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Two Serious Cases of Infection with *Clostridium celatum* after 40 Years in Hiding?

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Clostridium celatum [ce.la'tum. L. adj. *celatum* hidden] has been known since 1974, when it was isolated from human feces. In 40 years, no association with human infection has been reported. In this work, we present two serious cases of infection with the anaerobic Gram-positive rod *Clostridium celatum*.

CASE REPORTS

Case 1. A 53-year-old man presented at the emergency department 4 days after open surgery with removal of the gallbladder. The patient had been experiencing increasing pain in the upper abdomen and the right shoulder. He had no prior medical history and was taking no medication.

On physical examination, the patient's temperature was 37.8°C, heart rate was 86 bpm, blood pressure was 167/100 mm Hg, and oxygen saturation level was 100% without supplemental oxygen. An abdominal computed tomography (CT) scan revealed intra-abdominal fluid. Six hours later, the patient became septic, with a temperature of 38.4°C, a respiratory frequency of 28 breaths per min, a heart rate of 102 bpm, and an oxygen saturation level of 90%. His abdominal pain increased despite treatment with morphine. The leukocyte count was 22×10^9 /liter (normal value range, 3.5×10^9 /liter to 8.8×10^9 /liter), and the C-reactive protein (CRP) level was 142 mg/liter (normal value range, <6.0 mg/liter). Two sets of blood cultures (BacT/Alert using standard aerobic and anaerobic culture bottles; bioMérieux, Marcy l'Etoile, France), 40 ml in total, were drawn, and treatment was initiated with intravenous cefuroxime (1.5 g) and metronidazole (500 mg) administered three times daily. The next morning, intraperitoneal drainage was instituted by ultrasonic guidance and 300 ml of bloody bile was removed. Subsequent endoscopic retrograde cholangiopancreatography revealed a leak in an aberrant bile duct, and two stents were inserted in the ductus choledochus.

On hospital day 11, a rise in temperature, a CRP elevation from 143 to 211 mg/liter, and an increase in leukocyte count from 18×10^9 /liter to 39×10^9 /liter prompted a CT scan of the abdomen and a change of antibiotic treatment from cefuroxime to intravenous meropenem (1 g) three times daily. The CT scan revealed progression of the intra-abdominal fluid, and 300 ml of purulent bile was evacuated by catheter insertion. The discharge slowly decreased, and the intra-abdominal catheter was finally removed on hospital day 25. The following day, antibiotic treatment was stopped and the patient, having received a total of 24 days of treatment with intravenous antibiotics metronidazole (days 1 to 24), cefuroxime (days 1 to 10), and meropenem (days 10 to 24), was discharged.

Blood cultures showed growth of anaerobic Gram-variable rods from both anaerobic bottles after 1 day of incubation. Growth on chocolate agar supplemented with vitamin K and cysteine (Statens Serum Institut [SSI], Hillerød, Denmark) was observed after another 24 h in an anaerobic atmosphere. No aerobic growth was observed. The isolates were subjected to matrix-as-

sisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using two different platforms (Vitek MS 1.4.2.B [bioMérieux, Marcy l'Etoile, France] and Biotyper 3.1-65 [Bruker Daltonics, Bremen, Germany]) with or without formic acid. The Biotyper platform suggested *Clostridium* sp. (score, 1.945) and *C. disporicum* (score, 1.778), and the Vitek MS platform suggested *C. clostridioforme* (score, 99.9) for both isolates. To further identify the isolates, partial 16S rRNA gene sequencing (MicroSeq 500 system; Perkin-Elmer, Applied Biosystems Division, Foster City, CA) was performed. Isolate consensus sequences (462 bp) were compared using the EzTaxon server and showed 99.49% and 99.74% matches (blood isolates 1 and 2, respectively) to *C. celatum* type strain DSM 1785(T) (GenBank accession no. [X77844](#)) (1). There was good separation from the second-best match, *C. disporicum* type strain DSM 5521(T) (97.84%) (GenBank accession no. [Y18176](#)).

