Mycobacterium chelonae-abscessus Complex Associated with Sinopulmonary Disease, Northeastern USA

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Analyze the *M. chelonae-abscessus* complex
- Distinguish the molecular identity of "*M. franklinii*"
- Identify the most common clinical source of "*M. franklinii*"
- Evaluate the antimicrobial susceptibility of "*M. franklinii*"

Editor

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Members of the *Mycobacterium chelonae-abscessus* complex represent *Mycobacterium* species that cause invasive infections in immunocompetent and immunocompromised hosts. We report the detection of a new pathogen that had been misidentified as *M. chelonae* with an atypical antimicrobial drug susceptibility profile. The discovery prompted a multicenter investigation of 26 patients. Almost all patients were from the northeastern United States, and most had underlying sinus or pulmonary disease. Infected patients had clinical features similar to those with *M. abscessus* infections. Taxonomically, the new pathogen shared molecular identity with members of the *M. chelonae-abscessus* complex. Multilocus DNA target sequencing, DNA-DNA hybridization, and deep multilocus sequencing (43 full-length genes) support a new taxon for these microorganisms. Because most isolates originated in Pennsylvania, we propose the name *M. franklinii* sp. nov. This investigation underscores the need for accurate identification of *Mycobacterium* spp. to detect new pathogens implicated in human disease.

Infections caused by members of the *Mycobacterium chelonae-abscessus* complex remain a serious public health problem, and their role has expanded, with growing numbers of therapeutic interventions that disrupt the competency of the human immune system. Before 2001, only 2 species, *M. chelonae* and *M. abscessus,* were recognized as members of the complex. Since that time, 4 new species have been added to the complex: *M. immunogenum*, *M. massiliense*, *M. bolletii,* and *M. salmoniphilum*. With the exception of *M. salmoniphilum*, all members of the complex have been implicated in human disease (*1–4*). Recently investigators have noted a lack of separation of *M. bolletii* and *M. massiliense* from *M. abscessus* and have proposed they be classified as a subspecies of *M. abscessus* (*5*).

Members of the *M. chelonae-abscessus* complex represent *Mycobacterium* species that cause invasive skin and soft tissue infections, pneumonia, bloodstream infections, and abscesses in immunocompetent and immunocompromised hosts (*6,7*). Definitive identification by the clinical laboratory is needed for outbreak detection and for performance of susceptibility testing for patient management. Currently, the taxonomic relationships among members of the *M. chelonae-abscessus* complex lack clarity. The species are biochemically inert and their genetic signatures by partial 16S rRNA gene sequencing are often similar, which makes identification a great challenge for clinical laboratories.

In 2007, we detected a group of clinical isolates that were misidentified as *M. chelonae* with an atypical antimicrobial drug susceptibility profile. All isolates were from Pennsylvania, and as an interim identification, we labeled these isolates as CV for *M. chelonae* variant. Our discovery prompted a large multistate investigation that involved obtaining clinical correlation, retrospective and prospective collections of isolates with similar CV characteristics, and examination of a large set of known clinical isolates and type strains from the *M. chelonae-abscessus* complex. Given the taxonomic complexities and current ambiguities within the *M. chelonae-abscessus* group, we performed a comprehensive analysis of the potentially new species, including DNA-DNA hybridization, multilocus sequencing, and deep multilocus sequencing. We describe a new pathogen, *M. franklinii* sp. nov., a proposed new member of the *M. chelonae-abscessus* complex that was isolated from 26 patients in the United States. We discuss its potential role in human disease.

Methods

Isolates

All available type strains of *M. chelonae-abscessus* complex were obtained from American Type Culture Collection (ATCC), Collection of Institut Pasteur, or Culture Collection, University of Göteborg, Sweden. Previously identified clinical isolates of *M. abscessus*, and *M. chelonae* by partial 16S rRNA gene sequencing and internal transcribed spacer (ITS) PCR were retrieved for comparative analysis. A subset of these isolates was described in a prior study (*8*). CVs were defined as isolates that were cefoxitin susceptible or showed intermediate resistance, and identified as *M. chelonae* by partial 16S rRNA gene sequencing and ITS PCR (*9*) by Associated Regional and University Pathologists Laboratories and the Hospital of the University of Pennsylvania or by failure to amplify *hsp65* by PCR restriction fragment length polymorphism analysis (*10,11*) by the University of Texas Health Science Center. Microbiologic and medical records were reviewed for clinical information for select isolates. Clinical case reviews were conducted under institutional review board approved protocols at Associated Regional and University Pathologists Laboratories, Children's Hospital of Philadelphia, University of Pennsylvania, and the University of Texas Health Science Center. Study isolates CV002 (ATCC [pending] and DSMZ 45524) and CV005 (ATCC [pending]) have been deposited into culture collections.

Multilocus Sequencing

DNA extractions, PCR, and sequencing reactions were performed as described (*8*). Amplifications and sequencing reactions were performed by using primers specific for ≈1,400 bp of the 16S rRNA 5F (5′-TTGGAGAGTTTGATCCTGGCTC-3′) and 1492R (5′-ACGGITACCTTGTTACGACTT-3′), ≈700 bp of

rpoB, ≈400 bp of *sodA* (*12*), ≈240 bp of the ITS (*13*), and ≈400 bp of *hsp65* (*11*) genes or region. Sequence alignments and phylogenetic trees were constructed by using neighbor-joining method with Kimura 2-parameter distance correction model and 1,000 bootstrap replications in MEGA4 (*14*). Only unique sequences (sequevars) were included in the trees.

Standard for Identification

Final species identifications were based on comparisons of sequences for the full 16S rRNA and the partial *rpoB* genes to GenBank references of type strains. Full-length 16S rRNA sequences were used in this study, and we used 99.5% shared identity for identification to a type strain sequence. Species identification for *rpoB* gene was based on an identity of 98.0%–100% as outlined by Adekambi et al. (*12*,*15*).

DNA-DNA Hybridization

Purified DNA of the type strains and the patient isolates CV002, CV004, CV005, CV006, and CV005 was prepared as described (*16*). CV002 and CV005 strains were labeled with [^{32}P] dCTP using the Nick Translation Kit (Invitrogen, Carlsbad, CA, USA). Labeled DNA from the CV002 was hybridized with unlabeled DNA from isolates CV002, CV004, CV005, CV006, and CV015 and with unlabeled DNA from the type strains. Labeled DNA from patient isolate CV005 was then hybridized with unlabeled DNA from isolates CV002 and CV005. The reciprocal experiment was performed because of the nearness of CV005 to the 70% cut-off designated by Wayne (*17*) and the 0% divergence obtained in the first experiment. Hybridization was performed as previously described (*18*). All reactions were performed in duplicate at 70°C. The relative binding ratio (RBR) was calculated by using the method of Brenner et al. (*19*). The percentage divergence (calculated to the nearest 0.5%) was determined by assuming that each degree of heteroduplex instability, when compared with the melting temperature of the homologous duplex, was caused by 1% unpaired bases (*19*).

