Otitis Media and Otomastoiditis Caused by

*Mycobacterium massiliense* (*Mycobacterium abscessus* subspecies *bolletii*)

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**Running title:** *M. massiliense* otologic infections
We described two patients with otologic infections caused by *Mycobacterium massiliense* (*M. abscessus* subspecies *bolletti*) which were identified using *erm*(41) PCR, 23S rRNA, and *rpoB* gene sequence analysis. They were middle-aged adults with underlying otologic diseases and were treated successfully with clarithromycin-based combination regimens for three and nine months, respectively.
Mycobacterium abscessus complex comprises three closely related Mycobacterium subspecies, namely M. massiliense, M. bolletii, and M. abscessus (sensu stricto) (1). Species-level identification of M. abscessus complex depends on sequencing analysis of several genes, including the \textit{erm}(41) gene, the 23S rRNA gene, and several housekeeping genes (e.g. \textit{rpoB} and \textit{hsp65}), which is not available in many laboratories (1, 7, 9). A previous report also indicated that \textit{erm}(41) PCR can be efficiently used to simply differentiate M. massiliense from M. abscessus and M. bolletii and inconsistency could be found between \textit{rpoB} and \textit{hsp65} sequence analysis (7). Recently, the taxonomic status of M. massiliense is under debate and currently M. abscessus subspecies bolletii is probably preferred (10). Infections due to M. massiliense (M. abscessus subspecies bolletii) include post-surgical infections, cutaneous infections, pulmonary infections, and central nervous system infections (3, 8, 11-12, 14). Otitis media and otomastoiditis caused by nontuberculous mycobacteria (NTM) are common in children and species in the M. abscessus complex are the most frequently isolated pathogens in patients with those diseases (5, 17). In a recent study of 10 patients with otomastoiditis, van Ingen et al. reported that the causative pathogen in all patients was M. abscessus (sensu stricto) (17).

During the period 2000-2010, eight patients with otitis media or otomastoiditis, whose clinical specimens (biopsy and ear discharge) were positive for M. abscessus complex, were treated at the National Taiwan University Hospital. The biopsy or ear discharge specimens from the eight patients were processed and pretreated for mycobacterial cultures as previously described (13). The isolates of M. abscessus complex were identified to the subspecies level by screening for the presence of the \textit{erm}(41) gene as well as sequencing of the 23S rRNA and \textit{rpoB} (306 bp) genes (7). Minimum inhibitory concentrations (MICs) of 15 antimicrobial agents against the
four *M. massiliense* (*M. abscessus* subspecies *bolletti*) isolates were determined using the Sensititre RAPMYCOI panel test (TREK Diagnostic Systems, Magellan Biosciences, West Sussex, UK). MICs of all agents tested were read on the fifth day after incubation and those of clarithromycin were read after extended incubation (on day 14) (15).

Among the eight isolates of *M. abscessus* complex, four were confirmed to be *M. abscessus* (*sensu stricto*) and the other four isolates were confirmed to be *M. massiliense* (*M. abscessus* subspecies *bolletti*) by *erm* (41) PCR (397 bp) and sequence analysis for 23S rRNA (accession number F1358489.1, similarity of 99%), and *rpoB* genes (similarity of 100%) (7). The four patients with *M. abscessus* (*sensu stricto*) otitis media have been previously reported (6).

The MIC values of the 15 agents against the four *M. massiliense* (*M. abscessus* subspecies *bolletti*) isolates are shown in Table 2. Amoxicillin-clavulanic acid, cephalosporins, imipenem, tetracyclines, linezolid, fluoroquinolones, and aminoglycosides were not active against all the four isolates. MICs of the four isolates to clarithromycin were 0.12-1.0 μg/ml. MIC of one isolate (a colonizer from Patient 3) to clarithromycin was >16 μg/ml and that of other three isolates was 0.5 μg/ml (read on 14th day of incubation).

Two of the four patients (Patients 3 and 4) with positive culture of *M. massiliense* (*M. abscessus* subspecies *bolletti*) from ear discharges were considered as contaminants or colonizations instead of infections due to the good response with topical treatment alone and negative acid-fast stain and one single positive culture from multiple specimens (two specimens from each of the two patients) (Table 1). Patient 1 was a 53-year-old male who suffered from *Aspergillus niger* chronic otitis media and received mastodectomy plus tympanoplasty one year ago. Otorrhea,
hearing impairment and vertigo developed three months after surgery. Three cultures from the right ear discharges all grew *M. abscessus*, which were then confirmed to be *M. massiliense* (*M. abscessus* subspecies *bolletti*). The patient received clarithromycin (500 mg twice daily) plus ciprofloxacin (750 mg twice daily) for nine months. Repeated culture from the right ear was negative at end of treatment and he remained uneventfully for 4 years without any sequelae.

Patient 2 was a 58-year-old male patient with past history of perforation of tympanic membrane. He presented to the hospital with otorrhea, hearing impairment and Gradenigo syndrome (6, 15). He first received petrosectomy, mastoidectomy and tympanoplasty, followed by a combination therapy with clarithromycin (500 mg twice daily), ciprofloxacin (750 mg twice daily) and ethambutol (800 mg per day) for three months. Acid-fast bacilli were found in one ear discharge specimen that was also culture positive for *M. massiliense* (*M. abscessus* subspecies *bolletti*). At the end of treatment, culture became negative for mycobacteria. The patient was complicated with abducens nerve palsy during the follow-up of three years after completion of treatment (6).

