

Desulfovibrio desulfuricans Bacteremia in an Immunocompromised Host with a Liver Graft and Ulcerative Colitis

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Desulfovibrio spp. are anaerobic, sulfate-reducing, nonfermenting, Gram-negative bacteria found in the digestive tract of humans. Identification of these species with conventional methods is difficult. The reported case of a *Desulfovibrio desulfuricans* bacteremia occurring in an immunocompromised host with ulcerative colitis confirms that this organism may be a possible opportunistic human pathogen.

CASE REPORT

n 2006, a diagnosis of primary sclerosing cholangitis (PSC) was made in a 69-year-old woman with longstanding type 2 diabetes mellitus. Endoscopic retrograde cholangio- and pancreatography (ERCP) showed type 1 intrahepatic stenoses without extrahepatic involvement. Since then, she experienced repetitive episodes of cholangitis with Escherichia coli, several of them complicated by septicemia. As often seen in patients with PSC, she also suffered from a concomitant inflammatory bowel disease, in her particular case ulcerative colitis (UC). The latter was treated consecutively with azathioprine, infliximab, and finally adalimumab, which also failed to be effective. In November 2009, she underwent an orthotopic liver transplantation because of recurrent cholangitis. In April 2010, her immunosuppressive therapy was switched from mycophenolate and tacrolimus to cyclosporine and azathioprine in conjunction with 32 mg of methylprednisolone because of increasing episodes of abdominal discomfort. Despite this switch in therapy, she had to be hospitalized shortly thereafter with worsening abdominal pain, diarrhea, dehydration, and fatigue. An ileocolonoscopy performed at that time demonstrated a severe right-sided colitis as well as a nonulcerative terminal ileitis. Following therapy with intravenous fluids, transfusion of packed cells, and analgetics, her condition improved rapidly. However, 1 month later she was readmitted because of fever, increasing abdominal discomfort, and bloody diarrhea two to three times a day. On clinical examination, she had a temperature of 38.8°C, diffusely tender abdomen, and bloody stools on digital rectal exam. Endoscopic reevaluation showed a moderately persisting rightsided colitis. The colon biopsy specimen procured at that time stained positive for cytomegalovirus (CMV). The diagnosis of CMV colitis was confirmed in the blood by a positive quantitative PCR (26,422 copies/ml [4.42 log copies/ml]). Additional biochemical analysis showed a severe anemia with a hemoglobin level of 8.6 g/dl (reference values, 12 to 16 g/dl), a left shift of the white blood cell count (86.0% neutrophils [reference values, 38 to 77%]), and a rise in C-reactive protein (CRP) of 27.6 mg/liter (\leq 5 mg/liter). Liver enzyme levels were normal with the exception of gamma glutamyl transferases, which were twice the upper limit (65 U/liter [\leq 35 U/liter]). Diagnosis of CMV colitis was made, ganciclovir was initiated, and the steroids were gradually reduced. Nevertheless, the patient experienced recurrent episodes of spiking fever during which 40 to 60 ml of blood was collected and

inoculated in 2 aerobic (BacT/Alert FA) and 2 anaerobic (Bact/ Alert FN) bottles (bioMérieux, Hazelwood, MO).

Both anaerobic bottles became positive after 101.2 h and 111.9 h of incubation. Gram-negative rods were seen on a Gram-stained smear. Piperacillin-tazobactam (4 g every 8 h [q8h] intravenously) was added empirically. Both positive anaerobic blood cultures were further subcultured on Columbia blood agar with 5% horse blood and on chocolate agar. Under aerobic conditions at 37°C with 5% CO₂, no growth was obtained. However, under anaerobic conditions, small colonies of motile bacilli were observed 48 h after inoculation. Gram staining of these colonies showed very small, curved, and spiral Gram-negative rods. Based on the morphology, a Campylobacter species was suspected and clarithromycin (500 mg q12h orally) was added to the therapy since no clinical improvement was observed under empirically initiated therapy. All efforts to induce growth on Campylobacterselective medium under microaerophilic conditions were not successful. Biochemical tests for catalase, indole, gelatin, and esculin hydrolysis were negative, whereas those for nitrate reduction and urease were positive. Growth around a disk with 1,000 μ g oxgall (Diatabs; Rosco Diagnostica, Taasrup, Denmark) was inconclusive. Tests for the fermentation of sucrose and glucose were doubtful, whereas those for fermentation of salicin, arabinose, xylose, and lactose were negative. The cultured species appeared to be resistant for colistin and had a negative cytochrome oxidase reaction and a positive reaction for urease, which made us recall to the initial suggestion of a Campylobacter species (11). Further identification of the species at this stage was lacking. The reactions for *o*-nitrophenyl- β -D-galactopyranoside (ONPG), α -fucosidase, and *N*-acetyl- β -glucosaminidase were all negative (7).

In order to identify the species, 16S rRNA gene sequencing was performed by Wauters et al. (12). A total of 1,207 continuous nucleotides of 16S rRNA were determined. After comparison of

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the 16S rRNA genes with those available in GenBank and EMBL using the BLAST program, a similarity of 99.9% was detected with the sequence (accession no. AF 192154.1) of the strain Desulfovibrio desulfuricans ATCC 27774. Final diagnosis of D. desulfuricans bacteremia occurring in an immunocompromised host with CMV colitis was made. Since on blood agar medium no growth could be observed, a brain heart infusion (BHI; Oxoid, Cambridge, United Kingdom) agar enriched with 5% horse blood and a density of 1 McFarland instead of the standard 0.5 McFarland was used to test the susceptibility. Etests after 120 h of anaerobic incubation at 37°C showed a susceptibility to clindamycin (MIC < 0.016 mg/liter), metronidazole (MIC < 0.016 mg/liter),erythromycin (MIC, 1.0 mg/liter), amoxicillin-clavulanic acid (MIC, 0.047 mg/liter), and meropenem (MIC, 0.006 mg/liter) and resistance to piperacillin-tazobactam, with a MIC of >256 mg/ liter (MIC > 128 mg/liter [1]).

