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## *Corynebacterium ulcerans* in an Immunocompromised Patient with Diphtheria and Her Dog

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***Corynebacterium ulcerans* causes zoonotic infections, such as diphtheria and extrapharyngeal infections. We report here the first case of a diphtheria-like illness caused by *C. ulcerans* in France and transmitted likely by a dog to an immunocompromised woman.**

### CASE REPORT

A 47-year-old woman was admitted in the emergency room at the Bicêtre University Hospital (Le Kremlin-Bicêtre, France) for severe dyspnea in October 2003. She was immunocompromised due to treatment by prednisone at 6 mg/daily, tacrolimus at 12 mg/daily, and mycophenolate mofetil at 1 g/daily since she had undergone a kidney graft 1 year previously. Ten days prior to her admission to Bicêtre Hospital, the patient had been treated to no avail for sinusitis by oral amoxicillin-clavulanic acid (2 g/daily).

Physical examination revealed severe stridor. She had fever at 38°C and mild tachycardia. The endoscopic examination identified a pseudomembranous exudate covering the nasopharynx and laryngeal vestibulum, ulceration, and subglottic constriction. A blood test found an increase of C-reactive protein (100 µg/ml) and a white blood cell count of 3,500/µl. This severe dyspnea indicated intubation, and the patient was subsequently hospitalized in the intensive care unit.

Throat swab showed coryneform and gram-positive rods. After 24 h of incubation on sheep blood agar at 37°C in 5% CO<sub>2</sub>, shiny and whitish colonies grew that produced a slight hemolysis. Microscopic examination of these colonies revealed gram-positive, coryneform rods arranged in palisades. They were catalase positive, showed a positive reaction for urease, were negative for pyrazinamidase, and fermented glucose, ribose, maltose, and glycogen. These characteristics corresponded to those of *Corynebacterium ulcerans*. Biochemical identification was confirmed by partial sequencing of the 16S rRNA gene that showed 99% identity of the sequence of that strain with that of a *C. ulcerans* reference strain (11).

Since *C. ulcerans* may acquire lysogenic β-corynephages coding for a diphtheria-like toxin (DT), PCR test (using primers DT1 and DT2 for detection of *tox* gene of *C. diphtheriae* (6)) was performed that showed the presence of the DT-like gene

in *C. ulcerans*. Disk diffusion susceptibility testing was performed on Mueller-Hinton blood agar (supplemented with 5% [vol/vol] sheep blood) after overnight incubation at 35°C and 5% CO<sub>2</sub>. In the absence of standardized breakpoints for *Corynebacterium*, antibiotic susceptibility was determined by using the NCCLS criteria for *Streptococcus* spp. other than *Streptococcus pneumoniae* (10). *C. ulcerans* was susceptible to benzylpenicillin, amoxicillin, cephalothin, cefotaxime, imipenem, erythromycin, clindamycin, pristinamycin, gentamicin, trimethoprim, sulfonamides, ciprofloxacin, rifampin, and vancomycin and naturally resistant to fosfomycin, nalidixic acid, and colistin.

The patient (after treatment with intravenous amoxicillin-clavulanic acid for 5 days prior to the identification of *C. ulcerans*) was treated with intravenous benzylpenicillin adapted to her renal function during 9 days and one dose of diphtheria antiserum (40,000 U; Aventis, Romainville, France) as recommended (1). She was discharged without sequelae.

Although the patient had received diphtheria toxoid as a child, no diphtheria antibodies (<0.1 U/ml) were found in her serum sampled before the development of the disease and analyzed retrospectively by a commercially available enzyme immunoassay kit (Virotech, Russelsheim, Germany).

