Research Article
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Epidemic of surgical-site infections by a single clone of rapidly growing mycobacteria in Brazil

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Aim: Our aim is to investigate if the clusters of postsurgical mycobacterial infections, reported between 2004 and 2008 in seven geographically distant states in Brazil, were caused by a single mycobacterial strain. **Materials & methods:** Available information from 929 surgical patients was obtained from local health authorities. A total of 152 isolates from surgical patients were identified by PCR restriction enzyme analysis of the *hsp65* gene (PRA-*hsp65*) and sequencing of the *rpoB* gene. Isolates were typed by pulsed-field gel electrophoresis (PFGE) using two restriction enzymes, DraI and AseI. A total of 15 isolates not related to surgical cases were analyzed for comparison. **Results:** All isolates were identified as *Mycobacterium abscessus* ssp. *massiliense*. Isolates from surgical patients and one sputum isolate grouped in a single PFGE cluster, composed of two closely related patterns, with one band difference. A total of 14 other isolates unrelated to surgical cases showed distinctive PFGE patterns. **Conclusion:** A particular strain of *M. abscessus* ssp. *massiliense* was associated with a prolonged epidemic of postsurgical infections in seven Brazilian states, suggesting that this strain may be distributed in Brazilian territory and better adapted to cause surgical-site infections.

Infections by rapidly growing mycobacteria (RGM) in invasive procedures began to be reported to the Brazilian National Health Surveillance Agency (ANVISA) in 2000. In the period between 2000 and 2003, only 13 RGM infections were reported. A sudden increase in notifications occurred in 2004, reaching a total of 2128 cases in 2008 [101]. Most cases reported between 2004 and 2008 were related to video-laparoscopic and arthroscopic surgical procedures. No new cases were reported after 2008.

Surgical infections related to video surgeries first appeared in the city of Belém, state of Pará (PA), in north Brazil, between 2004 and 2005 [1,2]. Since 2005, similar cases were observed in other Brazilian states [3,4]. Patients presented with signs and symptoms of localized infection at surgical sites, and showed difficulty in wound healing, nodules or abscesses frequently with draining serous, bloody or purulent material, chronic development and lack of response to conventional treatment for common skin bacterial pathogens. Most surgeries were performed using laparoscopic or arthroscopic equipment, which was disinfected by immersion in 2% glutaraldehyde for 20–30 min between surgeries and used in different hospitals. The majority of cases (>75%) were concentrated in private hospitals. Isolates obtained from surgical cases in seven Brazilian states were RGM, showing a particular pattern by the molecular identification method of PCR restriction enzyme analysis of the *hsp65* gene (PRA-*hsp65*). This pattern was termed *Mycobacterium abscessus* 2, and was shown to be characteristic of *M. abscessus* ssp. *massiliense*, a recently proposed member of the *Mycobacterium chelonae–abscessus* group [5,6]. Surgical infections in three states, PA, Goiás

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- molecular typing
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- outbreak
- surgical infection

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(GO) and Rio de Janeiro (RJ), were monoclonal, with isolates showing indistinguishable pulsed-field gel electrophoresis (PFGE) patterns [2–4].

The *M. chelonae–abscessus* group of RGM has been responsible for an increase in healthcare-associated infections throughout the world. Exposure to these bacteria involves interactions with natural and man-made environments, rather than infected patients. There are multiple reports of infection after trauma and surgical or other invasive procedures, including acupuncture, injections of licit and illicit substances, intravenous catheter use, mesotherapy and pedicures [7,8]. Nosocomial outbreaks or pseudo-outbreaks due to *M. abscessus* and *M. chelonae* have been recognized since the mid-1970s [9–12]. The common presence of these organisms in hospital tap water, their relative resistance to glutaraldehyde, organomercurial agents and chlorine, their ability to survive and grow in distilled water and their presence in biofilms make it likely that these species will continue to be a threat in the hospital setting [13].

