

Peritonitis Due to *Brevibacterium otitidis* in a Patient Undergoing Continuous Ambulatory Peritoneal Dialysis

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Received 23 May 2000/Returned for modification 25 July 2000/Accepted 22 August 2000

***Brevibacterium otitidis* is a coryneform rod and, as far as is known, is isolated only from infected ears. We report the first known case of peritonitis caused by *B. otitidis* in a patient undergoing continuous ambulatory peritoneal dialysis.**

CASE REPORT

A 73-year-old woman was started on continuous ambulatory peritoneal dialysis (CAPD) in May 1997 because of renal insufficiency due to nephrosclerosis. In November 1998, peritonitis with methicillin-resistant, coagulase-negative *Staphylococcus* sp. was diagnosed and treated with intraperitoneally administered vancomycin, resulting in clinical resolution within 48 h.

In September 1999, the patient complained of moderate abdominal pain, her temperature was 37.5°C, and the dialysate effluent was cloudy. The subcutaneous tunnel and the exit site of the CAPD catheter were unremarkable. The cell count of the effluent disclosed 160 white blood cells/mm³, of which 46% were polymorphonuclear neutrophils. Her peripheral leukocyte count was 6,870/mm³, and the C-reactive protein level was 2.0 mg/dl. Other results disclosed a state of relative malnutrition, with an albuminemia of 3.2 g/dl and a total cholesterol level of 144 mg/dl.

Empirical intraperitoneal therapy with cefazolin and gentamicin was initiated, and rapid clearing of the effluent resulted. On follow-up dialysate examination 5 days after institution of therapy, no significant numbers of leukocytes were seen. Subsequently, we clearly identified the cause of the infection as inadequate manipulation of the bag connector with a screwdriver.

Microbiological findings. The dialysate was cultured on blood agar plates, and 10 ml was inoculated into a blood culture bottle (BACTEC plus aerobic/F*). All cultures grew a gram-positive coryneform rod. Colonies were smooth and slightly yellowish. There was no growth at 20°C. The organism was nonmotile, catalase positive, and urease negative and did not acidify any carbohydrates. Gelatin and casein were hydrolyzed. Methane-thiol production was rapidly positive. Nitrate was not reduced. There was no hydrolysis of esculin, xanthine, or tyrosine. No carbohydrates were utilized as shown by the API 50 CH panel (bioMérieux, Marcy l'Etoile, France). Pyrrolidonyl peptidase was strongly positive, but α -glucosidase was negative. The numerical code achieved by the API Coryne

strip (bioMérieux) was 6002004, corresponding to *Arthrobacter* spp. or *Brevibacterium* spp. The cellular fatty acids analyzed by gas-liquid chromatography (Delsi chromatograph, Intersmat, Brussels, Belgium) were predominantly of the branched type, with anteiso 15:0 and anteiso 17:0 accounting for more than 75% of the total. The diamino acid of the peptidoglycan was meso-diaminopimelic acid, as determined by N. Weiss at the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

The 16S rRNA gene sequence of the strain was studied using a set of primers for amplification. PCR products were purified from agarose gel by the QIAquick Gel Extraction kit (Qiagen, Westburg, The Netherlands). Analysis of the whole sequence was performed with an automatic DNA sequencer (Perkin-Elmer, Applied Biosystems, Foster City, Calif.) using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit. Each sequence was compared to the sequence data available in databases using BLAST (7). The 16S rRNA gene sequence of the strain exhibited 98.8% similarity to that of the type strain of *Brevibacterium otitidis*, DSM 10718^T.

The overall phenotypic findings and the chemotaxonomic characteristics of the strain were consistent with those of the genus *Brevibacterium* (3). The biochemical profile (Table 1) allowed us to assign it to the species *B. otitidis* (6). Although the 16S rRNA gene sequence similarity of 98.8% seems somewhat low, it is nevertheless compatible with isolates from the same species (8).

