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Prosthetic Valve Endocarditis Caused by *Corynebacterium afermentans* subsp. *lipophilum* (CDC Coryneform Group ANF-1)

DAVID L. SEWELL,[1]* MARIE B. COYLE,[2] AND GUIDO FUNKE[3]

Pathology and Laboratory Medicine Service, Veterans Affairs Medical Center, and Department of Pathology, Oregon Health Sciences University, Portland, Oregon 97201[1]; Department of Clinical Microbiology, Harborview Medical Center and University of Washington, Seattle, Washington, 98104[2]; and Department of Medical Microbiology, University of Zurich, CH-8028 Zurich, Switzerland[3]

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Corynebacteria are important causes of endocarditis in individuals with valvular prostheses. We report the first published case of prosthetic valve endocarditis caused by the newly defined species *Corynebacterium afermentans* subsp. *lipophilum* (former CDC coryneform group ANF-1). The isolate was recovered from a perivalvular abscess specimen and 5 of 15 Bactec blood cultures after 7 to 15 days of incubation. The isolation, identification, and susceptibility testing of *Corynebacterium* species are discussed.

Within the last few years there has been an increasing number of reports describing the pathogenic potential of irregular, asporogenous, gram-positive rods (IGPR), also referred to as coryneforms or diphtheroids (2). This may be due, in part, to a greater number of opportunistic infections occurring in patients who are immunosuppressed or implanted with prostheses as well as to the increased awareness of IGPR among clinical microbiologists. These organisms usually are identified on the basis of biochemical test criteria (6, 7) or by a commercial system (3). In addition, methods for the analysis of cell wall fatty acids by gas-liquid chromatography have become useful adjuncts to other identification systems (1). With the increased clinical importance of coryneforms, numerous reports have focused on the taxonomy of IGPR (2).

Documentation of endocarditis caused by IGPR is difficult because not all blood cultures become positive or they may require prolonged incubation, and the clinical significance is sometimes questionable because IGPR are frequent contaminants of blood cultures (11, 18). A recent review of the current literature on IGPR endocarditis identified 126 patients, with 73 (58%) cases of native valve endocarditis and 53 (42%) cases of prosthetic valve endocarditis (PVE) (15). *Corynebacterium jeikeium* and *Corynebacterium pseudodiphtheriticum* were the most common species causing PVE. Although most corynebacteria are susceptible to antimicrobial agents, resistance is unpredictable and must be determined by in vitro susceptibility testing (8, 9). This is the first published report of the newly defined species *Corynebacterium afermentans* subsp. *lipophilum* (former CDC coryneform group ANF-1) (17) causing PVE.

The isolate was recovered from the patient's blood in a Bactec 660 blood culture vial (Bactec Plus 26A; Becton Dickinson, Sparks, Md.) and from a specimen obtained from a perivalvular abscess. The blood and abscess isolates were subcultured on 5% sheep blood agar (SBA) (Trypticase soy agar base; Prepared Media Laboratories, Tualatin, Oreg.) and incubated at 35°C in 7.5% CO_2 for up to 5 days. An inoculum from the 5% SBA plates was used for determining conventional cultural and biochemical properties (6, 7) and inoculation of a Rapid CORYNE strip (Vitek Systems, St. Louis, Mo.) (3). Inoculum preparation and test reading were performed according to the manufacturer's instructions.

Growth on SBA was used to determine a profile of methyl esters of whole-cell fatty acids separated by gas-liquid chromatography with the Microbial Identification System (MIDI Inc., Newark, Del.). Data were analyzed by using the research program that provides two-dimensional dendogram plots of clusters of paired groups of organisms (10). The type strains, *C. afermentans* subsp. *afermentans* (CIP [Collection de bacteries de l'Institut Pasteur] 103499) and *C. afermentans* subsp. *lipophilum* (CIP 103500), were supplied by one of us (G.F.) and were tested in parallel with the patient's isolate.

The patient's blood isolate was tested for antimicrobial susceptibility by the disk diffusion method (12) and the broth microdilution method with Microscan Pos MIC Panel Type 6 (Microscan, Sacramento, Calif.). Vancomycin and penicillin G were tested for bactericidal activity by the broth macrodilution method (13). The susceptibility tests were read after 48 h of incubation instead of the recommended 18 to 24 h because of the slow growth of the isolate. The extended incubation period may have influenced the final result.

Case report. A 76-year-old male was admitted for evaluation and management of a left hemispheric cerebrovascular accident he had suffered 12 days earlier. His medical history was significant for a coronary artery bypass graft and porcine aortic valve replacement in 1991. On admission he had a temperature of 38.8°C and a leukocyte count of 12.9×10^9/liter (normal range, 4.8×10^9 to 10.8×10^9/liter) and was unable to follow verbal commands. The patient exhibited bilateral harsh carotid bruits.