Besides the classical species *M. chelonae* and *M. abscessus,* three other members of the *M. chelonae–abscessus* group were proposed in the 21st century: *Mycobacterium immunogenum* (2001), associated with pseudo-outbreaks by contaminated metalworking fluids [14]; '*Mycobacterium massiliense*' (2004), described after the study of a single strain from a patient with hemoptoic pneumonia [15]; and '*Mycobacterium bolletii*' (2006), characterized by multidrug resistance [16]. An extensive phenotypic and molecular characterization of bacteria from the *M. chelonae–abscessus* group led to the proposition that *M. abscessus*, '*M. massiliense*' and '*M. bolletii*' represent a single species, namely *M. abscessus.* Two subspecies were also proposed, *M. abscessus* ssp. *abscessus* and *M. abscessus* ssp. *massiliense* [6].

The objective of this study is to investigate if the strain of *M. abscessus* ssp. *massiliense* detected in surgical patients from PA, GO and RJ [2–4] was also responsible for cases in four other Brazilian states. Isolates of *M. abscessus* ssp. *massiliense* not related to outbreaks were characterized using the same molecular techniques for comparison.

Materials & methods

Demographic data of patients

Demographic data (age and sex) and type of surgery for 929 surgical patients were obtained from local health authorities in each state. A total of 14 patients unrelated to surgical cases, but collected in the same period, were included for comparison. Demographic information of these later patients was restricted to geographic location, sample origin and date of sample collection (Table 1).

Mycobacterial isolates

This retrospective study was carried out with 152 RGM isolates obtained from patients (one isolate per patient) reported to local authorities as having surgical-site infections between 2004 and 2008 in seven states in Brazil. One of these isolates, from PA, was deposited in the collection of the National Institute for Health Quality Control (INCQS) under number INCQS 594.

A total of 15 additional RGM isolates not related to surgical cases but showing the same PRA-*hsp65* pattern were also analyzed for comparison. A total of 14 were patient isolates – 13 from sputum and one from a postinjection abscess – and one was an environment isolate obtained from treated sewage (Table 2) [2,17].

Isolates were cultivated in Middlebrook 7H9 liquid medium or 7H10 agar plates supplemented with oleic acid, albumin, dextrose and catalase (OADC). Frozen stocks were stored at -80°C.

Species identification

All isolates were identified by analysis of phenotypic characteristics, growth rate and pigment production and by PRA-*hsp65*, as described by Chimara *et al.* [18]. Briefly, a 441-bp fragment of the *hsp65* gene was amplified using primers Tb11 (ACCAACGATGGTGTGTCCAT) and Tb12 (CTTGTCGAACCGCATACCCT), and digested separately with BstEII and HaeIII. The digestion patterns obtained were visualized after electrophoresis in 4% agarose gels stained with ethidium bromide and interpreted by comparison with published tables [18].

Species identification was confirmed by sequencing of the V region of the *rpoB* gene, amplified with primers MycoF (GGCAAGGTCACCCCGAAGGG) and MycoR (AGCGGCTGCTGGGTGATCATC), as described by Adékambi *et al.* [19]. Sequences obtained from 70 (46%) of 152 surgical isolates were randomly selected and the 15 nonsurgical isolates were identified based on similarity using Basic Local Alignment Tool (BLAST [102]) against the GenBank sequence database.

Pulsed-field gel electrophoresis

Isolates were typed by PFGE, as described by Viana-Niero *et al.* [2], with minor modifications. Single colonies were cultivated in Mueller–Hinton broth supplemented with 0.1%

Table 1. Demographic data of 929 patients reported with infections related to surgical procedures.

State	Age (years)	Sex (%)		Procedure (%)	Source of data	Ref.
	Average (min–max)	*M*	*F*			
PA	45 (10–89)	27.6	72.4	Cholecystectomy (58.52), hiatus hernia correction (7.07), diagnostic laparoscopy (4.82), bariatric surgery (3.54), other (26.05)	311 reported patients	[2]
GO	46 (21–71)	72.2	27.8	Arthroscopic surgery (77.8), laparoscopic surgery (22.2)	18 patients with positive cultures†	[4]
RJ	48 (14–89)	27	73	Cholecystectomy (56), diagnostic laparoscopy (7.8), appendectomy (7.1), arthroscopy (5.5), other (23.6)	146 patients with positive cultures†	[3]
ES	41 (8–83)	65	35	Cholecystectomy (33.4), bariatric surgery (20.8), arthroscopy (16.2), other (29.6)	263 reported patients	–
RS	52 (20–77)	40	60	Cholecystectomy (60), herniorraphy/ hernioplasty (30), diagnostic laparoscopy (10)	10 patients with positive cultures†	–
PR	46 (4–87)	27.3	72.7	Cholecystectomy (24.5),diagnostic laparoscopy (23.7), herniorraphy/ hernioplasty (13.1), bariatric surgery (16.6), appendectomy (11.4), other (10.7)	170 reported patients	–
SP	51 (28–83)	55	45	Cholecystectomy (92), arthroscopy (8)	11 reported patients	–

