ORIGINAL ARTICLE

Postcesarean section wound infection caused by *Mycobacterium massiliense*

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Abstract *Background/Purpose*: *Mycobacterium abscessus* subsp. *massiliense* (a subspecies of the *M. abscessus* complex) is a rare causative agent of surgical site infection after cesarean section (C section). We tried to seek the common source of infection and unravel the optimal treatment modalities.

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erm(41);
pulsed-field gel
   electrophoresis;
susceptibility testing

Methods: From September 2009 to October 2012, four postpartum women developed C-section wound infections caused by *M. massiliense*. Speciation of the four isolates was identified using of *hsp65*, *rpoB*, and *secA1* partial gene sequencing and the Basic Local Alignment Search Tool. The *erm*(41) and *rrl* genes were detected for the possibility of inducible macrolide resistance. Pulsed-field gel electrophoresis was used as a tool of molecular epidemiology. All patients underwent intensive intravenous and oral antimycobacterial regimens. Of these patients, three underwent debridement at least once.

Results: All four isolates were identified as *M. abscessus* subsp. *massiliense*. All of the isolates harbored a truncated *erm*(41) gene without *rrl* gene mutations, which explains the susceptibility to clarithromycin and azithromycin. Three isolates were indistinguishable by DNA strain typing, and the fourth strain was clonal with the other three strains. Their infections were not improved in spite of teicoplanin treatment initially. These patients underwent antimycobacterial regimens with/without surgery and were all cured.

Discussion: Teicoplanin treatment failure, painful cutaneous nodules, and persistent wound drainage alerted us to the possibility of nontuberculous mycobacterial skin and soft tissue infection. Accurate identification of subspecies, detection of drug resistance genes, susceptibility testing, and optimal antimycobacterial agents with/without surgical debridement are warranted for successful treatment.

Introduction

The *Mycobacterium abscessus* complex is ubiquitous in the environment, including in soil and water, and has often been incriminated in cases of wound and soft tissue infection.[1] Although the *M. abscessus* complex has frequently been described in cases of wound infection following cosmetic surgery, we only found one published case of postsurgical wound infection following cesarean section (C section) involving the *M. abscessus* complex.[2]

The current taxonomy of the *M. abscessus* complex is controversial. The complex is now thought to consist of three subspecies: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*.[3,4] In 2011, Leao et al[5] proposed the reclassification of subspecies *massiliense* and subspecies *bolletii* into one subspecies to be designated *M. bolletii*, following taxonomic rules of precedence. However, recent whole genomic studies have substantiated the presence of three separate subspecies, designated as *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, and *M. abscessus* subsp. *massiliense*.[3,4] Hereafter, for brevity, these subspecies will be referred to in this paper as *M. abscessus*, *M. bolletii*, and *M. massiliense*.[5,6] The importance of differentiating the *M. abscessus* complex into three subspecies has been emphasized recently especially with consideration of treatment regimens.[7] Following investigation of a rare wound infection case caused by *M. abscessus* in our hospital in September 2009, we focused on the causal relationship between *M. abscessus* complex and post C-section wound infections and identified four patients with cultures that tested positive for rapidly growing mycobacteria (RGM). Four clinical isolates were subsequently identified as *M. abscessus* subsp. *massiliense* using the partial *hsp65*, *rpoB*, and *secA1* genes sequencing, which were analyzed with Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) website.

Prior to the discovery of the *erm* gene, which is known to induce macrolide resistance in many species of rapidly growing mycobacteria including *M. abscessus*, clarithromycin was included in most treatment regimens for *M. abscessus*.[8,9] The presence of the *erm*(41) gene has been responsible for the induction of clarithromycin resistance in the majority of the isolates of *M. abscessus* subsp. *abscessus*. However, *erm*(41) is nonfunctional in isolates of *M. massiliense* rendering them macrolide susceptible, even after extended incubation.[10] In order to determine the optimal antimycobacterial regimens for *M. abscessus* subsp. *massiliense* soft tissue infection, we performed *in vitro* susceptibility testing on the four strains.

Recent advances in clinical mycobacteriology have focused on the molecular epidemiology, subspecies identification, the relationship between *erm*(41) gene and inducible macrolide resistance, and the selection of antimycobacterial agents in relation to clinical outcomes.