Deep Multilocus Sequencing

Single-end DNA libraries of *M. bolletii* CIP 108541^{T}, *M. chelonae* ATCC 35752^{T}, *M. immunogenum* CIP 106684^{T}, *M. massiliense* CCUG 48898^{T}, and CV002 were prepared using Illumina DNA Sample Kit (Illumina, Inc., San Diego, CA, USA) according to manufacturer's recommendations. Sequencing was performed in individual flow cell lanes on the Illumina Genome Analyzer (Illumina, Inc.) at the University of Utah Huntsman Cancer Institute Core Sequencing Facility.

De novo assembly of raw Illumina sequence data was achieved by using Velvet software (*20*). Velvet was run in 2 parts, velveth and velvetg. For velveth, the hash length was set at 23 (value was selected by calculations in the software manual); default settings were used for all other parameters. In velvetg, the –cov cutoff value was set to auto (setting allows software to automate appropriate coverage cutoff), and –min_contig_lgth was set to 100; all other settings were default.

The genome of *M. abscessus* CIP 104536^{T} (*21*) was used as the source reference set of 123 genes that were identified as likely core genome components for the phylum *Actinobacter* by Ventura et al. (*22*). The set of reference genes was randomly divided into 5 similarly sized sets to facilitate analysis. SeqMan (DNASTAR Inc., Madison, WI, USA) was used to align the assembled contigs from the sequenced species against each of the sets of reference genes. DNA and inferred amino acid sequences were aligned using MEGA. Only near full-length genes were used in future comparisons. Confirming the translation of the gene was in the correct reading frame relative to the different isolates substantiated quality of each assembled gene. The DNA and amino acid sequences were concatenated for each isolate and sequences alignments and phylogenetic trees were constructed in MEGA using the neighbor-joining method. Kimura 2-parameter distance correction was used for DNA and Poisson correction model was used for amino acid trees; 1,000 bootstrap replications were used for each tree constructed.

Susceptibility Testing

We determined antimicrobial susceptibility by broth microdilution using the recommended Clinical and Laboratory Standards Institute guidelines for rapidly growing *Mycobacterium* spp. (*23*). Some isolates were not tested for all antimicrobial agents and the concentrations of antimicrobial agents that were tested varied in the panels. MICs of clarithromycin were assessed at 3 days.

Results

Identification of Isolates by Multilocus Sequencing

We obtained 6 type strains representing all members of the *M. chelonae-abscessus* complex. All type strains and all 127 archived isolates underwent multilocus sequencing. For the 127 archived clinical isolates, we identified 64 *M. abscessus*, 58 *M. chelonae*, and 5 *M. massiliense* isolates. We designated an additional 26 isolates as CV on the basis of our case definition. All 26 CV isolates underwent *rpoB* gene sequencing, and a subset (n = 11) underwent multilocus sequencing. Unique sequevars are designated among the species *M. abscessus, M. massiliense, M. bolletii, M. chelonae,* and CV isolates.

16S rRNA Gene

Alignments of 1,341 bp of the 16S rRNA gene show 8 unique sequevars from 14 variable bp positions (Figure 1). *M. chelonae* isolates had the most variability with 4 sequevars and an intraspecies variability of 0.2% (1–3 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 2 sequevars differing by 1 bp. Two *M. massiliense* sequevars were observed and differed by 1 bp with 1 sequevar identical to an *M. abscessus* sequevar. A single sequevar was found for the CV isolates, and was identical to the type strain of *M. chelonae*.

ITS Region

Alignments of 233 bp of the ITS region show 18 unique sequevars from 30 variable positions (Figure 2). *M. chelonae* isolates had the most variability with 12 sequevars and an intraspecies variability of 1.7% (1–4 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates showed 4 sequevars with an intraspecies variability of 0.9% (1–2 bp). One *M. massiliense* sequevar was observed, which was identical to 1 of the *M. abscessus* sequevars. Two sequevars were found for the CV isolates and differed by 2 bp (0.9%). The CV sequevars were most closely associated with *M. salmoniphilum* ATCC 13758^T at 98.7% (3 bp).

hsp65 Gene

Alignments of 361 bp of the *hsp65* gene show 11 unique sequevars from 41 variable positions (Figure 3). *M. chelonae* isolates had the most variability with 5 sequevars and an intraspecies variability of 1.9% (1–7 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 3 sequevars with an intraspecies variability of 1.7% (1–6 bp). One *M. massiliense* sequevar was observed and identical to 1 of the *M. abscessus* sequevars. Two sequevars were found for the CV isolates and differed by 1 bp (0.3%). The CV sequevars were most closely associated with *M. immunogenum* CIP 106684^T at 98.6% (5 bp).

sodA Gene

Alignments of 426 bp of the *sodA* gene show 15 unique sequevars from 66 variable positions (Figure 4). *M. chelonae* isolates had the most variability with 9 sequevars and an intraspecies variability of 1.9% (1–8 bp). No *M. chelonae* clinical isolates shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 3 sequevars with an intraspecies variability of 0.7% (1–3 bp). One *M. massiliense* sequevar was observed, which was identical to 1 of the *M. abscessus* sequevars. Two sequevars, which differed by 11 bp (2.6%), were found for the CV isolates. The CV sequevars were most closely associated with *M. immunogenum* CIP 106684^T at 95.8% (18 bp).

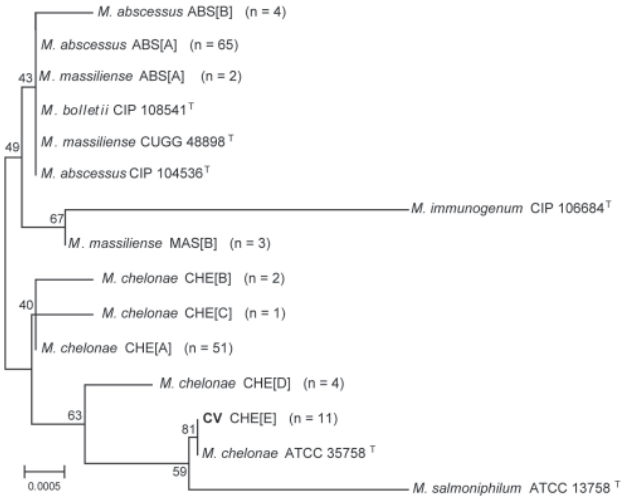

Figure 1. Neighbor-joining tree of a 1,341-bp region of unique 16S rRNA gene sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden; CV, *M. chelonae* variant; ATCC, American Type Culture Collection.

rpoB Gene

Alignments of 676 bp of region of the *rpoB* gene show 19 unique sequevars from 73 variable positions (Figure 5). *M. chelonae* isolates had the most variability with 9 sequevars and an intraspecies variability of 1.5% (1–10 bp). One *M. chelonae* clinical isolate shared 100% identity to the type strain ATCC 35758^T. *M. abscessus* isolates had 4 sequevars with an intraspecies variability of 0.7% (1–5 bp). The largest sequevar of *M. abscessus* clinical isolates had 100% identity to the type strain of *M. abscessus*. Two *M. massiliense* sequevars were observed and differed by 2 bp (0.3%). Neither sequevar shared 100% identity with the type strain for *M. massiliense*. Four sequevars were observed among the CV isolates. They had an interspecies variability of 1.8% (2–11 bp). The CV sequevars were most closely associated with *M. chelonae* CIP 106684^T at 95.1% (33 bp). Sequevar results are summarized in Table 1 in the online Technical Appendix (www.cdc.gov/EID/content/17/9/101667-Techapp.pdf).

