Outbreaks due to *Mycobacterium abscessus* subsp. *bolletii* in southern Brazil: persistence of a single clone from 2007 to 2011

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Received 25 February 2014
Accepted 16 July 2014

INTRODUCTION

The nontuberculous mycobacteria (NTM) species most commonly associated with localized infections of the skin and subcutaneous tissues include *Mycobacterium marinum* and *M. haemophilum* and the rapidly growing mycobacteria (RGM) (*M. abscessus, M. massiliense, M. chelonae, M. fortuitum*) (Kasperbauer & Huitt, 2013). RGM species are frequently involved in infections after traumatic injuries or surgery, and are of particular concern due to their tendency to dissemination in immunocompromised patients (Falkinham, 2013; Kothavade et al., 2013; Redelman-Sidi & Sepkowitz, 2010).

Abbreviations: AFB, acid-fast bacillus; GTA, glutaraldehyde; NTB, nontuberculous mycobacteria; RGM, rapidly growing mycobacteria.

There is a debate about the actual taxonomy of the genus; although there is evidence suggesting the presence of three subspecies of the *M. abscessus* complex, most studies in Brazil use only two subspecies, *M. abscessus* subsp. *bolletii* (which includes *M. massiliense* and *M. bolletii*) and *M. abscessus* subsp. *abscessus* (which includes *M. abscessus*) (Euzeby, 2014; Sampaio, 2010). The *M. chelonea-abscessus* group has emerged as an important cause of both community and hospital-acquired infections in humans (Koh et al., 2011). Several cases of infection due to this RGM have been reported in many countries, including Brazil (Cardoso et al., 2008; Cheng et al., 2013; Duarte et al., 2009; Léao et al., 2010; Monego et al., 2011; Viana-Niero et al., 2008).

The first outbreak of *M. abscessus* subsp. *bolletii* reported in Brazil occurred in 2004 and involved 68 patients who had
undergone laparoscopic surgery (Viana-Niero et al., 2008). A series of nosocomial outbreaks, all of them associated with invasive procedures, have since been reported in several Brazilian states (ANVISA, 2011). Representative isolates proved to be genetically related and were identified as a single clone, denoted BRA100 (Duarte et al., 2009). The BRA100 strain is known to be resistant to high glutaraldehyde (GTA) concentrations (up to 7%, after 15 to 30 min of exposure), which proves that this product is non-effective against specific strains of RGM (Duarte et al., 2009; Leão et al., 2010; Lorena et al., 2010).

The epidemiological situation in Brazil has made the government surveillance system adopt containment and control actions. These include analysis of sanitizing products in relation to the RGM susceptibility, deployment of a rapid response system for outbreak reporting, support of appropriate laboratory diagnosis of RGM infections, and an emphasis on the application of National Health Surveillance Agency guidelines (ANVISA, 2011).

Although RGM has already been reported in several Brazilian regions, scarce data are available from the state of Rio Grande do Sul (Leão et al., 2010). Thus, the aim of this study was to evaluate the susceptibility and molecular profile of RGM isolates involved in postsurgical infection outbreaks occurring in Rio Grande do Sul after the largest Brazilian epidemic (2006–2007).

**METHODS**

**Isolates and microbiological procedures.** From 2007 to 2010, four RGM outbreaks occurred in different cities, and one single case was reported in 2011, in the southernmost region of Brazil, all due to infections secondary to invasive procedures. During this period, a total of 109 clinical specimens (biopsy or aspiration of abscess fluids) obtained from patients with signs of postsurgical infections were sent to the Instituto de Pesquisas Biológicas–Laboratório Central de Saúde Pública (IPB-LACEN/RS) for investigation. The clinical specimens were processed for acid-fast bacillus (AFB) smears (Ziehl–Neelsen stain) and cultured on Ogawa-Kudoh (OK) solid medium for up to 4 weeks at 35–37 °C. Ultimately, only 43 specimens exhibited mycobacterial growth in culture, and were evaluated further.

**Species identification by PCR-restriction enzyme analysis (PRA).** DNA was extracted from colonies on OK and suspended in

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**Fig. 1.** Confirmed cases of *M. abscessus* subsp. *bolletii* from postsurgical infections in the state of Rio Grande do Sul, Brazil, 2007–2011.