†*Mycobacterium abscessus 2 PRA-hsp65 pattern.*
ES: Espírito Santo; GO: Goiás; PA: Pará; PR: Paraná; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SP: São Paulo.

Tween 80 and incubated at 37°C. When an optical density of 0.64 at 650 nm was attained, bacteria were centrifuged and resuspended in Sodium-Tris-EDTA-Tween (STET) buffer (100 mM NaCl, 10 mM Tris, pH 8.0, 50 mM EDTA, 0.1% Tween 80). The suspension was mixed with an equal volume of 1% low-melting preparative-grade agarose (BioRad Laboratories, CA, USA) and casted into plug molds. Plugs were treated with lysozyme (10 mg/ml) for 20 h. After lysis with 1% sarkosyl and proteinase K (2 mg/ml final concentration), DNA was digested with 30 U DraI (Promega, WI, USA) or AseI (Fermentas, Vinius, Lithuania) at 37°C. Plugs were loaded on a 1% pulsed-field certified agarose gel (BioRad) in 0.5% tris-borate-EDTA (TBE) buffer (45 mM Tris-HCl, 45 mM boric acid and 1 mM EDTA), and electrophoresis was carried out in a CHEF-DR III System (BioRad) at 14°C for 21 h at 6 V/cm, with a switch time of 1.6–21.3 s. Lambda Ladder PFG Marker (NewEngland BioLabs, MA, USA) was used as molecular standard. Type strains *M. abscessus* ATCC 19977, *M. chelonae* ATCC 35752, *M. immunogenum* ATCC 700505, '*M. massiliense*' CCUG 48898 and '*M. bolletii*' CCUG 50184 were included in the analysis of PFGE patterns.

Ethics

This study was approved by the Research Ethics Committees of the Universidade Federal de São Paulo (SP; approval numbers: CEP 1287/06 and CEP 0239/08), Instituto Evandro Chagas (PA; approval numbers: CEP/IEC 001108 and 0010/2008), Instituto Adolfo Lutz (SP; approval numbers: 0002D-BM1950/2008 and BM14/08), and Universidade Federal de Goiás (GO; approval number: 001/07). Informed consent was not required because patients' data were obtained from notifications received by local surveillance services as part of routine monitoring and were analyzed anonymously (Comissão Nacional de Ética em Pesquisa Resolution Conselho Nacional de Saúde 437/05).

Results

Demographic data of patients

Figure 1 shows the geographical localization of the cities in the seven Brazilian states showing

clusters of postsurgical infections by RGM and the distribution of case notifications to the local health authorities. Table 1 shows surgical patients' demographic data and type of surgery in each state.

Mycobacterial isolates & identification

Between 2004 and 2008, 532 isolates were obtained from patients submitted to surgical procedures in seven different states in Brazil. A total of 371 isolates were identified to the species level by PRA-*hsp65* or DNA sequencing in local laboratories. In total, 360 (97%) of the 371 isolates showed the *M. abscessus* 2 PRA-*hsp65* pattern with BstEII (235 and 210 bp) and HaeIII (200, 70, 60 and 50 bp). A total of 11 (3%) isolates were identified as *Mycobacterium fortuitum* (six), *Mycobacterium porcinum* (one), *Mycobacterium wolinskyi* (one), *M. avium* (one) and *M. neoaurum* (two) (Table 2). A total of 152 (42%) among 360 isolates showing the *M. abscessus* 2 PRA-*hsp65* pattern could be recovered and were included in this study: 58 from PA in the north region [2], 18 from GO in the central-west region [4], ten from Rio Grande do Sul (RS) and six from Paraná (PR), both states in the south region; and nine from São Paulo (SP), 29 from RJ [3] and 22 from Espírito Santo (ES) in the southeast region (Table 2).