Deep Multilocus Sequencing

Full-length genes were successfully assembled for 43 genes from 5 type strains and 1 representative clinical CV isolate. Gene names and corresponding GenBank accession

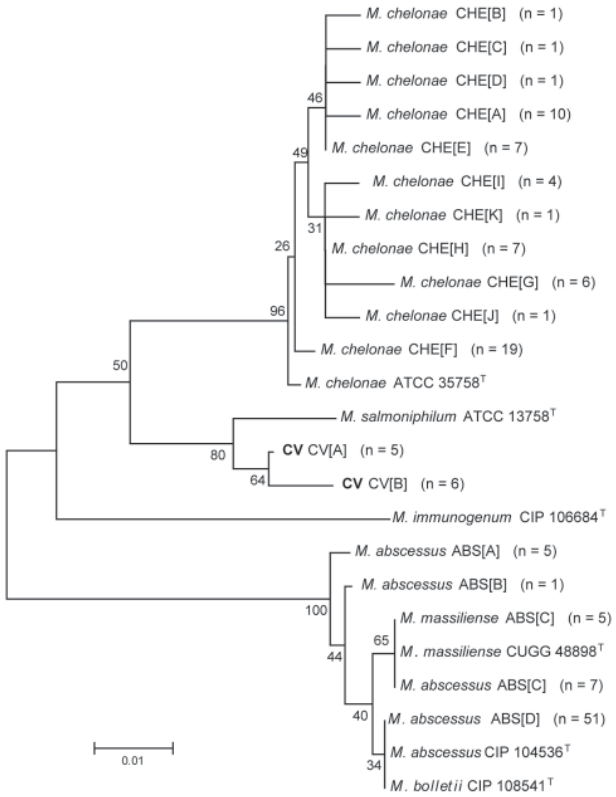

Figure 2. Neighbor-joining tree of 233 bp of unique internal transcribed spacer region sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CV, *M. chelonae* variant; CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden.

numbers are provided in the supplementary tables (online Technical Appendix Table 2). Pair-wise alignments of the DNA and amino acid sequences were performed for each isolate (online Technical Appendix Tables 3, 4). The 43 concatenated genes ranged from 41,580 to 41,619 bp and created an alignment of 41,792 nt including gaps. Comparisons of DNA percent identity ranged from 89.1% (*M. chelonae* to *M. abscessus*) to 98.3% (*M. abscessus* to *M. bolletii*). *M. massiliense*, *M. abscessus*, and *M. bolletii* showed a close association with percent identity range of 98.2%–98.3%. The novel CV isolate shared no greater than 90.5% identity with any type strain. Phylogenetic analysis shows closest relationship between *M. abscessus, M. massiliense*, and *M. bolletii* (Figure 6, panel A). The CV isolate is on a separate branch with nearly identical distances from *M. immunogenum* and *M. chelonae*.

Percent identity of amino acid comparisons ranged from 95.7% (*M. chelonae* to *M. abscessus*) to 99.6% (*M. abscessus* to *M. bolletii*). *M. massiliense*, *M. abscessus*, and *M. bolletii* showed a close association with percent identity range of 99.5%–99.6%. The novel CV isolate shared no greater than 96.1% identity with any type strain. Phylogenetic analysis shows closest relationship between *M. abscessus, M. massiliense*, and *M. bolletii* (Figure 6, panel B).

DNA-DNA Hybridization

DNA-DNA hybridization studies of clinical isolates CV004, CV005, CV006, and CV015 with labeled patient isolate CV002 showed RBRs of 66 to 96% and %D values of 0.0 to 2.0%. The reciprocal DNA-DNA hybridization of patient isolate CV002 with labeled isolate CV005 showed an RBR of 100% and a %D of 0.5%. The DNA-DNA hybridization studies of the phylogenetically (16S rRNA gene) related type strains *M. abscessus*, *M. bolletii*, *M. chelonae*, *M. immunogenum*, *M. massiliense*, and *M. salmoniphilum* performed with the patient isolate CV002 showed RBRs of 15%–69% and %D values of 5.0%–8.0%. DNA-DNA hybridization results are summarized in online Technical Appendix Table 5.

Susceptibility Testing

Susceptibilities by broth microdilution were available for most *M. chelonae, M. abscessus, M. massiliense,* and CV isolates. The MICs for the first 3 taxa were comparable

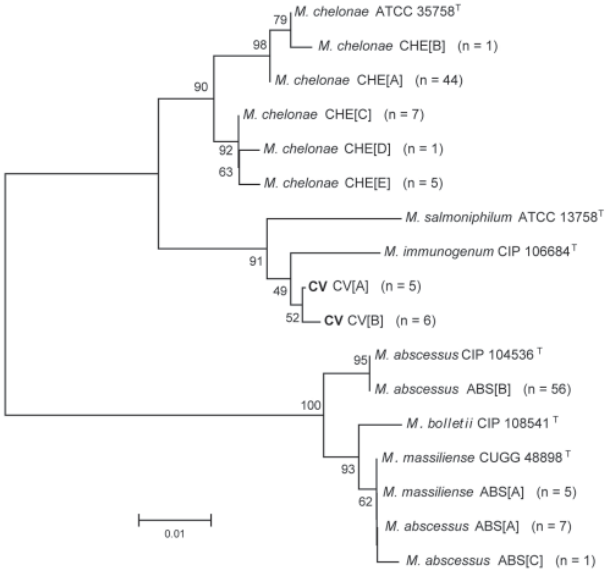

Figure 3. Neighbor-joining tree of a 361-bp region of unique heat-shock protein 65 gene sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CIP, Collection of Institute Pasteur; CV, *M. chelonae* variant; CCUG, Culture Collection, University of Göteborg, Sweden.