**Table 1.** Susceptibility profile of 43 *M. abscessus* subsp. *bolletii* from southern Brazil

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistant [n (%)]</th>
<th>Intermediate [n (%)]</th>
<th>Susceptible [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMK</td>
<td>0</td>
<td>0</td>
<td>43 (100)</td>
</tr>
<tr>
<td>FOX</td>
<td>0</td>
<td>12 (28)</td>
<td>31 (72)</td>
</tr>
<tr>
<td>CLR</td>
<td>6 (14)</td>
<td>0</td>
<td>37 (86)</td>
</tr>
<tr>
<td>CIP</td>
<td>43 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DOX</td>
<td>43 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SXT</td>
<td>43 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MXF</td>
<td>43 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOB</td>
<td>43 (100)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AMK, Amikacin; CIP, ciprofloxacin; CLR, clarithromycin; DOX, doxycycline; FOX, cefoxitin; MXF, moxifloxacin; SXT, trimethoprim-sulfamethoxazole; TOB, tobramycin.
250 μl of 1 × Tris-EDTA (TE), boiled for 20 min, incubated in an ultrasonic water bath for 15 min and frozen at −20 °C. All 43 isolates were initially analysed using PRA of the hsp65 gene (PRA-hsp65), as described by Telenti et al. (1993), with modifications. The primers Tb11 (5′-ACCAACGATGGTGTGTCCAT) and Tb12 (5′-CTTGTGCAGAACCGCATACCCT) were used to generate a 441 bp fragment of the hsp65 gene. The PCR mixture consisted of 20 ng of mycobacterial DNA, 25 pmol of Tb11 and Tb12 primers, 10^6 in the DNASTAR Lasergene 7 Genomics Suite, and homology analysis analysed in Chromas version 2.3. Sequence alignment was performed using the BLAST) algorithm. From each isolate with those deposited in GenBank, using theBLAST was performed by comparison of the consensus sequence obtained previously described in Brazil (Duarte et al., 2009), we compare the PFE pattern of isolate CRM-0019 (from the 2006–2007 Rio de Janeiro outbreak), which belongs to the BRA100 clone, with the pattern of our isolates, and they also proved to be clonally related. Although all isolates were clonally related, they did not have identical PFE patterns; the majority of isolates (n=37) presented 13 bands (cluster I), but six isolates presented 12 bands (cluster II). The dendrogram is shown in Fig. 2.

**Results**

One hundred and nine cases of postsurgical infections were reported in Rio Grande do Sul from 2007 to 2011. There were 34 in 2007 in the municipality of Santo Ângelo; 48 in 2008 in the municipalities of Tramandai (n=44) and Santa Maria (n=4); 26 in 2010 in the municipality of Carazinho; and one case in 2011 in the municipality of Torres (Fig. 1). Whereas only 13 clinical specimens (12 %) were positive on AFB staining, 43 (39 %) grew RGM in OK medium. All were identified as M. abscessus type 2 by PRA-hsp65 and were further confirmed as M. abscessus subsp. bolletii by rpoB gene sequencing (GenBank accession number KF360857).

The isolates were 100 % susceptible to amikacin but resistant to ciprofloxacin, doxycycline, moxifloxacin, trimethoprim-sulfamethoxazole and tobramycin. Furthermore, 31 (72 %) isolates were fully susceptible to cefotixin, and six isolates (14 %) were fully resistant to clarithromycin (Table 1).

All 43 M. abscessus subsp. bolletii specimens generated interpretable patterns (12 or 13 bands) on PFGE and proved to be clonally related (>85 % of similarity). To evaluate the relationship of this clone with the outbreaks previously described in Brazil (Duarte et al., 2009), we compared the PFE pattern of isolate CRM-0019 (from the 2006–2007 Rio de Janeiro outbreak), which belongs to the BRA100 clone, with the pattern of our isolates, and they also proved to be clonally related. Although all isolates were clonally related, they did not have identical PFE patterns; the majority of isolates (n=37) presented 13 bands (cluster I), but six isolates presented 12 bands (cluster II). The dendrogram is shown in Fig. 2.

To evaluate the discriminatory power of PFGE, we evaluated the patterns of five epidemiologically unrelated M. abscessus subsp. bolletii, and all were distinguishable.

We selected a set of representative isolates to be tested for susceptibility to 2 % GTA. There were five isolates from Santo Ângelo, seven isolates from Tramandai, one isolate from Santa Maria, four from Carazinho and the only isolate from Torres. In total, we tested 18 isolates that represented 42 % of the total isolates obtained (n=43). Eight isolates (44 %) were susceptible to 2 % GTA after 30 min exposure (isolates LACEN/RS-730320, LACEN/
RS-800112, LACEN/RS-800229, LACEN/RS-700429, LACEN/RS-730101, LACEN/RS-730102, LACEN/RS-730104 and LACEN/RS-730106) and 10 isolates (56%) were resistant to 2% GTA after 30 min exposure (isolates LACEN/RS-730342, LACEN/RS-800103, LACEN/RS-800113, LACEN/RS-800196, LACEN/RS-800532, LACEN/RS-100594, LACEN/RS-100595, LACEN/RS-100698, LACEN/RS-100727 and LACEN/RS-110636).