In a separate study performed in PA, RGM isolates were recovered from sputum specimens from 19 patients between 2004 and 2007 [17]. A total of 13 (68.4%) isolates showed the *M. abscessus* 2 PRA-*hsp65* pattern and were further analyzed in this study, together with one isolate from a postinjection abscess (B67) included in a previous study from our group [2].

In a study carried out in ES in 2005, aimed at isolating mycobacteria from treated sewage, a total of 21 RGM isolates were identified by PRA-*hsp65*. Only one isolate showed the *M. abscessus* 2 PRA-*hsp65* pattern, and was included in this study.

The PRA-*hsp65* pattern in the isolates of this study allowed a presumptive identification of *M. abscessus* ssp. *massiliense* [6]. This identification was confirmed by *rpoB* sequencing of a subset of 70 (46%) isolates from surgical patients and all other isolates not obtained from surgical cases. All sequences from surgical patients' isolates and the sequence from one sputum isolate (P10) showed 100% sequence similarity and were indistinguishable from the corresponding sequence from the INCQS 594 outbreak isolate (GenBank accession number EU117207). These sequences showed two characteristic substitutions (C[2683]T and T[2874]C) and a similarity of 99.72% with respect to the *rpoB* sequence of the deposited '*M. massiliense*' type strain (GenBank accession number AY593981.2).

In conjunction, the similarities of *rpoB* sequences from the 15 isolates not related to surgical cases ranged from 96.9 to 100% when compared with the corresponding sequence from

Table 2. Rapidly growing mycobacteria isolates recovered per state.

State	Isolates (n)	*Mycobacterium abscessus* 2 by PRA-*hsp65*	Studied (n; %)	Other species (n)	Not yet identified (n)	Period (year)
Surgical isolates						
PA	91	58	58 (100)	2[†]	31	2004–2005
GO	52	52	18 (34.6)	0	0	2005–2007
ES	145	79	22 (27.5)	1[‡]	65	2006–2007
RS	10	10	10 (100)	0	0	2007
RJ	148	144	29 (20.4)	5[§]	25	2007–2008
PR	52	9	6 (66.7)	3[†]	40	2007–2008
SP	10	10	9 (90)	0	0	2008
Total	532	360	152 (42)	11	161	2004–2008
Isolates not related to surgical cases						
PA	20	14	14 (70)[¶]	6	0	2004–2007
ES	21	1	1 (4.7)[#]	20	0	2005
Total	41	15	15	26	0	2004–2007

[†]Mycobacterium fortuitum.
[‡]Mycobacterium porcinum.
[§]Mycobacterium fortuitum *(1),* Mycobacterium wolinskyi *(1),* Mycobacterium avium *(1),* Mycobacterium neoaurum *(2).*
[¶]*Sputum (13), postinjection abscess (1).*
[#]*Treated sewage.*
ES: Espírito Santo; GO: Goiás; PA: Pará; PR: Paraná; PRA: PCR restriction enzyme analysis; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SP: São Paulo.

