An Outbreak of Keratitis Caused by Mycobacterium immunogenum

Jorge Luiz Mello Sampaio, Doraldo Nassar Junior, Denise de Freitas, Ana Luisa Höfling-Lima, Kozue Miyashiro, Fernando Lopes Alberto, and Sylvia Cardoso Leão

Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, São Paulo, Brazil; Departamento de Oftalmologia, Universidade Federal de São Paulo, São Paulo, Brazil; and Instituto Florey de Ensino e Pesquisa, São Paulo, Brazil

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From 8 October to 12 November 2003, 36 patients underwent surgical correction of myopia in a São Paulo, Brazil, clinic. Five patients had clinical signs of infectious keratitis, and a Mycobacterium species with previously unreported patterns determined by PCR restriction enzyme analysis of the hsp65 gene and PCR restriction enzyme analysis of the 16S-23S rRNA internal transcribed spacer (ITS) was isolated from corneal scrapings from four of these patients. Subsequent evaluation by phenotypic tests and partial sequencing of the hsp65, sodA, rpoB, and 16S rRNA genes and the ITS supported the species identification as a variant of Mycobacterium immunogenum. The source of infection was not determined. The outbreak was caused by a single clone, as evidenced by identical pulsed-field gel electrophoresis and enterobacterial repetitive intergenic consensus-PCR profiles. This is the first report of an outbreak where this species was isolated from infected tissues.

Species belonging to the Mycobacterium chelonae-abscessus group have been isolated from many environmental sources, including potable water and distribution systems, swimming pool water, metalworking fluids, and soil (9, 28, 30). Their survival was observed in some strains, and their ubiquity in the environment create favorable conditions for the occurrence of infections when sterility precautions or disinfection procedures are disregarded (29, 30). Many health care-associated outbreaks caused by M. abscessus or M. chelonae have been documented since the first report in 1969 (12). “Mycobacterium massiliense” and “Mycobacterium bolletii” have been recently described and proposed as M. chelonae-M. abscessus group members, but to date there are no reports concerning their isolation from environmental samples (1, 4). Mycobacterium immunogenum is a recently described rapidly growing species of the M. chelonae-M. abscessus group and occupies the same ecological niche as other M. chelonae-M. abscessus group members. Clinical isolates include those causing disseminated cutaneous infections, catheter-related infections, septic arthritis, chronic pneumonia, pacemaker-related sepsis, and possible keratitis (17, 31). A single genotype was isolated from metalworking fluids at 10 different sites in the United States, where hypersensitivity pneumonitis occurred among metal-grinding machinists, but M. immunogenum was not isolated from those patients (28). This species caused pseudo-outbreaks related to bronchoalveolar lavage procedures in Missouri and Maryland, but to date no reports of outbreaks of infections caused by this species have emerged (31). From 8 October to 12 November 2003, 36 patients—71 eyes—had LASIK performed in a private clinic located in the urban area of the city of São Paulo, Brazil. Among those patients, five had clinical signs of infectious keratitis, and a Mycobacterium species with a distinct PRA-hsp65 (PCR restriction enzyme analysis of the hsp65 gene) pattern was isolated from corneal scrapings from four of these patients. An epidemiological investigation was undertaken, and species identification was done by partial sequencing of multiple gene targets.

MATERIALS AND METHODS

Epidemiological study. A retrospective cohort study was conducted. All patients who underwent LASIK from 8 October to 12 November 2003 were followed up for 6 months after surgery. Any patient who had LASIK and presented with a corneal opacity was defined as a possible case of keratitis due to rapidly growing mycobacteria (RGM). Confirmed cases were those with a corneal scraping culture positive for RGM. The surgical procedures and sterilization process used were reviewed.