The incidence of otitis media and otomastoiditis caused by NTM is increasing (17). *M. abscessus* complex, *M. chelonae*, *M. fortuitum*, and *M. avium intracellulare* complex have been reported to cause otologic infections (4). In our previous report, species in the *M. abscessus* complex were the most common causes of otitis media followed by *M. chelonae* (6). In the present study, infection due to *M. massiliense* (*M. abscessus* subspecies *bolletti*) was found in one-third of the six patients with infection caused by *M. abscessus* complex (excluding Patients 3 and 4). This finding differs from that reported by van Ingen et al., who found that none of the otologic infections due to *M. abscessus* complex were caused by *M. massiliense* (*M. abscessus*...
Common features of otomastoiditis caused by *M. massiliense* (*M. abscessus* subspecies *bolletti*) and *M. abscessus* (*sensu stricto*) included the presence of underlying otologic disease and the need of prolonged antibiotic combination therapy along with surgical intervention (17). Most of the *M. massiliense* isolates were susceptible to clarithromycin (2, 7). Kim et al reported that clarithromycin-resistant *M. massiliense* isolates invariably had a point mutation at the adenine, A(2058) or A(2059), in the peptidyltransferase region of the 23S rRNA gene, which was quite different from *M. abscessus* and *M. bolletii* (7). Although one of the *M. massiliense* (*M. abscessus* subspecies *bolletti*) isolate in this report had high MIC value (>16 μg/ml) of clarithromycin, the described point mutations were not found in this isolate (7). Interestingly, otomastoiditis caused by *M. abscessus* (*sensu stricto*) has been reported predominately in children (17) while patients reported in this study and our previous study both disclosed the adult prevalence (6). Although tigecycline MICs were within 0.5-1 μg/ml in our study, the clinical efficacy of this agent needs to be investigated. Systemic antibiotics may be warranted if otomastoiditis, osteomyelitis, or central nervous system invasion develops (12, 16).

In conclusion, we discovered two patients with otologic infections due to *M. massiliense* (*M. abscessus* subspecies *bolletti*). *M. massiliense* (*M. abscessus* subspecies *bolletti*) should be considered when treating patients with otomastoiditis or otitis media and molecular techniques are warranted for species-level identification.
References


TABLE 1. Clinical manifestations of two patients with otologic infections (Patients 1 and 2) caused by *M. massiliense* (*M. abscessus* subspecies *bolletti*) and two patients (Patients 3 and 4) with colonization by the organism

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/sex</th>
<th>Initial presentation</th>
<th>Underlying medical condition</th>
<th>Predisposing factors</th>
<th>Topical antibiotics</th>
<th>Systemic antibiotics (duration)</th>
<th>Surgical intervention</th>
<th>Sequelae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53/M</td>
<td>Otorrhea, hearing impairment, and vertigo</td>
<td>No</td>
<td>Previously underwent tympanoplasty and/or mastoidectomy for chronic otitis media</td>
<td>No</td>
<td>Clarithromycin and ciprofloxacin (9 months)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>58/M</td>
<td>Otorrhea, hearing impairment, and Gradenigo’s syndrome</td>
<td>No</td>
<td>Tympanic membrane perforation</td>
<td>No</td>
<td>Ciprofloxacin, clarithromycin, and ethambutol (3 months)</td>
<td>Petrosectomy, mastoidectomy, and tympanoplasty</td>
<td>Abducens nerve palsy</td>
</tr>
<tr>
<td>3</td>
<td>45/M</td>
<td>Otorrhea, hearing impairment, and left facial palsy</td>
<td>No</td>
<td>No</td>
<td>Topical framycetin sulphate, and gramicidin</td>
<td>No</td>
<td>No</td>
<td>Left facial palsy</td>
</tr>
<tr>
<td>4</td>
<td>51/F</td>
<td>Otorrhea</td>
<td>No</td>
<td>Previously underwent tympanoplasty for chronic otitis media</td>
<td>Topical ofloxacin for two weeks</td>
<td>No</td>
<td>No</td>
<td>Lost to follow-up</td>
</tr>
</tbody>
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TABLE 2. Antimicrobial susceptibilities of four isolates of *M. massiliense* (*M. abscessus* subspecies *bolletti*) to 15 antimicrobial agents using the broth microdilution method

| Patient no./isolate no. | AMC   | FOX   | CRO   | FEP   | IPM   | CIP   | MXF   | DOX   | MIN   | LZD   | TOB   | AMK   | TGC   | SXT   | CLA  |
|------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|
| 1                      | >64/32| 64    | >64   | >32   | 32    | >4    | 8     | >16   | >8    | 32    | 16    | 8     | 1     | >8/152| ≤0.12/0.5|
| 2                      | >64/32| 64    | >64   | >32   | 64    | >4    | >8    | >16   | >8    | 32    | >16   | 64    | 0.5   | 8/152 | 0.12/0.5|
| 3                      | >64/32| 128   | >64   | >32   | 32    | >4    | >8    | >16   | >8    | 8     | >16   | >32   | 0.12  | ≤0.25/4.75| >16/>16 |
| 4                      | >64/32| 64    | >64   | >32   | 16    | >4    | >8    | >16   | >8    | >32   | 16    | 32    | 0.5   | >8/152| 0.25/0.5|

AMC, amoxicillin-clavulanate; FOX, cefoxitin; CRO, ceftriaxone; FEP, cefepime; IPM, imipenem; CIP, ciprofloxacin; MXF, moxifloxacin; DOX, doxycycline; MIN, minocycline; LZD, linezolid; TOB, tobramycin; AMK, amikacin; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole; CLA, clarithromycin.

*Designation of isolates from indicated patients are shown in Table 1.*