Although person-to-person transmission of *C. ulcerans* has not yet been reported, a contact investigation was initiated. *C. ulcerans* was searched in the throat of family members, patients of the hospital vicinity, and health care workers ( $n = 88$ ). A novel selection culture medium, one that used the property of the natural resistance of *Corynebacterium* spp. to fosfomycin, was used for this purpose. It was made of Columbia agar with sheep blood (5%) added and with colistin (10 µg/ml), nalidixic acid (15 µg/ml), and fosfomycin (125 µg/ml). This medium was utilized since the selective medium used for isolation of *C. diphtheriae* (Tinsdale medium) was not readily available. No other carrier of *C. ulcerans* was detected. All contact patients received prophylactic antibiotics (amoxicillin at 3 g/daily; allergic patients received erythromycin at 2 g/daily for 10 days) and diphtheria toxoid vaccination as recommended for persons exposed to diphtheria (1, 3).

Our patient had not traveled abroad recently in countries where diphtheria incidence remains high and had not direct

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FIG. 1. Chronic labial ulceration of the dog. The arrow points out the ulceration.

contact with dairy livestock or unpasteurized dairy products. However, she had a dog with at least a 3-year history of chronic labial ulceration, sneezing, and rhinorrhea (Fig. 1). It used to burrow in the ground. *C. ulcerans* strain was recovered from the tonsils, nose, and labial ulcerations of the animal. This strain could have been responsible for the chronic ulcerations but could have been considered as a colonizing agent of these chronic lesions. Automated ribotyping by using the Ribo-Printer (Qualicon, Wilmington, Md.) and the endonucleases BstEII and PvuII demonstrated that the patient and dog isolates corresponded to a single strain that was different from collection strains of *C. ulcerans* (Institut Pasteur, Paris, France) (Fig. 2). An amoxicillin-containing treatment (2 g/daily during 15 days) failed to eliminate the bacteria from the dog, and the animal was euthanized. Histopathologic examination of the dog tonsils showed gram-positive, coryneform rods partly arranged in palisades without sign of necrosis.

Finally, sequencing of the *tox* gene from the *C. ulcerans* strain identified a gene identical to that of *C. ulcerans* A6361, a strain isolated from a human skin ulceration in Germany (12). This gene encoded a DT that differed from *C. diphtheriae* toxin by 25 amino acid substitutions out of 501 amino acids. Therefore, DT from *C. ulcerans* and *C. diphtheriae* are 95% identical at both the nucleotide and the amino acid levels.

*C. ulcerans* was first isolated in 1926 by Gilbert and Stewart from human throat lesions (4). In 1995, Riegel et al. proposed

*C. ulcerans* as a distinct species within the *C. diphtheriae* group on the basis of molecular analysis of genomic DNA (11).

The main reservoir of *C. ulcerans* seems to be cattle, in which it may induce mastitis (9, 15). Our patient did not have risk factors associated with *C. ulcerans* infections, such as drinking raw milk or contact with farm animals or their waste (5, 9, 15). Recently, *C. ulcerans* producing diphtheria-like toxin was isolated from cats with nasal discharge in the United Kingdom (13). The dog was likely first colonized or infected since it had a long history of sneezing and chronic ulcerations and then may have transmitted the bacteria to its owner, who developed diphtheria. However, a common source of contamination and even a transmission from the woman to the dog cannot be ruled out.

The patient in the case study had received diphtheria toxoid as a child. Although it is well known that antibody titers in serum decrease in immunocompromised patients, including those who have undergone renal graft, diphtheria-like disease is not known in these patients. Moreover, the diphtheria toxoid is made of inactivated *C. diphtheriae* toxin that is different from *C. ulcerans* toxin. Thus, diphtheria antitoxin might be less efficient for preventing diphtheria due to *C. ulcerans* than that due to *C. diphtheriae*.