Environmental investigation. In December 2003, samples of tap water, anesthetic solution, povidone iodine solution, nonsurgical soap, commercial distilled water, water from the reservoir of a bench-top autoclave, and water from the air conditioning system drain were collected. The samples were first concentrated and decontaminated with N-acetylcysteine sodium hydroxide and then plated on Middlebrook 7H10 agar. The plates were incubated in ambient air at 30°C and observed weekly for 1 month.

Cultivation of corneal scrapings and Zielh-Neelsen staining. Corneal scrapings were collected with a Kimura spatula after topical anesthesia and plated directly on sheep blood agar, chocolate agar, and Sabouraud dextrose agar plates and in thioglycollate medium. Inoculated media were then incubated in ambient air at 35°C and observed daily for 2 weeks. Slides with corneal smears or bacterial smears were stained by the Zielh-Neelsen method as described elsewhere (6).

Phenotypic tests. Sodium chloride tolerance; utilization of citrate, mannitol, and sorbitol; growth rate; and pigment production were determined after incubation in ambient air at 30°C as described by Vincent et al. (27).

Susceptibility testing. Susceptibility to amikacin, tobramycin, doxycycline, ciprofloxacin, and clarithromycin was assessed by broth microdilution according to CLSI document M24-A (7). Staphylococcus aureus ATCC 29213 (ATCC is a trademark of the American Type Culture Collection) was used as a quality control strain.
Genomic DNA extraction. Genomic DNA was extracted from bacteria grown on solid media as described by Sampaio et al. (21). DNA concentration was estimated by spectrophotometry with GeneQuant (Pharmacia).

Amplification of the 16S-23S rRNA internal transcribed spacer for sequencing reactions and restriction enzyme analysis (PRA-ITS). The ITS fragment was amplified and sequenced with primers SP1 (5′ACCTCCTTTGTACTCAGGACGC) and SP2 (5′GATGCCTCAGCACACTATCCA) as previously described (20), except that Platinum Tag DNA polymerase (Invitrogen) was used and amplicons were digested separately with HaeIII, HhaI, or TaqI (Invitrogen) and then separated by electrophoresis in 2% Metaphor–1% SeaKem LE agarose gels (BioWhittaker Molecular Applications Inc.). PRA-ITS genotypes were assigned after estimation of fragment sizes by the BioNumerics program (Applied Maths). The interpretative criteria used were those of Roth et al. (20). Clinical isolates of M. chelonae ITS genotype II (F436) and M. abscessus ATCC 19977, and M. immunogenum ATCC 700505 were used as controls.

PCR and restriction enzyme analysis of the hsp65 gene (PRA-hsp65). A 441-bp fragment of the hsp65 gene was amplified with primers Tb11 (5′TGGTGATCATC) and Tb12 (5′CTTGTGCAAGGACATCTC) as previously described, with the following minor modifications (23). A total of 5 μl of each DNA solution (50 μg/ml) was added to 45 μl of a PCR mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM MgCl2, 1% enhancer (Invitrogen), 200 μM each 2′-deoxynucleoside 5′-triphosphate, 1 μM each primer, and 1.25 U of Taq DNA polymerase (Promega). Amplicons were digested separately with Metaphor (Promega) and HaeIII (Invitrogen), and restriction fragments were separated by electrophoresis in 2% Metaphor–1% SeaKem LE agarose gels. PRA genotypes were assigned after estimation of fragment sizes by the BioNumerics program v. 4.0 (Applied Maths). Clinical isolates of M. chelonae (F436) and M. abscessus (ATCC 19977) were used as controls.

