Application of four molecular typing methods for analysis of *Mycobacterium fortuitum* group strains causing post-mammaplasty infections

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**ABSTRACT**

A cluster of cases of post-augmentation mammaplasty surgical site infections occurred between 2002 and 2004 in Campinas, in the southern region of Brazil. Rapidly growing mycobacteria were isolated from samples from 12 patients. Eleven isolates were identified as *Mycobacterium fortuitum* and one as *Mycobacterium porcinum* by PCR–restriction digestion of the *hsp65* gene. These 12 isolates, plus six additional *M. fortuitum* isolates from non-related patients, were typed by pulsed-field gel electrophoresis (PFGE) and three PCR-based techniques: 16S–23S rRNA internal transcribed spacer (ITS) genotyping; randomly amplified polymorphic DNA (RAPD) PCR; and enterobacterial repetitive intergenic consensus (ERIC) PCR. Four novel *M. fortuitum* allelic variants were identified by restriction analysis of the ITS fragment. One major cluster, comprising six *M. fortuitum* isolates, and a second cluster of two isolates, were identified by the four methods. RAPD-PCR and ITS genotyping were less discriminative than ERIC-PCR. ERIC-PCR was comparable to PFGE as a valuable complementary tool for investigation of this type of outbreak.

**Keywords** Infections, mammaplasty, molecular epidemiology, *Mycobacterium fortuitum*, plastic surgery, typing

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**INTRODUCTION**

Rapidly growing mycobacteria (RGM) are normal inhabitants of a wide variety of environmental reservoirs, including natural and tap water, and soil [1]. They cause localised cutaneous infections following trauma, as well as outbreaks of nosocomial infection and pseudo-outbreaks [2]. Surgical site infections with RGM, most often caused by *Mycobacterium fortuitum*, *Mycobacterium chelonae* or *Mycobacterium abscessus*, have been associated with cardiac and ophthalmologic surgery and augmentation mammaplasty [3–6].

Augmentation mammaplasty is one of the most common cosmetic surgical procedures performed in Brazil. Twelve isolates of mycobacteria belonging to the *M. fortuitum* group were cultivated from patients who underwent augmentation mammaplasty in Campinas, São Paulo State, in the southern region of Brazil, between 2002 and 2004. There were no reports of post-mammaplasty mycobacterial infections in Campinas outside this period. An investigation was initiated to determine the character, source, mode of transmission and risk-factors for these infections.

Typing of isolates, best achieved by genetic comparison, is particularly useful for outbreak studies and other epidemiological investigations...
Phenotypic typing methodologies have limited discriminative capacities. Genotypic methods are usually more discriminative and have been useful in demonstrating that many outbreaks of infections with RGM involved multiple species and/or multiple strains of the same species [4,5,9,10]. Plasmid analysis has been used to characterise M. fortuitum isolates responsible for infections following augmentation mammoplasty and cardiac surgery in the USA [4,5]. Newer and more discriminative approaches include pulsed-field gel electrophoresis (PFGE), which has been used to type a range of different bacterial species, including M. fortuitum [7]. Several PCR-based techniques are also being used with increasing frequency to investigate outbreaks involving non-tuberculous mycobacteria. Randomly amplified polymorphic DNA (RAPD) PCR analysis has been used to type isolates of Mycobacterium avium, M. abscessus and Mycobacterium szulgai [8,11,12]. Enterobacterial repetitive intergenic consensus (ERIC) PCR analysis has been reported to be a useful tool for typing Mycobacterium tuberculosis isolates [13], and has been shown to differentiate Mycobacterium paratuberculosis from other species of mycobacteria, including M. fortuitum [14]; however, to date, there are no reports on the use of this technique to evaluate clonal relationships among RGM.

Since an outbreak setting could be a unique opportunity to validate molecular typing techniques for these organisms, and to define interpretative criteria based on epidemiological concordance, the present study used four different molecular typing techniques, 16S–23S rRNA gene spacer genotyping, RAPD-PCR, ERIC-PCR and PFGE, to compare post-mammoplasty and unrelated M. fortuitum isolates.

