CASE REPORTS

Etiological Misidentification by Routine Biochemical Tests of Bacteremia Caused by *Gordonia terrae* Infection in the Course of an Episode of Acute Cholecystitis

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Received 1 March 2006/Returned for modification 15 April 2006/Accepted 23 April 2006

***Gordonia terrae* has been reported to be a rare cause of bacteremia. We report the first case of bacteremia associated with acute cholecystitis. Commercial biochemical testing was not able to identify the strain at the genus level, classifying it instead as *Rhodococcus* sp. Definitive identification was obtained by sequencing of the 16S rRNA gene.**

CASE REPORT

A 61-year-old male patient with longstanding hepatitis C, hypertension, and calculous gallbladder presented with a fever (38.5°C) of 2 days' duration, lack of urinary sphincter control, and altered mental status, without other accompanying signs/symptoms at admission. Serum and urine laboratory data were unremarkable, a thorax chest film was normal, and abdominal sonographic findings consisted of dilated lumen of gallbladder and the presence of stones. Pretreatment cerebrospinal fluid and blood samples were taken for culturing, and antibiotic treatment with intravenous piperacillin-tazobactam (4 g every 8 h for 20 days) plus intravenous gentamicin (240 mg every 24 h for the first 4 days) was initiated due to a clinical diagnosis of acute cholecystitis. During the antibiotic course, the patient remained afebrile and asymptomatic. Afterward, cholecystectomy was performed; the gallbladder, of 11 by 6.5 cm, was sent to the laboratory for pathological study; and a bile sample was collected for culturing. After being discharged from the hospital, the patient remained asymptomatic upon outpatient follow-up.

Cerebrospinal fluid samples taken at admission showed no biochemical alterations, and culturing yielded no bacterial growth. The blood culture set taken at admission was incubated in BACTEC 9050 (Becton Dickinson, Cockeysville, Md.). On the fourth day of incubation, the aerobic bottle was positive for growth and subcultures in chocolate agar incubated at 37°C under aerobiosis with 5% CO₂ were performed. After 72 h, smooth, salmon-pink, catalase-positive and oxi-

dase-negative colonies were obtained. Microscopic examination showed gram-positive coccobacilli that were negative for Ziehl-Neelsen staining. API CORYNE (bioMérieux, Marcy-l'Etoile, France) was used for initial identification. The isolate gave a profile number of 1110004 and was identified as *Rhodococcus* sp. (98.1% identification; *T* index, 0.94). The strain was sent to the reference laboratory at Instituto Valenciano de Microbiología for further analysis.

Cultures of bile samples taken at surgery after 20 days of piperacillin-tazobactam treatment were negative.

Macroscopic and histological examination of the gallbladder wall revealed intense ulceration, calcification areas, adhesions of external surfaces to hepatic tissues, and moderate acute and chronic inflammation. A stone of 3 by 1.5 cm was found. A definite diagnosis of acute and chronic cholecystitis was made.

At the reference laboratory, the metabolic characteristics of the strain, determined by standard methods (3), were acid production from mannitol, rhamnose, galactose, raffinose, sorbitol, and citrate; hydrolysis of urea and esculin; reduction of nitrates; absence of beta-galactosidase; and absence of growth in lysozyme broth. With Kinyoun stain, a weakly acid-fast nature was shown. The following phenotypic identifications were discarded for the indicated reasons: *Corynebacterium* sp. and *Rhodococcus* sp., based on the weakly acid-fast nature of the strain; *Tsukamurella* sp., due to the absence of beta-galactosidase, the absence of growth in lysozyme broth, and the strain's capability for nitrate reduction; and *Nocardia* sp., due to the absence of beta-galactosidase, the absence of growth in lysozyme broth, and the absence of aerial filaments.

