Cardiobacterium Hominis Endocarditis: Description of Two Patients and Characterization of the Organism

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Two cases of endocarditis caused by *Cardiobacterium hominis* are reported. In both instances infection was subacute and characterized by (i) implantation on abnormal valves, (ii) chronic course lasting weeks to months before recognition, and (iii) rapid clinical and bacteriological response to penicillin, as well as other antibiotics commonly used to treat infections caused by gram-negative bacilli. Our isolates of *C. hominis* are compared with strains in the National Institutes of Health culture collection. Optimal growth requires yeast extract and incubation at 37°C with increased humidity and supplemental CO₂. The production of indole, a positive oxidase reaction, and characteristic sugar fermentations distinguish *C. hominis* from other slow-growing, gram-negative bacilli.

Cardiobacterium hominis, a slow-growing, gram-negative bacillus formerly designated as "group II-D" (17), was described by Slotnick and Dougherty in 1964 (12). Septicemia, usually associated with endocarditis, is the only disease state with which the organism has been unequivocally linked etiologically. This report describes two patients with endocarditis caused by *C. hominis* and compares bacteriological characteristics of the isolates to strains in the National Institutes of Health culture collection.

CASE STUDIES

Case 1. A 62-year-old man with asymmetric septal hypertrophy (ASH) was admitted to the hospital because of chest pain, chills, night sweats, fatigue, orthopnea, and weight loss (25 pounds) of 7-months duration. These symptoms followed a dental manipulation for which he received oral penicillin 3 days before and afterwards. Two months prior to admission he had an influenza-like illness treated with a single intramuscular injection of penicillin. He felt well for 2 weeks before his symptoms returned. At admission, he appeared chronically ill and dyspneic. The heart rate was 95 beats/min, respirations 22 per min, blood pressure 100/64 mm of Hg, and temperature 38.5°C. A Roth spot was present in the left ocular fundus. The heart was enlarged. A grade 5/6 apical holosystolic murmur and a grade 1/6 apical diastolic murmur were present. The spleen was palpable. The hematocrit was 28%, leukocyte count 8,200/mm³, and erythrocyte sedimentation rate 94

mm in 1 h. The rapid plasma reagin test for syphilis was positive, but the fluorescent treponemal antibody test was negative. Urinalysis was unremarkable. A chest roentgenogram showed increased pulmonary vascularity and enlargement of the left atrium and left ventricle. The electrocardiogram showed left bundle branch block. All 14 blood cultures taken on hospital days 2 through 9 revealed a gram-negative rod, later identified as *C. hominis*.

The patient was initially treated with gentamicin and carbenicillin. After determination that the organism was sensitive to penicillin at a minimum inhibitory concentration of less than 4 µg/ml, treatment with 3 million U of penicillin G every 6 h intravenously was initiated and continued as the only antibiotic for 4 weeks. After less than 1 week the patient became afebrile, the splenic size diminished, and his exercise tolerance increased concomitantly with the disappearance of chest pain and symptoms of congestive heart failure.

He was discharged after 46 days, but was readmitted 11 days later because of recurrent congestive heart failure and chest pain. Repeat blood cultures were negative. Asymmetric septal hypertrophy and severe mitral regurgitation were documented by cardiac catheterization. He underwent mitral valve replacement. Several chordae tendineae of the anterior leaflet of the mitral valve were ruptured and many were thickened (Fig. 1). The leaflets were diffusely thickened, and a large perforation was present in the anterior leaflet. The posterior

FIG. 1. Opened excised valve from case 1 showing diffusely thickened leaflets, with a large perforation in the anterior leaflet. Calcific nodules representing "healed vegetations" border the perforation.

leaflet was focally thickened. The aortic valve was normal. The patient had an uneventful recovery and after 20 months remains in functional class I.

