Clostridium clostridioforme and Atopobium minutum Clinical Isolates with VanB-Type Resistance in France

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Acquired vancomycin resistance in Gram-positive anaerobes has been reported only in Australia and Canada from rare vanB-positive stool samples in the absence of vancomycin-resistant enterococci (VRE). We report the emergence of VanB-type resistance in Clostridium clostridioforme and Atopobium minutum involved in human infections in France.

CASE REPORTS

Case 1. A 59-year-old man was admitted to the emergency room of the Central Hospital of Nancy in France for acute abdominal pain with vomiting. The patient had a past medical history that included aortobifemoral bypass surgery complicated by an abdominal wall eventration treated by placement of a prosthetic patch in 2000 and a cystoprostatectomy in 2008. Abdominal and pelvic computerized tomography (CT) indicated hydropneumoperitoneum. Exploratory laparotomy visualized stercoral peritonitis adjacent to a perforated loop of the intestine. The treatment included peritoneal lavage, ligation of ileal fistulas, and antibiotic therapy, including ceftriaxone and metronidazole. The patient left the intensive care unit (ICU) 9 days after antibiotic therapy, including ceftriaxone and metronidazole.

A. minutum was found to be resistant to vancomycin (MIC > 256 μg/ml) but remained susceptible to teicoplanin; in contrast, E. faecium and E. durans were susceptible to glycopeptides.

Case 2. A 45-year-old man was admitted to the Saint Joseph Hospital in Paris to cure a deep pressure sore with tissue necrosis. The patient had a long history of spina bifida which led to a chronic sacral bed sore. CT visualized profound bony destruction, and the patient was treated by surgical debridement combined with ceftriaxone and minocycline, leading to clinical improvement. Bacterial culture of deep infected tissue yielded Atopobium minutum, Enterococcus faecalis, Enterobacter cloacae, Prevotella oralis, and Staphylococcus aureus. Interestingly, A. minutum was found to be resistant to vancomycin and susceptible to teicoplanin.

A. minutum, formerly designated Lactobacillus minutus, is a Gram-positive, non-spore-forming, nonmotile, and strictly anaerobic bacillus that has been classified in the family Coriobacteriaceae (4). Atopobium spp. are members of the human commensal microbiota which have been reported only rarely in oral infections, abdominal wounds, blood, and pelvic abscesses, and in most instances, these bacteria were found associated with other microorganisms (15).

Microbiological data. C. clostridioforme strain CIP (Collection of Institut Pasteur) 110249 was identified by API 20A and Rapid ID 32 A strips (bioMérieux, Marcy l’Etoile, France). Identification of CIP 110250, an obligatory anaerobic, Gram-positive, non-spore-forming rod-shaped bacterium, could not be achieved biochemically. Definitive identification of the organisms was performed by sequencing a 1,483-bp PCR fragment from 16S rRNA using universal primers B27F (5′-AGAGTTTGAT CCTGGCCTCAG) and U1492R (5′-GGTTACCTGTAGTTACG ACTT) (18). A sequence of 1,327 bp from the CIP 110250 PCR fragment was identical to the corresponding portion of the 16S rRNA gene of A. minutum type strain DSM 20586 (GenBank accession number FN178468.2). A sequence of 1,351 bp from the CIP 110249 PCR product was closely related to the 16S rRNA gene from type strain C. clostridioforme ATCC 25537 (DSM 933) (99.7% identity) (GenBank accession number M59089 and DNA sequence revisited in this study).

C. clostridioforme CIP 110249 and A. minutum CIP 110250 were grown under anaerobic conditions at 37°C on prereduced brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI) and BHI agar supplemented with 5% horse blood. The MICs of vancomycin were determined by the Etest procedure (AB Biodisk, Solna, Sweden) and by 2-fold serial dilution in blood agar according to CLSI guidelines. Both strains were highly resistant to vancomycin (MIC > 256 μg/ml and MIC = 64 μg/ml for C. clostridioforme CIP 110249 and A. minutum CIP 110250, respectively) but susceptible to teicoplanin (MIC = 1 μg/ml, suggesting VanB-type resistance.

The C. clostridioforme group includes Clostridium bolteae, C. clostridioforme, and Clostridium hathewayi (9), and more recently, Clostridium aldenense and Clostridium citroniae have been added (19). All these species have been reported from human clinical infections, most often associated with other bacteria from peritoneal fluid in patients with peritonitis. C. clostridioforme bacteria have been also isolated from osteomy-
not able to transfer vancomycin resistance in D-Ala-D-Lac (VanA, VanB, VanD, and VanM) or D-Ala-D-Ser (VanC, VanE, VanG, VanL, and VanN), which have low affinity for glycopeptides. In addition, the elimination of the pentapeptide precursors produced by the ligase of the host ending in d-Ala-d-Ala is required (1, 20). VanA and VanB are the most common types and are responsible for more than 95% of the vancomycin-resistant enterococcus (VRE) isolates. The origin of the van genes remains hypothetical; however, recent studies indicated that vanA might originate from soil microorganisms (12), whereas vanB might arise from gene transfer from human intestinal microbiota (2). Anaerobes that could represent the reservoir of vanB, including C. bolteae, C. hathewayi, Clostridium innocuum-like, Clostridium lavalense, C. symbiosum, E. lenta, and Ruminococcus lactaris-like, have been identified from stool samples positive for vanB in the absence of VRE (7, 8, 11, 14, 16). However, these rare strains are limited to Australia and Canada. We report here the description of two vanB anaerobes isolated from clinical samples in France. Interestingly, the presence of VRE was not detected from stool specimens in our patients. Several studies have demonstrated a lack of correlation between the fecal detection of vanB by PCR or real-time PCR and the carriage of VRE. This result was attributed to the presence of vanB-containing anaerobic bacilli (11). Although the vanB reservoir has probably spread worldwide, until now, Australian and Canadian authors were the only ones to successfully identify different vanB-carrying anaerobes, probably due to the difficulty in cultivating certain strains. The isolation of two vanB-carrying anaerobes from infection sites in humans exemplifies the risk of dissemination of VanB-type resistance from the intestinal microbiota, which constitutes a reservoir for antibiotic resistance.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the 16S rRNA genes from isolates CIP 110250, CIP 110249, and ATCC 25537 were deposited in GenBank under accession numbers JF313107, JF313108, and JF313109, respectively. The nucleotide sequences of the vanB ligase genes from isolates CIP 110249 and CIP 110250 were deposited in GenBank under accession numbers JF313105 and JF313106, respectively.

**REFERENCES**


