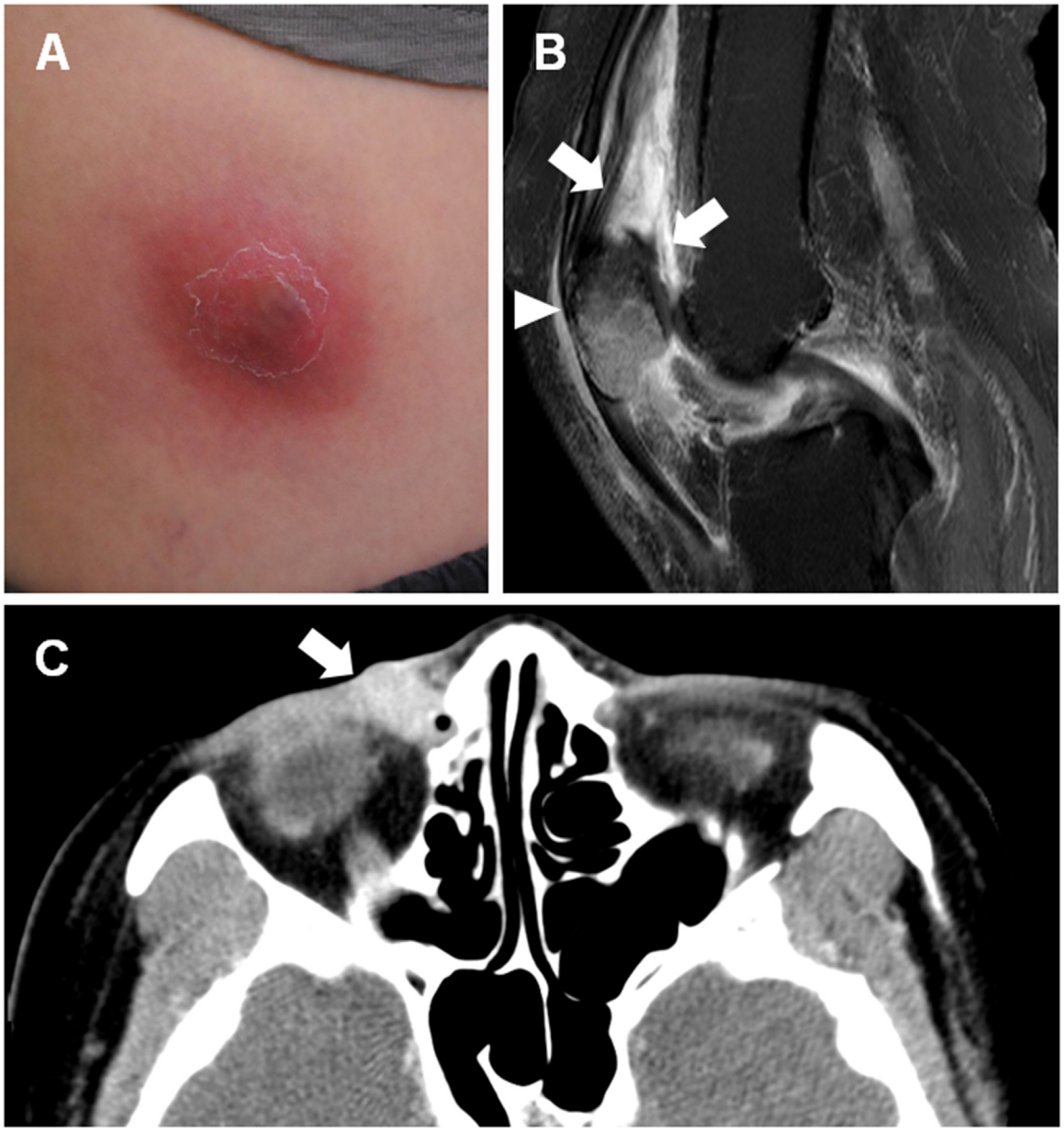

Figure 1. Representative pictures of extrapulmonary *Mycobacterium abscessus* complex infections. (A) Skin and soft tissue infections caused by *M. abscessus* subspecies *massiliense* after injection in a 37-year-old female patient. There is an erythematous nodule on the proximal thigh. (B) Knee joint and bone infections caused by *M. abscessus* subspecies *massiliense* after needle aspiration and injection in a 57-year-old female patient. T1-weighted sagittal magnetic resonance imaging of the right knee shows enhanced thickened synovium in the suprapatellar bursa (arrow). Additionally, there is osteomyelitis in the patella (arrow head). (C) Eye infection caused by *M. abscessus* subspecies *abscessus* in a 54-year-old female patient with right peri-orbital swelling and pain after surgery to the lacrimal sac. The computed tomography scan shows an enhancing soft tissue mass in the right medial canthal area of the orbit.