Case 2. A 56-year-old man known to have a precordial murmur since childhood developed increasing dyspnea and palpitations after an upper respiratory infection. Examination disclosed overt congestive heart failure. The murmur was believed to be due to congenital aortic valvular disease. Four months later he suddenly developed expressive and sensory aphasia, which was attributed to a cerebral embolus. He recovered slowly, but, 5 months after the cerebrovascular accident, he again developed congestive heart failure. Because of fever and splenomegaly he was admitted to the hospital. The heart rate was 110 beats/min, respirations 16 per min, blood pressure 100/60 mm of Hg, and temperature 38.1°C. The heart was enlarged. There was a grade 3/6 holosystolic murmur at the left sternal border and apex. Both the liver and spleen were palpable. The hematocrit was 31%, leukocyte count was 6,500/mm³ with a normal differential, and the erythrocyte sedimentation rate was 44 mm in 1 h. Urinalysis revealed 4+ albumin and 20 leukocytes and 100 erythrocytes per high-power field. Gram-negative bacilli, later identified as *C. hominis*, were recovered from six of six blood cultures. By disk sensitivity testing, the organism was determined to be susceptible to penicillin, ampicillin, chloramphenicol, tetracycline, streptomycin, kanamycin, gentamicin, colistin, and carbenicillin. Serum agglutinins with a titer of 1:80 were demonstrated against

Brucella melitensis. Because of initial suspicion of brucella endocarditis, therapy was begun with tetracycline and streptomycin. He promptly became afebrile, but after 5 days the therapy was changed to aqueous penicillin G, 10 million U intravenously per day, for 18 days. Oral therapy was continued for another 3 weeks with penicillin V, 5 g daily, plus probenecid. The patient remained afebrile and recovered uneventfully. Three months later he underwent cardiac catheterization, which documented a peak systolic gradient of 17 mm of Hg across the aortic valve. Left ventricular angiography revealed a slightly calcified aortic valve. There was a marked reduction in overall myocardial contractility. The congestive heart failure progressed, and he died 30 months later without clinical or bacteriological evidence of recurrent infective endocarditis; however, no autopsy was done.

MATERIALS AND METHODS

Blood culture technique. In case 1, 10 ml of venous blood collected by syringe and needle was inoculated into each of two screw-capped vacuum bottles containing 100 ml of brain heart infusion (BHI) broth with sodium polyanetholsulfonate (SPS), *p*-aminobenzoic acid, and CO₂ (BBL). Upon receipt in the laboratory, one bottle was air-vented by a needle attached to a Swinney filter. This bottle was considered aerobic, and the unvented bottle was considered anaerobic. Both were incubated at 35°C and inspected macroscopically daily for evidence of growth. Twenty-four-hour subcultures from both anaerobic and aerobic bottles were made onto BYE agar (1) and incubated anaerobically or under 10% CO₂, respectively. In case 2, 5 ml of venous blood was inoculated into each of two vacuum bottles. One

contained 100 ml of casein soy broth with SPS and CO₂. The second contained 100 ml of thioglycolate broth (without indicator) as well as SPS and CO₂. Each bottle was incubated without venting at 35°C. Routine blind subcultures to chocolate agar slants were made at 48 h and on day 10. Positive chocolate slants were sent to the National Institutes of Health for characterization. These subcultures were then handled as the subcultures from case 1. Organisms recovered from blood cultures in case 1 and case 2 were identified using the analysis outlined by Weaver et al. (19).

C. hominis bacterial strains. Three strains of *C. hominis* (NIH CPD no. 5, 7, and 8) were obtained from the National Institutes of Health culture collection maintained by the Microbiology Service of the Clinical Pathology Department. Strain NIH CPD no. 8 was isolated by Irving Slotnick, University of Florida, and has been suggested as a reference strain for *C. hominis* (3). It corresponds to Slotnick's strain 6573.

Media. All *C. hominis* cultures were streaked on 5% horse or sheep blood in Columbia base (Difco), phenylethyl alcohol medium, MacConkey agar (Difco), and two types of modified blood agars (1, 5). Growth at 22, 37, and 42°C was observed in Trypticase soy broth (TSB, Difco) at 24 and 48 h. Motility was determined in semisolid motility medium (Difco) at 37 and 22°C.

Growth requirements. Growth of *C. hominis* with and without 5 to 10% CO₂ was compared at 37°C. A moisture chamber was used at 37°C to determine whether increased humidity enhanced growth. The requirement for accessory growth factors was assessed by placing paper strips containing X factor (heme), V factor (coenzyme NAD), and both X and V factors on a Columbia agar plate previously inoculated with the test organism.

