Outbreak of nontuberculous *Mycobacteria* subcutaneous infections related to multiple mesotherapy injections

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**Running title**: Mycobacteria infection after mesotherapy
Abstract:
We describe an outbreak of severe subcutaneous infections due to nontuberculous mycobacteria (NTM) following mesotherapy. Epidemiological study and molecular comparison of *Mycobacterium chelonae* strains between patients and environment suggested that contamination could be associated with inappropriate cleaning of the multiple injection device using tap water.
Case Report:

Mesotherapy is a nonacademic healthcare-related practice involving subcutaneous injections of minute quantities of various medical drugs. First indicated for medical traumatic purposes, the practice was extended to various cosmetic and non cosmetic reasons including fat reduction, body contouring, rheumatism pain, or psycho-neurological disorders. This procedure is performed worldwide mostly by physicians, and has gained wider popularity while mostly used for aesthetic fat reduction.

In January 2007, a general practitioner notified to the health authorities and the regional centre for nosocomial infection control a cluster of subcutaneous infections due to non-tuberculosis Mycobacteria (NTM) following mesotherapy.

A complete screening was designed in all potentially exposed patients who underwent mesotherapy with the practitioner from October 1st, 2006 (first date of mesotherapy practice in the suspected medical room) to January 15, 2007 (date of disruption of mesotherapy practice). Each patient was contacted both by phone and mailing and urged to be examined by a specialist at the Department of infectious diseases of a tertiary-care reference hospital in Paris. A retrospective cohort study was performed in all exposed patients to describe the temporal and spatial distribution of cases and identify risk factors. A certain case was defined as an exposed patient with clinical subcutaneous lesions at the site of mesotherapy injections associated with positive cultures for NTM. A probable case was defined as an exposed patient with clinical subcutaneous lesions with negative smear and culture for NTM. An assessment study of hygiene practices was performed by an infection control practitioner at the outpatient clinic to determine potential risk factors to be tested in a comparative epidemiological study. Risk factors included the day and rank of outpatient visits, the site and reasons for injections. For each case, the incubation period was estimated as the time between the last mesotherapy session before symptoms and the date of the first symptoms of NTM infection. Comparisons
of means and proportions were calculated with standard statistics. As the clinic was closed on Wednesday, Saturday and Sunday, the number of visits on the day after closure, i.e. Monday or Thursday, expressed for 100 patient-visits was considered as a potential risk factor.

Multivariate analysis was performed using stepwise logistic regression with a p-to-enter and p-to-remove at 0.20. Hosmer-Lemeshow statistic was used to test the goodness of fit of the model. All calculations were performed using SAS software release 8.02 (SAS Institute, Cary, USA) and considered significant at p < 0.05.

Tap water of the medical examination room was sampled for search of mycobacteria as well as the injection device and topical creams. Products used for mesotherapy injections recovered in the office were analyzed by the laboratory of the French Agency for Sanitary Safety in Health Products (AFSSAPS). The first throw of cold water (between 18°C and 20°C) was sampled in the practitioner clinic on February 1, 2007. Detection of rapidly growing mycobacteria was performed after membrane filtration (5) and decontamination by laurylsulfate-NaOH. Typing of *Mycobacterium chelonae* strains were performed using Pulsed Field Gel Electrophoresis (PFGE) with *XbaI* as restriction enzyme at the National Reference Centre laboratory, as described (6,8,9). Gel images were analyzed by GelCompar version 3.0 (Applied Maths). The band-based Dice-unweighted Pair Group Method with Arithmetic Mean (UPGMA) method was used to prepare dendrogram of PFGE patterns and to calculate similarity indexes for *M. chelonae* isolates. To evaluate the clonality of strains from patients and environment, 9 epidemiologically unrelated strains received at the national reference centre for mycobacteria between 2004 and 2007 were tested as controls.