Following the initiation of clarithromycin, the patient recovered gradually. Therapy with piperacillin-tazobactam was stopped immediately after obtaining the results of the susceptibility testing, whereas ganciclovir therapy was discontinued after 18 days following two consecutive negative CMV PCR results. Clarithromycin was continued *in toto* for 10 days. Two sets of follow-up blood cultures 3 weeks after the onset of symptoms remained negative.

The reported human case is the second one of a monobacterial bacteremia with D. desulfuricans identified at the species level but the first one in an immunocompromised liver transplant recipient with ulcerative colitis. The first case was reported by Porschen and Chan in 1977. They described the occurrence of a Vibrio-like organism cultured from the blood of a patient with fever and nausea (9). In that particular case, D. desulfuricans was described as a catalase-positive, nitrate-inconclusive, and urease-negative bacillus. However, in 2005, Warren et al. showed that only Desulfovibrio fairfieldensis is characterized by positive catalase and nitrate tests, whereas D. desulfuricans is positive for nitrate and urease but negative for catalase (11). This suggests that the case in 1977 was due to D. fairfieldensis and not D. desulfuricans as suggested by the authors (1a, 6). A second case of D. desulfuricans bacteremia was published by Goldstein et al. in 2003 in an otherwise healthy man with a diarrheal disease 10 days prior to bacteremia. The authors hypothesized that the bacteremia was secondary to intestinal colonization with subsequent transmural invasion (3). Desulfovibrio spp. have been found in four other cases of bacteremia: in three cases the organism was determined to be D. fairfieldensis, and in one case the organism was identified only to the genus level (3). Loubinoux et al. suggested that compared to the other Desulfovibrio spp., D. fairfieldensis possesses a higher pathogenic potential since the majority of monobacterial infections are due to this organism (4).

Desulfovibrio spp. are ubiquitous and can be found in soil, water, and sewage, as well as in the digestive tracts of animals and humans. They are sulfate-reducing, nonfermenting, anaerobic, Gram-negative bacilli characterized by the presence of a pigment, desulfoviridin, which can be easily detected using a rapid test (11). Isolation from clinical specimens by conventional methods is difficult because of the indolent growth pattern lasting 4 to 7 days. For this reason, molecular techniques similar to those we used are often necessary for identification of the species. So far, four *Desul*- *fovibrio* spp. (*D. fairfieldensis*, *D. desulfuricans*, *D. piger*, and *D. vulgaris*) have been associated with human (mostly abdominal) infections (8, 11). Sulfate-reducing bacteria (SRB) have been implicated in the etiopathogenesis of ulcerative colitis because of their ability to produce hydrogen sulfide, which might lead to mucosal inflammation and ulceration of the intestinal mucosa (10). Moreover, Gibson et al. discovered in 1991 that the incidence of fecal carriage of active SRB is much higher in patients with UC (2). However, it remains a matter of debate at present whether these bacteria are the culprit agents for UC or whether they just take advantage of the mucosal inflammation and tissue destruction to cause bacteremia (1a).

In our case, one might postulate that the preceding CMV infection and UC with disintegration of the intestinal mucosa could have induced invasion of Desulfovibrio into the bloodstream. Also the use of a cocktail of immunosuppressive agents might have been playing a major role. The infrequent nature of the organism as a human pathogen (and therefore a limited number of reports) as well as the difficulty to determine its susceptibility to antibiotics due to its slow growth makes it so that there exists no clear consensus about the optimal antibiotic treatment. Lozniewski et al. tested susceptibility of 16 clinical isolates of Desulfovibrio spp. without identification to the species level (5). They documented broad MIC ranges for a wide range of antimicrobial agents. Warren et al. reported susceptibility to clindamycin and metronidazole, two drugs commonly used for anaerobic infections (13). D. fairfieldensis is more resistant to piperacillin-tazobactam and ceftriaxone than the other Desulfovibrio spp. (6, 11). Nakao et al. showed a high susceptibility of D. desulfuricans using the Etest (bioMérieux) of all six strains to five antianaerobic agents with low MIC₉₀ values: sulbactam-ampicillin, clindamycin, meropenem, metronidazole, and chloramphenicol. All strains were also susceptible to erythromycin. In contrast, these strains showed high MIC₉₀ values compared to those of other antibiotics, like piperacillin-tazobactam and cefotaxime (8). In our patient, clarithromycine was initiated, which led to clinical recovery.

In conclusion, *Desulfovibrio* spp. infrequently cause infections in humans. Nevertheless, their incidence is presumably being underestimated due to their slow growth, difficult identification, and the fact that one easily overlooks them in a mixed culture. Identification at the genus or species level therefore often requires additional molecular analysis. Clinically, *Desulfovibrio* spp. have been associated mostly with abdominal infections. Our case illustrates the first bacteremia of *D. desulfuricans* in an immunocompromised liver transplant recipient with UC and concurring CMV colitis, which might have facilitated its emergence. *Desulfovibrio* spp. is to be considered in analogous situations where empirical broad-spectrum antibiotic therapy fails.

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