Osteosynthesis-Associated Bone Infection Caused by a Nonproteolytic, Nontoxigenic \textit{Clostridium botulinum}-Like Strain

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A nonproteolytic, nontoxigenic \textit{Clostridium botulinum} strain identified by conventional and molecular techniques as type B-, E-, or F-like (BEF-like) was isolated from a human postsurgical wound. All previous reports of such strains have been from environmental sources. Since toxin production is the main taxonomic denominator for \textit{C. botulinum}, a new name is needed for nonproteolytic, nontoxigenic BEF-like clinical isolates.

CASE REPORT

A 50-year-old man was hospitalized in June 2002 for an open supracondylar fracture of the right humerus. His past medical history included several fractures between 1984 and 1999, psoriatic arthritis, and ankylosing spondylitis treated by corticotherapy up to 2001. The fracture was treated by open reduction and internal fixation with a Lecestre-type plate. The following week, the patient presented an inflammatory scar with fistula formation. Laboratory investigations revealed an erythrocyte sedimentation rate of 105 mm/h and a C-reactive protein value of 68 mg/liter. Surgical excision of infected tissues and drainage were performed. The patient was treated with intravenous ofloxacin (200 mg twice daily) and rifampin (1 g twice daily). Biopsy specimens of the triceps and articular capsule revealed after culture the presence of \textit{Clostridium perfringens} and \textit{Sphingomonas paucimobilis}. The patient was then treated with intravenous ceftriaxone (2 g once daily) and oral ofloxacin (200 mg twice daily) and metronidazole (200 mg twice daily) for 1 week. The symptoms gradually resolved, and the patient left the hospital on day 29 with trimethoprim-sulfamethoxazole (two tablets three times daily), ofloxacin (200 mg twice daily), and metronidazole (500 mg three times daily) prescribed. One month later, the patient developed an osteosynthesis-associated bone infection. Extensive debridement was performed, and all purulent-appearing bone was resected. The plate was removed and reinserted by using a gentamicin- and vancomycin-loaded spacer. The same antibiotic therapy was continued, and all purulent-appearing bone was resected. The plate was removed and reinserted by using a gentamicin- and vancomycin-loaded spacer. The same antibiotic therapy was continued, and the patient was discharged on day 74. A strictly anaerobic, gram-positive, motile, spore-forming rod, designated AIP 355.02, was the only bacterium isolated from all samples obtained during surgery. It was found to be susceptible to all antibiotics used except for trimethoprim-sulfamethoxazole and gentamicin.

Strain AIP 355.02 was characterized according to conventional tests (13). Metabolic end products were assayed by quantitative gas chromatography as described previously (5).

The organism was lecithinase negative and lipase positive on egg yolk agar plates. Gelatin was liquefied, and milk was not modified. Production of urease and indole was not detected. Acid was produced from glucose, fructose, maltose, mannose, ribose, starch, and sorbitol. Acid was not produced from arabino-lose, cellobiose, esculin, galactose, glycerol, inositol, lactose, mannitol, melezitose, melibiose, raffinose, rhamnose, sucrose, salicin, trehalose, and xylose. The strain hydrolyzes starch but not esculin. Abundant gas was produced. The major metabolic end products were acetic acid and butyric acid. These cultural and biochemical properties were consistent with those of nonproteolytic \textit{Clostridium botulinum} (group II). However, a mouse toxicity test for botulinum toxin was negative.