A transesophageal echocardiogram revealed a porcine aortic valve with thickened leaflets, moderately reduced leaflet motion, and a 0.5-cm echodensity associated with the leaflets in the left ventricular outflow tract consistent with a thrombus or vegetation. A primary atrioventricular block was noted on electrocardiography, which raised the issue of a perivalvular abscess. Cardiac catheterization revealed an unstable aortic valve annular ring and a large vegetation in the aortic leaflet.

A coccobacillary gram-positive rod was recovered from both aerobic blood culture sets drawn on admission. Bacterial endocarditis was diagnosed, and the patient started on vancomycin. Prior to surgery, gentamicin and metronidazole were added. At surgery, a prosthetic valve endocarditis with a perivalvular abscess was observed. The Hancock aortic valve was

* Corresponding author. Mailing address: Pathology and Laboratory Medicine Service, Veterans Affairs Medical Center, 3710 SW Veterans Rd., Portland, OR 97201. Phone: (503) 273-5363. Fax: (503) 721-7823.

TABLE 1. Differential reactions of *Corynebacterium* species and *T. otitidis*[a]

Taxon	Lipid requirement for optimal growth	Nitrate reduction	Urea hydrolysis	Acid from glucose	CAMP reaction[b]	Presence of TBSA[c]
C. jeikeium	+	−	−	+	−	−
C. urealyticum	+	−	+	−	−	+
C. pseudodiphtheriticum	−	+	+	−	−	−
C. afermentans subsp. *afermentans*	−	−	−	−	V[d]	−
C. afermentans subsp. *lipophilum*	+	−	−	−	−	−
C. propinquum (CDC group ANF-3)	−	+	−	−	−	−
T. otitidis	−	−	−	−	+	+

[a] Data were compiled from references 1, 4, 6, 16, and 17.
[b] A positive CAMP reaction is defined by enhanced hemolysis with *Staphylococcus aureus* ATCC 25923 (4).
[c] TBSA, tuberculostearic acid.
[d] V, variable.

replaced with a Carpentier-Edwards porcine valve. Gentamicin and metronidazole were discontinued 4 days after the valve replacement.

Five of 15 blood cultures became positive within 7 to 15 days of incubation for a gram-positive bacillus, later identified as *C. afermentans* subsp. *lipophilum*. The specimen from the perivalvular abscess contained the identical organism. On the basis of 48-h susceptibility tests, antimicrobial therapy was switched to intravenous penicillin G for four weeks and then oral penicillin for an additional 6 weeks. The MIC and MBC of penicillin and vancomycin after 48 h incubation were 0.03 and 0.5 μg/ml, respectively. The patient's recovery was uneventful, and he was discharged to a nursing home.

Bacteriology. Colonies on SBA plates incubated for 24 h in 7.5% CO_2 were nonhemolytic, pinpoint in size (<0.5 mm in diameter), and glassy grayish. The type strain *C. afermentans* subsp. *lipophilum* had identical cultural characteristics, whereas the colonies of the type strain of *C. afermentans* subsp. *afermentans* were larger (0.5 to 1.5 mm in diameter) and white. On SBA supplemented with 1% Tween 80 or SBA overlaid with 0.1 ml of sterile olive oil, the colonies from the patient's isolate and from the type strain of *C. afermentans* subsp. *lipophilum* were similar in size and color to colonies of *C. afermentans* subsp. *afermentans*. On Gram staining, the patient's isolate was a gram-positive, coccoid to club-shaped rod.

The Rapid CORYNE strip produced an identification code (2100004) that was the same for the patient's isolate and the two type strains of *C. afermentans*. The patient's isolate was positive only for pyrazinamidase, alkaline phosphatase, and catalase. In conventional biochemical tests, the isolate and the two type strains produced catalase but their reactions were negative for nitrate reduction, motility at 25°C, urease, gelatin and esculin hydrolysis, growth on Simmons citrate, and all carbohydrate substrates tested (glucose, xylose, mannitol, lactose, sucrose, and maltose).

The cellular fatty acid profile was analyzed with the MIDI research software as an adjunct method of identification. The profiles generated for the patient's isolate and the type strain of *C. afermentans* subsp. *lipophilum* were nearly identical and both isolates contained, as major fatty acids, hexadecanoic acid (C16:O), *cis*-9-octadecenoic acid (C18:1w9c), and octadecanoic acid (C18:O). The computer-generated two-dimensional plot placed our isolate in a tight cluster with three reference strains of CDC coryneform group ANF-1.