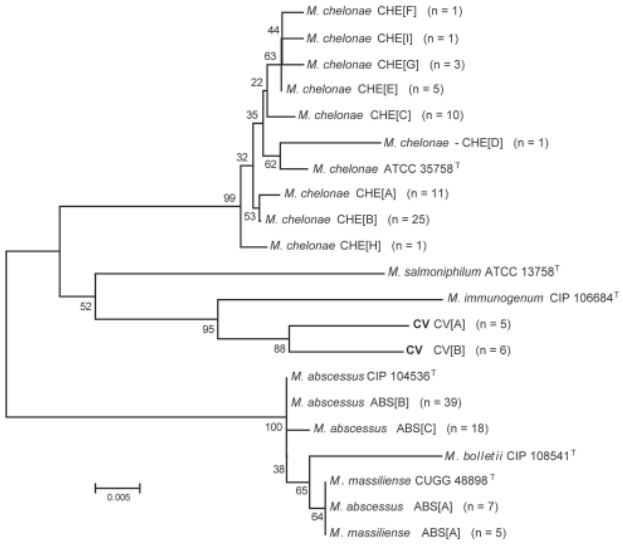

Figure 4. Neighbor-joining tree of a 426-bp region of unique *sodA* gene sequences of 138 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CIP, Collection of Institute Pasteur; CV, *M. chelonae* variant; CCUG, Culture Collection, University of Göteborg, Sweden.

those in to previous reports (*24*). With the exception of 1 isolate, all isolates of *M. chelonae* were cefoxitin resistant (MIC >128 μg/mL), and *M. abscessus* and *M. massiliense* were cefoxitin intermediate (MIC 32–64 μg/mL). All 3 taxa were minocycline resistant. Unlike *M. chelonae*, CV isolates were intermediate (88%) or susceptible (12%) to cefoxitin.

Clinical Spectrum and Characteristics of CV Isolates

The most common source for the CV isolates was respiratory (n = 20) (online Technical Appendix Table 6). The remaining 6 CV isolates had clinical sources that included skin (n = 2), granulomatous liver lesion (n = 1), central line infections (n = 2), and an unspecified body fluid. Most isolates (n = 15) were recovered from patients seen in 4 different hospitals or clinics in Pennsylvania. Eight patients with CV infection were seen at the Hospital of the University of Pennsylvania and 7 of 8 patients were adult females (ages 41–74 years) who acquired the mycobacterial infection as outpatients. Six charts were available for review, and partial information was available for 2 additional patients (patients CV007, CV008, CV010, CV012–CV014, CV034, and CV036).

The medical histories of the 6 patients with complete information fell into 2 groups: those with chronic sinusitis (2 patients) and those with lower respiratory symptoms (4 patients). All patients with lower respiratory symptoms and cultures positive for CV had underlying pulmonary disease (cystic fibrosis, primary ciliary dyskinesia, lung cancer, chronic obstructive pulmonary disease, recurrent pneumonia/bronchiectasis). No patients with lower respiratory symptoms received specific antibiotic therapy aimed at treating rapidly growing mycobacterial infection, although 1 patient received long term antimicrobial drug therapy for concomitant *M. avium* infection. Two patients with sinusitis were not treated with antimicrobial drugs, but both had sinus surgery with symptomatic improvement. One patient had 3 positive sputum cultures for a rapidly growing *Mycobacterium* over a 3-year period, 2 of which were shown by genetic sequencing to be CV organisms (CV014 was selected for further study). No other patient had >1 positive culture for CV, if follow-up cultures were performed. Three patients had sequential or concomitant infections with *M. abscessus, M. avium-intracellulare,* or both bacteria.

The first isolate identified as a CV was discovered in 2005 from a patient in New York. The next isolate was not identified until 2007 from Pennsylvania. Overall, 23 (88%) of 26 were isolated in patients in the northeastern United States, and the remaining 3 isolates were recovered from patients in Minnesota, Oregon, and Colorado.

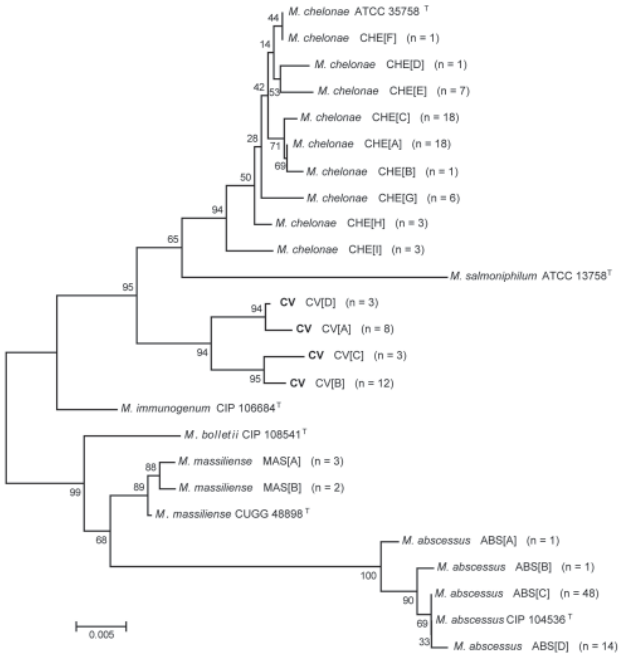

Figure 5. Neighbor-joining tree of a 676-bp region of unique *rpoB* gene sequences of 153 clinical isolates and reference strains of the *Mycobacterium chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Clinical isolates are labeled by the identification, followed by the sequevar group and the number of isolates. Scale bar indicates nucleotide substitutions per site. ATCC, American Type Culture Collection; CV, *M. chelonae* variant; CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden.

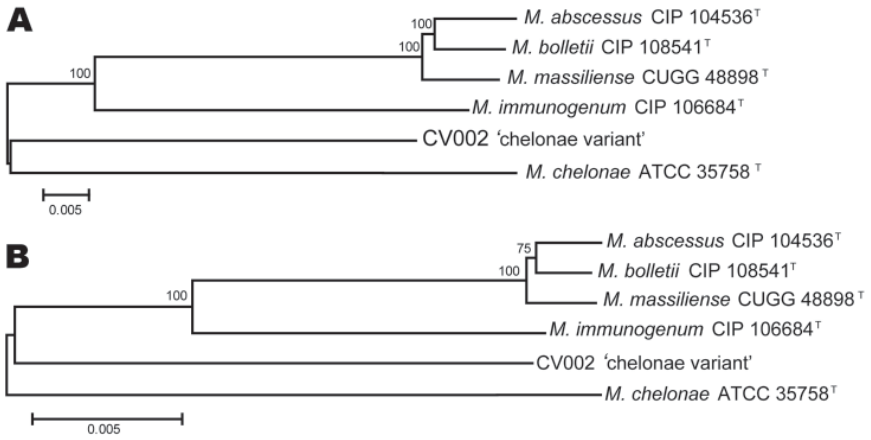

Figure 6. Neighbor-joining tree of DNA (A) and amino acid (B) concatenated gene sequences of *Mycobacterium chelonae* variant (CV) isolates and reference strains of the *M. chelonae-abscessus* complex. Branch support is recorded at nodes as a percentage of 1,000 bootstrap iterations. Upper scale bar indicates nucleotide substitutions per site and lower scale bar indicates amino acid substitutions per site. CIP, Collection of Institute Pasteur; CCUG, Culture Collection, University of Göteborg, Sweden; CV, *M. chelonae* variant; ATCC, American Type Culture Collection.