Among 105 exposed patients, 48 responded to the mailing and were examined by the infectious disease specialist. Overall, 16 cases were identified during an 8-month period (attack rate: 15.2%), including 12 certain cases (10 positive for *Mycobacterium chelonae* and...
1 for *Mycobacterium frederiksbergense* and 1 positive for both mycobacteria) and 4 probable cases for which abscess culture was negative for NTM. The search for other pathogens was systematically performed on clinical samples. The results were not concordant with clinical findings. The cases were in average 33 years old (range: 24 to 58 years), mainly female (15/16) and presented with 10 to 120 skin lesions, predominantly on hip area, upper legs and abdomen (figure 1). Between October, 2006 and January, 2007, the number of cases varied from 1 to 3 per week. Two latter cases were diagnosed in April and May 2007. The median incubation period was 9 days (range: 7 to 152 days). The median number of mesotherapy courses per case before infection diagnosis was 5 (range: 1 to 8) and did not significantly differ with the median number of mesotherapy courses in non infected patients (4.8 vs 5.17 respectively, p = 0.76). In the univariate analysis (table 1), NTM infection incidence rate was higher in patients having Monday or Thursday visits, being at the 2\textsuperscript{nd} rank in a series of patients on the same day to receive the injections, having cosmetic purpose for weight loss or more injections on abdomen, upper leg or hip. In the multivariate analysis, being at the 2\textsuperscript{nd} rank during the session and having a higher rate of visits on Monday or Thursday remained the only independent risk factors of NTM infection.

The hygiene practices assessment showed inappropriate cleaning of the automatic repetitive injector with non sterile tap water. Indeed, the injector was often soiled with injected products leakage out of the syringe which may favor cleaning of the material with tap water and soap. No clear recommendation for cleaning this injector was given by the manufacturer. No other breach in hygiene practices like skin disinfection or hand-hygiene was observed. The injected products were sterile and disposable injection material was for single use. Tap water sample from the room where mesotherapy acts were practiced since October 2006 showed 2400 CFU/L of *M. chelonae*. Products used for mesotherapy treatment recovered in the clinic were negative for NTM. Pulsed-field gel electrophoresis patterns of *M. chelonae* isolates from 11
patients of mesotherapy and tap water of the medical examination room showed 100% similarity indexes by Dice analysis and were considered indistinguishable (Figure 2.A and B), while non epidemiologically related control strains showed 60 to 89% of similarity indexes with the *M. chelonae* outbreak isolate (Figure 1.A). No further cases occurred after implementation of control measures.

Although outbreaks of mesotherapy-associated skin complications have already been reported (1), this is the first time that a cluster of NTM infections is clearly related to a device-prone transmission during mesotherapy cares. Our study based on epidemiological and microbiological data demonstrated a relationship with incorrect use of injecting material. Although the practitioner concerned by the current outbreak respected standard precautions of hygiene and used only sterile products, the automatic repetitive injector was evidenced as the vehicle of transmission. Such injecting device is a non-sterile material which is commonly shared and reused consecutively by the practitioner for each patient. The NTM contamination was likely to occur between two patient cares when the device was soiled during rinsing with contaminated non sterile tap water. As insufficiently dried before reuse, residual water onto the surface could suffuse along the needle and then contaminate further injections. This hypothesis is supported by other studies describing similar mechanism of NTM transmission related to non sterile water during the disinfection process of devices used for disco-vertebral or plastic surgery (2,3).

Epidemiological combined with microbiological molecular analysis provide strong evidence of contaminated tap water as a source of NTM infection. First, to be treated at the second rank in the session after the first tap water flow used to clean the injector and to have visits the day after the clinic closure could be related to prolonged water stagnation in the pipe, then
favoring NTM multiplication. Second, the presence of similar NTM profile, predominantly
M. chelonae, found in tap water and samples from subcutaneous abscesses of the cases
confirms this environmental source. In addition, comparison with control strains demonstrated
the highly discriminative power of PFGE method to identify clonal origin. Other reports have
suggested the potential role of tap water for NTM infection in non medical practices such
pedicure or beauty cares (7,10). Recently, an outbreak of Mycobacterium abscessus wound
infection was reported among lipotourists from the United States who underwent
abdominoplasty, suggesting a link with tap water used to irrigate the wound (4).