Susceptibility of the strain was tested by the disk diffusion method on Mueller-Hinton blood agar incubated at 37°C for 24 h. Paper disks (Becton Dickinson, Cockeysville, Md.) containing penicillin, ampicillin, cefotaxime, cephalothin, erythromycin, ciprofloxacin, gentamicin, and vancomycin were used, and the results were interpreted in accordance with the criteria established for staphylococci by the National Committee for Clinical Laboratory Standards in 1997 (5a). The isolate was susceptible to all of the antibiotics tested.

Discussion. Poor socioeconomic conditions and the low education level of the patient, resulting in inadequate manipulation of the bag connector, may explain the recurrent episodes of peritonitis during her CAPD treatment. Her relative malnutrition may have also contributed to the infection.

Coryneform bacteria belonging to the genus *Brevibacterium* have been increasingly involved as opportunistic pathogens in various clinical, mostly nosocomial, settings (3). The vast majority of the isolates are *B. casei* (2). *Brevibacteria* have already been isolated in cases of CAPD peritonitis (4). Four strains

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TABLE 1. Differential characteristics of *Brevibacterium* species of human origin^a

Characteristic	<i>B. casei</i>	<i>B. epidermidis</i>	<i>B. iodinum</i>	<i>B. otitidis</i>	<i>B. mcbrellneri</i>
Colony appearance	Smooth, white-grayish	Smooth, white-yellow	Smooth, grayish	Smooth, yellowish	Dry-friable, grayish
Growth at 20°C	+	+	+	—	—
Nitrate reduction	± ^b	± ^c	+	—	—
α-Glucosidase	± ^c	—	—	—	—
Pyrrolidone peptidase	V	—	—	+	—
Xanthine hydrolysis	+	+	+	—	V
Utilization of:					
D-Arabinose	+	—	—	—	—
Mannitol	—	+	—	—	—
Gluconate	+	+	—	—	—

^a Data are from reference 6 and this study. +, positive; —, negative; V, variable.

^b Most strains negative.

^c Most strains positive.

studied by Funke and Carlotti and isolated from peritoneal dialysate belonged to the species *B. casei* (2). One strain of *B. iodinum* was isolated from the peritoneal fluid of a CAPD patient with peritonitis associated with acute urticaria (1). *B. iodinum* has, to the best of our knowledge, never been isolated from humans in other instances, and it must be noted that the description of the isolate in the foregoing study was very limited and that the species identification was based only on the oxidase test. *B. otitidis* is a new species recently described and has so far been isolated only from infected human ears (6). Our isolate is the first of *B. otitidis* recovered as a relevant pathogen outside the ear.

Brevibacterium sp. should be differentiated from other non-fermentative coryneform bacteria, especially *Arthrobacter* spp., nonfermenting *Microbacterium* (formerly *Aureobacterium*) species, and "*Corynebacterium aquaticum*." Chemotaxonomic properties are useful for this purpose, as the peptidoglycan of these genera does not contain *meso*-diaminopimelic acid (3). Some *Arthrobacter* species may be difficult to distinguish from *Brevibacterium* sp. routinely. Strong and rapid methane-thiol production is helpful to confirm brevibacteria. The former *Aureobacterium* species, recently transferred to the genus *Microbacterium* (9), and "*C. aquaticum*" are also yellow pigmented, but they oxidatively acidify carbohydrates, in contrast to brevibacteria. *B. otitidis* is metabolically less active than *B. casei* and *B. epidermidis* and is similar in this respect to *B. mcbrellneri* (6). However, colonies of *B. otitidis* are smooth and yellowish while those of *B. mcbrellneri* are dry and crumbly (5). In addition, pyrrolidonyl peptidase is strongly positive in *B. otitidis* and negative in *B. mcbrellneri*. The main differential

characteristics of *Brevibacterium* species isolated from clinical specimens are reported in Table 1.

This observation emphasizes the need to identify coryneform bacteria more accurately for better assessment of their pathogenic role in opportunistic infections.

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