# Outbreak of *Mycobacterium haemophilum* Infections after Permanent Makeup of the Eyebrows

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**We report a *Mycobacterium haemophilum* outbreak after permanent make-up of the eyebrows performed by the same freelance artist. Twelve patients presented an eyebrow lesion and cervical lymphadenitis. All were treated with antibiotics. Surgery was required in 10 cases. *M. haemophilum* DNA was identified in the make-up ink.**

Mycobacterial infections, mainly due to rapid growing mycobacteria, are increasingly recognized as a complication of cosmetic procedures. Outbreaks have been described after liposuction [1] and mesotherapy [2] as well as after nonmedical cosmetic procedures, such as tattooing [3, 4]. Here we describe an outbreak of 12 cases of *Mycobacterium haemophilum* skin infection and lymphadenitis occurring after permanent make-up of the eyebrows, a procedure that involves the introduction of pigment droplets into the superficial layer of the dermis [5].

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## Methods

Patient specimens were obtained by skin biopsy of the eyebrows or lymph node fine needle aspiration under ultrasound control for direct examination (auramine-rhodamine acid fast staining), culture and molecular diagnosis. Broad-spectrum mycobacterial polymerase chain reaction (PCR) targeting the 16S rRNA encoding gene was used directly on native samples to amplify mycobacterial DNA [6]. In order to enhance the sensitivity, a 2 step nested-PCR was performed (forward nested-primer 271 5'-CTTAACACATGCAAGTCGAAC-3'; reverse nested-primer 259 5'-TTTCACGAACAACGCGACAA-3') amplifying a 550 bp product, followed by direct sequencing.

As *Mycobacterium haemophilum* was strongly suspected on the basis of PCR results, culture was set up in MGIT tubes supplemented with 0.2 mL of hemin (Sigma-Aldrich), at a concentration of 1.6 mg/mL, as well as on chocolate blood agar plates. All cultures were incubated at 30°C for 8 weeks. For positive cultures, identification of the mycobacterial isolates was performed by sequencing [7].

## Results

The index patient was referred to our infectious diseases outpatient clinic in April 2009 because of a skin lesion of the eyebrow and ipsilateral suppurative lymphadenitis that appeared 5 weeks after permanent make-up of the eyebrows (Figure 1*A* and 1*B*). Acid-fast bacilli were seen on the smear of the lymph node aspiration. A broad-spectrum mycobacterial PCR was positive, and *Mycobacterium haemophilum* was identified by sequencing. *Mycobacterium haemophilum* grew after 14 days in the hemin-enriched broth. During the following 8 months, 11 additional cases of eyebrow lesions associated with lymphadenitis were identified and referred to our clinic. All arose after permanent makeup of the eyebrows. Figure 2 summarizes the timing of the outbreak.

All 12 patients were female, median age 56 years. None of the patients was immunosuppressed. Median incubation time was 3 weeks (range 2–7 weeks). Patients presented an inflammatory lesion of one eyebrow, consisting of a few red papules or pustules or an erythematous plaque. In all cases the lesion was associated with ipsilateral lymphadenopathy in the parotid region, affecting 1 or more lymph nodes (median 2, range 1–5). Eight patients presented with an abscess (which later developed in to a fistula in 7 cases). None of the patients reported systemic symptoms.