Case 2. An 87-year-old woman with a history of osteoporosis, knee arthrosis, and total right-side knee arthroplasty was admitted to the orthopedic department with a painful, swollen, and warm left knee. She had undergone a total knee arthroplasty of the left knee 23 days prior to admission.

On admittance, her temperature was 38.3°C. The total leukocyte count was 10.7×10^9 /liter, and the CRP level was 115 mg/liter. Antibiotic treatment with oral amoxicillin-clavulanic acid (500 and 125 mg, respectively) and dicloxacillin (1 g) three times daily was initiated. A culture of a sterile knee puncture on hospital day 3 was without aerobic or anaerobic growth. Partial 16S rRNA gene sequencing was performed on the sample but with no positive result. Amoxicillin-clavulanic acid and dicloxacillin were discontinued in favor of intravenous cefuroxime (1.5 g three times a day). The patient still showed symptoms from the knee on hospital day 13, and open surgery was performed. Synovial fluid was cloudy, and soft tissue including the synovial tissue appeared gray

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and infected. A total of eight biopsy specimens were taken. Five were bone biopsy specimens, and three were from soft tissue. Antibiotic treatment was changed to intravenous meropenem (1 g three times a day) and vancomycin (1 g twice a day). No aerobic or anaerobic growth was observed after 5 days of incubation, and the bone biopsy specimens were submitted for partial 16S rRNA gene sequencing (MicroSeq 500 system). 16S rRNA gene analysis of the five culture-negative bone biopsy specimens resulted in five sequences of 453 to 462 bp. These were compared using the EzTaxon server, and all sequences had a 100% match to *C. celatum* type strain DSM 1785(T) (GenBank accession no. X77844).

The three soft tissue samples, which had been suspended in a thioglycolate broth, were recultured. On hospital day 24, 11 days after synovectomy, the three recultured tissue biopsy specimens revealed anaerobic growth on chocolate agar supplemented with vitamin K and cysteine (SSI). Treatment with vancomycin was changed to metronidazole (500 mg three times a day). The meropenem treatment was continued. The isolates (tissue isolates 1 to 3) were subjected to MALDI-TOF MS on the two different platforms (Vitek MS and Biotyper) with or without formic acid. The Biotyper suggested *C. disporicum*, with scores of 1.785, 2.018, and 1.808, respectively, and the Vitek MS suggested *C. paraputrificum* (99.9), *C. paraputrificum* (99.5), and *C. clostridioforme* (98.9), respectively. One of the isolates (tissue isolate 3) was subjected to partial 16S rRNA gene sequencing. The consensus sequence (460 bp) had a 100% match to *C. celatum* type strain DSM 1785(T) (GenBank accession no. X77844).

The patient was discharged after completing a 24-day course of intravenous antibiotics: cefuroxime (admission days 4 to 13), meropenem (admission days 13 to 26), vancomycin (admission days 13 to 24), and metronidazole (admission days 24 to 28). She made an uneventful recovery.

The antimicrobial susceptibility of blood isolate 1 and tissue isolate 3 to seven different antimicrobial agents was determined using the Etest gradient method (bioMérieux, Lyon, France) and M.I.C. Evaluators (Oxoid, Hants, United Kingdom) on brucella blood agar supplemented with hemin and vitamin K (Becton, Dickinson GmbH, BD Diagnostics, Heidelberg, Germany) according to the manufacturers' instructions. The isolates were susceptible to penicillin, piperacillin-tazobactam, meropenem, metronidazole, clindamycin, moxifloxacin, and vancomycin according to CLSI breakpoints for anaerobic bacteria (Table 1).

There have been no reports on human infection with *C. celatum*, and only one publication was retrieved on PubMed describing how *C. celatum* is unable to reduce the dye indigo for industrial purposes (2, 3). Within a period of 1 week, we isolated *C. celatum* from blood and synovial tissue from two different patients admitted at two different hospitals.