**DISCUSSION**

This study investigated 109 patients with postsurgical infections reported from 2007 to 2011 in five different municipalities of the state of Rio Grande do Sul, Brazil. Only 43 of the collected specimens presented mycobacterial growth in the OK medium, possibly due to the poor quality of the specimen, to storage conditions, or to the low sensitivity of the culture method for diagnosis of biopsy specimens and surgical wound aspirates, especially during antimicrobial treatment. In fact, the American Thoracic Society (Griffith et al., 2007) issued diagnostic criteria in 2007, which highlight the importance of drawing culture specimens from sterile body sites when attempting to establish true infection by NTM.

Both methods used here for species identification presented identical results for all isolates. Although PRA-hsp65 can be used as a quick and reliable method to identify NTM in the clinical laboratory (Chimara et al., 2008), a more complete characterization was obtained by sequencing of the rpoB gene, allowing subspecies identification.

Interestingly, we found 14% resistance to clarithromycin among the isolates studied, demonstrating a different susceptibility profile compared with the BRA100 clone from other Brazilian regions (Duarte et al., 2009; Leão et al., 2009; Meneño et al., 2011). This result is of particular relevance because macrolide-based regimes are often used for treatment of *M. abscessus* disease (Brown-Elliott et al., 2012; Griffith et al., 2007). Moreover, clarithromycin is usually the option for therapy combined with amikacin (Griffith et al., 2007). As resistance to clarithromycin is usually induced (Brown-Elliott et al., 2012), increased use of this antibiotic may have led to the emergence of resistant RGM. The susceptibility profiles observed in our isolates for amikacin, ciprofloxacin, doxycycline, trimethoprim-sulfamethoxazole and tobramycin were the same susceptibility pattern as previously reported for the BRA100 clone (Duarte et al., 2009; Meneño et al., 2011). However, it is of note that most isolates of our study were susceptible to cefoxitin in contrast to the BRA100 from Rio de Janeiro, which tended to be resistant to this antibiotic (Duarte et al., 2009).

Our results confirmed the discriminatory power of PFGE, considering that distinct molecular profiles were observed among the isolates unrelated to the outbreaks. Although all isolates were clonally related, they did not exhibit identical PFGE patterns, resulting in two clusters with only one band (~50 kb) of difference between them. The band is thought to reflect the presence of a pMAB01-like plasmid but this was not confirmed experimentally.

Interestingly, the 43 strains (cluster I, 13 bands and cluster II, 12 bands) exhibited the same profile of the BRA100 clone, as previously reported in Brazilian outbreaks. The spread of a single clone of *M. abscessus* subsp. *bolletii* in different regions of the country seems to be related to a common source of infection, reinforcing the concept that the BRA100 clone is an emergent pathogen in the Brazilian hospital environment (Leão et al., 2010). Furthermore, recent studies indicated a molecular relationship between the BRA100 clone and strains from Taiwan, Malaysia, UK and USA, demonstrating that this clone is disseminated worldwide (Cheng et al., 2013; Davidson et al., 2013; Aitken et al., 2012). Some aspects that may explain the persistence of BRA100 in the environment are its virulence and biofilm formation, which is particularly associated with *M. abscessus* (Donlan, 2001; Duarte et al., 2009; Shang et al., 2011; Simoes et al., 2005). The main factor related specifically to Brazilian outbreaks was tolerance to 2% GTA (Duarte et al., 2009; Lorena et al., 2010), although several mitigating measures have been recommended by the National Health Surveillance Agency, as mentioned above. We found that 56% (10/18) of the BRA100 clone isolates survive abundantly after 30 min of exposure to 2% GTA, which may have contributed to the possible dissemination of this biocide-tolerant micro-organism in different hospitals.

In conclusion, our study has shown the persistence of a single clone of *M. abscessus* subsp. *bolletii*, BRA100, which was responsible for outbreaks occurring in distinct periods from 2007 to 2011 in Rio Grande do Sul. It should be stressed that this is the first report of clarithromycin resistance among isolates belonging to BRA100, which indicates an important change in the susceptibility profile of this clone. *M. abscessus* subsp. *bolletii* BRA100 has persisted despite the control measures adopted by the Brazilian public health surveillance system, indicating that these actions alone are not being effective in preventing dissemination or that they are not being implemented rigorously enough by health institutions. Therefore, our study contributes to the knowledge of the epidemiological profile and the magnitude of infections attributable to BRA100 in Brazil.

**ACKNOWLEDGEMENTS**

This study received financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico, FIP/HCPC 09-0653, ICORHTA 5 U2R TW006883-02 and ENSP-011-LIV-10-2-3 (MCT/CNPq; Edital Universal, process no. 480789/2010-0 MCT/CNPq 14/2010). The authors would like to thank the staff from Seção de Micobactérias, IPB/LACEN—FEPPS and Laboratório de Micobactérias, Instituto de Microbiologia—UFRJ, for their assistance with sample processing and culture management; Marilina Bercini of Divisão de Vigilância Epidemiológica; and Ana Luiza Ramme of Divisão de Vigilância Sanitária, Centro Estadual de Vigilância em Saúde/RS.
**REFERENCES**