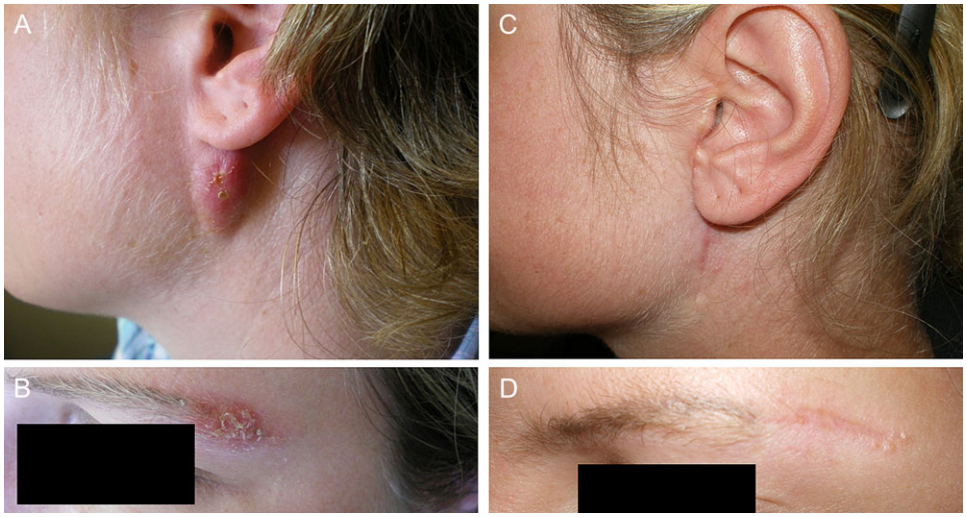


**Figure 1.** Typical clinical manifestation of *M. haemophilum* infection after permanent makeup. *A,* Inflammatory lymphadenopathy affecting the parotid region. *B,* Erythematous plaque over the eyebrow tattoo. *C,D,* Outcome after surgery and antibiotic treatment.

Ten patients had a microbiological diagnosis of *M. haemophilum*. For the remaining 2, diagnosis was based on clinical presentation and epidemiological link (permanent make-up performed by the same artist). Lymph node fine needle aspirate and skin biopsy of the eyebrow were positive for *M. haemophilum* in 9 and 2 patients, respectively. Microscopic examination showed acid-fast bacilli in 8 patients, whereas broad spectrum mycobacterial PCR was positive for *M. haemophilum* in 5 patients. Culture supplemented with hemin was positive in 10 patients after 9–70 days (median, 32 days) and the microorganism was identified as *M. haemophilum* in all positive cultures.

All the strains showed the same susceptibility/resistance pattern: susceptibility to amikacin at a concentration of 10 mg/L, to clarithromycin at 4 mg/L, to moxifloxacin at 0.5 mg/L, to ofloxacin at 10 mg/L, to rifabutin at 1 mg/L and to rifampin at 10 mg/L, whereas they were resistant to linezolid at a concentration of 4 mg/L.

Systemic antibiotic treatment consisting of a triple-drug combination of clarithromycin, ciprofloxacin, and rifabutin was started after diagnosis of *M. haemophilum* infection. Patients unable to tolerate rifabutin were treated with rifampin. Once susceptibility testing was completed, ciprofloxacin was changed to moxifloxacin.

Patients were scheduled for surgery if they did not respond to antibiotic treatment after 2 months (7 cases) or if they could not tolerate combined therapy (2 cases). Patient 5 underwent parotidectomy without preoperative antibiotic treatment. Of the 10 patients treated surgically, 9 underwent partial parotidectomy, local eyebrow excision and selective neck dissection. Since the lymphadenopathy was located within the parotid gland, parotidectomy was required to allow facial nerve dissection. Patient 6 underwent lymph node and fistula resection without parotidectomy. No major complications of surgery were observed and cosmetic results were satisfactory (Figure 1*C* and 1*D*).

Antibiotic treatment was continued for 3 months after surgery or, if no surgery was performed, until complete remission. Median duration of antibiotic treatment was 30 weeks (range 15–46 weeks). There were 10 treatment modifications because of side effects. Seven patients stopped rifabutin because of the following toxicities: moderate to severe neutropenia (2 patients), liver enzyme elevation (2 patients), severe uveitis (1 patient), severe malaise with myalgias (1 patient), and nausea (1 patient). Two patients developed a rash (1 on rifampin and 1 on clarithromycin). Finally, 1 patient developed tachycardia due to a clarithromycin-salmeterol interaction.

