**Relapsing *Brevibacterium casei*
Peritonitis: Value of 16S rRNA
Gene Sequencing in Accurate
Species Identification**

Peritonitis is a frequent and potentially serious complication of peritoneal dialysis (PD), contributing substantially to treatment-related adverse events and treatment failure in PD patients (1,2). The most common pathogens are staphylococci and Enterobacteriaceae, but other pathogens are involved as well (3). In this short report, we describe a case of relapsing peritonitis with repeated isolation of *Brevibacterium casei*, a very rare causative pathogen identified by 16S rRNA gene sequencing.

CASE REPORT

A 37-year-old Caucasian man on PD was admitted to our hospital because of progressively increasing abdominal pain. His medical history consisted of congenital kidney hypoplasia, leading to end-stage renal failure in September 2008, and continuous cycling PD (HomeChoice: Baxter Healthcare Corporation, Deerfield, IL, USA). He had experienced 3 previous episodes of peritonitis in August 2009, October 2010, and January 2011 caused by *Staphylococcus aureus*, *Serratia marcescens*, and *Klebsiella pneumoniae* respectively. Because of documented skin hypersensitivity to cetrimonium bromide, this patient's exit-site disinfection was performed using chlorhexidine 0.5 mg/mL.

Clinical examination revealed a diffusely tender abdomen in an otherwise not acutely ill patient. Blood pressure was 116/82 mmHg, temperature was 36.2°C. An effluent sample revealed a white blood cell count of 6301/μL, confirming the diagnosis of peritonitis. Empiric treatment was begun with intraperitoneal ceftazidime (250 mg/L in each dwell) and a single intraperitoneal dose of vancomycin 2 g.

METHODS AND RESULTS

On staining of the effluent, gram-positive rods were detected. Cultures showed growth of grey-whitish colonies with a shiny surface, producing a distinctive “Camembert cheese-like” odor. Staining of the colonies confirmed the presence of coryneform gram-positive rods. Biochemical identification was performed using the API Coryne system (bioMérieux, Marcy l’Etoile, France), but resulted in a code (6112004) with a low-percentage identification (55.2% in favor of *Brevibacterium* species, 41.9% in favor of *Arthrobacter* species), meaning that reliable discrimination between the two genera could not be made. The cheesy smell and the coryneform shape on Gram staining suggested that the strain was a *Brevibacterium* species (4). Although the 55.2% identification—which could be considered a surrogate for the likelihood of the organism being *Brevibacterium*—was comparable to a previously reported identification percentage for *Brevibacterium*, the API system has been shown to be imperfect for a definite identification in this setting (5). Identification of the strain was therefore confirmed by a more specific method using 16S rRNA gene sequencing,

according to interpretative criteria defined by the U.S. Clinical and Laboratory Standards Institute (CLSI), a worldwide authoritative source of guidelines in the field of clinical microbiology (6,7). We made use of the freely accessible GenBank database (U.S. National Institutes of Health, Bethesda, MD, USA), a public sequence database, and the BLAST algorithm for sequence alignment. Our strain exhibited 99.4% similarity (497 of 500 bases) in 16S rRNA sequence to the GenBank sequence of the type strain of *Brevibacterium casei* (ATCC 35513^T). Furthermore, our strain differed in 16S rRNA gene sequence by more than 1% from other species. We were thus able to identify the coryneform rods as *Brevibacterium casei*.

Susceptibility to antibiotics was tested by the disk diffusion method using Rosco Neo-Sensitabs (Rosco Diagnostica, Taastrup, Denmark). Zone diameters were interpreted according to the Neo-Sensitabs standardized CLSI 2009 guidelines for *Staphylococcus* species (8), because zone diameter breakpoints for *Brevibacterium* species are lacking. Additionally, minimal inhibitory concentration values were determined by E-tests (AB Biodisk, Solna, Sweden). Table 1 gives the minimal inhibitory concentration values for antibiotics and the

TABLE 1
Characteristics of the *Brevibacterium casei* Cultured in the Present Study

Antibiotic	Zone diameter (mm)	Interpretation ^a	MIC (mg/L)	Interpretation ^a
Penicillin	18	R	2	I
Oxacillin	9	R	—	—
Cefotaxime	—	— ^b	12	R
Meropenem	—	— ^b	0.064	S
Ciprofloxacin	—	— ^b	0.75	S
Levofloxacin	30	S	—	—
Gentamicin	39	S	0.75	S
Tetracycline	39	S	0.19	S
Minocycline	51	S	—	—
Teicoplanin	24	S	0.125	— ^d
Vancomycin	—	—	0.38	S
Others				
Erythromycin	30	S ^c	0.19	S
Clindamycin	32	R ^c	1.5	I
Trimethoprim–sulfamethoxazole	46	S	—	—
Linezolid	—	— ^b	0.38	S

MIC = minimal inhibitory concentration.

^a According to Clinical and Laboratory Standards Institute (CLSI) guidelines (6). Depending on the MIC of the strain *in vitro*, it is considered to be sensitive (S), intermediately sensitive [I (only intravenous therapy might be effective)], or resistant (R) to a given antibiotic therapy.

^b No breakpoints, defined by the Neo-Sensitabs (Rosco Diagnostica, Taastrup, Denmark) standardized modified CLSI 2009 criteria (8).