Biochemical reactions. The methods for biochemical characterization followed standard microbiological techniques (16). Fermentation reactions were determined in phenol red broth base (Difco) containing 1% carbohydrate. H₂S production was tested by suspending lead acetate-impregnated paper over TSI slants (Difco) and observing both the TSI butt and paper strip. Production of indole was tested in indole test broth (tryptone broth, Difco) by using both Kovacs' reagent and Ehrlich reagent. A complete test for nitrate reduction was performed after growth in nitrate beef extract broth (BBL). The presence of catalase was demonstrated by the evolution of gas from 3% H₂O₂ placed directly on colonies of the test organism on BHI agar. The oxidase reaction was demonstrated by the method of Kovacs as elaborated by Steel (15). The Voges-Proskauer test for acetylmethylcarbinol and the methyl red test were performed on a 48-h growth in MRVP medium (Difco). Simmons citrate agar (Difco) was employed to test the ability to utilize citrate as the sole source of carbon and energy. Urease production was determined at 48 h on urea slants (Difco). Esculin hydrolysis in the presence of 40% bile was observed on bile esculin slants (Difco). Amino acid decarboxylase/dihydrolase activity was detected in Moeller medium (BBL) containing either L-arginine monohy-

drochloride, L-ornithine dihydrochloride, or L-lysine dihydrochloride. The tubes were inoculated by stabbing deeply and then were overlaid with a 1-cm layer of sterile mineral oil.

Antibiotic susceptibility. Susceptibility to antibiotics of the isolates from cases 1 and 2 and the three available strains of *C. hominis* was determined by a serial dilution microtiter technique (7). The broth medium employed was one routinely used for testing hemophilus: 10% Fildes reagent, 0.5% yeast extract, and 2% IsoVitaleX in TSB base.

RESULTS

C. hominis was recovered from all blood cultures drawn in each patient, but its characteristically slow growth resulted in large numbers of cultures being taken (14 in case 1 and 6 in case 2) before growth was detected 6 days after initial collection. Previous authors have emphasized the pleomorphism of *C. hominis* and its irregular staining (12, 13, 16). We confirmed this when the organisms were grown on agar without yeast. However, when the organisms were grown on agar containing yeast extract they appeared mainly as uniform gram-negative rods (0.5 by 2 µm) (Fig. 2). Both supplemental CO₂ and increased humidity, tested separately, enhanced growth.

Biochemical characteristics of our isolates are compared with reference strains in Table 1. *C. hominis* is reported to produce indole. However, we found that indole production was slight and could be missed unless concentrated by xylene extraction before adding Kovacs' or Ehrlich reagent for detection. *C. hominis* may resemble *Actinobacillus actinomycetemcomitans* or *Haemophilus aphrophilus*, but the latter two are oxidase negative and reduce nitrate to nitrite. Other *Haemophilus* species require V factor and may also require X factor. Pasteurella is distinguished by catalase positivity. Brucella and *Bordetella bronchiseptica* are also catalase positive and, moreover, differ from *C. hominis* in their ability to produce urease and reduce nitrates and in their failure to produce indole. *Bordetella parapertussis*, unlike *C. hominis*, is oxidase negative and catalase positive and produces beta hemolysis on blood agar. *Eikenella corrodens*, a gram-negative rod recently recognized as a pathogen, has been implicated as a cause of endocarditis (4). Colonies have a typical corroding morphology and do not produce acid by fermentation or oxidation from carbohydrates (2).

The *C. hominis* strains tested were uniformly susceptible to penicillin G as well as antibiotics commonly used to treat gram-negative bacillary infections (Table 2). Minimal inhibitory concentrations were less than 2 µg/ml when the two isolates and the reference strains

FIG. 2. *Cardiobacterium hominis* organisms appearing as uniform gram-negative rods when grown on agar containing yeast extract.

were tested against ampicillin, carbenicillin, cephalothin, tetracycline, chloramphenicol, streptomycin, kanamycin, gentamicin, and colistin.