Clostridium celatum is a Gram-positive, nonmotile, anaerobic rod with large endospores that was first isolated from human feces 4 decades ago. The bacterium was found in 14 of 60 fecal specimens from healthy adults, and the species was as numerous as *C. perfringens* in normal stool (2).

Our isolates grew as large (2 to 4 mm in diameter), circular, flat, opaque, and pale gray colonies with a small central crest and irregular edges on chocolate agar under conditions of anaerobic incubation. Wet-mount microscopy performed on two isolates

TABLE 1 Results from identification and antimicrobial susceptibility testing

Parameter	Result(s)	
	Blood isolate 1	Tissue isolate 3
MALDI-TOF MS		
Vitek MS (% match)	<i>C. clostridioforme</i> (99.9)	<i>C. clostridioforme</i> (98.9)
Biotyper (score)	<i>C. disporicum</i> (1.767)	<i>C. disporicum</i> (1.808)
API RapidID 32A		
(% match)	<i>C. tertium</i> (80.3)	<i>C. clostridioforme</i> (99.3)
Numerical profile	4701004000	4161004000
Urease		
API RapidID 32A	Negative	Negative
Specific urease test	Negative	Negative
Etest gradient MIC (mg/liter)		
Penicillin	0.032	0.25
Piperacillin-tazobactam	1.5	2.0
Meropenem	0.015	0.125
Metronidazole	0.125	0.5
Clindamycin	0.015	0.015
Moxifloxacin	0.25	0.5
Vancomycin	0.5	0.5

(blood isolate 1 and tissue isolate 3) showed nonmotile rods with large spores. Hauschild and Holdeman found that *C. celatum* produced nitrite and sulfide, and, of note, 4 of 14 strains were tested for production of urease and all tested positive (growth from egg yolk agar incubated in peptone yeast-urea broth). Hauschild and Holdeman also found that *C. celatum* ferments a number of carbohydrates, including mannose (2). We tested both isolates for urease production with the API RapidID 32A test (bioMérieux, Marcy l'Etoile, France) and a specific urease test (SSI Diagnostica, Hillerød, Denmark) and found them negative in both urease tests (Table 1). Both isolates tested negative for nitrite production and negative for fermentation of mannose with the API RapidID 32A. The isolates were also subjected to the API 20A test, and no fermentation of carbohydrates, including mannose, was observed. The Culture Collection of the University of Göteborg holds a *C. celatum* isolate, CCUG 34252, which has also tested negative for urease and nitrite production and negative for fermentation of mannose. The CCUG 34252 isolate was found in intestinal mucosa from an 89-year-old woman in 1995. It was previously identified as *C. clostridioforme*, but the identification was changed after 16S rRNA gene sequence analysis.

The genus *Clostridium* is large and heterogeneous and includes more than 200 species. Accurate identification of these bacteria has been difficult, and *Clostridium* spp. might very well have been misidentified with phenotypic methods. In recent years, several new species have been recognized and others reclassified using newer molecular methods, such as 16S rRNA gene sequencing (4).

Among the medically significant *Clostridium* spp., *C. perfringens* is the dominating pathogen in cases of bacteremia. The major portal of entry is the hepatobiliary and gastrointestinal tract. Reports suggest that bacteremia with non-*C. perfringens* spp. affects younger patients and is more frequently hospital acquired than *C. perfringens* bacteremia (5). Septic arthritis due to *Clostridium* spp., on the other hand, is not a frequent occurrence. Among the re-