**MATERIALS AND METHODS**

**Mycobacterial isolates**

Between February 2003 and April 2004, 33 (6.7%) of 492 patients who underwent augmentation mammoplasty at Campinas presented with a clinical picture suggestive of surgical site infection, characterised by local pain, increased temperature, swelling, fluid accumulation, and sometimes spontaneous drainage. Nineteen surgical site samples were collected for bacteriological examination. Acid-fast bacilli were isolated in cultures from 11 patients and sent to regional reference laboratories for identification. Ten isolates were recovered for this study (P3–P12; Table 1). During the epidemiological investigation, two additional cases were diagnosed who underwent surgery in Campinas before this period, in July and November 2002, respectively, and the corresponding isolates (P1 and P2, Table 1) were included in the study. Isolates from four unrelated patients from different geographical regions with post-augmentation mammoplasty M. fortuitum infections diagnosed in the same period were also included. Two patients were from Salvador, Bahia (C1 and C3), one was from Goiania, Goiás (C2), and one was from Itu, São Paulo (C4) These cities are located 1982 km, 835 km and 46 km, respectively, from Campinas. M. fortuitum was also isolated in cultures from 11 patients and sent to regional reference laboratories for identification. Ten isolates were recovered for this study (P3–P12; Table 1). During the epidemiological investigation, two additional cases were diagnosed who underwent surgery in Campinas before this period, in July and November 2002, respectively, and the corresponding isolates (P1 and P2, Table 1) were included in the study. Isolates from four unrelated patients from different geographical regions with post-augmentation mammoplasty M. fortuitum infections diagnosed in the same period were also included. Two patients were from Salvador, Bahia (C1 and C3), one was from Goiania, Goiás (C2), and one was from Itu, São Paulo (C4) These cities are located 1982 km, 835 km and 46 km, respectively, from Campinas. M. fortuitum was also isolated in cultures from 11 patients and sent to regional reference laboratories for identification. Ten isolates were recovered for this study (P3–P12; Table 1). During the epidemiological investigation, two additional cases were diagnosed who underwent surgery in Campinas before this period, in July and November 2002, respectively, and the corresponding isolates (P1 and P2, Table 1) were included in the study. Isolates from four unrelated patients from different geographical regions with post-augmentation mammoplasty M. fortuitum infections diagnosed in the same period were also included. Two patients were from Salvador, Bahia (C1 and C3), one was from Goiania, Goiás (C2), and one was from Itu, São Paulo (C4) These cities are located 1982 km, 835 km and 46 km, respectively, from Campinas. M. fortuitum was also

**Table 1.** Data and genotypic patterns for 12 isolates of rapidly growing mycobacteria from 12 patients (P1–P12) with augmentation mammoplasty surgical site infections at Campinas, São Paulo, and six unrelated patients (C1–C6) with surgical site infections following other types of surgery and/or from different cities

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>City</th>
<th>Hospital</th>
<th>Type of prosthesis</th>
<th>Surgeon</th>
<th>Surgery date (day/month/year)</th>
<th>Sample collection date (day/month/year)</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Campinas</td>
<td>3</td>
<td>1</td>
<td>G</td>
<td>19/7/2002</td>
<td>18/11/2003</td>
<td>XI E5 R5 PF5</td>
</tr>
<tr>
<td>P2</td>
<td>Campinas</td>
<td>1</td>
<td>3</td>
<td>B</td>
<td>18/10/2002</td>
<td>16/5/2003</td>
<td>V E1 R1 PF1a</td>
</tr>
<tr>
<td>P3</td>
<td>Campinas</td>
<td>2</td>
<td>3</td>
<td>A</td>
<td>27/2/2003</td>
<td>17/9/2003</td>
<td>XI E2 R2 PF2a</td>
</tr>
<tr>
<td>P4</td>
<td>Campinas</td>
<td>1</td>
<td>3</td>
<td>H</td>
<td>28/2/2003</td>
<td>1/3/2004</td>
<td>V E1 R1 PF1b</td>
</tr>
<tr>
<td>P5</td>
<td>Campinas</td>
<td>1</td>
<td>3</td>
<td>D</td>
<td>10/4/2003</td>
<td>29/9/2003</td>
<td>V E1 R1 PF1b</td>
</tr>
<tr>
<td>P7</td>
<td>Campinas</td>
<td>1</td>
<td>1</td>
<td>C</td>
<td>16/6/2003</td>
<td>18/9/2003</td>
<td>M. porcinum E9 R9 ND</td>
</tr>
<tr>
<td>P8</td>
<td>Campinas</td>
<td>1</td>
<td>3</td>
<td>E</td>
<td>19/6/2003</td>
<td>7/10/2003</td>
<td>V E1 R1 PF1b</td>
</tr>
<tr>
<td>P10</td>
<td>Campinas</td>
<td>1</td>
<td>2</td>
<td>J</td>
<td>26/8/2003</td>
<td>26/9/2003</td>
<td>V E1 R1 PF1a</td>
</tr>
<tr>
<td>P11</td>
<td>Campinas</td>
<td>2</td>
<td>4</td>
<td>A</td>
<td>9/1/2004</td>
<td>10/5/2004</td>
<td>XI E2 R2a PF2b</td>
</tr>
<tr>
<td>P12</td>
<td>Campinas</td>
<td>1</td>
<td>3</td>
<td>I</td>
<td>9/4/2004</td>
<td>4/5/2004</td>
<td>V E1 R1 PF1b</td>
</tr>
</tbody>
</table>