Methods

Patients/clinical data

Wound or tissue cultures collected at Chang Gung Memorial Hospital—Linkou Medical Center, Kwei-Shan, Taoyuan, Taiwan from September 1, 2009 to October 31, 2012 from four postpartum patients were positive for *M. massiliense*. The first isolate was collected in 2009, and the other three were collected in 2012. Demographic data including age, comorbidities, symptoms and signs, the incubation period (from the date of operation to the date of onset of symptoms), human immunodeficiency virus antigen and antibody (HIV Ag/Ab) tests data, and treatment modalities were abstracted from the medical records. The antimycobacterial agents included the following: oral azithromycin (250 mg) two tablets once daily, or oral

clarithromycin (500 mg) one tablet twice daily, oral moxifloxacin (400 mg) one tablet once daily, imipenem/cilastatin (500 mg/500 mg) intravenous infusion one vial every 6 hours, tigecycline (50 mg) intravenous infusion two vials for loading, then one vial every 12 hours, and amikacin (500 mg) intravenous infusion one vial for loading, and 7.5 mg/kg every 12 hours. Surgical therapy, including fasciotomy for deep-seated surgical site infection, and incision and drainage for superficial surgical site infection was performed. The classification of surgical site infection is according to the definition proposed by the Centers for Disease Control and Prevention.[11] Outcomes were classified as follows: (1) successful—complete remission of tissue erythema, tenderness, swelling without residual pus discharge, and good healing of C-section wound; (2) failed—incomplete remission of tissue erythema, tenderness, swelling, or persistent residual pus discharge, and poor healing of C-section wound.

Microbiological identification by *hsp65, rpoB,* and *secA1* partial gene sequencing

The forward and reverse primers for *hsp65* were Tb11 (5′-ACCAACGATGGTGTGTCCAT-3′) and Tb12 (5′-CTTGTCGA ACCGCATACCCT-3′) amplified a 439-bp fragment; for *rpoB*, the primers were Myco-F (5′-GGCAAGGTCACCCCGAAGGG-3′) and Myco-R (5′-AGCGGCTGCTGGGTGA-TCATC-3′); for *secA1*, the primers were Mtu.For1 (5′-GA-CAGY-GAGTGGATGGGYCGSGTGCACCG-3′) and Mtu.Rev490 (5′-GCGGACGATGTARTCCTTGTCSCG-3′). The polymerase chain reaction conditions were the same as those in the methods described by Zelazny et al.[12] The amplicons were sent to Tri-I Biotech, Inc. (New Taipei, Taiwan) for sequencing. The isolates were sequenced using an ABI 3730 genetic analyzer (Applied Biosystem, Carlsbad, CA, USA). Genetic sequences were analyzed (http://blast.ncbi.nlm.nih.gov) and compared to the NCBI database using BLAST.

Partial *erm*(41) and *rrl* genes sequencing

Partial *erm*(41) sequencing followed the method described by Maurer et al.[13] with the forward primer (5′-GGCCTTCTTCGTGATCTATCG-3′) and reverse primer (5′-CACCGATTCCACCGGTTAG-3′). The *rrl* gene was amplified using primers SP1 (5′-CCTGCACGAATGGCGTAACG-3′) and SP2mod (5′-CACCAGAGGTTCGTCCGTC-3′). The amplified fragments were purified using the EasyPure PCR/Gel Extraction Kit (Bioman Corp., New Taipei, Taiwan). The purified fragments were sequenced in the Applied Biosystems 3730 genetic analyzer (Applied Biosystems, Carlsbad, CA, USA). Genetic sequences were analyzed using nucleotide BLAST offered by the NCBI.

In vitro susceptibility testing

Using broth microdilution, we determined the minimum inhibitory concentrations (MICs) according to the guidelines proposed by the Clinical and Laboratory Standards Institute (CLSI).[14] Isolates were subcultured on Middlebrook 7H11

agar plates and incubated at 30°C for 3 days. The inoculum was prepared in cation-adjusted Mueller—Hinton broth to match the density of a 0.5 McFarland standard. Following dilution to a $1-5 \times 10^6$ per mL suspension, 10 μL was dispensed into 100 μL of cation-adjusted Mueller—Hinton broth and subsequently inoculated into 96-well plates to yield a final density of $1-5 \times 10^4$ in each well.