PCR and restriction enzyme analysis of the hsp65 gene (PRA-hsp65). A 441-bp fragment of the hsp65 gene was amplified with primers Tb11 (5′TGGTGATCATC) and Tb12 (5′CTTGTGCAAGGACATCTC) as previously described, with the following minor modifications (23). A total of 5 μl of each DNA solution (50 μg/ml) was added to 45 μl of a PCR mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM MgCl2, 1% enhancer (Invitrogen), 200 μM each 2′-deoxynucleoside 5′-triphosphate, 1 μM each primer, and 1.25 U of Platinum Tag DNA polymerase (Invitrogen). Amplification conditions were as follows: 2 min at 95°C fol-

Amplification of the hsp65 gene for sequencing reactions. External primers hsp667FW (5′GGGCCAAGCAGATGGTGCCAT) and hsp667RV (5′GGAGCTGGCCAAGACAATTGCGTACG) were used to amplify and sequence a 667-bp fragment (positions 145 to 585 of the hsp65 gene). A total of 5 μl of each DNA solution (50 μg/ml) was added to 45 μl of a PCR mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM MgCl2, 200 μM each 2′-deoxynucleoside 5′-triphosphate, 1 μM each primer, and 1.0 U of Platinum Tag DNA polymerase. Reaction mixtures were submitted to the following cycling conditions: 2 min at 95°C followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min, with a final extension step of 72°C for 5 min.

RESULTS

Outbreak cohort. The surgical facility, located in the urban area of the city of São Paulo, Brazil, started its practice in March 1999, and up until 7 October 2003, approximately 2,214 patients underwent LASIK there. No possible cases of keratitis were detected in this population. From 8 October to 12 November 2003, 36 patients—71 eyes—underwent LASIK performed by the same surgeon at this surgical facility. Surgeries were performed in batches every 2 or 3 weeks. Among those patients, five had clinical signs of infectious keratitis. The median attack rate was 7.04 per 100 eyes or 13.89 per 100 patients. The median interval from exposure to clinically apparent infection was 20 days. The 31 patients who were potentially exposed and did not show clinical signs of keratitis were recalled for a follow-up visit in the third month following surgery. None of them had clinical signs of keratitis or corneal infiltrates 3 months after surgery. Patients 1 and 2 underwent LASIK on both eyes on 8 October (Tables 1 and 2). On 28 October, patient 1 presented with a small white infiltrate on the right cornea. A sample was not collected, and the patient improved with empirical treatment based on amikacin (14 mg/ml) and clarithromycin (10 mg/ml) eye drops plus oral clarithromycin at 500 mg twice daily for 2 months. On 30 October, patient 1 presented with a small white infiltrate on the right cornea. The patient was treated with ciprofloxacin eye drops but had no clinical improvement. On 20 November, a corneal scraping was collected and sent to the laboratory for acid-fast staining and culture. The direct smear was positive, and the culture was positive for RGM. Empirical treatment based on amikacin (14 mg/ml) and clarithromycin (10 mg/ml) eye drops plus oral clarithromycin 500 mg twice daily was initiated, but clinical improvement was only achieved after corneal flap removal and maintenance with topical and systemic drugs for 3 months.

Patients 3 and 4 underwent LASIK on both eyes on 29 October (Tables 1 and 2). On 20 November, patient 3 presented with photophobia and conjunctival hyperemia. He was treated with ciprofloxacin eye drops but had no clinical improvement. On 27 November, multiple white infiltrates were noted on his left cornea (Fig. 1) and corneal scrapings were
TABLE 1. Summary of epidemiological investigation of three groups of patients with M. immunogenum postoperative keratitis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of surgery</td>
<td>10/08/2003</td>
<td>10/29/2003</td>
<td>11/12/2003</td>
</tr>
<tr>
<td>No. of patients</td>
<td>13</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>No. of surgeries</td>
<td>25</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>No. of cases of postoperative keratitis</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Operating room: OR1, Surgeon S1, Assistant A1, Claril (nafazolin hydrochloride-feniramime maleate)

Antiseptic (povidone iodine) Yes Yes Yes
Local anesthetic (Anestalcon [proparacain hydrochloride]) Yes Yes Yes
LASIK corneal marker Yes Yes Yes
Buffered salt solution irrigation Yes Yes Yes
Merocel surgical sponge Yes Yes Yes
Postoperative tobramycin plus dexamethasone eye drops Yes Yes Yes

*OR1, operating room 1.
*S1, surgeon 1.
*A1, assistant 1.