Discussion

We describe the discovery of a new human pathogen with clinical features similar to *M. abscessus* that is implicated as a cause of infection for patients with chronic lung diseases, intravascular catheters, and chronic sinusitis. On the basis of our investigations, we propose CV isolates become a new member of the *M. chelonae-abscessus* complex and be named *Mycobacterium franklinii* sp. nov. *M. franklinii* (frank li′ ni I, N.L. masc. gen. n. *franklinii* of Franklin, pertaining to Benjamin Franklin, statesman, founder of the University of Pennsylvania, inventor, and scientist who helped create the nation's first public hospital in Philadelphia, Pennsylvania, USA, the origin of the isolates).

The microorganisms are acid-fast, gram-positive bacilli, and colony morphology alone is not sufficient for differentiation from other rapidly growing *Mycobacterium* spp. Colonies are nonpigmented appearing on 5% sheep blood agar, Middlebrook 7H10 agar and egg-based Lowenstein-Jensen slants in 2–5 days at temperatures between 24 and 37°C (optimally at 30°C). Even with molecular techniques, underrecognition of this new pathogen is not surprising because it shares 100% full 16S rRNA gene identity with *M. chelonae* and has sequence variation in the *hsp65* gene that results in inconsistent amplification with typical diagnostic primers for sequence or PCR restriction fragment length polymorphism analysis (*10*,*11*). However, diagnosis of *M. franklinii* infection is essential because it is more susceptible to antimicrobial drugs than other members of the *M. chelonae-abscessus* complex, and its susceptibility pattern with cefoxitin was a distinguishing characteristic leading to its discovery.

Multilocus sequencing on a population of *M. chelonae-abscessus* complex isolates using 5 DNA regions enabled us to examine a population of closely related isolates to accurately assess species variability. *M. franklinii* shared complete 16S rRNA gene sequence identity with the type strain of *M. chelonae*, but was differentiated from *M. chelonae* and other members of the *M. chelonae-abscessus* complex by partial sequencing of *rpoB, hsp65, sodA,* and ITS DNA targets. Concatenated analysis of 43 genes (≈40,000 bp) from deeper sequencing of *M. franklinii* demonstrated that this novel species shares ≤90.5% identity with any other *M. chelonae-abscessus* group member. DNA-DNA hybridization analysis also supports the novel classification with its low relative binding ratios and higher percent divergence from all other *M. chelonae-abscessus* complex type strains. Cefoxitin susceptibility or intermediate susceptibility is another distinguishing feature. Preliminary testing on 6 *M. franklinii* isolates revealed inducible resistance (data not shown) in 50% of the isolates upon prolonged clarithromycin incubation (14 days). This finding suggests that similar to *M. abscessus*, isolates of this species may have an inducible *erm* gene.

The pathophysiology of diseases associated with *M. franklinii* is largely unknown. Most *M. franklinii* isolates were from respiratory sources and from patients with underlying lung conditions. Three of these disorders (cystic fibrosis, primary ciliary dyskinesia, and recurrent pneumonia) had associated bronchiectasis, which is a known risk factor for *M. abscessus* and *M. massiliense* nodular lung disease but not for *M. chelonae* (*6*,*25*). It is unclear whether this microorganism causes respiratory tract disease, or simply colonizes damaged airways and sinuses. Similar to patients with *M. abscessus* and *M. chelonae* infections, we found 2 cases each of sinusitis and catheter-associated infection from *M. franklinii* (*26*). The association with chronic sinusitis presumably relates to sinus washes using tap water rinses in previously diseased sinuses. Although the exact reservoir of *M. franklinii* is unknown, a recent study in the Netherlands by Van Ingen et al. reported 2 isolates from tap and shower water based on *rpoB* gene sequence (*27*), and these 2 isolates shared 99.6% identity to our *M. franklinii* sequevar A *rpoB* gene

sequence. The observation in the Netherlands suggests an environmental source for this organism, and it is likely that the novel species is regionally specific and can survive in municipal water sources. This hypothesis would be supported by the large number of cases in a focused region in Pennsylvania.

Our population analyses of clinical isolates of *M. chelonae* demonstrate a lack of taxonomic clarity. Additionally, our investigations lend further evidence that species distinctions for *M. bolletii* and *M. massiliense* may be inappropriate and support the recent proposal to modify their classifications (*5*,*28*). Taxonomic uncertainty likely arises as our understanding of microbial phylogeny expands with rapid advances in technologies and often results in inconsistent standards being applied for species designations. For example, DNA-DNA hybridization is a relatively standard technique, but upon review of 14 species descriptions in 2009 only 5 were supported by using DNA-DNA hybridization (*29*–*36*).

The discovery of an emerging pathogen should be taken in the context of microbial ecology and evolution, the interaction between host and microbe, and factors of virulence. This investigation underscores the need for accurate identification of *Mycobacterium* spp. for detection of a new pathogen. The interplay between colonization and disease is not clearly defined, but we demonstrate its role in central line infections and for patients with sinopulmonary disease. *M. franklinii* may have newly emerged as a human pathogen over the past 5 years, or it has been involved in human disease previously and was unrecognized. In order to further our understanding of this pathogen and its role in disease, greater surveillance and awareness is necessary. At this time, clinical laboratories can identify *M. franklinii* by sequencing based assays that target either the ITS region (between 16S and 23S rRNA genes), *hsp65*, *rpoB*, and *sodA* genes, or by complete 16S rRNA gene sequence analysis in conjunction with cefoxitin and minocycline susceptibility patterns. The type strain, CV002 (ATCC [pending] and DSMZ 45524), was isolated from a skin lesion.

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Mycobacterium chelonae-abscessus Complex Associated with Sinopulmonary Disease, Northeastern USA

Technical Appendix

Technical Appendix Table 1. Study isolates and sequevar group for the 16S rRNA, *rpoB, sodA, hsp65* genes and ITS region of *the Mycobacterium chelonae-abscessus* complex *

Isolate no.	16S rRNA sequevar group	*rpoB* sequavar group	*sodA* sequevar group	*hsp65* sequevar group	*ITS* sequevar group
M. abscessus isolates					
A001	ABS[A]	ABS[C]	ABS[A]	ABS[A]	ABS[C]
A003	ABS[A]	ABS[A]	ABS[A]	ABS[A]	ABS[C]
A004	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A005	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A006	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A007	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A010	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A011	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A014	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A015	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A017	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A020	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A021	ABS[B]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A023	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A024	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A029	ABS[A]	ABS[C]	ABS[A]	ABS[A]	ABS[C]
A030	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A032	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A033	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A034	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A035	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A036	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A039	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A041	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A042	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A043	ABS[A]	ABS[C]	ABS[A]	ABS[A]	ABS[C]
A044	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A046	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A047	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A048	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A049	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A050	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A051	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A052	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A053	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A054	ABS[A]	ABS[B]	ABS[B]	ABS[B]	ABS[A]
A056	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A057	ABS[A]	ABS[C]	ABS[A]	ABS[A]	ABS[C]
A058	ABS[A]	ABS[C]	ABS[A]	ABS[A]	ABS[C]
A059	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A060	ABS[A]	ABS[C]	ABS[A]	ABS[A]	ABS[C]
A061	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A063	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[B]
A064	ABS[A]	ABS[C]	ABS[C]	ABS[B]	ABS[D]

