Mycobacterium chelonae is a non-tuberculous Mycobacterium spp. classified in the rapidly growing mycobacteria group. M. chelonae has been isolated from various environmental sources, including tap water, water tanks, industrial sources, and even medical instruments. Disruption of the skin barrier caused by trauma, surgical intervention or cosmetic procedures facilitates the entry of different bacteria that can produce localized or, usually in immunosuppressed patients, systemic infections. We describe here an outbreak of M. chelonae infection in five immunocompetent patients who were being tattooed by the same artist.

CASE REPORTS

Five otherwise healthy patients attended our clinic between September 2008 and April 2009 because of the presence of skin lesions in tattooed areas, all of which had been recently administered in the same tattoo parlour. The series includes 3 men and 2 women with a mean age of 21 years (age range 18–23 years). More than one type of ink was used in every patient, but in all patients the lesions were restricted to the grey areas of the tattoos. The delay in onset of the clinical lesions varied from 3 to 30 days. Lesions consisted of red papules with remarkable superficial hyperkeratosis in most cases. Patient 3 also presented with frank pustules (Figs 1 and 2).

Histopathological examination showed a dense lymphohistiocytic inflammatory infiltrate mainly involving the superficial dermis in all cases. In cases 2 and 3, well-developed granulomas were easily found, with many Langhans-type giant cells and, occasionally, central necrosis. In cases 4 and 5, granulomas were also seen, but these were made up only of epithelioid histiocytes with few, if any, giant cells. Suppuration, with the presence of polymorphonuclear cells, was noted in some of the granulomas, but none showed central necrosis. In case 3, histopathological examination revealed a dense, band-like, lymphohistiocytic infiltration in the superficial dermis. Only after serial sections and careful scrutiny, poorly-formed, histiocytic, granulomas were seen. These showed neither central necrosis nor suppuration. Acid-fast staining for bacilli was negative in all cases.

Paraffinized samples were processed according to the technique described by Loeschke et al. (1). After deparaffinization, DNA was extracted and processed using GenoType CM Kit (Hain, Germany), according to the supplier’s protocol, except for a prolonged PCR second cycle (25 instead of 20 of the following cycles: 25 s at 95°C, 40 s at 53°C and 40 s at 70°C). PCR products were then hybridized with the GenoType CM strips according to the manufacturer’s instructions. Hybridization was positive for positions 1, 2, 3, 5 and 10, which are the positions for identification of M. chelonae. Skin biopsy specimens were cultured in BACTEC MGIT 960 System (Becton Dickinson Microbiology Systems, Spersk, MD, USA) and Coletos (Pronadisa/lab Conda) medium. Positive mycobacterial cultures were obtained in 3 cases (patients 1, 4 and 5). The molecular methods described above were used for the identification of M. chelonae in patients 2 and 3, being positive in both cases. The susceptibility testing was performed by the Etest® (AB Biodisk, Solna, Sweden) on Muller–Hinton agar with blood. The minimal inhibitory concentrations (MIC) of the isolates (in mg/l) were clarithromycin 2, imipenem > 32, amikacin 4, cefoxitin > 32, doxycycline > 256, cotrimoxazole > 32, and ciprofloxacin > 32.

Three patients were initially treated with clobetasol propionate ointment due to suspicion of an allergic reaction to the tattoo ink. Clarithromycin 500 mg twice a day was administered in patients 1 and 4 until resolution (after 3 and 5 months, respectively). In two patients antibiotic therapy either had to be suspended after 1 week (patient 5) or reduced to a half-dose after the initial 3 weeks of therapy, for an additional 11 weeks.
of treatment (patient 3), due to gastric intolerance. In addition, topical gentamicin twice a day was also prescribed in all patients. Patient 2 refused any treatment and did not attend the follow-up visit, but reported complete resolution of the lesions after being contacted by telephone.

Two other clients of the same parlour who presented with similar lesions in the grey areas of their tattoos were evaluated in our clinic, but were lost to follow-up after the first visit.

DISCUSSION

Infectious complications are the most important potential sequelae in association with tattoos when the aseptic conditions of the procedure are not maintained or when the dye used for injection is not sterile. *Staphylococcus* and *Streptococcus* infections have an historical interest as causes of erysipelas, cellulitis and gangrene (2). Local skin lesions, such as verruca vulgaris (3), molluscum contagiosum (4) or superficial mycosis, have also been described. Systemic infections are the biggest concern and they include hepatitis (5), HIV (6), syphilis (5), leprosy (7) or tuberculosis (8). Atypical mycobacteria infections secondary to tattooing have been reported previously (2, 9–12).

In our series, the source of infection may have been either the water used to dilute the ink for obtaining the grey colour or the containers used for mixing the ink. The owner of the establishment stated that he always used distilled water in tattoo procedures, therefore a possible origin in the tap water used to wash the mixing containers can be speculated.

The detection of atypical mycobacteria in clinical samples raises concern about evaluation of their significance. These organisms are environmental; thus they could appear in these samples as the result of contamination or colonization (13). Detection of these organisms using molecular tools can increase this problem because of the high sensitivity of these techniques. However, detection of *M. chelonae* in extrapulmonary samples is frequently significative (14). Moreover, the detection of these organisms in an outbreak setting (such as the one described here) is difficult to interpret as potential contamination, especially because the pathology and outcome of the patients is similar to confirmed cases, and because our molecular study is a retrospective one using samples that were highly suspicious of being *M. chelonae* infections.

There is some controversy regarding susceptibility testing in rapidly growing mycobacteria because of the difficulties in standardization and reproducibility of the results, but the American Thoracic Society recommends the broth microdilution MIC determination (15). Even when this method was not used in our patients, clarithromycin was prescribed according to the Etest® with a good clinical response in all treated patients.

Although auto-resolution of an atypical mycobacterium infection in an immunocompetent patient is possible, the clearance of lesions in patient 2 could not be confirmed as she was only contacted by telephone. A potential concern with such untreated, but apparently cured patients with subcutaneous atypical mycobacterium infection, is the possibility of reactivation if their immune status changes. We suggest that long-term follow-up of these patients would be good practice.

We report here five well-documented cases and other two non-confirmed cases of *M. chelonae* infection related to the grey areas of tattoos administered in the same tattoo parlour. Atypical mycobacterial infection should be considered when skin lesions develop following a tattoo or any other procedure that affects the subcutaneous tissue.

REFERENCES