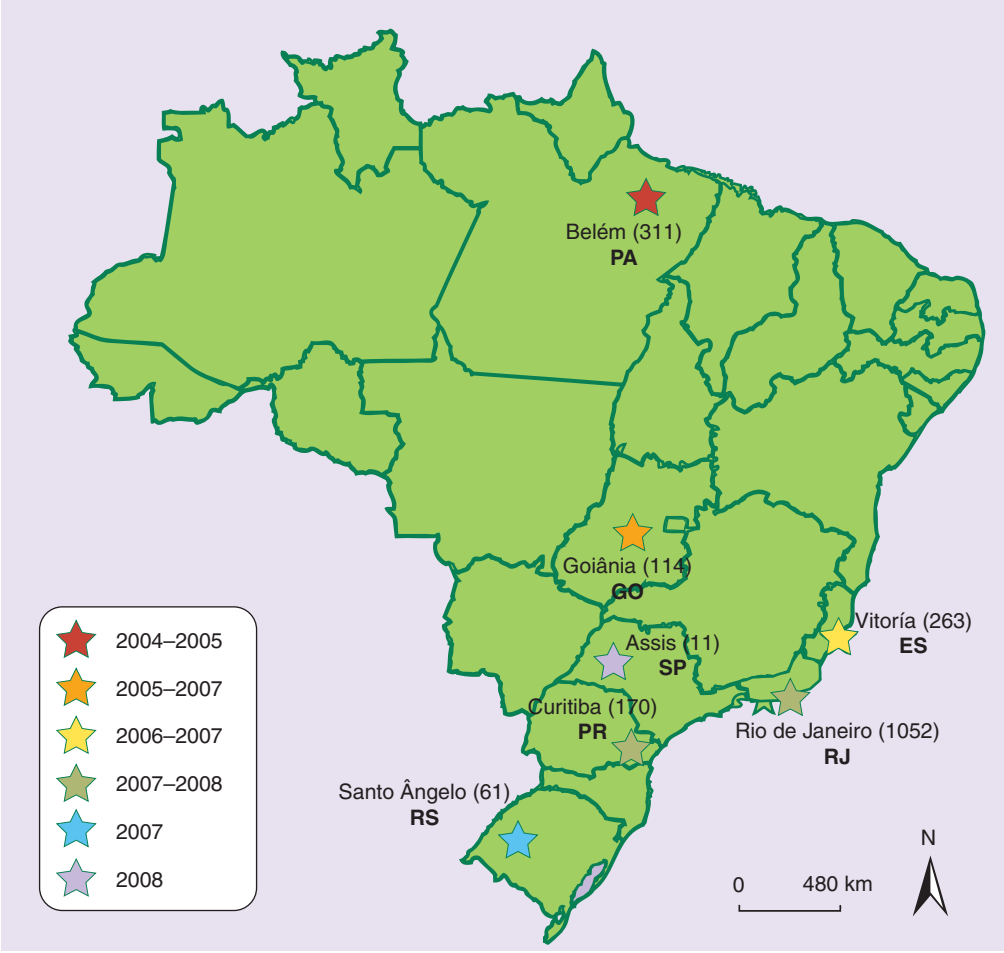

Figure 1. Cities in seven Brazilian states in which postsurgical infections by rapidly growing mycobacteria occurred. The outbreak period in each state is shown in the accompanying chart. The total number of cases reported to the local health authorities is shown in parentheses.

the '*M. massiliense*' type strain (accession number AY593981.2), from 95.8 to 100% with respect to the '*M. bolletii*' type strain *rpoB* sequence (accession number AY859692), and from 95.4 to 99.6% compared to the *M. abscessus* type strain sequence (accession number AY147164) (Table 3). The isolate from treated sewage (MG3-6) showed 100% sequence similarity with the '*M. massiliense*' type strain. One isolate from sputum (P54) was identified as *M. abscessus* 2 by PRA-*hsp65*, but the *rpoB* sequence analysis showed highest similarity (99.58%) to the *M. abscessus* type strain sequence (accession number AY147164). Additional sequencing of *hsp65*, *gyrA* and *gyrB* genes confirmed the species assignment of isolate P54 as *M. abscessus* ssp. *massiliense* (data not shown).

The *rpoB* sequences from the 15 isolates not related to surgical cases were deposited in GenBank under accession numbers EU220421, FJ590437–FJ590442, FJ590444, FJ590447, FJ590448, FJ859889–FJ859892 and GQ178075.

Molecular typing

All isolates in this study produced interpretable PFGE patterns. PFGE patterns of DraI-digested DNA for the 152 surgical isolates formed a cluster with two closely related patterns differing in a single band of approximately 50 kb (Figure 2). PFGE of AseI-digested DNA was carried out with a subset of 11 surgical isolates randomly selected, distributed among the seven states. The results confirmed the clonality demonstrated with PFGE of DraI-digested DNA. Interestingly, isolates showing the 50-kb band with PFGE of DraI-digested DNA showed a band of approximately the same size as with PFGE of AseI-digested DNA (Figure 2).

Isolates from patients unrelated to surgical cases showed DraI and AseI PFGE patterns distinct from those of the surgical isolates, except for one sputum isolate (P10), whose PFGE patterns were indistinguishable from the patterns lacking the 50-kb band obtained with the surgical isolates (Figure 2).