| C1          | Salvador     | NI       | NI                 | NI      | 14/5/2002                     | II E7 R7 PF7    |
| C3          | Salvador     | NI       | NI                 | NI      | 26/5/2004                     | II E10 R7a PF10 |
| C4          | Itu          | NI       | 1                  | NI      | 24/10/2003                    | XIV E8 R8 PF8   |

| C5          | Campinas     | 5        | NA                 | NI      | 20/2/2002                     | V E11 R8 PF11   |
| C6          | Resende      | NI       | NA                 | NI      | 9/9/2003                      | V E1a R1 PF12   |

NI, not investigated; NA, not applicable; ND, not done.
isolated from two patients following, respectively, abdominal wall plastic surgery undertaken at Campinas (C5) 5 months before the first case of augmentation mammoplasty infection, and reductive mammoplasty, performed in October 2003 at Resende, Rio de Janeiro (C6), a city located 260 km from Campinas. Environmental samples and samples from surgical equipment were not available for analysis.

**Primary isolation and preliminary tests**

Clinical specimens (mammary abscess aspirates, mammary tissue fragments, surgical site secretions) were examined for the presence of acid-fast bacilli by Ziehl–Neelsen staining, and were cultivated on Löwenstein–Jensen and sheep blood agar, and in the MGIT system (Becton Dickinson, Franklin Lakes, NJ, USA), at both 30°C and 37°C. The presence of mycobacteria in positive cultures was confirmed by Ziehl–Neelsen staining. Cultures were exposed to light to check for pigment production.

**DNA extraction**

One loopful of bacteria grown on solid medium was transferred to 100 μL of sterile distilled water, boiled for 10 min and frozen at −20°C until use. Supernatants (5–10 μL) were used as templates for PCR.

**Species identification**

Species were identified according to basic cultural characteristics (rate of growth and pigmentation) and PCR restriction enzyme analysis (RPA) [15]. In brief, a 441-bp fragment of the *hsp65* gene was amplified using primers Tb11 5′-ACC-AAACGATGGTTGTCCAT and Tb12 5′-CTTGTGCAACCCG-CATACCCCT and was used as the molecular size standard. Interpretative criteria used for species identification were those of Brunello et al. [16].

**16S–23S rRNA gene spacer genotyping**

Amplification of the 16S–23S internal transcribed spacer (ITS) was performed as described previously [17], with minor modifications, using primers SP1 5′-ACCTCCTTCTTAAAG-GAGCCACC and SP2 5′-GATGCCTGCAAACACATATCCCA. PCRs contained 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM MgCl₂, Triton X-100 0.1% w/v, 200 μM each dNTP, 0.22 μM each primer, and 1.0 U of Platinum Taq DNA polymerase (Invitrogen). PCRs comprised 5 min at 96°C, followed by 38 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplified products were digested separately with HaeIII and CfoI (Invitrogen) at 37°C for 270 min. Amplification products and restriction fragments were separated by electrophoresis in Metaphor 2% w/v and SeaKem LE 1% w/v agarose gels. Size markers were 25-bp (Invitrogen) and 50-bp DNA ladders. Isolates were assigned to ITS genotypes after estimation of fragment sizes using BioNumerics v. 4 (Applied Maths, Sint-Martens-Latem, Belgium).

