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SHORT REPORT
Source investigation of two outbreaks of skin and soft tissue infection by *Mycobacterium abscessus* subsp. *abscessus* in Venezuela

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SUMMARY

Outbreaks of soft tissue or skin infection due to non-tuberculous mycobacteria are reported frequently in scientific journals but in general the infection source in these outbreaks remains unknown. In Venezuela, in two distinct outbreaks, one after breast augmentation surgery and another after hydrolipoclasy therapy, 16 patients contracted a soft tissue infection due to *Mycobacterium abscessus* subsp. *abscessus.* Searching for the possible environmental infection sources in these outbreaks, initially the tap water (in the hydrolipoclasy therapy outbreak) and a surgical skin marker (in the breast implant surgery outbreak), were identified as the infection sources. Molecular typing of the strains with a variable number tandem repeat typing assay confirmed the tap water as the infection source but the molecular typing technique excluded the skin marker. We discuss the results and make a call for the implementation of stringent hygiene and disinfection guidelines for cosmetic procedures in Venezuela.

Key words: Breast augmentation surgery, hydrolipoclasy therapy, infection source, *Mycobacterium abscessus*, VNTR typing.

Non-tuberculous mycobacteria (NTM) are omnipresent inhabitants of a wide variety of environmental reservoirs, including natural and tap water and soil [1]. NTM skin and soft tissue infections (SSTIs) have an increasing incidence; infection occurs by inoculation of the bacteria following trauma of the skin. In some cases the infection sources for outbreaks of skin and soft tissue caused by NTM have been associated with solutions contaminated with NTM that were used in the invasive procedure or with inadequately sterilized medical equipment which became contaminated with NTM, most probably through tap water [1]. However, in most outbreaks the infection source remains unknown.

The species of the *Mycobacterium abscessus* group are an important cause of several outbreaks of disseminated or localized SSTI in humans [2]. *M. abscessus* encompasses three subspecies: *M. abscessus* subsp. *abscessus, M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* [2]. Antimicrobial treatment for an infection with a member of the *M. abscessus* group is difficult, due to both natural and acquired resistance to most of the currently available antibiotics [2–4]. In Venezuela, infection with *M. abscessus* group in skin

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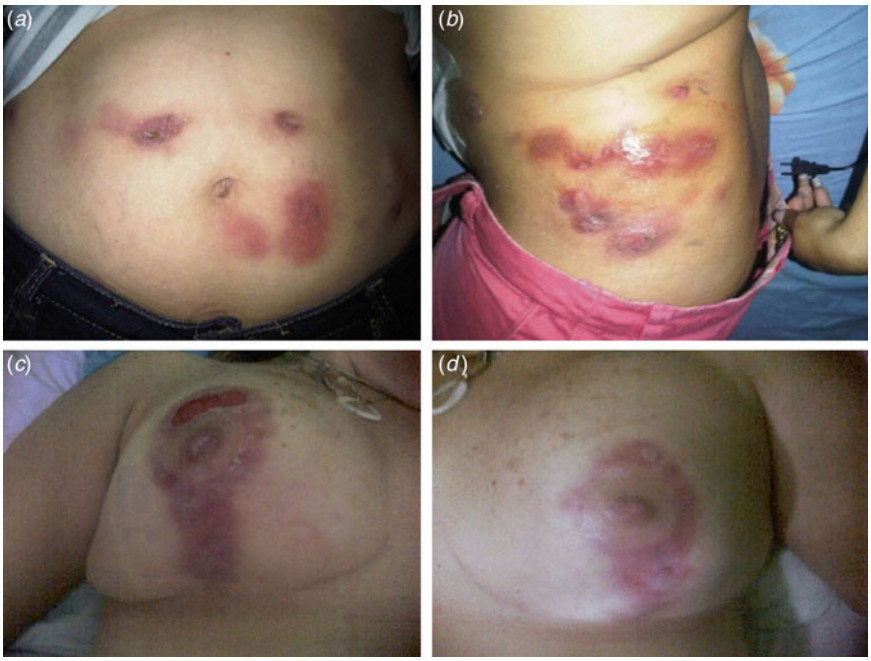

Fig. 1. Two different female patients infected with *M. abscessus* subsp. *abscessus* after hydrolipoclasy therapy. (*a*) Abdomen of the first patient. (*b*) Left flank of the second patient. (*c, d*) A female patient affected in both breasts by a *M. abscessus* subsp. *abscessus* infection after a breast augmentation operation.

