Mycobacterium marseillense Infection in Human Skin, China, 2018

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We describe a case of facial skin infection and sinusitis caused by Mycobacterium marseillense in an immunocompetent woman in China in 2018. The infection was cleared with clarithromycin, moxifloxacin, and amikacin. Antimicrobial drug treatments could not be predicted by genetic analyses; further genetic characterization would be required to do so.

Mycobacterium marseillense is a member of the M. avium complex (1) that has caused infections with lymphatic or pulmonary involvement sporadically in humans (2–4). We report M. marseillense infection involving facial skin in an immunocompetent woman in eastern China.

These authors contributed equally to this article.

In April 2018, a 59-year-old woman was referred to our institute (Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, China) for a 4-year history of an erythematous plaque with ulceration located on the right cheek. The primary lesion was a small erythematous patch that gradually developed into an asymptomatic ulcerative plaque (i.e., the plaque had no heat, swelling, pain, or pruritus). She also reported occasional bloody, purulent nasal discharge over the course of 2 years. Two years before visiting our hospital, cutaneous tuberculosis was suspected, so she received treatment for tuberculosis (rifampin, isoniazid, ethambutol, pyrazinamide) for 10 months. No obvious improvement was observed with this treatment. Her medical history was otherwise unremarkable.

On physical examination, an infiltrated erythematous plaque with yellow scales and crusting on the right cheek was visible (Figure, panel A). Routine laboratory tests showed no remarkable findings. The results of autoantibody and HIV tests were negative, and immune subset cell counts were unremarkable. Histologic examination showed infiltration of a large number of lymphocytes, plasma cells, and neutrophils and some tissue cells in the dermis (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/25/10-19-0695-App1.pdf). Computed tomography scan of the paranasal sinuses showed bilateral maxillary, right ethmoid, and frontal sinusitis (Figure, panel C). Culture and PCR for mycobacteria in nasal discharge yielded negative findings.

After 3 weeks of skin tissue culture at 32°C in Löwenstein–Jensen medium, we observed smooth, yolk-yellow bacterial colonies (Appendix Figure 2). Ziehl-Neelsen staining confirmed the cultured organism was acid-fast bacilli. Sequence analysis indicated that the complete genetic sequence of 16S rRNA was 99.0%, hsp65 100%, and rpoB 99.8% homologous with M. marseillense strain FLAC0026. Phylogenetic analysis of the 16S rRNA sequence showed the isolate clustered with M. chimaera and M. intracellulare (Figure, panel D). Although the 16S rRNA gene sequence of the isolate was 100% similar to M. intracellulare subsp. yongonense 05-1390, the sequence similarities to hsp65 and rpoB were relatively low. Sequence analyses suggested M. marseillense infection.

Referring to the guidelines for pulmonary M. avium complex disease, we treated the patient with the antimicrobial drugs clarithromycin, rifampin, and ethambutol (5). Afterward, in vitro drug susceptibility testing showed the isolate was sensitive to clarithromycin, azithromycin, and amikacin; moderately sensitive to moxifloxacin; and resistant to ethambutol and rifampin. Therefore, 3 months after initiating treatment, we changed the regimen to clarithromycin, moxifloxacin, and amikacin, which she received for 2 months. The patient’s skin lesions healed gradually, and nasal symptoms disappeared, but a scar and erythema
remained (Figure, panel B). Computed tomography scans of the paranasal sinuses showed the reduction of sinusitis (Figure, panel C). No recurrence was observed during 4 months of monitoring.

We characterized this isolate’s genome (GenBank accession no. VAS10000000) further to help determine the cause of its virulence and resistance (Appendix Figure 3). Genetic analyses indicated the genome (≈5,706,022 bp) contained 5,343 predicted genes, 3 rRNAs, and 48 tRNAs and had a GC content of 67.73%. We annotated the genes functionally through multiple databases (Appendix Table 1, Figure 4). Using the Virulence Factors of Pathogenic Bacteria database, we identified 137 potential virulence genes (identity >95.0%, E value <1 × 10⁻⁵; Appendix Table 2), mtrA modulates antimicrobial drug efflux, murA encodes the fosfomycin resistance protein, and gyrA encodes the fluoroquinolone resistance protein.

*M. marseillense* infections are rare in humans. Our case demonstrates that *M. marseillense* can cause infections in immunocompetent persons. For facial skin infection with *M. marseillense*, this and similar (7) reports indicate the need for vigilance of paranasal sinus infection. Although many potential virulence factors could be detected by genomic analysis, cases of infection and transmission with this bacterium are rarely reported, suggesting the presence of other influencing factors.

The drug resistance mechanisms of *M. marseillense* have not been completely elucidated. The drug susceptibility test results and treatment response we observed were generally consistent with those previously reported for...
cases of pulmonary infection, although sensitivity to rifampin and quinolones yielded various results (2–4). Drug susceptibility testing indicated that the isolate we obtained was resistant to ethambutol and rifampin. However, in genetic analyses, mutations associated with ethambutol and rifampin resistance were not detected. According to the Comprehensive Antibiotic Resistance Database, our isolate was resistant to fluoroquinolone, but drug susceptibility test results were inconsistent. Our results indicate that drug susceptibility testing should be performed for *M. marseil- lense* to guide antimicrobial drug treatment. If drug susceptibility results are absent, treatments including macrolides and amikacin appear to be reasonable.