The strain was presumptively identified as *Gordonia terrae* based on colony characteristics and the profile of utilization of sugars as the sole carbon source. Due to the very infrequent isolation rate of *G. terrae* in human specimens by clinical mi-

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crobiology laboratories, the strain was finally identified by genomic DNA extraction from pure culture colonies and sequence analysis of the 16S rRNA gene (10). Bacterial DNA was extracted, amplified with previously described primers (17), and gel purified by using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Sequencing was performed with an ABI Prism 310 automated sequencer (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The nucleotide sequence was analyzed with the use of a BLAST search of the National Institutes of Health GenBank database (BLASTN, version 2.2.13). The strain was definitively identified as *G. terrae*, as 100% similarity with the *G. terrae* type strain (sequence accession no. DQ180334, corresponding to strain NML88-1049) was obtained. Susceptibility tests were performed by agar dilution in Mueller-Hinton agar according to CLSI guidelines for aerobes (6). MICs were 0.03, 0.25, 0.125, 0.015, and 0.25 µg/ml for penicillin, vancomycin, cefotaxime, piperacillin-tazobactam, and gentamicin, respectively.

Hepatitis C virus infection facilitates gallstone formation (5). The diagnosis of obstructive cholecystitis is based on clinical and sonographic findings with specificity and sensitivity values of greater than 90% (12), as other signs, such as moderate temperature elevation and minimal icterus, may not occur. Moreover, laboratory data are rarely required for diagnosis because there are usually only moderately elevated leukocyte counts, and mild elevations in bilirubin, aspartate aminotransferase, alkaline phosphatase, and serum amylase occur in only 50%, 40%, 25%, and 10% of patients, respectively (12). Elderly patients with calculous cholecystitis appear to be more susceptible to the presence of bacteria in bile (12) and bacteremia (9). The presence of bacteria in bile is assumed to occur via an ascending route from the duodenum, when high bacterial counts occur transiently after meals under conditions that allow overgrowth. Elderly patients and/or those with infectious complications should be treated early for infection; piperacillin plus an aminoglycoside is an appropriate initial antibiotic regimen (12), and delayed surgery has been advocated based on the conservative management opinion that morbidity is thereby decreased (12).

The bacterial genus *Gordonia* includes 21 species (www.bacterio.cict.fr) of mycolic acid-containing actinomycetes and belongs to the supragenetic group, which also includes the genera *Mycobacterium*, *Corynebacterium*, *Nocardia*, *Rhodococcus*, *Tsukamurella*, and *Dietzia* (8). *Gordonia* species have been isolated from the environment and from the intestinal contents of mammals (3). To our knowledge, 11 infections by *G. terrae* have been previously described: six catheter-related bacteremias (4, 11, 13), two brain abscesses (7, 8), one skin infection with lymphadenitis (11), one mycetoma (1), and one granulomatous mastitis (18). The isolation of *Gordonia* strains requires 3 to 4 days of incubation; this delay can provide false-negative results and underestimations of infections caused by this genus (14) and can lead to easy confusion with *Rhodococcus* spp., with no single commercially available biochemical testing kit able to differentiate them (13, 15). Penicillins and aminoglycosides are active against *Gordonia* species (2), and treatment of previous bacteremias caused by species from this

genus suggests that a combination of penicillins and aminoglycosides can be a suitable therapy (14).

Here, we describe a *G. terrae* bacteremia in the course of an episode of acute cholecystitis (the definitive anatomopathological diagnosis) in a patient with chronic cholecystitis and hepatitis C successfully treated with piperacillin-tazobactam (20 days) plus gentamicin (4 days). *G. terrae* was isolated only from the one blood culture set taken prior to treatment initiation; it was not recovered from the surgical specimen. The lack of isolation in the bile sample may be due to the high susceptibility of the isolate (consistent with the susceptibility of a *G. terrae* isolate reported previously [13]), as well as to the antibiotics used as treatment (MIC of piperacillin-tazobactam, 0.015 µg/ml) and to the length of the treatment prior to cholecystectomy. Previous reports of patients undergoing cholecystectomy have shown very high mean concentrations of piperacillin-tazobactam in serum (69.1-9.9 µg/ml, respectively), in gallbladder bile (342.3-7.7 µg/ml, respectively), and in the gallbladder wall (49.3-2.9 µg/ml, respectively) after a single dose of 4 g of piperacillin and 0.5 g of tazobactam (16).

The small reported number of infections by *Gordonia* species, together with the time-consuming, labor-intensive, and inconclusive results of biochemical identifications of aerobic actinomycetes, suggest the need for methods such as 16S rRNA sequencing for definitive identification (15). The fact that these highly complex methods rely on specialized equipment, facilities, and personnel suggests that suspected isolates should be sent to reference laboratories (14).

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