Patient 4 presented with photophobia and conjunctival hyperemia on 18 November. On 20 November, corneal scrapings were collected and sent to the laboratory for acid-fast staining and culture. The direct smear was positive, and the culture was positive for RGM.

Patient 5 underwent LASIK on both eyes on 12 November (Tables 1 and 2). On 28 November, he presented with photophobia and conjunctival hyperemia in his left eye. Amikacin (14 mg/ml) and clarithromycin (10 mg/ml) eye drops plus oral clarithromycin were initiated. On 4 December, corneal scraping and culture. The direct smear was positive, and the culture was positive for RGM.

Patient 5 underwent LASIK on both eyes on 12 November (Tables 1 and 2). On 28 November, he presented with photophobia and conjunctival hyperemia in his left eye. Amikacin (14 mg/ml) and clarithromycin (10 mg/ml) eye drops plus oral clarithromycin were initiated. On 4 December, corneal scraping and culture. The direct smear was positive, and the culture was positive for RGM.

TABLE 2. Characteristics of five patients belonging to the three groups described in Table 1*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
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<td>32</td>
<td>36</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Surgery time</td>
<td>13:05</td>
<td>13:26</td>
<td>12:36</td>
<td>12:45</td>
<td>13:03</td>
</tr>
<tr>
<td>Surgery in both eyes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Surgery sequence no.</td>
<td>5th, 6th</td>
<td>7th, 8th</td>
<td>9th, 10th</td>
<td>11th, 12th</td>
<td>5th, 6th</td>
</tr>
<tr>
<td>Date clinical signs appeared</td>
<td>10/28/03</td>
<td>10/30/03</td>
<td>11/20/03</td>
<td>11/18/03</td>
<td>11/26/03</td>
</tr>
<tr>
<td>Affected eye</td>
<td>RE</td>
<td>RE</td>
<td>RE</td>
<td>RE</td>
<td>LE</td>
</tr>
<tr>
<td>Corneal scraping collection date</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Isolate no.</td>
<td>F1111</td>
<td>F1114</td>
<td>F1112</td>
<td>F1113</td>
<td>F1113</td>
</tr>
</tbody>
</table>

*Patients 1 and 2 belonged to group 1 in Table 1, patients 3 and 4 belonged to group 2 in Table 1, and patient 5 belonged to group 3 in Table 1.

Phenotypic tests. All outbreak isolates were nonpigmented, rapidly growing, acid-fast bacilli. They were unable to grow on Lowenstein-Jensen medium containing 5% sodium chloride or to utilize citrate, D-mannitol or D-sorbitol as the sole carbon source. The MICs of amikacin, tobramycin, ciprofloxacin, and clarithromycin were, respectively, 32 μg/ml, 16 μg/ml, >16 μg/ml, >16 μg/ml, and 2 μg/ml for all isolates.

Analysis of partial sequences and PRA-ITS and PRA-hsp65 patterns. For all of the targets examined, sequences from outbreak isolates had 100% similarity. When partial sequences of the hsp65, 16S rRNA, sodA, and rpoB genes and ITS were compared to those available in the GenBank database, the highest similarity indices obtained were with sequences belonging to M. immunogenum (Table 3). The highest similarity index with the hsp65 gene sequence available in the GenBank database was with strain M-JY14, an environmental strain of M. immunogenum (14), followed by M. immunogenum ATCC 700505 and ATCC 700506 (Table 3). When sequences from outbreak isolates were aligned with that from M. immunogenum ATCC 700506 (GenBank accession no. AY498741), base substitutions were observed at positions 291, 292, 306, 358, 413, and 575 (M. tuberculosis H37Rv genome). Substitutions at positions 291 and 306 occurred at regions corresponding to the HaeIII recognition sites in M. immunogenum ATCC 700505 or ATCC 700506, resulting in a new PRA-hsp65 pattern (Fig. 2) with 325- and 130-bp bands after BseBI digestion and 200-, 70-, 58-, and 55-bp bands after HaeIII digestion. Type strain M.
immunogenum ATCC 700505 had a PRA-hsp65 pattern with 325- and 130-bp bands after BstEII digestion and 145-, 70-, 58-, and 55-bp bands after HaeIII digestion (Fig. 3).