That *C. ulcerans* infections can mirror classical cases of diphtheria may be linked to the ability of *C. ulcerans* to produce a DT similar to that of *C. diphtheriae* (and *C. pseudotuberculosis*), although it is expressed at a lower level (12). Here, the *tox* gene of this *C. ulcerans* strain was identical to that of *C. ulcerans*

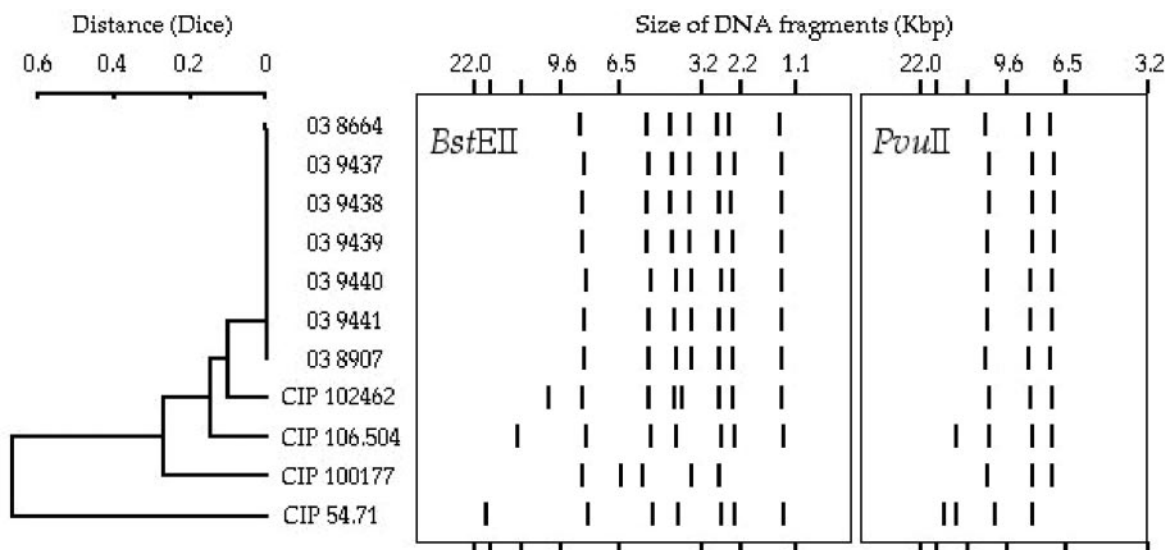


FIG. 2. Ribotype analysis by using RiboPrinter data and software Taxotron. The analysis considered two bands as identical when their size difference was <5% of their size. The complement of the Dice coefficient was used for calculating distances (averaged for both enzyme data) and clustering (unweighted pair-group method with arithmetic averages). The patient strain was 03 8664. All other numbers (03 9437, 03 9438, 03 9439, 03 9440, 03 9441, and 03 8907) were dog isolates. Collection de l'Institut Pasteur strains were epidemiologically unrelated *C. ulcerans* isolates.

A6361, a strain isolated from extrapharyngeal disease in Germany (12). This result may indicate that *C. ulcerans* toxins could be identical in strains responsible for extrapharyngeal infections and in those responsible for diphtheria and that *C. ulcerans* toxins are different from *C. diphtheriae* toxins (12). These results have the following implications: (i) *C. ulcerans* diphtheria probably does not result from a simple transfer of phages coding for the *C. diphtheriae* toxin from *C. diphtheriae* to *C. ulcerans*; (ii) toxins of *C. ulcerans* may induce either extrapharyngeal infection or diphtheria, depending more of the immunological status of the patient rather than on the nature of the toxin; and (iii) animals may be an environmental reservoir for sporadic cases of *C. ulcerans* diphtheria.

The incidence of *C. ulcerans* infections in humans remains low; only a few cases have been reported in Denmark, Germany, Switzerland, The Netherlands, the United States, and Japan (2, 5, 7, 8, 14). In the United Kingdom, 24 cases of toxigenic *C. ulcerans* infections have been reported from 1993 to 1999 (8). To our knowledge, this is the first case of diphtheria due to *C. ulcerans* in France. The rarity of cases might be due to a low pathogenicity of *C. ulcerans* or its toxin (12).

Human-to-human transmission of *C. ulcerans* has not been reported, but many researchers recommend isolation of infected patients (2, 9). Rapid identification of carriers should benefit from the use of this novel fosfomicin-containing culture medium, which can be conveniently prepared in any routine laboratory.

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