All patients who underwent parotidectomy ($n = 9$) and the patient who had adenectomy only were free of infection after a median follow-up of 6 months after surgery, as assessed by clinical and ultrasound evaluation. The 2 patients treated with antibiotics alone (patients 2 and 10) were also free of infection after a treatment duration of 9 and 8 months, respectively. Thus, only 2 of 11 patients had a response to antibiotic treatment alone (patient 5 had no preoperative antibiotic treatment).

Limited outbreak investigation showed that all procedures were performed by the same tattoo artist, who worked freelance in different cosmetic studios. No major infractions of standard hygienic rules were identified. The tattoo artist stated that she had performed permanent make-up on ~400 women during the outbreak period, but it was not possible to identify and contact these women. Microbiological investigations of oil and cold sterilizing agents were all negative. Concerning the inks, direct examination was negative in 18 samples. However, in 6 of these

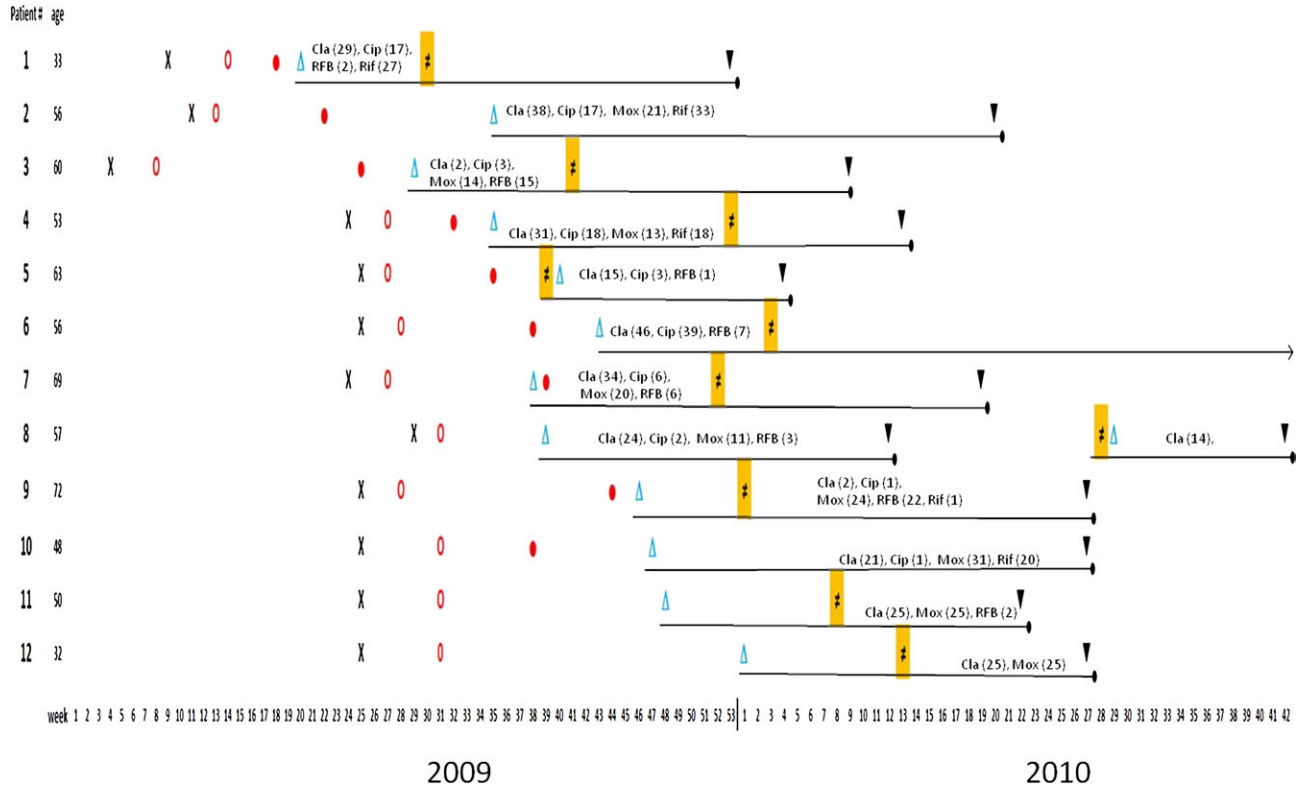


**Figure 2.** Timescale of permanent makeup application, onset of clinical manifestations, and treatment in the 12 patients described in the text. Legend: X: Permanent make-up application; 0: : Onset of symptoms; ●: Diagnosis (first contributive clinical specimen); Δ: Start of antibiotic treatment; ▼: End of antibiotic treatment; ≠: Surgery; Antibiotics (treatment duration in weeks): Cla: clarithromycin; Cip: ciprofloxacin; Mox: moxifloxacin; RFB: rifabutin; Rif: rifampin

samples, broad-spectrum PCR was positive for *M. haemophilum*. All hemin-supplemented cultures were negative.

## Discussion

Non-tuberculous mycobacteria (NTM) are an emerging cause of infections after cosmetic procedures [1, 2]. Here we report an outbreak of *M. haemophilum* lymphadenitis after permanent make-up of the eyebrows occurring in 12 women. Although the source of contamination could not be identified definitively, the makeup ink used by the tattoo artist was shown to be contaminated with *M. haemophilum* DNA by PCR. Since the natural habitat of *M. haemophilum* might be water [8], we hypothesize that the ink was contaminated by tap water. Unfortunately, the tattoo instruments and the tap water could not be investigated for mycobacteria, as they were discarded or unavailable for culture. One further limit of epidemiological investigation was the inability to identify and contact the women who had permanent makeup by the same artist during the outbreak period.