Table 3. Similarity of *rpoB* sequences (region V) from surgical and nonsurgical rapidly growing mycobacteria isolates with the corresponding sequences from *'Mycobacterium massiliense'* (accession number AY593981.2), *'Mycobacterium bolletii'* (accession number AY859692) and *Mycobacterium abscessus* (accession number AY147164) type strains.

State	Isolate	Similarity (%)				*rpoB* accession number
		'Mycobacterium massiliense' CIP 108297	*'Mycobacterium bolletii'* CIP 108541	*Mycobacterium abscessus* CIP 104536	Epidemic strain INCQS 594	
PA, GO, RJ, RS, ES, PR, SP	INCQS 594	99.72	98.45	96.48	100.00	EU117207
ES	MG3-6†	100	98.45	96.48	99.72	FJ859889
PA	P07‡	100	98.45	96.48	99.72	FJ590441
PA	P53‡	100	98.45	96.48	99.72	FJ859892
PA	P13‡	100	98.45	96.48	99.72	FJ590447
PA	P14‡	99.86	98.31	96.48	99.58	FJ590448
PA	P08‡	99.86	98.31	96.48	99.58	FJ590442
PA	B67§	99.86	98.31	96.34	99.56	EU220421
PA	P10‡	99.72	98.45	96.48	100	FJ590444
PA	P03‡	98.45	100	95.64	98.45	FJ590437
PA	P52‡	98.31	99.86	95.50	98.31	FJ859891
PA	P06‡	98.31	99.86	95.50	98.31	FJ590440
PA	P05‡	98.31	99.86	95.50	98.31	FJ590439
PA	P51‡	98.17	99.72	95.36	98.17	FJ859890
PA	P04‡	98.45	99.72	95.64	98.45	FJ590438
PA	P54‡	96.90	95.78	99.58	96.62	GQ178075

†*Isolate from sewage.*
‡ *Pulmonary isolates.*
§*Postinjection abscess isolate.*
ES: Espírito Santo; GO: Goiás; PA: Pará; PR: Paraná; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SP: São Paulo.

Discussion

This is a cross-sectional study of the relatedness of isolates from seven states using a convenience sample of 152 patient isolates from outbreaks affecting 929 patients. Results of identification and typing were consistent in confirming that most infections were caused by a single RGM strain. With the progression of the outbreak, patients began to be treated based on clinical and epidemiological information and/or evidence of RGM surgical-site infection. Such evidence could be either a positive culture or the observation of acid-fast bacilli in clinical samples or biopsies, assuming that the infections were most probably caused by that particular strain. This, according to new guidelines from ANVISA, was enough to initiate patient treatment. Therefore, although a total of 532 isolates were obtained, only 371 were identified to the species level and the remaining 161 isolates were not subjected to further molecular identification.

The results of PRA-*hsp65*, DNA sequencing and PFGE using two different restriction enzymes in conjunction confirmed that 152 isolates obtained from surgical patients in seven different states belonged to a single strain, with a particular *rpoB* sequevar and two highly similar PFGE patterns, showing one band difference. By Tenover criteria, PFGE patterns showing three or fewer band differences are considered highly related [20]. The clonality of the surgical isolates was also confirmed by randomly amplified polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus (ERIC) [21,22] with a subset of isolates from PA, GO and RJ (data not shown). By contrast, isolates not obtained from surgical cases showed a considerable PFGE clonal variation and only one sputum isolate (P10) grouped with the isolates from surgical patients. PFGE appears to be the gold standard for typing RGM, and stable fingerprint patterns have been obtained with this technique [23]. High PFGE discriminatory indexes were obtained for *M. chelonae* (0.993) and *M. abscessus* (0.972) [21].

No specific investigation aimed at isolating mycobacteria from medical devices or environmental sources was performed during the

outbreaks, and the source(s) of infections of these surgical infections could not be identified yet. A precise evaluation of the transmission dynamics of these NTM in this geographical region could not be performed.