^c Disc diffusion method showed the presence of erythromycin-induced clindamycin resistance.

^d No breakpoints, defined by CLSI 2010 criteria (9).

related interpretations according to the novel CLSI 2010 breakpoints for *Brevibacterium* species (9).

Guided by susceptibility testing, antibiotic treatment was narrowed to vancomycin for 2 weeks, with a target serum trough level of 15 mg/L. The symptoms cleared rapidly, and within 48 hours, the patient's white blood cell count had declined to 97/ μ L.

Four weeks after cessation of vancomycin, abdominal discomfort reappeared, with clinical signs suggesting peritonitis, which was confirmed by a white blood cell count in effluent of 3727/ μ L. Empiric treatment with ceftazidime and vancomycin was started. Again, *Brevibacterium casei* was isolated from the effluent. Treatment was continued with vancomycin, resulting in rapid clinical resolution and complete disappearance of white blood cells in effluent in 72 hours.

Because of the rapid relapse of the peritonitis, colonization of the catheter was suspected. In an attempt to eliminate biofilm, urokinase 100 000 IU was instilled into the catheter on 3 consecutive days. Vancomycin treatment was continued for 3 weeks. Peritonitis again relapsed within 4 weeks after cessation of antibiotics, and *Brevibacterium casei* was again isolated from the effluent. It was decided at that point to remove the peritoneal catheter and to switch the patient to hemodialysis. Culture of the removed dialysis catheter failed to demonstrate the presence of *Brevibacterium casei*.

DISCUSSION

Coryneform bacteria are aerobically growing gram-positive rods with distinctive irregular morphology on staining. Taxonomically, they are a heterozygous group consisting of various genera, including the genus *Corynebacterium* and the genus *Brevibacterium* (7). Because most of these organisms are part of the normal flora of skin and mucous membranes, coryneform bacteria isolated from clinical specimens are usually thought to be contaminants, and thus to be of minor importance as a cause of infection in the setting of patients on PD. However, some reports have indicated that *Brevibacterium* species should not be neglected as a cause of peritonitis in patients on continuous ambulatory PD (CAPD). In 1993, Gruner *et al.* were first to report *Brevibacterium* species as a possible causative agent in this setting (5). One year later, Funke and Carlotti identified the strain as *Brevibacterium casei* (10). After the introduction of 16S rRNA gene sequencing revolutionized bacterial taxonomy, with the description of many new species, Wauters *et al.* revised Funke's 2004 species identification, re-identifying their strain as *Brevibacterium sanguinis* (11). Because the latter species exhibits a very

high biochemical similarity to *Brevibacterium casei*, most biochemical tests lack the sensitivity to discriminate the two species, possibly resulting in misidentification (11). However, GenBank database and CLSI guidelines have made possible the use, by microbiology laboratories, of 16S rRNA gene sequencing for reliable and affordable identifications, and the present report used 16S rRNA gene sequencing to accurately identify *Brevibacterium casei* as the cause of peritonitis in our CAPD patient. Other reports have found *Brevibacterium iodinum* (12) and *Brevibacterium otitidis* (13) in dialysate of CAPD patients with peritonitis.

Susceptibility testing of *Brevibacterium casei* used to be based on nonspecific criteria—for example, criteria for *Staphylococcus* species (14). To the best of our knowledge, the present report is first to interpret the susceptibility of a *Brevibacterium* species in accordance with the recently published specific CLSI criteria for coryneform bacteria. As previous reports have (15), we noted reduced susceptibility to beta-lactam antibiotics, but full susceptibility to quinolones, aminoglycosides, tetracyclines, glycopeptides, and linezolid (Table 1). However, contrary to previous reports that interpreted *Brevibacterium casei* as having sensitivity to third-generation cephalosporins (cefotaxime and ceftriaxone) (16), our report found resistance (Table 1). Based upon the novel CLSI criteria, it is justifiable to treat *Brevibacterium casei* infection with glycopeptides or, alternatively, with quinolones, as has previously been described (15). Beta-lactam antibiotics should be avoided because of the reduced susceptibility demonstrated. Given that there was more clinical evidence available of combating *Brevibacterium* infections with vancomycin (17), we accordingly chose that agent for use in treatment.

Beukinga *et al.* reported relapse in a patient with long-term catheter-related *Brevibacterium* bacteremia (18). Relapse of peritonitis attributable to *Brevibacterium* species in CAPD patients has never before been unambiguously published. In our case, relapse of the infection occurred twice despite microbiologically and clinically effective vancomycin treatment. Definite eradication of infection was obtained only after removal of the CAPD catheter. No pathogenic strains were cultured from the catheter, probably as a result of the ongoing antibiotic treatment and the high susceptibility of this *Brevibacterium* strain to vancomycin.

CONCLUSIONS

The present report emphasizes how important it is for nephrologists and clinical microbiologists to pay attention to coryneform bacteria such as *Brevibacterium*

species isolated from effluent. Because biochemical identification of *Brevibacterium* to the species level might be aspecific, we advocate the use of 16S rRNA gene sequencing as a valuable method for accurate species identification.

DISCLOSURES

The authors have no actual or potential financial conflicts of interest.

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