*M. haemophilum* was first recognized as a pathogen in immunocompromized patients, with a preference for skin infections because it grows at 30°C [9]. Cervical lymphadenitis in children is the other classical clinical manifestation and recent series demonstrate that *M. haemophilum* is the second most common cause of lymphadenitis after *M. avium* in the pediatric population [10]. Recently, 7 cases of *M. haemophilum* cervical lymphadenitis among immunocompetent adults were published [11].

Because the organism does not grow on standard media, some cases of *M. haemophilum* infection may be missed. In the present case, broad-spectrum mycobacterial PCR proved to be extremely useful for outbreak recognition. However, since it is not widely available, special cultures for *M. haemophilum* are recommended in patients presenting with chronic lymphadenitis [7, 11].

As for many other NTM, there are no standardized susceptibility methods for *M. haemophilum*. However, antimycobacterial drugs that appear to be active in vitro are clarithromycin, rifabutin, rifampin, and fluoroquinolones, whereas isoniazid and ethambutol are usually inactive and amikacin and sulfonamides variably active [7, 9]. In a randomized controlled trial involving 100 children, surgery was found to have a higher success rate than antibiotic treatment alone [12]. There are almost no data on the treatment of lymphadenitis among immunocompetent adults.

Our cases confirm that the management of *M. haemophilum* infection among immunocompetent adults is primarily surgical. All operated patients were free of infection, whereas only 2 of 11 patients had a response to antimicrobial treatment. Surgery appeared to be safe in our patients, but it carries a risk of temporary or permanent damage to the facial nerve with a reported incidence as high as 12% [12]. In our patients, antibiotic treatment was poorly tolerated (10 interruptions due to side effects). As we observed no recurrence following adequate surgical excision, and given the results of the randomized trial in children [12], we propose that postoperative antibiotic treatment may be avoided.

In conclusion, we describe an outbreak of *M. haemophilum* lymphadenitis after permanent make-up of eyebrows occurring in 12 women. The index case was diagnosed by direct broad-range mycobacterial PCR. Surgery was required in most cases. Finally, as we observed both low efficacy and poor tolerance of antibiotic treatment, its precise role merits further study.

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***Potential conflicts of interest.*** All authors: no conflicts.

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