Despite some flaws as potential loss of patients (less than 50% response to our mailing), a few
cases with non documented microbiological data, or temporal discrepancy between the
occurrence of cases and NTM found in the environment, this outbreak investigation highlights
that failure in disinfection of injecting material could generate severe infections with NTM
related to non regular medical cares. Efforts should focus on information of practitioners on
hygiene practices based on appropriate guideline recommendations, especially for invasive
procedures in non hospital settings. In addition, guidelines for use and disinfection of the
automatic repetitive injector are warranted.

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References


Table 1: Epidemiological analysis of risk factors for NTM infections.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>No of exposed patients</th>
<th>Incidence per 100 patients</th>
<th>p</th>
<th>ORa**</th>
<th>95% CI**</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one visit on Monday or Thursday</td>
<td>yes</td>
<td>79</td>
<td>20.3</td>
<td>0.03</td>
<td>3.1</td>
<td>1.0-9.6</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0.9-10.6</td>
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<tr>
<td>At least one visit at the second rank</td>
<td>yes</td>
<td>32</td>
<td>28.1</td>
<td>0.03</td>
<td>3.1</td>
<td>1.0-9.6</td>
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<td></td>
<td>no</td>
<td>73</td>
<td>9.6</td>
<td>0.001</td>
<td>1.0</td>
<td>0.9-10.6</td>
</tr>
<tr>
<td>Treatment for weight loss purpose</td>
<td>yes</td>
<td>68</td>
<td>20.6</td>
<td>0.04</td>
<td>3.1</td>
<td>1.0-9.6</td>
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<tr>
<td></td>
<td>no</td>
<td>37</td>
<td>5.4</td>
<td>0.001</td>
<td>1.0</td>
<td>0.9-10.6</td>
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<tr>
<td>Injections on abdomen, upper leg or hip</td>
<td>yes</td>
<td>66</td>
<td>24.2</td>
<td>0.001</td>
<td>9.7</td>
<td>1.2-77.8</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>39</td>
<td>0</td>
<td>0.001</td>
<td>1.0</td>
<td>0.9-10.6</td>
</tr>
<tr>
<td>Rate of risk visits &gt; P 50 *</td>
<td>yes</td>
<td>53</td>
<td>26.4</td>
<td>0.001</td>
<td>9.7</td>
<td>1.2-77.8</td>
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<tr>
<td></td>
<td>no</td>
<td>52</td>
<td>3.8</td>
<td>0.001</td>
<td>1.0</td>
<td>0.9-10.6</td>
</tr>
</tbody>
</table>

* (No of visits on Monday or Thursday / total No of visits /patient)*100.
This variable is categorised by two classes less or more than the median value P50 = 33.3%

** Multivariate logistic regression estimates adjusted odds ratio and 95% confidence interval. Hosmer-Lemeshow test = 0.48
Legend to Figures:

Fig. 1. Picture of multiple subcutaneous abscesses due to *Mycobacterium chelonae* after injections for mesotherapy.

Fig. 2. *M. chelonae* PFGE patterns

Dendrogram of PFGE patterns of 20 *M. chelonae* isolates (from 11 patients of mesotherapy, tap water of the medical examination room, and 9 control strains) prepared using the Dice-UPGMA method. (B) PFGE patterns of *M. chelonae* genomic DNA digested with *XbaI*. Lane A, *M. chelonae* control strain 4; lanes B to L, *M. chelonae* isolates from patients of mesotherapy; lane M, *M. chelonae* isolate from tap water of the medical examination room; lane N, Lambda ladder PFG marker. Molecular sizes (in kilobases) of the DNA standards are given at the right side of the gel.
Figure 1:
Figure 2

A B    C  D  E    F   G   H   I     J   K  L   M    A    N
A B    C  D  E    F   G   H   I     J   K  L   M    A    N
A B    C  D  E    F   G   H   I     J   K  L   M    A    N

patient 1   (B)
patient 2   (C)
patient 3   (D)
patient 4   (E)
patient 5   (F)
patient 6   (G)
patient 7   (H)
patient 8   (I)
patient 9   (J)
patient 10  (K)
patient 11  (L)
tap water  (M)
control 1   
control 6   
control 7   
control 4   (A)
control 9   
control 2   
control 8   
control 5   
control 3   

194.0
145.5
97.0
48.5