A065	ABS[A]	ABS[C]	ABS[C]	ABS[B]	ABS[D]
A066	ABS[A]	ABS[C]	ABS[C]	ABS[B]	ABS[D]
A067	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A068	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A069	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A070	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A071	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A072	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A073	ABS[A]	ABS[C]	ABS[D]	ABS[C]	ABS[D]
A074	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[A]
A075	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[A]
A076	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[A]
A077	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A078	ABS[A]	ABS[D]	ABS[C]	ABS[B]	ABS[A]
A079	ABS[A]	ABS[C]	ABS[C]	ABS[B]	ABS[D]
A080	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
A081	ABS[B]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A082	ABS[B]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
A083	ABS[B]	ABS[D]	ABS[C]	ABS[B]	ABS[D]
M. abscessus CIP 104536^{T}	ABS[A]	ABS[C]	ABS[B]	ABS[B]	ABS[D]
M. bolletii isolates					
M. bolletii CIP 108541^{T}	ABS[A]	BOL	BOL	BOL	ABS[D]
M. chelonae isolates					
C001	CHE[A]	CHE[G]	CHE[E]	CHE[C]	CHE[G]
C002	CHE[A]	CHE[A]	CHE[B]	CHE[A]	CHE[E]
C003	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C004	CHE[A]	CHE[B]	CHE[A]	CHE[A]	CHE[F]
C005	CHE[A]	CHE[E]	CHE[C]	CHE[A]	CHE[H]
C006	CHE[A]	CHE[A]	CHE[B]	CHE[A]	CHE[E]
C009	CHE[A]	CHE[G]	CHE[E]	CHE[C]	CHE[G]
C010	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C011	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C012	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C013	CHE[A]	CHE[F]	CHE[D]	CHE[A]	CHE[C]
C014	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C015	CHE[A]	CHE[G]	CHE[E]	CHE[C]	CHE[G]
C016	CHE[D]	CHE[H]	CHE[E]	CHE[C]	CHE[I]
C017	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C018	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C019	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C020	CHE[A]	CHE[E]	CHE[C]	CHE[B]	CHE[J]
C021	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C022	CHE[D]	CHE[I]	CHE[G]	CHE[C]	CHE[I]
C023	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C024	CHE[A]	CHE[A]	CHE[B]	CHE[A]	CHE[E]
C025	CHE[B]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C026	CHE[A]	CHE[H]	CHE[E]	CHE[D]	CHE[D]
C027	CHE[A]	CHE[E]	CHE[C]	CHE[A]	CHE[H]
C029	CHE[A]	CHE[G]	CHE[C]	CHE[E]	CHE[G]
C031	CHE[A]	CHE[A]	CHE[B]	CHE[A]	CHE[E]
C032	CHE[C]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C033	CHE[A]	CHE[G]	CHE[C]	CHE[E]	CHE[G]
C034	CHE[B]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C035	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C038	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C039	CHE[A]	CHE[A]	CHE[B]	CHE[A]	CHE[B]
C040	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C041	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C042	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C043	CHE[A]	CHE[A]	CHE[H]	CHE[A]	CHE[E]
C044	CHE[D]	CHE[I]	CHE[G]	CHE[C]	CHE[I]
C045	CHE[A]	CHE[E]	CHE[C]	CHE[A]	CHE[H]
C047	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C048	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C050	CHE[A]	CHE[D]	CHE[C]	CHE[E]	CHE[G]
C051	CHE[D]	CHE[I]	CHE[G]	CHE[C]	CHE[I]

C052	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C053	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C054	CHE[A]	CHE[A]	CHE[B]	CHE[A]	CHE[E]
C055	CHE[A]	CHE[E]	CHE[C]	CHE[A]	CHE[H]
C056	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C057	CHE[A]	CHE[A]	CHE[A]	CHE[A]	CHE[A]
C058	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C059	CHE[A]	CHE[A]	CHE[B]	CHE[A]	CHE[E]
C060	CHE[A]	CHE[G]	CHE[F]	CHE[E]	CHE[H]
C061	CHE[A]	CHE[E]	CHE[C]	CHE[A]	CHE[F]
C062	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[H]
C063	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
C064	CHE[A]	CHE[E]	CHE[C]	CHE[A]	CHE[H]
C065	CHE[A]	CHE[H]	CHE[I]	CHE[E]	CHE[K]
C066	CHE[A]	CHE[C]	CHE[B]	CHE[A]	CHE[F]
M. chelonae ATCC 35758[T]	CHE[E]	CHE[J]	CHE[J]	CHE[F]	CHE[L]
M.immunogenum isolates					
M. immunogenum CIP 106684[T]	IMM	IMM	IMM	IMM	IMM
M. massiliense isolates					
M001	MAS[B]	MAS[A]	ABS[A]	ABS[A]	ABS[C]
M002	MAS[B]	MAS[A]	ABS[A]	ABS[A]	ABS[C]
M003	ABS[A]	MAS[B]	ABS[A]	ABS[A]	ABS[C]
M004	ABS[A]	MAS[B]	ABS[A]	ABS[A]	ABS[C]
M005	MAS[B]	MAS[A]	ABS[A]	ABS[A]	ABS[C]
M. massiliense CCUG 48898[T]	ABS[A]	MAS[C]	ABS[A]	ABS[A]	ABS[C]
Novel species					
CV002	CHE[E]	CV[B]	CV[B]	CV[B]	CV[B]
CV004	CHE[E]	CV [A]	CV [A]	CV [A]	CV [A]
CV005	CHE[E]	CV[B]	CV[B]	CV[B]	CV[B]
CV006	CHE[E]	CV [A]	CV [A]	CV [A]	CV [A]
CV007	CHE[E]	CV[B]	CV[B]	CV[B]	CV[B]
CV008	CHE[E]	CV[B]	CV[B]	CV[B]	CV[B]
CV010	CHE[E]	CV[B]	CV[B]	CV[B]	CV[B]
CV012	CHE[E]	CV[B]	CV[B]	CV[B]	CV[B]
CV013	CHE[E]	CV [A]	CV [A]	CV [A]	CV [A]
CV014	CHE[E]	CV [A]	CV [A]	CV [A]	CV [A]
CV015	CHE[E]	CV [A]	CV [A]	CV [A]	CV [A]
CV021	NA	CV[B]	NA	NA	NA
CV022	NA	CV[D]	NA	NA	NA
CV023	NA	CV[D]	NA	NA	NA
CV024	NA	CV [A]	NA	NA	NA
CV025	NA	CV[C]	NA	NA	NA
CV026	NA	CV[D]	NA	NA	NA
CV027	NA	CV [A]	NA	NA	N/
CV028	NA	CV[B]	NA	NA	NA
CV030	NA	CV[B]	NA	NA	NA
CV031	NA	CV[C]	NA	NA	NA
CV032	NA	CV[C]	NA	NA	NA
CV033	NA	CV[B]	NA	NA	NA
CV034	NA	CV[B]	NA	NA	NA
CV035	NA	CV[B]	NA	NA	NA
CV036	NA	CV[A]	NA	NA	NA

*ITS, internal transcribed spacer; CIP, Collection of Institut Pasteur; NA, not applicable.