The antimicrobial test agents included amikacin (AMK), isepamicin (ISP), azithromycin (AZI), clarithromycin (CLR), doxycycline (DOX), minocycline (MIN), tigecycline (TGC), cefoxitin (FOX), ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF), imipenem (IPM), meropenem (MPM), trimethoprim/sulfamethoxazole (SXT), and linezolid (LZD).

Panels were incubated for 3 days at 30°C in room air. Using a mirrored light box, the MIC was read as the first well in which there was no growth, except for SXT, for which the MIC was 80% inhibition of growth compared to the control growth. The intermediate breakpoints (in μg/mL) of these antibiotics (except AZI, ISP, LVX, and TGC, which do not have CLSI breakpoints), were those proposed by the CLSI, with AMK = 32, FOX = 32−64, CIP = 2, CLR = 4, DOX = 2−4, IPM = 8−16, LZD = 16, MPM = 8−16, and MXF = 2.

Mycobacterium peregrinum ATCC 700686 was used for quality control of the panels with acceptable MIC ranges recorded in μg/mL as follows: AMK ≤ 1−4, FOX = 4−32, CIP ≤0.12−0.5, CLR ≤0.06−0.5, DOX = 0.12−0.5, IPM = 2−16, LZD = 1−8, MPM = 2−16, MIN = 0.12−0.5, MXF ≤0.06−0.25, and SXT ≤0.25/4.8−2/38 following CLSI recommendations.[14]

Pulsed-field gel electrophoresis

The protocols proposed by Zhang et al.[15] were followed with minor modifications. Briefly, *M. massiliense* isolates cast into low-melting-point agarose plugs were lysed with lysozyme, sodium dodecyl sulfate, and proteinase K. The genomic DNA contained in the plugs was digested with restriction endonucleases *Ase*I (New England BioLabs, Ipswich, MA, USA) and separated by pulsed-field gel electrophoresis (PFGE) with a CHEF Mapper system (Bio-Rad Laboratories, Richmond, CA, USA). PFGE gels were run in regular 0.5× Tris—borate—EDTA buffer (50mM Tris, 45mM boric acid, and 0.5mM EDTA) plus 200μM thiourea. An autoalgorithm mode was chosen with the running molecular weights ranging from 20 kb to 380 kb. The gel was stained with ethidium bromide and photographed under UV illumination for analysis. The band sizes, in kilobase (kb) pairs, were estimated using Phoretix 1D Pro software (TotalLab Ltd., Newcastle upon Tyne, NE, UK) with a lambda ladder and a 48.5-kb ladder as the molecular size standards.

Differences between PFGE profiles were determined, and strain relatedness was determined by use of a modification of the definitions recommended by Tenover et al.[16] Isolates were considered indistinguishable, closely related, or possibly related if they exhibited zero, two to three, or four to six band differences, respectively. In all these circumstances, the isolates were considered clonal. Isolates were considered not related if they exhibited seven or more band differences.

Ethics statement

The study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital—Linkou Medical Center in September 2012. Patients were requested to give written informed consent for the storage and use of their data. No linkage of these data with other sources was done. No patient identifiers were included in the dataset used for this analysis. All bacterial strains were obtained from the Bacteria Bank, Department of Laboratory Medicine, Chang Gung Memorial Hospital.

Results

Patients/clinical data

Four patients' demographic data, HIV Ag/Ab status, treatment, and outcomes were collected. The patients' ages ranged from 30 years to 34 years. The indications of C-section were two breech presentations, one cephalopelvic disproportion, and one chorioamnionitis. Two of them had thalassemia, one of whom was also a hepatitis B carrier. The presentations of these patients' surgical site infections were four painful subcutaneous nodules, two wounds with marked erythema, and one surgical wound dehiscence (Figure 1). The incubation period (from the date of the operation to the date of onset of symptoms) of four patients was from 53 days to 97 days. Three patients had superficial surgical site infections. One patient developed a deep surgical site infection. Three of the patients underwent surgical debridement one to three times in addition to antimycobacterial therapy. The fourth patient received only antimycobacterial therapy without surgical debridement. Two of the patients had coagulase negative *Staphylococcus* coinfection; one patient had methicillin-resistant *Staphylococcus aureus* coinfection. These three patients underwent teicoplanin treatment for 2 weeks (data not shown in Table 1) but continued with persistent wound drainage. All four patients received

Figure 1. The cesarean section wound infected with *Mycobacterium massiliense*. Arrow = wound erythema; arrowhead = wound dehiscence and drainage site; star = painful cutaneous nodule.