On the basis of nonstandardized broth microdilution susceptibility tests (48 h of incubation instead of 24 h), the patient's isolate was interpreted as susceptible to penicillin (MIC and MBC, 0.03 μg/ml) and vancomycin (MIC and MBC, 0.5 μg/ml). It also demonstrated apparent susceptibility to ampicillin (MIC, ≤0.12 μg/ml), cefazolin (MIC, ≤2 μg/ml), ceftriaxone (MIC, ≤4 μg/ml), gentamicin (MIC, ≤1 μg/ml), erythromycin (MIC, ≤0.25 μg/ml), ciprofloxacin (MIC, ≤1 μg/ml), and imipenem (MIC, ≤1 μg/ml). The organism was resistant to clindamycin (MIC, ≥2 μg/ml) and trimethoprim-sulfamethoxazole (MIC, ≥2/38 μg/ml). Zone sizes were difficult to read at 24 h on blood Mueller-Hinton agar and unreadable on plain Mueller-Hinton agar. At 48 h the zone sizes were similar for both media. The lack of recommended breakpoints for facultatively anaerobic gram-positive rods other then *Listeria* spp. and the extended incubation period (48 h) preclude a reliable interpretation of zone sizes from the disk diffusion tests (12).

Coryneform organisms are uncommon causes of infections and as commensals of the skin and mucous membranes are often dismissed as culture contaminants, especially when recovered from blood cultures. However, there are now over 100 published reports of endocarditis due to *Corynebacterium* species and other coryneform groups, mostly involving prosthetic or damaged native heart valves (15). The increasing number of cases of endocarditis due to coryneform organisms suggests that laboratories have become more capable of overcoming the difficulties associated with the isolation, identification, and susceptibility testing of the coryneforms. The need for multiple blood cultures and prolonged incubation of blood and routine cultures is reflected in this case and other published reports (11, 18, 20). Bactec blood cultures required a range of 7 to 15 days to become positive, and the initial subcultures required 3 to 4 days for visible growth. Our experience with this strain of *C. afermentans* subsp. *lipophilum* suggests that the isolation rate and rapidity of isolation could be increased with the use of special media. The growth of this isolate was enhanced by subculture to SBA + 1% Tween 80 (17) and to SBA overlaid with sterile olive oil.

Riegel et al. (17) proposed *C. afermentans* subsp. *afermentans* and *C. afermentans* subsp. *lipophilum* for CDC coryneform ANF-1 bacteria on the basis of rRNA gene restriction patterns and DNA-DNA relatedness studies. All of the ANF-1 strains used in the Riegel study were isolated from blood cultures, but their role in disease remained uncertain (17). Phenotypically both subspecies of *C. afermentans* do not ferment any of the carbohydrates used in the routine testing of IGPR (6) or reduce nitrate or hydrolyze urea (Table 1). The most distinctive feature is the colony size and appearance on SBA: *C. afermentans* subsp. *afermentans* colonies are white and 1 to 2 mm in diameter on SBA after 24 h of incubation whereas *C. afermentans* subsp. *lipophilum* colonies are more glassy and grayish and less than 0.5 mm in diameter (17). When SBA is supplemented with 1% Tween 80, colonies of *C. afermentans* subsp. *lipophi-*

lum become whitish beige and reach about the same size as *C. afermentans* subsp. *afermentans* colonies on SBA.

The differentiation of *C. afermentans* from other related nonfermenting IGPR can be achieved with the tests outlined in Table 1. The detection of tuberculostearic acid (e.g., with the MIDI system) as a major criterion for distinguishing between *C. afermentans* subsp. *afermentans* and *Turicella otitidis* (5) may not be available in the routine laboratory. However, *T. otitidis* has been isolated only from patients with otitis media to date (4). If the API (Rapid) CORYNE strip is used for initial biochemical testing it will most likely generate the correct identification (19). However, some strains of *Brevibacterium* spp. share the same numerical code (2100004) with *C. afermentans* (4) but this taxon can easily be separated on the basis of its large colonies (>2 mm on SBA) and its distinct cheese-like smell.

The clinical significance of the isolation of a *Corynebacterium* species from blood is dependent on laboratory and clinical data such as numbers of positive cultures, intravenous drug abuse, alcoholism, and preexistent heart disease (15). The treatment of endocarditis due to *Corynebacterium* species can be difficult, and vancomycin (with or without gentamicin) or penicillin plus gentamicin are suggested drugs of choice for nonsurgical treatment (8, 15). In vitro standardized susceptibility testing is problematic because of the slow growth and fastidious nature of the coryneforms and the lack of recommended breakpoints for interpretation of test results. Testing may have to be performed with media other than Mueller-Hinton (brain heart infusion or Isosensitest media) or with a 48-h incubation (8, 15). Susceptibility tests on the patient's isolate and two type strains were performed on Mueller-Hinton media but had to be incubated for 48 h before the endpoints could be read.

Minimum bacterial activity of the antimicrobial agent should be determined, because of tolerance and lack of bactericidal activity associated with some coryneforms (8, 14). Finally, this case serves as a reminder that IGPR are important causes of endocarditis and require multiple blood cultures and prolonged incubation for recovery of these microorganisms from infected patients.

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