Technical Appendix Table 2. Gene designations and corresponding GenBank accession numbers of study isolates and reference sequences from *Mycobacterium abscessus**

Gene name	Alignment gene length, bp	*M. abscessus* ORF designation	*M. abscessus* (NC_010397) Protein ID#	GenBank accession nos. for study isolates
Translation initiation factor IF-3 (*infC*)	531	MAB_2321	YP_001703056	HQ661899– HQ661904
S-adenosyl-methyltransferase (*mraW*)	1014	MAB_1998	YP_001702734	HQ661905– HQ661910
Phosphatidate cytidylyltransferase (*cdsA*)	879	MAB_3186c	YP_001703917	HQ661911– HQ661916
Phosphoribosylaminoimidazole carboxylase (*purE*)	501	MAB_3619c	YP_001704347	HQ661917– HQ661922
Preprotein translocase subunit (*secY*)	1260	MAB_3784c	YP_001704513	HQ661923– HQ661928
(HSP-70 cofactor (*grpE*)	753	MAB_4272c	YP_001704999	HQ661929– HQ661934
Recombination protein (*recR*)	609	MAB_0320	YP_001701073	HQ661935–HQ661940
Ribonuclease P protein component (*rnpA*)	384	MAB_4954c	YP_001705676	HQ661941–HQ661946
Elongation factor Ts (*tsf*)	834	MAB_3195c	YP_001703926	HQ661947–HQ661952
Hypothetical protein Lactamase_B	1692	MAB_3083c	YP_001703815	HQ661953–HQ661958
50S ribosomal protein L7/L12 (*rplL*)	390	MAB_3876c	YP_001704604	HQ661959–HQ661964
50S ribosomal protein L13 (*rplM*)	444	MAB_3752c	YP_001704480	HQ661965–HQ661970
Co-chaperonin GroES (*groE*S)	300	MAB_3732c	YP_001704460	HQ661971–HQ661976
30S ribosomal protein S1 *(rpsA*)	1446	MAB_2296	YP_001703031	HQ661977–HQ661982
Phosphoribosylformylglycinamidine synthase II (*purL*)	2286	MAB_0707	YP_001701457	HQ661983–HQ661988
Putative Mrp homolog protein	1137	MAB_1366c	YP_001702106	HQ661989–HQ661994
Putative metalloprotease	513	MAB_1669	YP_001702408	HQ661995–HQ662000
50S ribosomal protein L20 (*rplT*)	390	MAB_2323	YP_001703058	HQ662001–HQ662006
ABC transporter ATP-binding protein	720	MAB_2747c	YP_001703480	HQ662007–HQ662012
Guanylate kinase	501	MAB_2823c	YP_001703556	HQ662013–HQ662018
Elongation factor P (*ef*P)	564	MAB_2837c	YP_001703570	HQ662019–HQ662024
Deoxyuridine 5'-triphosphate nucleotidohydrolase (*dut*)	453	MAB_3003c	YP_001703735	HQ662025–HQ662030
30s ribosomal protein S2 (*rpsB*)	849	MAB_3196c	YP_001703927	HQ662031–HQ662036
Aspartyl/glutamyl-tRNA amidotransferase, B subunit (*gatB*)	1482	MAB_3334c	YP_001704064	HQ662037–HQ662042
30s ribosomal protein S9 (*rpsI*)	534	MAB_3751c	YP_001704479	HQ662043–HQ662048
50S ribosomal protein L6 (*rplF*)	540	MAB_3797c	YP_001704526	HQ662049–HQ662054
Transcription antitermination protein NusG (*nusG*)	804	MAB_3894c	YP_001704622	HQ662055–HQ662060
Adenylosuccinate synthetase (*purA*)	1296	MAB_4249c	YP_001704976	HQ662061–HQ662066
Cell division protein FtsZ *(ftsZ*)	1164	MAB_2009	YP_001702745	HQ662067–HQ662072
Recombinase A (*recA*)	1041	MAB_3060c	YP_001703792	HQ662073–HQ662078
Tyrosyl-tRNA synthetase *(tyrS*)	1293	MAB_2354	YP_001703089	HQ662079–HQ662084
UDP-N-acetylglucosamine pyrophosphorylase (*glmU*)	1452	MAB_1148c	YP_001701890	HQ662085–HQ662090
Valyl-tRNA synthetase (*valS*)	2643	MAB_1603	YP_001702342	HQ662091–HQ662096
Crossover junction endodeoxyribonuclease (*ruvC*)	510	MAB_2884c	YP_001703617	HQ662097–HQ662102
DNA-directed RNA polymerase subunit beta (*rpoC*)	3960	MAB_3868c	YP_001704596	HQ662103–HQ662108
DNA repair protein RecN (*recN*)	1770	MAB_2361	YP_001703096	HQ662109–HQ662114
GTP-dependent nucleic acid-binding protein (*ychF*)	1074	MAB_1266	YP_001702008	HQ662115–HQ662120
GTP-binding protein Era (*era*)	915	MAB_1672	YP_001702411	HQ662121–HQ662126
Hypothetical protein MAB2781c	978	MAB_2781c	YP_001703514	HQ662127–HQ662132
Nicotinate-nucleotide adenylyltransferase (*nadD*)	638	MAB_1621	YP_001702360	HQ662133–HQ662138
50S ribosomal protein L10 (*rplJ*)	531	MAB_3877c	YP_001704605	HQ662139–HQ662144
50S ribosomal protein L35 (*rpmI*)	195	MAB_2322	YP_001703057	HQ662145–HQ662150
Putative Holliday junction resolvase	522	MAB_2850c	YP_001703583	HQ662151–HQ662156

*ORF, open reading frame; ID, identification

Technical Appendix Table 3. Similarity table of the average (range) of percent identity between concatenated DNA sequences of 43 genes of the *Mycobacterium chelonae-abscessus* complex type strains and CV002*

Strains	Average % identity (range)					
	M. abscessus CIP 104536Ta	*M. bolletii* CIP 108541^{T}	*M. chelonae* ATCC 35752^{T}	CV002 (*M. chelonae* variant)	*M. massiliense* CCUG 48898^{T}	*M. immunogenum* CIP 106684^{T}
M. abscessus CIP 104536Ta	–	98.3 (94.4–99.8)	89.1 (80.7–97.3)	90.0 (83.5–97.3)	98.2 (92.2–100)	91.4 (83.9–98.0)
M. bolletii CIP 108541^{T}		–	89.3 (80.7–98.0)	90.1 (83.8–98.0)	98.2 (95.1–100)	91.5 (84.1–98.3)
M. chelonae ATCC 35752^{T}			–	90.0 (82.2–98.3)	89.3 (81.0–97.7)	89.6 (81.0–98.0)
CV02				–	90.1 (83.8–97.7)	90.5 (83.4–99.7)
M. massiliense CCUG 48898^{T}		+			–	91.7 (83.9–99.2)

*Sequences derived from GenBank accession no. NC_010397. CIP, Collection of Institut Pasteur.