or soft tissue has been reported after cosmetic or surgical procedures such as mesotherapy, acupuncture, breast augmentation surgery, subcutaneous or intramuscular injection and liposuction, often coming in outbreaks involving from two up to dozens of persons [3, 4]. In 2010, we reported an outbreak due to *M. abscessus* subsp. *abscessus* after mesotherapy affecting 68 patients who had been treated by the same therapist and in the same office, the largest registered outbreak ever associated with this alternative therapy [4]. In this outbreak, while searching for the infection source, *M. abscessus* was isolated from an environmental sample in the clinic. However, polymerase chain reaction (PCR)-based strain typing techniques (ERIC-PCR, BOXA1R, RAPD) showed that the patient's isolates were indistinguishable from one another indicating a common source, but different from the environmental isolate. No medical preparations used in this mesotherapy outbreak were available for analysis and we concluded that this outbreak was most likely caused by a contaminated injectable mesotherapy product and not by mycobacteria present in the clinic environment where the therapy had been practised [4].

Here we report two other distinct outbreaks due to *M. abscessus* subsp. *abscessus* that occurred recently in Venezuela. In the period between April and May 2011, we received in our laboratory six samples of secretions from abscesses of the abdominal wall of six female patients (age range 25–40 years), who underwent hydrolipoclasy therapy in a beauty salon located in the city of Merida, Venezuela. All patients had been treated in February of that year by the same therapist and in the same office. We registered another outbreak in November 2012 in which ten patients (age range 22–41 years) presented with infection in the surgical wounds after breast augmentation procedures in a private clinic in Caracas, the capital city. Interviews with the patients and the personnel of the clinic determined that all patients had been operated on by the same plastic surgeon and in the same operation room 3 months earlier, in September 2012. Samples were obtained from five patients for mycobacterial culture.

In both outbreaks, all patients showed multiple lesions consisting of indurated erythematous papules and nodules that progressed to fluctuant abscess formation (Fig. 1). The localization of the lesions coincided with the sites where the injections were applied for the hydrolipoclasy therapy or with the incisions made by the surgeon for breast augmentation (Fig. 1). There was no lymphadenopathy and the results of routine laboratory tests were normal. Patients presented onset of the lesions 20–60 days (average 30 days) after they had undergone a hydrolipoclasy therapy or breast surgery.

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Most (14/16) patients had earlier bacterial cultures that were negative for growth after 48 h of incubation or grew opportunistic bacteria. In both outbreaks, all 16 patients had undergone at least one antibiotic therapy course without improvement of the symptoms. Nine fast growing NTM isolates were obtained from the clinical samples (five isolates of the breast augmentation outbreak and four of the hydrolipoclasy therapy outbreak). The nine NTM strains were identified as *M. abscessus* subsp. *abscessus* by the PCR-restriction enzyme analysis (PRA)-*hsp65* technique [5]. All patients were treated with surgical debridement along with a combination of amikacin and clarithromycin for 3 weeks followed by a maintenance phase of 4 months treatment with clarithromycin only. Five of the ten breast augmentation patients had their implants removed because deeper tissue layers were compromised by the infection and we suspected biofilm formation on the breast implants. All patients evolved satisfactorily and were declared cured after this treatment period.

To determine the infection source, the surgeon and the hydrolipoclasy therapist were interviewed. The plastic surgeon revealed that he had operated in two other clinics in Caracas at the time of the outbreak and that in these clinics no infection with NTM had occurred in the patients who underwent breast implant operations, a strong indication of an internal infection source in the outbreak clinic. The hydrolipoclasy therapist did not contribute information useful for outbreak research. Environmental samples were taken from the operating room where the breast operations were performed and from the office of the hydrolipoclasy therapist. Samples included tap water, environmental swabs, disinfectants, lidocaine, surgical material, gauzes, anaesthetics and surgical skin markers (Methylene Blue and Gentian Violet). For the isolation of mycobacteria the medical instruments, gauzes and environmental swabs were soaked overnight in a minimal volume of sterile distilled water. This water and also the tap water of the medical offices were decontaminated for 20 min with 1-hexadecylpyridinium chloride (HPC, Sigma, USA) in a final concentration of 0·05% (w/v) and filtered through a 0·45 µm cellulose membrane. The filters were washed with sterile water and placed upside down on tryptone soy agar plates supplemented with 5 % (v/v) glycerol and PANTA [6] and incubated at 30 °C and 37 °C. After 2 days of incubation, the membranes were removed from the plates and the culture plates were checked during a period of 6 weeks for the growth of acid-fast bacilli. Disinfectants, skin markers and other medical solutions were centrifuged (20 min at 4000 ***g***) and the sediments were inoculated directly on culture media. Two NTM isolates were obtained, one from the surgical skin marker (breast augmentation outbreak) and one from tap water (hydrolipoclasy therapy), and both isolates were identified as *M. abscessus* subsp. *abscessus* by the PRA-*hsp65* technique [5].

