First Isolation of *Clostridium amygdalinum* from a Patient with Chronic Osteitis

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Received 12 June 2006/Returned for modification 22 June 2006/Accepted 27 July 2006

We describe a case of osteitis caused by a new and unusual *Clostridium* species, *Clostridium amygdalinum*, an environmental, moderately thermophilic bacterium. This is the first documented report of human infection caused by this organism.

CASE REPORT

A 34-year-old man sustained in November 2003 a bifocal open fracture of the right tibial shaft (rated Gustilo IIIB). The index trauma was a motorcycle accident, and the patient’s open leg fracture stayed in contact with the ground for about 6 h before he was admitted to the primary care institution. In the emergency unit, the fracture was treated by external fixation, and the wound was closed with the help of plastic surgery (local muscle flap and skin graft). The patient was admitted to our institution in October 2005 because of septic nonunion of the right tibia shaft despite the fact that the external fixation was maintained. The diagnosis of septic nonunion was reached due to the two productive fistulas located at the anterior aspect of the leg combined with failure of external fixation and the persistence of the fracture on X-ray images. At that time, surgical management consisted of setting up a new extended external fixation combined with a surgical excision at the level of the right tibia (soft tissue debridement and removal of 10 cm of necrotic tibial bone). During that surgery, 14 biopsy specimens were obtained. Eight of them revealed after culture only the presence of the same undefined strain AIP 321.05. Bacterial growth was not seen in two biopsy specimens. The patient was then treated with linezolid (600 mg twice daily) for 2 months. Two months later, the patient had not regained hyperthermia, and no bony consolidation was observed. A second reconstructive surgery was performed, consisting of a vascularized fibular bone graft. Several biopsies were performed. Two revealed after culture only the presence of the same unidentified strain AIP 321.05, as evidenced by 16S rRNA gene sequencing. *C. sporogenes* was not found. The same antibiotic treatment was continued. The evolution was favorable, with increasing features of bone union on X-ray images and no recurrence of infection. The recovery of the strain AIP 321.05 in six of the operative specimens at a 2-month interval led us to conclude that this bacterium was the cause of the chronicity of the infection for this patient.

Strain AIP 321.05 was characterized by routine biochemical tests (10). Metabolic end products were assayed by quantitative gas chromatography as described previously (3). The bacterium was lecithinase and lipase negative on egg yolk agar plates. Gelatin and milk were not modified. Production of catalase and indole was not detected. Sulfite was reduced but not nitrate. Desulfoviridin was not produced. In Trypticase-glucose-yeast extract broth abundant gas was produced. The major metabolic end products were acetic acid, propionic acid, and butyric acid. Acid was produced from cellobiose, galactose, glucose, fructose, maltose, mannitol, melibiose, raffinose, ribose, sucrose, and trehalose. Acid was not produced from arabinose, esculin, inositol, lactose, mannose, melezitose, rhamnose, salicin, sorbitol, and xylan. The strain hydrolyzed esculin. Terminal spores were observed. Culture supernatant fluids were nontoxic to mice. According to the criteria of the Comité de l’Antibiogramme de la Société Française de Microbiologie for susceptibility testing of anaerobes (8), the organism was considered susceptible to penicillin, ampicillin, amoxicillin, amoxicillin-clavulanate, cephalexin, cefotaxin, imipenem, metronidazole, tetracycline, and rifampin. Taken together, the physiological properties did not enable affiliation of the isolate with any defined taxon. The 16S rRNA gene sequence was determined as described previously (4). The 1,362-nucleotide sequence was compared to all eubacterial 16S rRNA gene sequences available in the GenBank database by using the multisquence Advanced BLAST comparison software from the National Center for Biotechnology Information (1). Alignments were done with CLUSTAL W (13). The phylogenetic tree revealed that strain AIP 321.05 falls into cluster XIVa of *Clostridium* (7), with highest sequence similarity (99.5%) to an environmental strain, namely, *Clostridium amygdalinum* (11) (Fig. 1). Closely similar degrees of 16S rRNA gene sequence identity (98.7 to 98.5%) were observed with two sulfate-reducing strains of *Clostridium* spp. isolated from a hot...
spring in Bolivia. However, except for their capacity to degrade xylan and to reduce sulfate, no description was available for these organisms, which were designated "Clostridium sulfatireducens" 38-1, and "Clostridium boliviensis" E-1, under GenBank accession numbers AY943861 and AY943862, respectively. Strain AIP 321.05 also showed 98.2% sequence similarity with the Clostridium saccharolyticum type strain DSM 2544 (12) and Clostridium sp. strain DR7, which has not been identified to the species level (GenBank accession numbers Y18185 and Y10030, respectively).

Strain AIP 321.05 has been deposited in the Collection de l'Institut Pasteur under accession number CIP 109224, and in GenBank the accession number for the 16S rRNA sequence is DQ507245.

Table 1 presents a comparison of the biochemical reactions between the isolate and related species. Clostridium saccharolyticum differs from strain AIP 321.05 in that it is a gram-negative and nonmotile rod. Clostridium amygdalinum is a newly described gram-positive, spore-forming rod (11) that has been isolated from an anaerobic-digester sludge. The authors of that study reported that this species is moderately thermophilic with optimum growth at 45°C and uses benzyaldehyde as an electron acceptor to form benzyl alcohol. Our isolate also grows at 45°C. Moreover, in Trypticase-yeast extract broth (without glucose) supplemented with 10 mmol of benzyaldehyde/liter, it forms benzyl alcohol in approximately equal amounts as determined by gas chromatography (data not shown). To our knowledge, this property has been reported only for two species previously described, namely, Clostridium amygdalinum and Soehngenia saccharolytica (11), and for Clostridium acetobutylicum (9). However, a concentration of 10 mmol of benzyaldehyde/liter inhibited the growth of C. acetobutylicum. Moreover, this bacterium required the presence of glucose and butyrate in the medium (9). Thus, the present isolate resembles Clostridium amygdalinum in phenotypic and phylogenic characteristics, except that it does not produce indole and produces larger amounts of propionate and butyrate.

The genus Clostridium is widely distributed in nature, existing primarily in soil, sewage sediments or as part of the gastrointestinal bacterial flora of humans and animals. At present, there are more than 160 recognized species, but only a few species have been implicated in human illness. In general, toxigenic or endogenous species are recognized as causative agents of food poisoning, gas gangrene, tetanus, botulism, ulcerative colitis, septicemia, and postoperative infections in humans. In contrast, most environmental clostridia are considered seemingly harmless nonpathogenic bacteria. However, it should be noted that such bacteria have already been implicated in a serious human infection (5). In addition, unidentified spore-forming anaerobic bacteria are often isolated from

![FIG. 1. Dendrogram showing the phylogenetic position of strain AIP 321.05 among representatives of cluster XIVa of Clostridium based on 16S rRNA gene sequences. The numbers above the branches are bootstrap percentages from 100 resampled data sets. The reference sequences were obtained from the GenBank/EMBL databases. Accession numbers are given. The bar represents a 1% sequence difference.](image-url)
clinical specimens. For example, Brook (2) found that 1,543 samples processed for anaerobes yielded 113 isolates of Clostridium spp., 38% of which were unidentified. Therefore, in order to help the clinician, it is important to make a description as complete as possible of these uncommon organisms when recovered. Our case report highlights the importance of C. amygdalinum as a potential human pathogen.

REFERENCES