Eubacterium callanderi Bacteremia: Report of the First Case

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***Eubacterium callanderi* is an environmental anaerobic rod-shaped bacterium first isolated in 1998 from an industrial anaerobic digester. We report on the first clinical isolate of *E. callanderi*, which was recovered from the blood of a patient with a bladder carcinoma. Identification of the organism was made by cell fatty acid chromatographic analysis and 16S rRNA gene sequencing.**

CASE REPORT

An 80-year-old man underwent a cystoprostatectomy with urethrostomy for a bladder carcinoma in March 1999. Pulmonary metastases and local recurrence of the tumor were diagnosed in October 1999. One month later treatment with gemcitabine (1,000 mg/m², days 1, 8, and 15) was initiated. On day 8 of the second cure, fever (39°C) and marbling of the lower limbs developed, and a full blood count showed neutrophilic leukocytosis (17 g/liter). On the same day, four blood cultures were performed with blood collected aseptically: one pair of blood samples was obtained from the peripheral circulation, and one pair of blood samples was obtained from the central infusion implant. The patient was then treated with ceftriaxone at 2 g once a day in combination with amikacin at 1 g once a day. After 1 day a methicillin-resistant *Staphylococcus epidermidis* strain was isolated in one aerobic culture of blood from the central infusion implant. After 10 days, a gram-positive bacterium was isolated from one anaerobic culture of blood from the infusion implant and one anaerobic culture of from blood from the peripheral circulation. There was no growth in the fourth blood culture. The infusion implant was removed 1 day after the fever had started. It was cultured, and a methicillin-susceptible *S. epidermidis* strain was isolated. Vancomycin was added to the patient's treatment regimen, and the dose was adjusted to give a concentration in serum of 20 to 40 µg/ml. There were no indications of endocarditis on transesophageal echocardiography, and several subsequent blood cultures remained negative. The patient's condition, however, deteriorated and he died in January 2000.

Growth of the gram-positive bacterium was observed in a Lytic 10 BACTEC anaerobic bottle (Becton Dickinson Diagnostic Instrument Systems, Franklin Lakes, N.J.) after 10 days of incubation in a BACTEC 9240 automated instrument. The isolate was subcultured on blood agar plates (bioMérieux, Marcy l'Etoile, France) incubated at 37°C in an anaerobic

atmosphere. After 15 days small colonies were seen, and staining revealed a gram-positive, non-acid-fast bacillus. A test for catalase was negative. The biochemical characteristics of the organism determined with the API 20A, API STREPTO 20E, and API 20E systems (bioMérieux) are presented in Table 1.

The antibiotic susceptibility of the isolate was determined by the standard disk diffusion method on Mueller-Hinton blood agar (bioMérieux). The bacterial strain was classified as susceptible, intermediate, or resistant by the growth inhibition zones according to the guidelines of the AntibioGram Committee of the French Society for Microbiology (1). The *Eubacterium callanderi* strain that was isolated from blood from the peripheral circulation was found to be susceptible to penicillin G, amoxicillin plus clavulanic acid, imipenem, metronidazole, vancomycin, and clindamycin. It was resistant to cefotetan.

The cell wall fatty acid composition was determined by gas chromatography (5890 series II; Hewlett-Packard, Palo Alto, Calif.) (L. Miller and T. Berger, Bacterial identification by chromatography of whole cell fatty acids, Hewlett-Packard application note 228-241, Hewlett-Packard, Avondale, Pa., 1985) with a culture grown for 15 days on blood agar and was as follows: C_{14:0} fatty acid methyl ester (FAME), 11.90%; C_{16:0} aldehyde, 4.50%; C_{16:0} FAME, 18.65%; C_{16:0} dimethyl acetyl (DMA), 17.74%; C_{18:2ω9,12} FAME, 4.84%; C_{18:1ω9} FAME, 10.38%; C_{18:0} FAME, 7.23%; C_{18:0} DMA, 4.06%.

The mean ± standard deviation of the G+C content after five determinations by high-pressure liquid chromatography (L-6200 system pump; Merck Clevenot, Nogent-sur-Marne, France) was 54.8 ± 0.6 mol. The G+C content of the type species, *Eubacterium limosum*, is 47% (7), although the G+C content is quite variable in the genus *Eubacterium*, ranging from 30% in *Eubacterium rectale* to 55% in *Eubacterium suis* (7).