complex or *Mycobacterium xenopi*, whereas causation by MABC is rare, and additionally, most previous studies have not performed subspecies identification (Garcia et al., 2013; Kim et al., 2016).

NTM ocular infections have increasingly been reported over the past few decades and are typically linked to medical interventions, trauma, and implants (Kheir et al., 2015). The most common causative organism is *Mycobacterium chelonae*, with MABC accounting for 10% of NTM ocular infections (Kheir et al., 2015). Some patients recover fully after antibiotic treatment, whereas others need therapeutic surgical interventions (Kheir et al., 2015), and unlike other extrapulmonary NTM infections, NTM ocular infections can be successfully treated with topical antibiotics (Abshire et al., 2004).

MABC is traditionally considered to be resistant to multiple antibiotics, and data supporting effective drugs or regimens are very limited. However, it is unclear why extrapulmonary MABC infections are highly likely to be cured after 4–6 months of antibiotic therapy, whereas MABC pulmonary disease is regarded as an incurable infection even after more than 12–24 months of multiple-antibiotic therapy (Griffith et al., 2007). The present study clearly demonstrated that 50% (10/20) of extrapulmonary MABC infections were caused by *M. massiliense*, and 20% (2/10) of *M. abscessus* isolates were of the C28 sequevar and were susceptible to clarithromycin. All 10 patients infected with *M. massiliense* and two patients infected with the *M. abscessus* C28 sequevar showed favorable outcomes, whereas all three

patients in this study with unfavorable responses were infected with the *M. abscessus* T28 sequevar. Interestingly, 63% (5/8) of the patients with the *M. abscessus* T28 sequevar showed favorable outcomes despite inducible resistance of the infecting organism to macrolides. The effect of antibiotics other than macrolides and combined surgical intervention, as well as the low mycobacterial burden at sites of extrapulmonary infection, might contribute to this relatively high rate of favorable outcome in patients with extrapulmonary MABC infections. However, in MABC pulmonary disease, a substantial proportion (25–42%) of cases caused by the *M. abscessus* T28 sequevar also achieve successful treatment outcomes (Koh et al., 2011; Lyu et al., 2014).

There are several limitations to this study. First, the study was conducted at a single referral center in South Korea, and, as the proportion of *M. massiliense* among MABC isolates and of clarithromycin-susceptible isolates (C28 sequevar) among *M. abscessus* isolates may vary by geographic region, some of the findings may not be generalizable. Second, treatment regimens for extrapulmonary MABC infections were not standardized in the institution during the study period. Third, the pathogenesis, severity, and optimal treatment modalities could differ depending on the site of infection in extrapulmonary MABC infections. Finally, it was difficult to compare the clinical efficacy of the different treatment regimens because the treatment regimens were non-standardized, the number of patients was small, and the clinical manifestations differed. Further studies with larger numbers of patients are needed to determine the optimal antibiotic strategies for the treatment of extrapulmonary MABC infections according to the site of infection.

In conclusion, 50% of extrapulmonary MABC infections were caused by *M. massiliense*, which is susceptible to macrolides, and although 80% of *M. abscessus* isolates were inducibly resistant to macrolides (T28 sequevar), the remaining 20% of *M. abscessus* isolates were susceptible (C28 sequevar). This prevalence of macrolide-susceptible strains may in part explain why extrapulmonary MABC infections are more likely to be cured than MABC pulmonary disease, and indeed all patients with *M. massiliense* and the *M. abscessus* C28 sequevar showed favorable treatment outcomes. Precise species and subspecies identification and the determination of macrolide susceptibility are highly recommended for the optimal management of patients with extrapulmonary MABC infections.

Conflict of interest

We have no conflicts of interest to declare.

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