Factors that could have played important roles in the origin of this epidemic were previously discussed [2–4]. The tolerance of the epidemic strain to 2% glutaraldehyde, used for disinfection of the surgical equipment between surgeries, was demonstrated [3]. Moreover, the absence of standardized cleaning protocols and the use of high-level disinfection instead of sterilization procedures for critical instruments, and the lack of quality control of the glutaraldehyde solution in use were identified in hospitals with surgical cases (data not shown). Another relevant issue was the tendency of laparoscopic and arthroscopic equipment usually being the physician's property and frequently being transported and used in different hospitals. These factors in conjunct could have contributed to the occurrence of the large number of infections caused by a single clone of an organism particularly fit to cause these outbreaks. It was observed that adjustments in the procedures for cleaning and sterilization of surgical equipment and the obligation that any external equipment must be sterilized by the hospital staff led to resolution of cases in most institutions (data not shown).

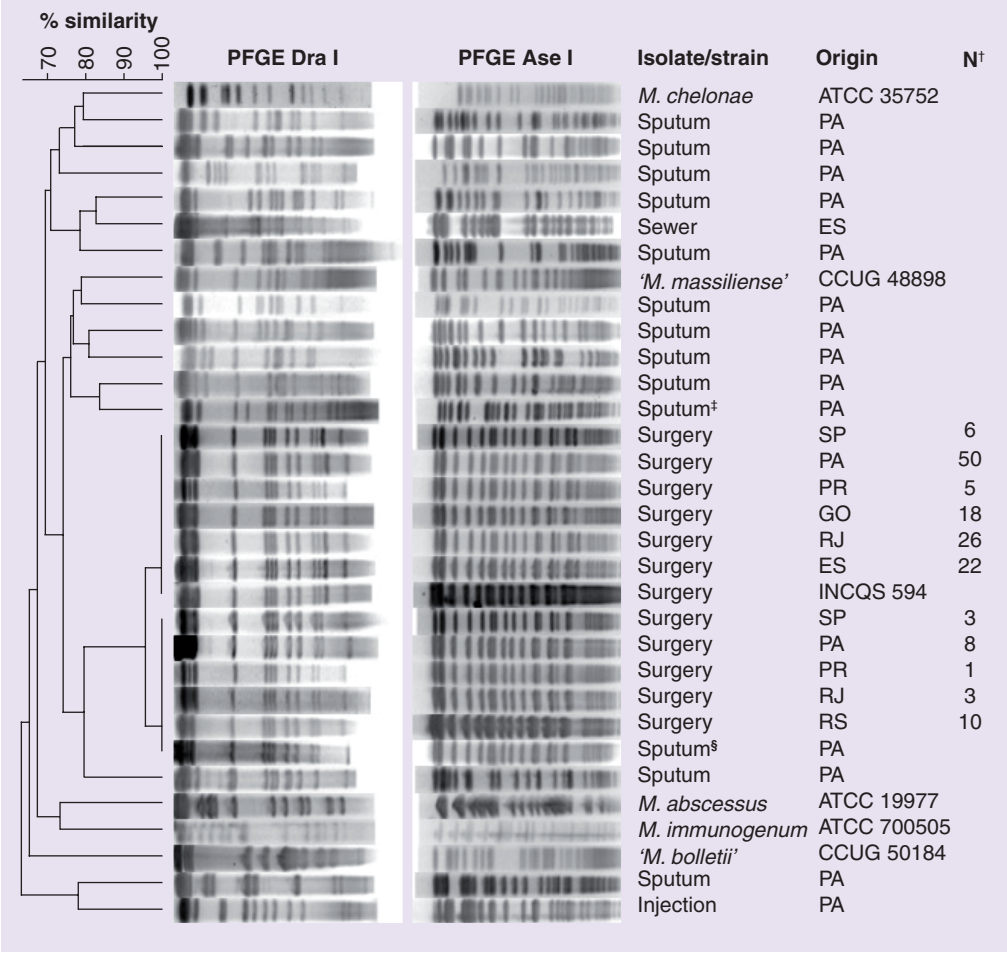

Figure 2. Composite cluster analysis of DraI and AseI pulsed-field gel electrophoresis patterns of 152 surgical isolates and the collection isolate INCQS 594, 15 isolates not related to surgical cases, and the five type strains from the *Mycobacterium chelonae–abscessus* group. Dendrograms were prepared using the BioNumerics program v. 5.1 (Applied Maths, Belgium) by the band-based Dice unweighted pair group method with arithmetic mean, based on 2% optimization and position tolerance.

†Number of surgical isolates presenting this PFGE pattern in each state.

‡Isolate P54 showing discordant identification results by PRA-*hsp65* and *rpoB* sequencing.