When the partial ITS sequence from isolate F1112 was aligned with that of *M. immunogenum* ATCC 700505 and ATCC 700506 (accession no. AY497531 and AY593977, respectively), 10 base substitutions, three insertions, and one deletion were observed. Substitutions at positions 28 and 105 (*M. tuberculosis* H37Rv genome) resulted in recognition sites for HhaI. A deletion at position 114 and a substitution at position 115 resulted in loss of a restriction site recognized by TaqI, while a substitution at position 153 resulted in a recognition site for this restriction endonuclease, resulting in a new PRA-ITS pattern (Fig. 4). Outbreak isolates had an ITS pattern with an amplicon of 269 bp, no restriction with HaeIII (data not shown), 157-, 71-, and 41-bp fragments after HhaI digestion, and 157- and 112-bp fragments after restriction with TaqI. Type strain *M. immunogenum* ATCC 700505 had an amplicon of 267 bp, no restriction with HaeIII or HhaI, and 148- and 119-bp fragments after restriction with TaqI (Fig. 5).

Partial sequences of the 16S rRNA gene from outbreak isolates F1111 and F1112 gave evidence of a single substitution.

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**TABLE 3. Summary of BLAST results obtained with sequences from outbreak isolate F1112**

<table>
<thead>
<tr>
<th>Gene or intergenic region</th>
<th>GenBank accession no. for isolate F1112</th>
<th>GenBank accession no. of sequence with highest similarity</th>
<th>Strain</th>
<th>No. of identical nucleotides/total</th>
<th>Index of similarity (%) to <em>M. immunogenum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>hsp65</em></td>
<td>DQ288262</td>
<td>AY615711</td>
<td>ATCC 700506</td>
<td>436/441</td>
<td>98.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AY498741</td>
<td>ATCC 700506</td>
<td>435/441</td>
<td>98.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AY458081</td>
<td>ATCC 700505</td>
<td>417/424</td>
<td>98.35</td>
</tr>
<tr>
<td>ITS</td>
<td>DQ288266</td>
<td>AY593977</td>
<td>ATCC 700505</td>
<td>214/225</td>
<td>95.53</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>DQ288263</td>
<td>AY457080</td>
<td>ATCC 700505</td>
<td>1,466/1,467</td>
<td>99.93</td>
</tr>
<tr>
<td>sodA</td>
<td>DQ288265</td>
<td>AY458106</td>
<td>ATCC 700505</td>
<td>410/417</td>
<td>98.32</td>
</tr>
<tr>
<td>rpoB</td>
<td>DQ288264</td>
<td>AY262739</td>
<td>ATCC 700505</td>
<td>647/651</td>
<td>99.38</td>
</tr>
</tbody>
</table>

* ATCC 700505 (American Type Culture Collection), CIP106684 (Institut Pasteur Collection, Paris, France), DSM44764 (German National Resource Centre for Biological Material), and CCUG47286 (Culture Collection, University of Göteborg, Göteborg, Sweden) are culture collection codes for the type strain of *M. immunogenum*. BLAST results obtained in July 2006.