To determine an epidemiological link between the patients and the environmental isolates all *M. abscessus* subsp. *abscessus* strains were analysed with a variable number tandem repeat (VNTR) typing assay, which evaluates 18 different loci [7]. This technique has been shown to have a high discriminatory power [7]. Two control strains were included in the analysis: *M. abscessus* ATCC 19 977 and a clinical isolate of *M. abscessus* subsp. *bolletii*. Based on the patterns obtained with VNTR typing, all breast implant patients were infected with the same strain (VNTR pattern **1**,**3**,**0**,**3**,2,3,4,3,**1**,**8**,**2**,**1**,**5**,**2**,2,**2**,3,2). However, the *M. abscessus* strain isolated from the surgical skin marker (VNTR pattern **6**,**2**,**1**,**2**,2,3,4,3,**2**,**3**,**3**,**4**,**3**,**5**,2,**4**,3,2) did not match the pattern of the strains isolated from the breast implant patients (non-matching loci are highlighted in bold). The isolates from the patients and tap water in the hydrolipoclasy outbreak were indistinguishable by VNTR typing (VNTR pattern 6,2,**3**,2,2,3,4,3,2,3,3,4,3,**4**,2,4,3,2) but different from the patients' strains isolated in the breast augmentation outbreak (non-matching loci are highlighted in bold), strongly suggesting that this outbreak was related to contaminated tap water.

Antibiotic susceptibility testing using the microdilution method, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards) [8], confirmed the relationship between the strains isolated from the hydrolipoclasy outbreak and tap water from the therapist's office. All these isolates were susceptible to amikacin [minimum inhibitory concentration (MIC) $\leqslant 1$ µg/ml], clarithromycin (MIC $\leqslant 2$ µg/ml), imipenem (MIC 4 µg/ml) and intermediately susceptible to linezolid (MIC >16 µg/ml). In the breast augmentation outbreak, the susceptibility patterns differed between the patients' isolates and the isolate from the skin marker. The skin marker strain was susceptible to ciprofloxacin (MIC 1 µg/ml) while the patients' strains were resistant to this drug (MIC 4 µg/ml).

In conclusion, although the surgical skin marker initially was assumed to be the source of infection for the

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breast augmentation patients, this could not be confirmed by VNTR typing and therefore the origin of this outbreak remains unknown. The source of infection in the hydrolipoclasy outbreak was most likely the tap water that contaminated the injected products or the medical devices used in the procedure. Moreover, all the patients' lesions were initially misdiagnosed as pyogenic abscesses and in both outbreaks all had an unnecessary delay of approximately 2 months before a definitive diagnosis of a NTM infection was made. NTM infection should be considered in patients who develop late-onset SSTI after injection or surgical intervention, particularly if they do not respond to conventional antibiotic treatment as was the case in both outbreaks described here.

As far as we know this is the first report of an outbreak with NTM after hydrolipoclasy therapy. A thorough review of the literature revealed only one prior publication on mycobacterial infection associated with this procedure; a case report of a woman in Brazil who became infected with *M. fortuitum* after undergoing this alternative therapy [9]. Hydrolipoclasy therapy is widely applied in the United States and Europe and it has been reported as a viable alternative for the treatment of localized fat deposits without the side-effects of liposuction procedures. It is less invasive and uses the injection of a normal saline or hypotonic solution and ultrasound waves that act directly on local adiposity.

We show that molecular VNTR typing is a powerful tool for the study of the infection source in outbreaks caused by the *M. abscessus* group. This technique has a high discriminatory power and thus is helpful for the investigation of outbreaks or pseudo-outbreaks. VNTR typing has been used in molecular fingerprinting of *M. abscessus* group isolated from sputum, bronchoalveolar lavage and paediatric cystic fibrosis patients [10] but never before in the characterization of outbreaks of SSTIs. In order to avoid future outbreaks due to infections with NTM, we call for the implementation of stringent hygiene and disinfection protocols in cosmetic procedures used in Venezuela.

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DECLARATION OF INTEREST

None.

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