§ Isolate P10 showing PFGE patterns indistinguishable from the patterns lacking the 50-kb band obtained with the surgical isolates.

ES: Espírito Santo; GO: Goiás; PA: Pará; PFGE: Pulsed-field gel electrophoresis; PR: Paraná; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SP: São Paulo.

The analysis of the 161 isolates from surgical patients not identified yet (Table 2) will allow a more precise evaluation of the distribution of different mycobacterial species in surgical patients in the different states, and could be an indirect estimation of the influence of the cleaning procedures in the maintenance of the outbreak. The role of equipment contamination in the distribution of cases to different hospitals also deserves more investigation.

It is well accepted that infections by nontuberculous mycobacteria are acquired from the environment, or from contaminated water, reagents, solutions, needles or invasive equipment. The finding of isolates identified as *M. abscessus* ssp. *massiliense* in 13 pulmonary, one postinjection abscess and one sewage specimen in two different states suggests that this species may be ubiquitously present in the environment in Brazil. Moreover, one sputum isolate obtained in PA belonged to the particular strain of the surgical cases. No epidemiological relation of this pulmonary case to the surgical outbreak was found in a preliminary investigation. Bronchoscopes were not used, suggesting that this patient acquired the infection with this particular strain from other environmental sources. The presence of the epidemic strain in specimens unrelated to surgery would favor the hypothesis of the environmental origin of these infections. On the other hand, the epidemic strain was identified in only one of the 15 isolates not related to surgical cases obtained in the same period of the outbreak in PA.

Isolate P54 showed equivocal results in identification by PRA-*hsp65* and *rpoB* sequencing. This lack of congruence of molecular identification results was reported by other authors. Kim *et al*. described two isolates identified as *M. massiliense* by *rpoB* sequence analysis and *M. abscessus* by *hsp65* sequence analysis [24]. On the other hand, Zelazny *et al*. reported five isolates identified as *M. abscessus* by *rpoB* sequence analysis and '*M. massiliense*' by *hsp65* sequence analysis [25]. Both studies identified these isolates as '*M. massiliense*' by analysis of additional DNA targets. The same was observed in the isolate in this study. These results strongly suggest that the separation of *M. abscessus*, *M. massiliense* and *M. bolletii* as different species is not supported by *rpoB* sequence analysis alone, as was also recently confirmed by our group [6].

Conclusion

Molecular epidemiology techniques helped to identify that a single RGM strain of *M. abscessus* ssp. *massiliense* was responsible for most hospital infections across a large, diverse country. Our results reinforce the hypothesis that this strain may show specific biological characteristics, such as higher adaptive properties in the environment and tolerance to high-level disinfectants, which could have favored its selection in nosocomial settings. This kind of important and sudden distribution for this single strain could also indicate that a previous isolate was subjected to some sort of selective pressure and became more pathogenic. Additional studies are needed to appraise these hypotheses.

Future perspective

We have demonstrated that an organism emerged that was well suited to the medical practice of the time. With corrective actions it has diminished at specific institutions and, if actions are widely disseminated, it should diminish throughout Brazil.

In the future, it is likely that surgical mycobacterial infections will be detected and controlled sooner in Brazil and elsewhere. In fact, molecular methods for the identification and typing of mycobacteria are evolving, and the identification of genetic markers of disease is an important goal and the subject of investigation in our group. The experience acquired with this epidemic will be essential for the future management of mycobacterial infections.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

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Executive summary

Objectives of the study

- To compare mycobacterial isolates from patients with surgical infections reported to Brazilian health authorities between 2004 and 2008.
- To investigate the clonality of these isolates.
- To compare surgical isolates with isolates not related to surgical cases.

Methods

- Identification of isolates by PCR restriction enzyme analysis (PRA-*hsp65*) and *rpoB* gene sequencing.
- Typing of isolates by pulsed-field gel electrophoresis.
- Comparison of the results obtained with isolates from surgical patients and isolates not related to surgical infections.

Conclusion

- A unique strain of *Mycobacterium abscessus* ssp. *massiliense* was responsible for the majority of surgical-site infections in seven different states in Brazil between 2004 and 2008.
- Isolates from patients unrelated to surgical cases demonstrated significant clonal variation.

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