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**FIG. 2. Alignment of hsp65 gene partial sequences from species belonging to the *M. chelonae-M. abscessus* group and *M. immunogenum* outbreak isolate F1112. Gray boxes correspond to restriction sites recognized by the HaeIII endonuclease. Note that substitutions at positions 291 and 306 (*M. tuberculosis* H37Rv genome) in isolate F1112 resulted in loss of restriction sites.**
at position 183 (M. tuberculosis H37Rv genome) compared to that of M. immunogenum ATCC 700505 (results not shown). When partial sequences of the sodA gene from outbreak isolates F1111 and F1112 were aligned with that from the type strain, substitutions were observed at positions 33, 81, 129, 201, 378, 438, and 451 (M. tuberculosis H37Rv genome). In comparing partial sequences of the rpoB gene from outbreak isolates F1111 and F1112 to that from the M. immunogenum type strain (GenBank accession no. AY262739), base substitutions at positions 2538, 2544, 2883, and 3073 and deletions at positions 28 and 105 (M. tuberculosis H37Rv genome) resulted in restriction sites recognized by HhaI; a deletion at position 114, an insertion at position 117, and a substitution at position 153 resulted, respectively, in loss and gain of restriction sites recognized by TaqI.

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**FIG. 3.** PRA-hsp65 patterns after BstEII (lanes 1 to 6) or HaeIII (lanes 7 to 12) digestion. Lanes: 1 and 7, M. abscessus I ATCC 19977; 2 and 8, M. abscessus II strain F649; 3 and 9, M. chelonae strain F436; 4 and 10, M. immunogenum ATCC 700505; 5 and 11, M. immunogenum outbreak isolate F1111; 6 and 12, M. immunogenum outbreak isolate F1112.

**FIG. 4.** Alignment of partial ITS sequences from species belonging to the M. chelonae-M. abscessus group, including three M. chelonae genotypes, and M. immunogenum outbreak isolate F1112. Gray boxes and white boxes correspond to restriction sites recognized by the HhaI and TaqI endonucleases, respectively. Note that in the sequence corresponding to isolate F1112, substitutions at positions 28 and 105 (M. tuberculosis H37Rv genome) resulted in restriction sites recognized by HhaI; a deletion at position 114, an insertion at position 117, and a substitution at position 153 resulted, respectively, in loss and gain of restriction sites recognized by TaqI. The “M. bolletii” ITS sequence was not included since it is not yet available in the GenBank database.

**FIG. 5.** PRA-ITS patterns after HaeIII (lanes 1 to 5), HhaI (lanes 6 to 10), or TaqI (lanes 11 to 15) digestion. Lanes: 1, 6, and 11, M. abscessus ATCC 19977; 2, 7, and 12, M. chelonae ITS genotype II strain F436; 3, 8, and 13, M. immunogenum ATCC 700505; 4, 9, and 14, outbreak isolate F1111; 5, 10, and 15, outbreak isolate F1112. Note that there are no restriction fragments after HaeIII digestion in any of the isolates or strains (lanes 1 to 5) and no restriction fragments after HhaI digestion in M. abscessus, M. chelonae, or M. immunogenum ATCC 700505 (lanes 6 to 8).
PFGE and ERIC-PCR. Outbreak isolates were undistinguishable by PFGE and ERIC-PCR, while the ERIC-PCR profile obtained with *M. immunogenum* ATCC 700505 was different from those obtained with outbreak isolates (Fig. 6).

**DISCUSSION**

Although infectious keratitis is a rare complication of LASIK, RGM are the most frequent bacterial agents isolated in such cases (13). This is the third reported outbreak of keratitis caused by RGM following LASIK in Brazil (5, 10), adding to the growing list of 25 cases and outbreaks of these infections which have been published and indexed in PubMed since the first description in 1998 (19). In this outbreak, the source of contamination was not identified. Failure to isolate *M. chelonae-M. abscessus* group species from environmental samples may be due to insufficient sample volume or to collection of environmental samples after the operating room was thoroughly cleaned. Outbreak isolates were initially evaluated by PRA-*hsp65* and shown to have a pattern similar to that of *M. chelonae*. The high MIC of tobramycin (16 µg/ml) and the inability to use citrate as the sole carbon source were inconsistent with *M. chelonae*, which prompted us to perform additional identification tests.