**Randomly amplified polymorphic DNA PCR**

Primer RAPDI 5′-TGGTCCGCGCA [18] was used according to the procedure described by Zhang et al. [8], with minor modifications. PCRs contained 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM MgCl₂, 250 μM each dNTP, 1.0 μM primer, and 0.6 U of Taq DNA polymerase (Promega). PCRs comprised 4 min at 94°C, followed by 40 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min, with a final extension at 72°C for 10 min. Amplified products were separated by electrophoresis at 5 V/cm in SeaKem LE 2% w/v agarose gels. A mixture of λ DNA/HindIII fragments and ϕX174 RF DNA/HaeIII fragments (Invitrogen) was used as the size standard.

**Enterobacterial repetitive intergenic consensus PCR**

ERIC-PCR was performed using primers ERIC1R 5′-ATG-TAACGTCTCTGGGATTAC and ERIC2 5′-AAGTAA-GTACTGCGGTGAGCC as described by Sechi et al. [13], with minor modifications. PCRs were performed in a volume of 30 μL containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM MgCl₂, 200 μM each dNTP, 1.0 μM each primer, and 0.6 U of Taq DNA polymerase (Promega). PCRs comprised 2 min at 94°C, followed by 35 cycles of 94°C for 45 s, 52°C for 1 min and 70°C for 10 min, with a final extension at 70°C for 20 min. Amplified products were separated by electrophoresis at 5 V/cm in SeaKem LE 2% w/v agarose gels. A mixture of λ DNA/HindIII fragments and ϕX174 RF DNA/HaeIII fragments (Invitrogen) was used as the reference marker.

**Pulsed-field gel electrophoresis**

Single colonies were transferred to 10 mL of Mueller–Hinton broth supplemented with Tween-80 0.1% w/v and incubated at 37°C with agitation (140 rpm) to an OD₂₅₀ of 0.65. Cells were centrifuged, resuspended in Triton X-100 1% w/v in 0.05 M EDTA, and incubated for 2 h at 37°C. The cells were then washed in 0.05 M EDTA, centrifuged, and resuspended in TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) containing lysozyme 1 mg/mL. The suspension was then mixed with an equal volume of low-melt preparative grade agarose (Bio-Rad, Richmond, CA, USA) 2% w/v in 125 mM EDTA held at 53°C, and cast into plug moulds. Plugs were transferred to 24-well cell culture plates containing 2.5 mL of EDTA and lysozyme 1 mg/mL, and incubated overnight at 37°C. The plugs were then transferred to 0.5 M EDTA containing Sarkosyl 1% w/v and held at 4°C for 1 h, and then incubated at 55°C for 24 h in the same solution containing proteinase K 2 mg/mL. The plugs were washed in TE and then incubated in TE containing phenylmethylsulfonylfluoride (Sigma, St Louis, MO, USA) 4 mg/mL for 1 h at 55°C. Finally, the plugs were washed extensively in TE. The DNA contained in the plugs was digested overnight at 37°C with 10 U of XhoI (Invitrogen). After digestion, plugs were loaded into the slots...
of a Pulsed-Field Certified Agarose (Bio-Rad) 1% w/v gel prepared in 0.5 × TBE buffer (45 mM Tris-HCl, 45 mM boric acid, 1 mM EDTA) and subjected to PFGE in 0.5 × TBE buffer containing 75 μM thiourea. PFGE was performed in a CHEF-DR III system (Bio-Rad) at 14°C for 20 h at 6 V/cm, with a ramped pulse time of 5–20 s. Polymerised bacteriophage lambda DNA (New England Biolabs, Ipswich, MA, USA) was used as the molecular size standard. After staining with ethidium bromide, gels were photographed on a UV transilluminator.

Computer-assisted analysis of patterns

All gel images were analysed using BioNumerics v. 4. Dendrograms to illustrate the pattern relatedness among the isolates were constructed using the Dice coefficient and the unweighted pair-group method with the arithmetic averages clustering method (UPGMA).

Statistical analysis

Fisher’s exact test was used to assess the association between clonal identity and hospital, surgeon or type of prosthesis.