The identity of strain AIP 355.02 was subsequently confirmed by the sequence of the 16S rRNA gene. A PCR using universal 16S ribosomal DNA (rDNA) primers p584 (5′-AGA GTTGTGATCATGGCCTCAG-3′; 8-27f by the \textit{Escherichia coli} numbering system) and p995 (5′-TACGGCTACCTTGTTAC GACTT-3′; 1492-1513r) was performed. The PCR product was sequenced on an Applied Biosystems automatic sequencer (Genome Express, Meylan, France) in both directions by using forward and reverse primers. The 1,323-nucleotide sequence was compared with 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). The alignment was done with CLUSTAL W (18). The highest sequence similarity value (100%) was obtained with the 16S rDNA sequence of the nonproteolytic \textit{C. botulinum} type B strain (Ekland 17B; ATCC 25765T). Lower percentages of identity (99%) were observed with the other \textit{C. botulinum} strains belonging to group II, namely, \textit{C. botulinum} type E (ATCC 9564T and Iwanai strains) and \textit{C. botulinum} type F (ATCC 23387T), after which, the more closely related species was \textit{Clostridium uliginosum}, which exhibited a more distant affinity (97%). From the distance matrix values, a dendrogram was constructed by using the Fitch and Margoliash algorithm (9). The phylogenetic tree revealed that strain AIP 355.02 belongs to group II (nonproteolytic \textit{C. botulinum} types B, E, and F) and is genetically highly related to the toxigenic \textit{C. botulinum} type B strain, ATCC 25765T (Ekland 17B) (Fig. 1). This close genetic affinity is consistent with...
the phenotypic characters. Although the very high levels of rDNA sequence similarities (99 to 100% identity) do not allow definition with certainty of the toxigenic type to which this bacterium is related, these phylogenetic and biochemical characteristics clearly demonstrate that AIP 355.02 is an authentic nonproteolytic C. botulinum strain despite its nontoxigenic property. The possible presence of unexpressed neurotoxin genes as reported for C. botulinum type A (7, 10) was evaluated by PCR using specific primers (8). No type A, B, E, F, and G neurotoxin genes were detected.

Currently the species C. botulinum is defined on the basis of production of botulinic neurotoxins (types A to G) and encompasses four distinct metabolic groups (I to IV). Groups I and II are toxic to humans. Group I includes C. botulinum toxin type A and proteolytic strains of C. botulinum toxin types B and F. Group II contains all toxin type E strains and the nonproteolytic strains of toxin types B and F (12). Clostridium sporogenes displays very high 16S rRNA sequence homology and DNA relatedness with proteolytic C. botulinum toxin types A, B, and F and is considered as the nontoxigenic counterpart of group I (14). This species has been isolated from environmental sources in addition to multiple human infections, such as bacteremia, cutaneous abscesses, postsurgical wounds, leg or foot ulcers, and other pyogenic infections (2, 6). Similarly, the isolation of nontoxigenic Clostridium strains phenotypically highly related to group II has been frequently reported (4, 16, 17). All strains described hitherto were obtained from fish and environmental samples. So far, none had been isolated from human clinical specimens, except for one not documented isolate, obtained from an ear (11). Here we report one case of postoperative osteoarticular infection caused by a nonproteolytic, nontoxigenic C. botulinum-like strain.

For the clinician, the results described above create a problem for bacterial identification. They also highlight the nomenclatural problem evoked by Campbell et al. (3) for defining nonproteolytic C. botulinum and closely related nontoxigenic strains. Presently, there is no species name for these bacteria, which are often designated as nontoxic, nonproteolytic C. botulinum-like. Although unsatisfactory from a taxonomic point of view, this status can be sufficient for the environmental strains. However, the occurrence of this organism in a postoperative infection creates an urgent need to give a name to these nontoxic variants, to avoid confusion for the medical staff and microbiologists. In the present classification, the strains belonging to group I (proteolytic strains) are designated by two species names. The name C. botulinum must be conserved for toxigenic strains, while C. sporogenes is retained for nontoxigenic strains (15). A similar distinction between toxigenic strains and their nontoxigenic counterparts would be very useful within metabolic group II.

**Nucleotide sequence accession number.** Strain AIP 355.02 has been deposited in Collection de l’Institut Pasteur under accession no. CIP 107861. The GenBank accession no. for the 16S rDNA sequence is AY303799.
REFERENCES