DISCUSSION

C. hominis has caused bacterial endocarditis in nine previously documented cases (6, 8, 10, 14, 17, 20). In two additional cases the organism was recovered from blood cultures: a newborn with septicemia, and an adult with positive blood cultures (17). Thus, where adequate clinical information has been supplied, no disease state other than bacterial endocarditis has been related to *C. hominis*. The source of infection and mode of transmission have been unclear in all cases, although the organism is presumed to inhabit the upper respiratory tract and feces of most humans of all age groups (13). *C. hominis* appears to possess low virulence. This hypothesis is in keeping with the observation that the

endocarditis has a subacute course, and in at least six of the previously reported cases, infection occurred in patients with preexisting disease of the mitral or aortic valves or a ventricular septal defect.

Our patients likewise had underlying abnormalities of their heart valves. In ASH, the underlying heart disease in case 1, the mitral leaflets and chordae tendineae are nearly always focally but extensively thickened by fibrous tissue, an abnormality that at least partly results from trauma caused by abutment of this leaflet against the hypertrophied ventricular septum (9, 10). In case 2, the patient had congenital aortic stenosis, and the infective endocarditis most likely affected this valve.

C. hominis isolates from each of the two patients were highly susceptible to penicillin derivatives. Delays in identification and susceptibility testing caused by slow growth of the bacterium resulted in initial treatment with antibiotics generally more active against other

TABLE 1. Key biochemical^a characteristics of five strains of *C. hominis*

Determination	NIH CPD strain			Case 1	Case 2
	5	7	8		
Oxidase	+	+w ^b	+	+w	+
Catalase	—	—	—	—	—
Dextrose	A ^c	A	A	A	A
Lactose	—	—	—	—	—
Sucrose	A	A	A	A	A
Maltose	A	A	A	A	A
Decarboxylase reactions	—	—	—	—	—
Indole	+	+	+w	+	+
Growth					
10% CO ₂	4+	4+	4+	4+	4+
Aerobic	0	0	0	0	0
Anaerobic	1+	1+	1+	1+	1+
Nitrate reduction	—	—	—	—	—

^a The organisms grew poorly on triple sugar iron media and in MR-VP broth. They did not require X and V factors, were nonmotile, and did not produce gas from glucose.

^b +w denotes a weakly positive reaction.

^c A denotes acidification.

TABLE 2. Antibiotic susceptibilities of five strains of *C. hominis*^a

Antibiotic	NIH CPD strain			Case 1	Case 2
	5	7	8		
Penicillin G	<0.4	<0.4	<0.4	<0.4	<0.4
Ampicillin	<0.4	<0.4	<0.4	<0.4	<0.4
Carbenicillin	<1.6	<1.6	<1.6	<1.6	<1.6
Cephalothin	<0.4	<0.4	<0.4	<0.4	<0.4
Tetracycline	1.6	0.8	0.8	0.8	<0.4
Chloramphenicol	1.6	0.8	0.8	1.6	<0.4
Streptomycin	0.8	1.6	0.8	1.6	0.8
Kanamycin	<0.4	0.8	<0.4	<0.4	0.8
Gentamicin	0.8	0.8	0.2	0.4	0.8
Colistin	<0.4	<0.4	<0.4	<0.4	<0.4

^a Numbers represent minimal inhibitory concentration (MIC) in micrograms per milliliter.

gram-negative bacilli. After identification of the organism, however, both patients received penicillin in high doses intravenously, and bacteriological cure resulted.

The morphological characteristics of *C. hominis* were dependent on whether or not yeast extract was incorporated into the growth medium. This organism has characteristically been described as an irregularly straining, highly pleomorphic gram-negative rod. We found that on two types of modified blood agar, both containing yeast extract, the organism appears homogeneously stained and of uniform dimensions with occasional irregular forms. These properties suggest to us that, in addition

to supplemental CO₂ and increased humidity, yeast extract promotes optimal growth. Without yeast extract highly pleomorphic, irregularly staining rods were seen. The biochemical reactions of our two isolates are identical with Slotnick's reference strain 6573, as well as two additional strains of *C. hominis* available for comparison. Among the most important biochemical reactions which distinguish *C. hominis* from other slow-growing, gram-negative bacilli with which it may be initially confused are indole production, the oxidase reaction, and the sugar fermentation pattern. The production of indole, although slight, appears invariable and is best demonstrated by xylene extraction of the test medium.

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