Table 1 Demographic and clinical data of four postcesarean section cases infected with *Mycobacterium massiliense*.

No.	Age (y)	Comorbidity	Symptoms & signs	Incubation period (d)	Type of surgical site infection	Debridement (times)	Parenteral antimycobacterial agents (d)	Oral antimycobacterial agents (d)	Outcome
1	32	None	Wound dehiscence, painful nodules, wound erythema	96	Superficial	2	MXF (22), TGC (27)	CLR (172), MXF (150)	Cured
2	32	Thalassemia, HBV carrier	Painful nodules	53	Deep	1	AMK (40), TGC (9)	CLR (158), MXF (168)	Cured
3	30	None	Painful nodules	79	Superficial	3	AMK (91), IPM (15), TGC (31)	AZI (182), CLR (3), MXF (136)	Cured
4	34	Thalassemia	Painful nodules, wound erythema	97	Superficial	0	AMK (28), IPM (28)	CLR (186), MXF (148)	Cured

AMK = amikacin; AZI = azithromycin; CLR = clarithromycin; HBV = hepatitis B virus; IPM = imipenem/cilastatin; MXF = moxifloxacin; TGC = tigecycline.

antimycobacterial therapy for ∼6 months. The regimens included oral macrolides (azithromycin or clarithromycin), oral moxifloxacin or intravenous moxifloxacin, intravenous amikacin, intravenous imipenem/cilastatin, and intravenous tigecycline. Fortunately, all of the patients were cured with antimycobacterial therapy with/without surgical debridement.

Microbiological identification

The four isolates were identified as *M. massiliense* using the partial *hsp65*, the *rpoB*, and *secA1* genes sequencing. The data were analyzed using BLAST on the NCBI website.

Partial *erm*(41) and *rrl* genes sequencing

The *erm*(41) gene sequences of all four isolates were of the same length (249 bp), which is shorter than that of *M. abscessus* ATCC 19977[T]. Compared with the sequence of *M. abscessus* ATCC 19977[T], the *erm*(41) sequence of *M. massiliense* lacked 273 bp length because of two characteristic deletions (bases 61–62 and 159–429). The *rrl* gene sequencing revealed the same wild type as that of *M. abscessus* ATCC 19977[T].

Antimicrobial susceptibility testing

All four isolates of *M. massiliense* were resistant to DOX, MIN, CIP, MXF, MPM, SXT, and LZD, and susceptible to AMK and CLR. We regarded all four isolates as susceptible to AZI as CLR is the class drug for the new macrolides including AZI.[14] CIP is the class drug for CIP and LVX; thus, the CIP MIC predicts LVX MICs but not MXF. Isolates 1, 2, and 3 were susceptible to FOX and IPM. Isolate 4 was intermediate susceptible to FOX and IPM. There is no breakpoint of TGC for RGM; however, all isolates had TGC MICs (1 μg/mL).
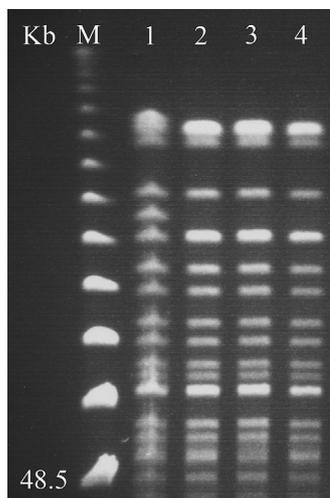

Figure 2. Pulsed-field gel electrophoresis of four presenting *Mycobacterium massiliense* isolates from patients with postpartum cesarean section wound infections.

Genotyping

The PFGE patterns of the three isolates of *M. massiliense* were indistinguishable, and the fourth was clonal with the other three isolates (Figure 2). Lane 1 of Figure 2 represents the genotype of an isolate from a case outside the time of the other three cases.

