Outbreak of Mesotherapy-Associated Cutaneous Infections Caused by
Mycobacterium chelonae in Colombia

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Mesotherapy is a popular but controversial therapeutic technique, often presented as a safe non-surgical alternative to liposuction (1). It consists of mesodermal injections of various substances used to treat conditions that span from wrinkles to arthritis pain. Concerns have been raised about safety of the procedure as outbreaks of dermal infections by non-tuberculous mycobacteria (NTM) have been reported. Rapidly growing mycobacterial species such as Mycobacterium fortuitum, M. peregrinum, M. chelonae, M. abscessus, M. simiae, M. massiliense, M. bolletii, M. cosmeticum, and M. immunogenenum have been linked to such infections (2–8). The source of mycobacteria was most often traced to infected injectable solutions. We report 29 cases of NTM subcutaneous post-mesotherapy infections. Our study includes the microbiological characterization of the isolates and molecular typing using the enterobacterial repetitive intergenic consensus (ERIC) and polymerase chain reaction restriction enzyme analysis (PRA) techniques.

This study was conducted from October 2004 through February 2005 at the Corporación para Investigaciones Biológicas in Medellín, a national reference center for mycobacterial diagnostics in Colombia. Seventy patients were recruited from essentially private dermatological practices in Medellín and from several hospitals within the city. All subjects had undergone mesotherapy between October 2004 and February 2005 in different aesthetic centers (87%) or at home (13%). One practitioner had performed mesotherapy on 46 of the patients (66%). The patients were 68 females (97%) and 2 males (3%),
ranging in age from 16 to 55 years (median, 35 years old).

Skin lesions had developed from 8 to 60 days (mean, 30 days) after mesotherapy. Fewer than 5 lesions were encountered in 26 patients (37%), between 6 and 10 lesions in 19 patients (27%), between 11 and 15 lesions in 13 patients (19%), and more than 16 lesions in 12 patients (17%). Skin lesions were mapped to where the injections had been applied: gluteus in 44 patients (63%), legs in 50 patients (71%), abdomen in 61 patients (87%), arms in 42 patients (60%), and back in 36 patients (51%). The clinical presentation was characterized by pain in 58 patients (83%), erythema in 70 patients (100%), secretion in 39 patients (56%), and nodules in 58 patients (83%). Multiple symptoms were present simultaneously in 26 patients (37%).

Forty-eight subjects (69%) had received antibiotics prior to specimen culture; of these, 14 (20%) had received penicillin, 13 (19%) ciprofloxacin, and 12 (17%) cephalosporin. Additionally, 35 patients (50%) had undergone a non-surgical percutaneous drainage of the lesions.

Clinical samples were obtained by needle aspiration or biopsy from 1 lesion of each of the 70 patients. Acid-fast bacilli (AFB) staining was performed as well as culture in a liquid MGIT®-BACTEC 960 automated system (Becton Dickinson, Franklin Lakes, N.J., USA), and solid media such as Lowenstein-Jensen (LJ) medium or Middlebrook 7H11 thin layer agar. Solid media were incubated at regular oxygen atmosphere at 32°C and 37°C.

Mycobacteria recovered from either culture medium were identified according to the recommended biochemical procedures listed in Table 1 (9). Identification of the species was confirmed by PRA based on the amplification of 441-bp fragments from the hsp65 gene and restriction with BstEII and HaeIII enzymes (10). ERIC was performed as recommended by Sampaio et al. (11). ERIC results were analyzed by BioNumerics v. 4.0 (Applied Maths, Sint-Martens-Latem, Belgium) and dendrograms were based on the Dice-UPGMA method, with 1% optimization and position tolerance.

AFB were identified by direct staining in 3 cases (4%) while 29 cases (41%) had positive cultures for NTM. Among these, previously established biochemical tests allowed the identification of 26 isolates as \textit{M. chelonae}, 2 as \textit{M. abscessus}, and 1 as \textit{M. fortuitum}. Confirmation was provided by the analysis of PRA data using PRASITE at http://app.chuv.ch/prasite. A HaeIII restriction pattern of 145-, 70-, 60-, and 55-bp bands followed by 235- and 210-bp bands after BstEII digestion was characteristic of \textit{M. abscessus}, while \textit{M. chelonae} isolates gave bands at 200, 60, 55, and 50 bp with HaeIII followed by 320 and 130 bp bands after BstEII. Finally, \textit{M. fortuitum} was characterized by its typical restriction pattern of 145-, 120-, 60-, and 55-bp bands with HaeIII and 235-, 120-, and 85-bp bands with BstEII.

Drug susceptibility tests against amikacin, tetracycline, ofloxacin, and clarithromycin were performed using a microdilution broth method on Middlebrook 7H9 medium and analyzed following the Clinical and Laboratory Standards Institute (Wayne, Pa., USA) interpretive criteria (12). All 26 \textit{M. chelonae} isolates were found to be susceptible to amikacin and clarithromycin with minimal inhibitory concentrations (MICs) of \(\leq 1\) \(\mu g/mL\) and \(\leq 2\) \(\mu g/mL\), respectively (Table 2). Nineteen of these 26 isolates (73%) were susceptible to tetracycline with a MIC of \(\leq 1\) \(\mu g/mL\), 4 (15%) were classified as intermediate with a MIC falling into the 2-8 \(\mu g/mL\) range, and the remaining 3 (12%) were resistant with a MIC of \(\geq 16\) \(\mu g/mL\). Thirteen of the \textit{M. chelonae} isolates (50%) were susceptible to ofloxacin with a MIC of \(\leq 1\) \(\mu g/mL\), 7 (27%) were intermediate with a MIC falling between 1 \(\mu g/mL\) and 2 \(\mu g/mL\), and 6 (23%) were resistant with a MIC of \(\geq 4\) \(\mu g/mL\). The 2 \textit{M. abscessus} isolates were resistant to tetracycline but susceptible to the other 3 antibiotics. The \textit{M. fortuitum} isolate was resistant to both tetracycline and clarithromycin and susceptible to amikacin and ofloxacin.