Technical Appendix Table 4. Similarity table of the average (range) of percent identity between concatenated amino acid sequences of 43 genes of the *Mycobacterium chelonae-abscessus* complex type strains and CV002*

Strains	Average % identity (range)					
	M. abscessus CIP 104536^{T}	*M. bolletii* CIP 108541^{T}	*M. chelonae* ATCC 35752^{T}	CV002 (*M. chelonae* variant)	*M. massiliense* CCUG 48898^{T}	*M. immunogenum* CIP 106684^{T}
M. abscessus CIP 104536^{T}	–	99.6 (97.6–100)	95.6 (83.7–100)	95.9 (87.3–100)	99.5 (97.8–100)	97.2 (88.3–100)
M. bolletii CIP 108541^{T}		–	95.7 (84.5–100)	95.9 (88.0–100)	99.5 (98.2–100)	97.2 (88.3–100)
M. chelonae ATCC 35752^{T}			–	95.8 (84.1–100)	95.6 (84.5–100)	95.9 (84.1–100)
CV002				–	95.9 (88.0–100)	96.1 (86.5–100)
M. massiliense CCUG 48898^{T}					–	97.2 (88.3–100)

*CIP, Collection of Institut Pasteur.

Technical Appendix Table 5. Comparison of DNA-DNA hybridization results of patient isolates and phylogenetically related type strains of *the Mycobacterium chelonae-abscessus* complex*

Unlabeled DNA	% similarity to radiolabeled CV002		% similarity to radiolabeled CV005	
	RBR at 70°C	% Divergence	RBR at 70°C	% Divergence
CV002	100	0.0	100	0.5
CV004	80	1.5		
CV005	66	0.0	100	0.0
CV006	96	2.0		
CV015	94	2.0		
M. abscessus CIP 104536^{T}	66	6.0		
M. bolletii CIP 108541^{T}	15	6.0		
M. chelonae ATCC 35752^{T}	42	8.0		
M. immunogenum CIP 106684^{T}	65	5.0		
M. massiliense CCUG 48898^{T}	69	6.5		
M. salmoniphilum ATCC 13758^{T}	41	6.5		

*RBR, relative binding ratio (see text for full definition); CIP, Collection of Institut Pasteur.

Technical Appendix Table 6. Case histories of patients with CV recovered during study*

Isolate no.	Year isolated	Patient age, y/sex	State	Source	Underlying condition	Symptoms	Treatment	Outcome
CV002	2005	9/M	NY	Skin lesion	Unknown	Unknown	Unknown	Unknown
CV004	2007	5/M	PA	Central line port	Medulloblastoma, chemotherapy	Erythema and purulent discharge at central line site	Levofloxacin, cefoxitin, clarithromycin	Infection resolved
CV005	2007	2/F	PA	Bronchial wash	Esotropia, vocal cord paralysis, and gastro-duodenal fistula	Cough	Unknown	Infection resolved
CV006	2007	10/F	PA	Liver biopsy	Relapsed acute lymphoblastoma leukemia	Biopsy-confirmed granulomatous liver lesions	Clindamycin, ceftazidime	Infection resolved
CV007	2007	41/F	PA	Sputum	Primary ciliary dyskinesia, bronchiectasis and prior *Mycobacterium avium-intracellulare* and *M. abscessus* infection	Dyspnea and increased sputum for several months	Intravenous cefepime for 3 weeks	Symptoms improved
CV008	2007	64/F	PA	Sputum	Prednisone therapy, bronchiectasis, multiple episodes of pneumonia and autoimmune hepatitis	Dyspnea and increased sputum production for 3 weeks	10-d course of fluoroquinolone	Respiratory function subjectively and radiographically improved
CV010	2007	72/F	PA	Sputum	Unknown	Unknown	Unknown	Unknown
CV012	2007	63/F	PA	Maxillary sinus	Unknown	Unknown	Unknown	Unknown
CV013	2007	45/F	PA	Sputum	Cystic fibrosis and prior *Mycobacterium avuim* infection	Worsening cough, sputum production and dyspnea. Chest CT scan showed bilateral bronchiectasis and tree in bud opacities	At time of presentation, receiving azithromycin, ethambutol and rifampin for *M. avium*; continued on same regimen for several months	Symptoms subjectively improved and follow up cultures were repeatedly negative for *M. avium* and rapidly growing mycobacteria
CV014	2007	74/F	PA	Sputum	Unknown	Three isolates recovered over 3- year period	Unknown	Unknown
CV015	2008	10/M	PA	Sputum	Eczema	Fever, sore throat, stomatitis, and drooling. HSV-1 PCR sputum/saliva positive	Acyclovir	Lost to follow-up
CV021	2008	76/M	NJ	Bronchial wash	Unknown	Unknown	Unknown	Unknown
CV022	2008	58/F	MN	Sputum	Unknown	Unknown	Unknown	Unknown
CV023	2009	69/M	VA	Respiratory	Unknown	Unknown	Unknown	Unknown
CV024	2009	78/F	ME	Respiratory	Unknown	Unknown	Unknown	Unknown
CV025	2009	73/F	OR	Body fluid	Unknown	Unknown	Unknown	Unknown
CV026	2009	18/M	CO	Sputum	Unknown	Unknown	Unknown	Unknown
CV027	2009	18/M	PA	Sputum	Diabetes	Lung injury following smoke inhalation from house fire. Diagnosed with pulmonary mucormycosis by BAL. Weeks later developed respiratory failure and pulmonary hemorrhage	Unknown	Patient died
CV028	2009	38/F	ME	Sputum	Unknown	Unknown	Unknown	Unknown
CV030	2009	48/F	ME	Sputum	Unknown	Unknown	Unknown	Unknown
CV031	2009	53/F	ND	Bronchial lavage	Unknown	Unknown	Unknown	Unknown

CV032	2009	1/M	NJ	Blood	Unknown	Unknown	Unknown	Unknown
CV033	2009	73/M	PA	Maxillary sinus	Unknown	Unknown	Unknown	Unknown
CV034	2009	44/F	PA	Maxillary sinus	Maxillary sinus surgery for chronic sinusitis	Two weeks postoperatively had routine follow-up and complained of nasal drainage.	None	Nasal drainage resolved over several weeks without antimicrobial therapy
CV035	2010	66/F	PA	Thigh lesion	Diabetes	Erythematous 2-cm indurated plaque to a 3.5- x 3.5-cm lesion. First skin biopsy showed granulomatous reaction. Subsequent biopsies showed abscess and adjacent leukocytoclastic vasculitis	Unknown	Unknown
CV036	2010	47/M	PA	Maxillary sinus	Unknown	Unknown	Unknown	Unknown

*CV, *Mycobacterium chelonae* variant; CT, computed tomography; HSV-1, herpes simplex virus 1.