RESULTS

Species identification

The 12 specimens from mammaplasty patients from Campinas and the six specimens from unrelated patients who underwent surgery at Hospital 1 in Campinas, and isolates cultivated from two unrelated patients following non-prosthetic plastic surgery (C5 and C6), had 257-bp ITS amplicons that yielded HaellIII restriction fragments of 148 bp and 109 bp, but no digestion with CfoI, corresponding to ITS genotype V described by Roth et al. [17]. Two isolates from unrelated patients in Salvador (C1 and C3) had 280-bp ITS amplicons that yielded HaellIII restriction fragments of 172 bp and 108 bp, but no digestion with CfoI, corresponding to ITS genotype II. Four isolates from patients from Campinas (P1, P3, P9 and P11) had two ITS amplicons of 294 bp and 252 bp, with HaellIII restriction fragments of 144 bp, 108 bp and 84 bp, and CfoI fragments of 173 bp, 150 bp, 134 bp and 80 bp. This pattern did not correspond to any of the ten M. fortuitum genotypes described by Roth et al. [17] and was named genotype XI. One isolate (P6) presented a further novel pattern, named genotype XII, with two ITS amplicons of 282 bp and 252 bp, yielding restriction fragments after HaellIII digestion of 169 bp, 143 bp and 108 bp, and CfoI fragments of 282 bp, 172 bp and 80 bp. Two geographically unrelated isolates (C2 and C4) had novel ITS patterns that were named genotypes XIII and XIV, respectively. The isolate from patient P7 yielded the M. porcinum ITS pattern reported by Roth et al. [17] (Table 1; Fig. 1).

Fig. 1. 16S–23S rRNA internal transcribed spacer (ITS) genotyping results for Mycobacterium fortuitum isolates from 12 cases of augmentation mammaplasty surgical wound infections from Campinas, Sao Paulo (P1–P12) and six unrelated patients (C1–C6) following other types of surgery and/or from different cities.
ITS genotype V isolates P2, P4, P5, P8, P10 and P12 had indistinguishable ERIC-PCR and RAPD-PCR patterns (E1 and R1, respectively) (Table 1; Fig. 2). PFGE results showed two band differences in isolates P2 and P10 compared with the other four isolates (Table 1; Fig. 3). Isolate C6, also belonging to ITS genotype V, had an indistinguishable RAPD-PCR pattern, but was discriminated from the other isolates in this group by PFGE and ERIC-PCR analysis.

Two isolates (P3, P11) had indistinguishable ITS and RAPD-PCR patterns, and a single band difference in ERIC-PCR and PFGE patterns (Table 1; Figs 2 and 3). The remaining isolates had unique genotypes by RAPD-PCR, ERIC-PCR and PFGE, except for isolates C4 and C5, which had indistinguishable three-band RAPD-PCR patterns, but different ITS, ERIC-PCR and PFGE patterns. The *M. porcinum* isolate (P7) was discriminated easily from the *M. fortuitum* isolates by all the genotyping techniques used.

**Statistical analysis**

There was a significant association between the clonal group of six isolates (P2, P4, P5, P8, P10 and P12) and surgical procedures performed at Hospital 1 (two-tailed p 0.002), but there was no significant association with particular surgeons (p ≥ 0.545) or type of prosthesis (p ≥ 0.242). No statistical analysis was performed on the clonal group from Hospital 2, since only two cases were detected, but the same surgeon performed surgery on both patients and used different types of implant.

**DISCUSSION**

Post-augmentation mammaplasty infection, with an estimated annual risk of 1–2% [19], can be a devastating occurrence, resulting in additional scars, capsular contracture, surgical wound dehiscence and implant exposure, so that implant removal becomes a therapeutic requirement. These infections may also have great psychological impact when implant removal is necessary, because replacement must be postponed for months to years. There were no reports or notifications before 2003 of post-mammaplasty infections in Campinas caused by the *M. fortuitum* group of organisms. However, 11 patients presented with confirmed surgical site infections.
caused by these organisms between February 2003 and April 2004. Two additional cases were diagnosed retrospectively following surgery in 2002. Typing results proved that one of these two isolates was indistinguishable from five isolates obtained during the investigation period, indicating that the outbreak period was broader than initially suspected, possibly extending back to July 2002. There have been no further cases of post-mammaplasty infections caused by mycobacteria in Campinas since April 2004.

The time from surgery to microbiological diagnosis ranged from 1 to 16 months. Since empirical therapeutic approaches used commonly to treat surgical site infections are not effective against RGM, early clinical suspicion and microbiological diagnosis are key factors in reducing morbidity. Diagnosis is sometimes delayed because of incomplete or inadequate microbiological test requests. Acid-fast staining, a rapid and simple diagnostic tool for mycobacterial infections, is not performed routinely unless requested explicitly. Although RGM can grow in solid and liquid media used commonly in routine bacteriology, isolation requires incubation for ≥3 days and, for some species, e.g., *M. chelonae*, lower incubation temperatures (30°C).