Once the mycobacterial species were identified, ERIC-PCR was applied to determine the relatedness of the 29 isolates. The dendrogram was constructed with the aid of BioNumerics v. 4.0, using Dice-UPGMA method coefficients with 1% tolerance (Figure 1). In the first cluster, 24 \textit{M. chelonae} isolates (83%) had identical patterns and were therefore clearly related to each other. The second cluster was formed by the 2 related \textit{M. abscessus} isolates (7%). The remaining 2 \textit{M. chelonae} isolates and the single \textit{M. fortuitum} isolate showed different ERIC patterns and were unrelated to the other isolates. Interestingly, the same practitioner had performed mesotherapy on 12 (50%) of the patients belonging to cluster 1, the 2 patients (100%) of cluster 2 and 1 of the patients with no matching isolate. Mesotherapy was applied by different practitioners to the remaining patients of the outbreak.

In the present study, we investigated 70 cases of infections linked to mesotherapy that occurred between October 2004 and February 2005, leading the Medellin Health Authorities to announce an epidemic alert on April 19th, 2005. Positive

<table>
<thead>
<tr>
<th>Drug and MIC in (\mu g/mL)</th>
<th>No. of \textit{M. chelonae} isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amikacin</strong></td>
<td></td>
</tr>
<tr>
<td>Susceptible, (\leq 16)</td>
<td>26 (100)</td>
</tr>
<tr>
<td>Intermediate, (\geq 16)</td>
<td></td>
</tr>
<tr>
<td><strong>Tetracycline</strong></td>
<td></td>
</tr>
<tr>
<td>Susceptible, (\leq 1)</td>
<td>19 (73.0)</td>
</tr>
<tr>
<td>Intermediate, (2-8)</td>
<td>4 (15.5)</td>
</tr>
<tr>
<td>Resistant, (\geq 16)</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td><strong>Ofloxacin</strong></td>
<td></td>
</tr>
<tr>
<td>Susceptible, (\leq 1)</td>
<td>13 (50.0)</td>
</tr>
<tr>
<td>Intermediate, (1-2)</td>
<td>7 (27.0)</td>
</tr>
<tr>
<td>Resistant, (\geq 4)</td>
<td>6 (23.0)</td>
</tr>
<tr>
<td><strong>Clarithromycin</strong></td>
<td></td>
</tr>
<tr>
<td>Susceptible, (\leq 2)</td>
<td>26 (100)</td>
</tr>
</tbody>
</table>

All the \textit{M. chelonae} were tested against amikacin, tetracycline, ofloxacin, and clarithromycin and the minimum inhibitory concentrations (MICs) were determined for each antibiotic.
NTM cultures in 29 (41%) patients revealed *M. chelonae*, *M. abscessus*, and *M. fortuitum* as the causative agents.

In the *M. chelonae* and *M. abscessus* isolates, the highest percentage of drug-resistance was to ofloxacin (24%), followed by tetracycline (11%) and clarithromycin (4%). All of these isolates were susceptible to amikacin. These results differ from others reported in the United States, according to which, in 44 *M. chelonae* and *M. abscessus* isolates, the resistance to amikacin and ciprofloxacin was 10 and 86%, respectively, while all were susceptible to clarithromycin (13%). The high percentage of resistance to ofloxacin in the present outbreak could be related to the frequent use of quinolone for the treatment of bacterial infections in Medellin. An explanation for the lack of isolation of atypical etiologic agents in more than 50% of the samples could be the use of antibiotics in 68% of the cases before the samples were taken. Specifically, 19% of the subjects had received ciprofloxacin, which is active against *M. chelonae*.

The present ERIC results strongly suggest a common source of infection, possibly the injected compounds. Unfortunately, these had been discarded and were not available for culture. ERIC is a useful tool for defining the relatedness of outbreak isolates.

The simultaneous use of 3 culture methods (MGIT®, Middlebrook 7H11 thin layer agar and LJ) appeared to be critical as not all 3 media gave a positive result for each sample. Also, the dual incubation of the solid media at 32°C and 37°C is recommended. This underscores the importance of culturing clinical specimens derived from this type of lesion in both liquid and solid media.

The results presented here highlight the need to remain vigilant against potential NTM outbreaks and call for more stringent regulations to be imposed on mesotherapy practices.

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REFERENCES


Fig. 1. DNA enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis of non-tuberculous mycobacteria (NTM) isolated from 29 patients in a mesotherapy-associated outbreak, October 2004–February 2005, Medellín, Colombia. Cluster 1 is formed by *M. chelonae* (24 isolates, 10 shown: PRA39, -41, -42, -44, -52, -60, -62, -68, and -74) and cluster 2 is formed by *M. abscessus* (2 isolates, PRA51 and PRA54). *M. fortuitum* (PRA40) and other *M. chelonae* (PRA45 and PRA75) displayed different ERIC patterns.