Discussion

M. massiliense skin and soft tissue infection is also difficult to differentiate from other nontuberculous mycobacterial cutaneous infection or nocardia soft tissue infection,[17,18] as the clinical presentations, such as persistent subcutaneous tender nodule, skin erythema, and unhealing wound, are similar. All four isolates were identified as *M. massiliense* and possessed a truncated *erm*(41) gene without *rrl* gene mutations, rendering the gene nonfunctional and incapable of inducing resistance to clarithromycin and azithromycin in contrast to *M. abscessus*, for which the majority of isolates harbor an inducible *erm*(41) gene, and *M. bolletii*, which is intrinsically resistant to macrolides. This finding is the same as that reported by Kim et al.[19] and Maurer et al.[20]

By PFGE, three of the four isolates of *M. massiliense* were indistinguishable and the first was clonal with the other three isolates. The latter three isolates presented one cluster, which reminded us of the importance of seeking for the common source of infection. With the help of Infection Control Practitioners, environmental specimens such as tap water, swabs of inner wall of washbasins, gel for fetal heart beats sonogram, cleaning liquid soap for abdomen, providone–iodine solution, and sterilized saline solution for irrigation for peritoneal cavity were all collected for bacterial and mycobacterial cultures. Bacterial cultures of gel for fetal heartbeat sonogram yielded *Escherichia coli* and glucose-nonfermenting gram-negative bacilli. *M. massiliense* was not detected in any of the environmental cultures. As the identification of a single common infection focus is difficult, strict compliance with infection control measures is of paramount importance to prevent surgical site infection.

The most common causative microbes for C-section wound infection were *Streptococci*, followed by *E. coli* and *Bacteroides* species.[21] Although publications regarding plastic surgery or cosmetic surgery related to *M. abscessus* surgical site infections are plentiful,[22–24] we found only one previous publication related to *M. abscessus* in a C section-related surgical site infection.[2] In the article, 13 laboratory-confirmed *M. abscessus* infected post C-section patients were treated by oral clarithromycin, intravenous imipenem/cilastatin, and intravenous amikacin. Among these 13 patients, 10 had satisfactory outcomes, but three had unknown posttreatment outcomes. Antimicrobial susceptibility patterns and *erm*(41) gene polymorphisms were not known in this study. The longest treatment course used in this study was 24 months. The American Thoracic Society/Infectious Disease Society of America recommends combination therapy for the treatment of most postsurgical infections involving the *M. abscessus* complex.[8] In most prior cases of published *M. massiliense* infections,

combination therapy was used. In other studies, the authors reported successful experiences in treatment of *M. abscessus* complex infections with the concomitant use of macrolides with amikacin, fluoroquinolones, imipenem/cilastatin, or cefoxitin.[25–28] A few cases were successfully treated with only oral clarithromycin.[29,30] Most published regimens included clarithromycin alone or in combination therapy.

In our study, we implemented a strategy including oral clarithromycin, intravenous tigecycline (or imipenem/cilastatin, or moxifloxacin), and intravenous amikacin for the treatment of patients with post C-section infections. The patients were subsequently maintained with oral clarithromycin, and moxifloxacin with/without daily intramuscular injection of amikacin. All four patients underwent antimycobacterial therapy as long as 6 months, and three of them underwent surgical debridement. One patient only received antimycobacterial agents without surgical debridement. All patients had satisfactory outcomes without any sequelae. We chose tigecycline as one member of our combination regimens, because it is not only effective against RGM but is also effective for complicated skin and soft tissue infections.[31] In a clinical trial initiated by Wallace et al.,[32] tigecycline-containing regimens were successfully prescribed to treat six of eight (75%) cases of complicated *M. abscess* complex or *Mycobacterium chelonae* skin and soft tissue infections. It is noteworthy that a high percentage of adverse reactions such as nausea and/or vomiting is present in tigecycline-containing regimens. In certain situations, once daily dose or prolonged infusion perhaps can decrease the adverse reactions, but it was not proven clinically effective against *M. massiliense* skin and soft tissue infections. Sequential oral macrolide and moxifloxacin therapy is reasonable because treatment with clarithromycin and fluoroquinolone produced satisfactory outcomes in *M. massiliense* pulmonary infection as reported by Koh et al.[7,26]

In conclusion, subspecies identification, drug-resistant gene detection, susceptibility testing, and selection of optimal antimycobacterial agents are warranted for the successful treatment of *M. massiliense* skin and soft tissue infections.

Conflicts of interests

The authors declare that they have no financial or nonfinancial conflicts of interest related to the subject matter or materials discussed in the manuscript.

Acknowledgments

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