For sequencing, the organism was incubated for 1 h at 37°C and then the DNA was extracted in 180 µl of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, 0.1 M NaCl) at pH 8.0. Digestion was carried out with 40 µl of proteinase K solution (25 mg/ml) and 25 µl of 10% sodium dodecyl sulfate for 1 h at 55°C. The digestion was stopped by the addition of 200 µl of 4 M guanidine thiocyanate, and the mixture was left at room temperature for 1 h before it was heated to 100°C for 10 min following the addition of 50 µl of 0.5 M NaOH. Final extraction of nucleic acid was carried out with a QIAmp kit (Qiagen,

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TABLE 1. Phenotypic characteristics of *E. callanderi*

Characteristic or test	Result
Indole	—
Nitrates	—
Voges-Proskauer	—
Citrate	—
Esculin	+
Gelatin	+
Pyrolidonyl	+
Arylamidase	+
α -Galactosidase	—
β -Glucuronidase	—
β -Galactosidase	+
Alkaline phosphatase	+
Leucine arylamidase	+
Arginine dihydrolase	—
Lysine decarboxylase	—
Ornithine decarboxylase	—
Acid from glucose	—

Hilden, Germany). PCR-mediated amplification of the 16S rRNA gene and sequence determination were performed with primers FD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and RP2 (5'-ACGGCTACCTTGTACGACTT-3') and controls (*Escherichia coli* DNA as a positive control and distilled water as a negative control), as described previously (10). The 1,462-bp sequence was aligned and compared with all eubacterial 16S rRNA gene sequences available in the GenBank database by using the multisequence Advanced BLAST comparison software from the National Center for Biotechnology Information (2). The highest sequence (16S rRNA gene) similarity value (99.3%) was obtained with the *E. callanderi* strain DSM 3662 16S rRNA gene sequence (GenBank/EMBL accession no. X969610.1).

Although our isolate was similar to *Eubacterium* spp. in morphology, G+C content, cell wall fatty acid profile, and antibiotic susceptibility (3, 4), it was the 16S rRNA gene sequence that enabled us to identify the organism to the species level.

The genus *Eubacterium* includes strictly anaerobic, non-spore-forming, nonmotile rod-shaped bacteria. *E. callanderi* is an environmental bacterium that was initially isolated from an anaerobic digester processing the contents from a wood fiber-to-alcohol fermentation plant (9). Among the strictly anaerobic, non-spore-forming, gram-positive rods, species of the genus *Eubacterium* have mostly been isolated in clinical samples: *Eubacterium lentum* and *Eubacterium agalactolyticum* from pleuropulmonary exudates and *Eubacterium aerofaciens*, *Eubacterium nodatum*, *Eubacterium sapheum*, and *Eubacterium timidum* from oral-dental exudates (5, 6). *E. aerofaciens* has been implicated in endocarditis, and *E. lentum* is frequently isolated from blood. Although bacteria of the genus *Eubacterium* have been found in cavities of humans and animals, the isolation of *E. callanderi* from blood has not been reported previously (7).

The fact that *E. callanderi* is not part of the common skin flora and the fact that we isolated the organism from independent cultures of blood collected at different sites shows that our isolate was not a contaminant introduced during blood collection. It was also not a laboratory contaminant, as we had not isolated *E. callanderi* in our laboratory over the previous 24 months and there was no amplification with the negative control. The isolate was recovered from blood collected when the patient presented with an acute onset of fever. While the route of entry of *E. callanderi* may have been the central infusion implant, the organism most likely entered the body from the bowel through the neoplasm. *E. callanderi* is a nonglucolytic bacterium (Table 1), and phenotypic identification of the organism is not reliable since it is fastidious and rarely available for most routine laboratory work. Moreover, the available phenotypic tests do not enable *E. callanderi* to be identified to the species level because it is not included in the API 20A system database (bioMérieux). Fatty acid analysis is not useful for the identification of *Eubacterium* species (8). Analysis of the 16S rRNA sequence of the organism, however, provides a reliable and straightforward identification tool, and the routine use of this method should increase our knowledge of the clinical spectrum of *E. callanderi* infections in people.

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