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References

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Geospatial Variation in Rotavirus Vaccination in Infants, United States, 2010–2017

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We evaluated rotavirus vaccination rates in the United States by using records from a nationwide health database. From data on 519,697 infants, we found 68.6% received the entire rotavirus vaccine series. We noted pockets of under-vaccination in many states, particularly in the Northeast and in some western states.

Vaccination coverage in the United States frequently is evaluated with telephone and mailed surveys (1). However, telephone response rates have declined over the past 2 decades (2) and parents who choose not to vaccinate their children might be less likely to participate in surveys (3).
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Appendix 1

Material and Methods

Ethic Statement

Informed consent was obtained from the patient. Ethics approval was obtained from the ethics review board of the Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, China.

Tissue Culture, Bacterial Strains, Genomic DNA Extraction and Sequencing

Skin tissue grinding fluid and nasal discharge from the patient were collected and cultured on Löwenstein–Jensen medium at 32°C and 37°C. The grown colonies of bacteria on Löwenstein–Jensen medium were then carefully transferred to Middlebrook 7H10 agar with Middlebrook oleic albumin dextrose catalase (OADC) enrichment and cultured at 37°C. *Mycobacterium marseillense* used in our study was isolated from the skin tissue of our patient. Genomic DNA Isolation was performed using a DNeasy Blood & Tissue kit (QIAGEN, The Netherlands) according to the manufacturer’s instructions. The DNA purity and concentration was measured by NanoDrop 2000 spectrophotometer. Genome sequencing was conducted by Novogene Technology Co. Ltd (Beijing, China) using Illumina PE150.
**Genome Analyses**

Circular genome maps were generated using Circos (Version 0.64) based on analysis of the coverage of sample sequencing reads, single-nucleotide polymorphisms (SNPs), and InDels. The coding genes were predicted by GeneMarkS software. CRISPRdigger was used to predict the CRISPRs (clustered regularly interspaced short palindromic repeats) of the genome. Scattered repeat sequences were predicted by RepeatMasker software, and TRF searched for tandem repeats in DNA sequences. Functional annotations were performed using the non-redundant protein (Nr) database, SwissProt, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, Cluster of Orthologous Groups of proteins (COG), Gene Ontology (GO), and protein families (Pfam). Antimicrobial resistance genes were detected based on the Antibiotic Resistance Genes Database (ARDB) and Comprehensive Antibiotic Resistance Database (CARD). Virulence factor annotation was carried out based on Virulence Factors of Pathogenic Bacteria database (VFDB) and Pathogen-Host Interactions database (PHI).

**Phylogenetic Analyses**

**Appendix Table 1. Information on *Mycobacterium marseillense***

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>nr</th>
<th>SwissProt</th>
<th>KEGG</th>
<th>COG</th>
<th>TCDB</th>
<th>GO</th>
<th>PHI</th>
<th>VFDB</th>
<th>ARDB</th>
<th>CARD</th>
<th>Secretory protein</th>
<th>T3SS</th>
<th>CAZY</th>
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</thead>
<tbody>
<tr>
<td><em>M. marseillense</em></td>
<td>5,220</td>
<td>2,309</td>
<td>5,164</td>
<td>3,836</td>
<td>295</td>
<td>3,467</td>
<td>292</td>
<td>454</td>
<td>0</td>
<td>27</td>
<td>108</td>
<td>186</td>
<td>140</td>
</tr>
</tbody>
</table>

*ARDB, Antibiotic Resistance Genes Database; CARD, Comprehensive Antibiotic Resistance Database; CAZY, Carbohydrate-Active enZYmes Database; COG, Cluster of Orthologous Group; GO, Gene Ontology; ID, identification; KEGG, Kyoto Encyclopedia of Genes and Genomes; NR, redundant protein database; PHI, Pathogen-Host Interactions database; T3SS, type 3 secretion system; TCDB, Transporter Classification Database; VFDB, Virulence Factors of Pathogenic Bacteria database.

**Appendix Table 2. Antimicrobial resistance genes found in *Mycobacterium marseillense* isolate from patient, China, 2018***

<table>
<thead>
<tr>
<th>ORF ID</th>
<th>Best Hit e value</th>
<th>Best Hit ARO</th>
<th>Best Identities</th>
<th>ARO</th>
<th>ARO name</th>
<th>Bit score</th>
<th>ARO category</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. marsei GM003653, locus Scaffold 12: 152238:153491:+</td>
<td>0</td>
<td>Mycobacterium 0.96875</td>
<td>ARO: 3003784</td>
<td>M. tuberculosis murA</td>
<td>fosfomycin resistance protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. marsei GM000974, locus Scaffold 3: 140161:142677:+</td>
<td>0</td>
<td>Mycobacterium 0.932994</td>
<td>ARO: 3003941</td>
<td>Mycobacterium 1572.76, resistant gene</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ARO, antibiotic resistance ontology; ID, identification; ORF, open reading frame.*
Appendix Figure 1. Hematoxylin and eosin stain of skin lesion (original magnification ×4). Inset shows part of the sample.
Appendix Figure 2. Tissue homogenate culture showing bacterial colonies after incubation on Löwenstein–Jensen medium.
Appendix Figure 3. Overview of *Mycobacterium marseillense* genome. From the outermost to the innermost circle: the position coordinate of the reference sequence, the InDel distribution of the sample, the single-nucleotide polymorphism (SNP) number distribution of the sample, the reads coverage depth of the sample, the GC content of the reference sequence genome, and the GC skew value distribution of the reference sequence genome, respectively.
Appendix Figure 4. Function categories of genes by Cluster of Orthologous Group annotation.