Identification of mycobacteria by gene sequencing has been mostly based on the *hsp65* and 16S rRNA genes (25). The finding of similarity indices of 98.35% and 99.93%, respectively, with partial sequences of the *hsp65* and 16S rRNA genes from *M. immunogenum* type strain ATCC 700505 would be enough to confirm species identification, since McNabb et al. (18) demonstrated that when comparing *hsp65* sequences, a correct species identification can be confidently made when the similarity index is ≥97%. The recent description of “*M. massilense*” and “*M. bolletii*,” in which *hsp65* partial sequences are shown to have 99.32% to 100% similarity indices compared to each other or to *M. abscessus* type II (GenBank accession no. AY859675, AY596465, and AY603554), indicates that the maximum similarity index of 98.35% obtained when comparing *hsp65* partial sequences from outbreak isolates to those available in the GenBank database is not enough to confirm identification of the species as *M. immunogenum*.

Drancourt and Raoult (8) have proposed that when analyzing rRNA gene sequences, a match of 99.5% or more would represent intraspecies variability. This criterion may even be used to differentiate *M. abscessus*, *M. chelonae*, and *M. immunogenum* from each other since similarity indices vary from 99.32% to 99.39% when sequences longer than 1,480 bp are compared. In contrast to that proposal, 16S rRNA gene sequences of *M. abscessus*, “*M. massilense*,” and “*M. bolletii*” have a 100% similarity index (GenBank accession no. AY593980, AY457071, and AY859681).

Partial sodA sequences from outbreak isolates had a match of 98.32% with those from *M. immunogenum*, which supports this species identification, in accordance with Adékambi and Drancourt (3), who found 98% to 100% similarity indices when partial sodA sequences from the same species were compared. Exceptions are the recently proposed species “*M. massilense*” and “*M. bolletii*,” in which the sodA sequences have matches of 98.64% and 98.41% (GenBank accession no. AY498743, AY862403, and AY596465) with that from *M. abscessus* ATCC 19977, respectively.

The analysis of a 651-bp region from the *rpoB* gene from outbreak isolates (Table 3) showed a 99.38% match with *M. immunogenum* ATCC 700505, an index higher than 98.3%, the minimal value obtained by Adékambi et al. (2) when comparing isolates of the same species. This region also has enough polymorphism to discriminate “*M. massilense*” and “*M. bolletii*” from other species belonging to the *M. chelonae-M. abscessus* group, since their similarity indices vary from 95% to 98.08% (GenBank accession no. AY147164, AY147163, AY262739, AY859692, and AY593981).

Although there is not a consensus for a cutoff for species identification when analyzing ITS partial sequences, the highest match (95.53%) for outbreak isolate F1112 was with *M. immunogenum* ATCC 700505, a value above the maximum match (94%) obtained when comparing ITS sequences from different species belonging to the *M. chelonae-M. abscessus* group (GenBank accession no. AJ314870, AJ314875, AJ291582, AJ291583, AJ291584, AY593978, and AY497531). The phenotypic and genotypic characteristics described here for outbreak isolates are concordant, and we propose that they are a variant of *M. immunogenum*. Outbreak isolates had indistinguishable PFGE profiles, indicating that they have the same clonal origin. This also suggests a common source of infection for all patients. ERIC-PCR grouped outbreak isolates into a single cluster and differentiated them from type strain *M. immunogenum* ATCC 700505. The discriminatory power of PFGE has not been determined for this species, although Wallace et al. (28) analyzed 15 epidemiologically unrelated clinical isolates and demonstrated that they were correctly classified as unrelated by PFGE according to the criteria of Tenover et al. (24). This outbreak was caused by a single clone of a variant of *M.
immunogenum, and to our knowledge this is the first report of an outbreak where this species was isolated from infected tissue.

ACKNOWLEDGMENTS

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REFERENCES


26. Reference deleted.