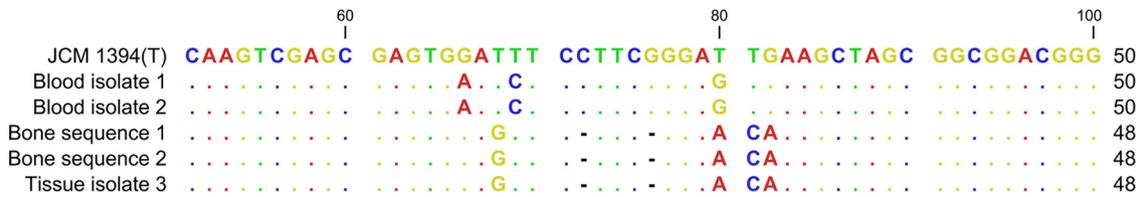


FIG 1 Partial 16S rRNA nucleotides 50 to 100 from the JCM 1394(T) type strain (top), case 1 (blood isolates 1 and 2), and case 2 (bone sequences 1 and 2 and tissue isolate 3). This region demonstrates the difference of six nucleotides and two deletions (black line) between the 16S rRNA gene sequences from case 1 and case 2 at the beginning of the sequence.

ported cases, the knee is the location most often involved, and several cases have occurred following invasive procedures performed on the joint, as was the case with one of our patients (6).

Obviously, the finding of two cases of infection with *C. celatum* after 40 years “in hiding” prompted further investigations to exclude laboratory contamination or mixing of samples. We concluded that the isolates were indeed not results of contamination or mixing of samples; blood cultures and tissues originated from patients admitted at two different hospitals, and the samples were received 4 days apart and never handled in the same sections of the laboratory. Furthermore, the blood and tissue isolates were submitted to several analyses in order to characterize and, if possible, differentiate them. We found that the blood and tissue isolates differed in levels of antimicrobial susceptibility and in biochemical reactions as well as in their 16S rRNA gene sequences (sequencing of samples from the two patients was performed on separate days).

Antimicrobial susceptibility testing revealed that the blood isolate was more susceptible to penicillin and meropenem than the tissue isolate (Table 1). The biochemical characteristics were determined by the API Rapid ID 32A test. The blood isolate tested positive for β -galactosidase-6-phosphate and α -glucosidase, while the tissue isolate tested positive for β -glucuronidase and α -arabinosidase. As *C. celatum* is not in the API database, the system was unable to identify the isolates to the species level. The closest matches were *C. tertium*, with an 80.3% score for blood isolate 1, and *C. clostridioforme*, with a 99.3% score for tissue isolate 3. *C. celatum* is not part of the current MALDI-TOF MS databases for the Vitek MS platform or the Biotyper platform. Consequently, the Biotyper platform suggested different species with scores close to the cutoff for species identification (≥ 2.000). In contrast, the Vitek MS platform suggested *C. clostridioforme* and *C. paraputrificum*, with matches of 99.9% and 98.9%, respectively (Table 1). Recently, Alby et al. reported numerous examples of misidentifications with the Vitek MS system, including several cases of misidentifications of anaerobic bacteria (7).

We routinely subject anaerobic bacteria from blood and other sterile body sites to partial 16S rRNA gene sequencing if MALDI-TOF MS is not able to identify an isolate with a score above the cutoff value (8). Partial 16S rRNA gene sequencing identified but also separated the two isolates, revealing differences of six nucleotides and two deletions between the isolates at the beginning of the sequence (Fig. 1). However, this did not correspond well to the excellent identification scores of the EzTaxon server, with 99.49% and 100% matches for the blood and tissue isolates, respectively, to *C. celatum* type strain DSM 1785(T) (GenBank accession no. X77844) (1). After downloading the X77844 sequence, we discov-

ered that the initial region of our 16S sequences was not covered by this sequence. Subsequently, we compared both of our sequences to the NCBI BLAST database and found a 99% match to *C. celatum* type strain JCM 1394(T) (GenBank accession no. AB971795) with 100% coverage. There was a total difference of five bases for the blood isolates and of four bases plus two deletions for the tissue isolates in comparison with the consensus sequence of *C. celatum* type strain JCM 1394(T).

To our knowledge, these are the first two documented cases of *Clostridium celatum* as a potential human pathogen. In these two cases, a MALDI-TOF MS platform and the API RapidID 32A suggested identification of non-*C. celatum* species with convincing scores. It is possible that *C. celatum* has been misidentified in the clinical microbiology laboratory for the last 4 decades.

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