*M. fortuitum* was the designation given by da Costa Cruz in 1938 to an organism isolated from a post-injection abscess in a Brazilian patient [20]. *M. fortuitum* is a member of the RGM, and is a ubiquitous contaminant and coloniser that can be isolated from environmental sources such as potable water systems and soil [21]. *M. porcinum*, isolated originally from pigs [22], was only recognised recently as an agent of human disease by Schinsky *et al.* [23], following re-evaluation of some isolates identified previously as *M. fortuitum* third biovariant. Members of the *M. fortuitum* complex can be identified to the species level by PRA, based on *hsp65* gene polymorphism [15]. *M. fortuitum hsp65* gene sequences deposited in GenBank consist of only one allelic variant, compatible with the PRA *hsp65* pattern described originally by Telenti *et al.* [15]. Two additional PRA *hsp65* patterns have been described in isolates from Brazil [24], but all isolates included in the present study displayed the original PRA profile [15].

ITS can be used for identification of *M. fortuitum* and for grouping isolates in ten allelic variants [17]. With use of ITS as a preliminary grouping tool, four additional *M. fortuitum* restriction banding patterns were identified, indicating that this genomic region is highly polymorphic in *M. fortuitum*. Nevertheless, the genotyping discriminative capacity is limited, as unrelated isolates were grouped in the same ITS genotype.

The present study found that *M. fortuitum* isolates from mammaplasty patients belonging to ITS genotype V had indistinguishable RAPD-PCR and ERIC-PCR patterns. By PFGE, four isolates belonged to genotype PF1b, and two belonged to genotype PF1a. Since these two genotypes differ by two fragments, they would be considered to be closely related [25], and probably formed part of the same outbreak. A positive correlation was found between this cluster and patients who received surgery at Hospital 1, where this strain persisted from at least October 2002 to April 2004. Environmental persistence of *M. fortuitum* strains that caused furunculosis associated with footbaths at a nail salon in the USA was documented for 6 months [7].

Isolate C6 also belonged to ITS genotype V and had a RAPD-PCR pattern indistinguishable from that of isolates from Hospital 1. The similarity index by ERIC-PCR was 93%, and the PFGE patterns differed by more than four fragments. C6 was isolated in a different geographical region, and no connection to the outbreak was detected in the epidemiological investigation. Considering these results, *M. fortuitum* PFGE genotypes differing by four or more fragments, and strains with ERIC-PCR profiles with a similarity index of ≤93%, should be considered unrelated.

Two isolates from Hospital 2, detected in patients who underwent mammaplasty with an interval of 10 months, had closely related PFGE genotypes, PF2a and PF2b, but were indistinguishable by ERIC-PCR (97% similarity) and RAPD-PCR. The same surgeon performed surgery on both patients, and also on a third patient (P6), who developed infection caused by a different *M. fortuitum* strain. The results confirmed that infections at other hospitals were caused by different *M. fortuitum* genotypes, and that there was no clonal dissemination between hospitals. Unfortunately, this was a retrospective study and possible environmental reservoirs were not sampled during the outbreak; thus, no reservoir for these organisms was identified.

The results obtained suggested that RAPD-PCR with the RAPD1 primer alone is not sufficiently
discriminative to evaluate *M. fortuitum* isolates from outbreaks. RAPD-PCR primers described by Zhang *et al.* [8] for *M. abscessus* typing were also tested during the study, but were less discriminative than primer RAPD1 (data not shown).

PFGE produced the most discriminatory patterns, but is technically difficult, labour-intensive, and requires expensive equipment. PCR-based techniques are less expensive, are easier to perform, generate results in a timely fashion, and require only well-standardised protocols and equipment available in most laboratories. ERIC-PCR generated profiles that formed the same clonal groups as those recognised by PFGE. ERIC-PCR probably works in mycobacteria more like an RAPD-PCR, as ERIC sequences have been detected only in enterobacterial species [26], but the use of higher annealing temperatures (52°C) seems to make it less sensitive to reaction conditions. It was sufficiently discriminative to be useful for the generation of reliable and timely results in the investigation of outbreaks caused by *M. fortuitum.*

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