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Endocarditis of Native Aortic and Mitral Valves Due to *Corynebacterium accolens*: Report of a Case and Application of Phenotypic and Genotypic Techniques for Identification

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Endocarditis of native aortic and mitral valves due to an organism identified as *Corynebacterium accolens* developed in a 73-year-old patient without predisposing factors. The organism was identified as *C. accolens* by biochemical identification, amplified rRNA gene restriction analysis, and DNA-DNA hybridization. This is the first case of *C. accolens* endocarditis reported, adding to the increasing number of *Corynebacterium*-related cases of endocarditis.

Cases of endocarditis are rarely caused by corynebacteria. A total of 126 cases have been reviewed recently (4). Fifty-three of these cases were related to the presence of prosthetic valves, and 107 of the organisms were related to *Corynebacterium* species living commensally on the skin. Both associations may be ascribed to surgery or to the injection of drugs. Furthermore, most of the cases occur in patients with preexisting heart disease or in patients who are parenteral drug users (4).

The increase in the number of reports concerning *Corynebacterium* infections may be due to several factors, e.g., better knowledge of the taxonomy of these organisms, better identification schemes, more awareness among clinicians and microbiologists of the possible importance of these organisms, and/or a growing population of immunodepressed patients.

Here we report a case of native valve endocarditis due to *Corynebacterium accolens*, which is known to be an inhabitant of the upper respiratory tract, in a patient without predisposing factors. An infectious focus could not be revealed at preoperative oral and otorhinolaryngeal examination.

It is shown that current biochemical identification of this organism may pose some problems and needs special attention.

Case report. A 73-year-old Caucasian male, returning from a journey to Australia, suffered from recurrent periods of acute fever with shivering, which was treated with various antibiotics.

Two and a half months later he was hospitalized in a general hospital for left heart decompensation. Endocarditis of the aortic and mitral valves was suspected at echocardiography and treated with amoxicillin-clavulanate IV (3 g/24 h). Blood cultures all remained negative.

Because of rapidly progressing cardiac decompensation and a deteriorating general condition he was transferred to a cardiac surgical unit in another general hospital for operative treatment. On admission a Swan-Ganz profile suggested septic shock. There was oligoanuria with a blood creatinine level of 4.7 mg/dl. The erythrocyte sedimentation rate after 1 h was 50 mm/h, the C-reactive protein level was 12.2 mg/dl, and the leukocyte count was 11,200/mm^3.

Antibiotic treatment was stopped on admission, and blood—16 samples in total—was taken every 4 h and cultured for a period of 10 days, all with negative results. Preoperative otorhinolaryngeal and oral investigation could not reveal an infectious focus. Transesophageal echocardiography of aortic and mitral valves showed severe insufficiency with vegetations on both valves. Surgery was performed on the second day after admission. No antibiotics were given at the time of induction of anesthesia. The aortic and mitral valves, both heavily affected, were excised, and each was replaced with a porcine valvular bioprosthesis.

Microscopic investigation of the excised valves revealed the presence of a gram-positive coccobacillus. On the third postoperative day an unusual gram-positive organism was cultured from the vegetations of the mitral valve.

A combination therapy of gentamicin (three doses of 80 mg/24 h intravenously) and flucloxacillin (six doses of 2 g/24 h intravenously) was instituted during surgery. Flucloxacillin was replaced by six doses of penicillin G (2×10^6 U) daily in association with gentamicin at 3 mg/kg of body weight per 24 h. Gentamicin administration was stopped at 15 days postoperation. Penicillin G treatment was continued for 6 weeks.

Because of persisting total atrioventricular blockage a definitive pacemaker was placed on the 10th postoperative day. At 6 weeks postoperation, the C-reactive protein level, the erythrocyte sedimentation rate, and the leukocyte count were within the normal range and antibiotic treatment was stopped. The patient was dismissed at 7 weeks postoperation. Three months later the patient did not show any sign of infection or decompensation, and he was physically active.

Bacteriology. At laboratory 2, negative cultures were obtained from the excised valves and vegetations of the aortic valve. Culture of the vegetations of the mitral valve on sheep blood agar plates incubated for 48 h in a candle extinction jar revealed slowly growing, nonhemolytic, greyish, transparent, small colonies. On Gram staining the cells appeared primarily

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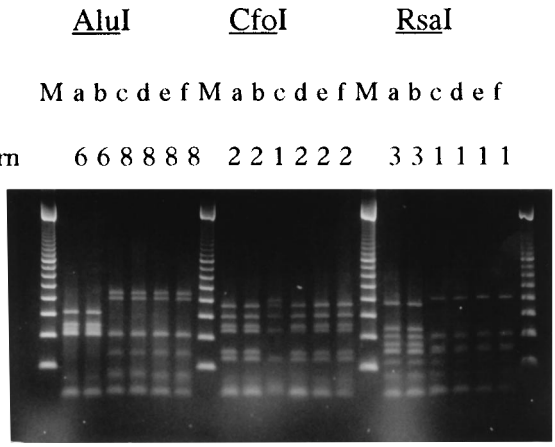

FIG. 1. Patterns observed after restriction of the amplified 16S rDNA with *Alu*I, *Cfo*I, and *Rsa*I for the endocarditis isolate in comparison with the patterns observed for reference strains of *C. accolens* and strains of *C. macginleyi* and *C. propinquum*. Lanes: M, 100-bp ladder (Pharmacia Biotech, Uppsala, Sweden); a, *C. propinquum*, IBS B501121 (Institute of Bacteriology of Strasbourg, Strasbourg, France [IBS]); b, *C. propinquum* 202147 (see reference 4); c, *C. macginleyi* CIP 104099^T (Collection Institut Pasteur); d, *C. accolens* IBS T 48463; e, *C. accolens* IBS B 80466; f, strain isolated from native valves in a case of endocarditis (this report). Pattern numbering is that of Vaneechoutte et al. (11).

as gram-positive coccobaccilli, single, in pairs, and in short chains. Catalase and oxidase tests were negative.

API20 Strep (BioMérieux, Marcy l'Etoile, France) identification with 24-h incubation was read by the ATB software as profile number 114000, with Vosges-Proskauer, leucine aminopeptidase, and pyrrolidonyl aminopeptidase as the only positive tests. This resulted in a doubtful identification: *Gemella morbillorum* (78.3% probability), *Streptococcus adjacens* (12.5% probability), or *Gemella haemolysans* (7.4% probability). The positive Voges-Proskauer and pyrrolidonyl aminopeptidase tests were contradictory to the identification as *G. morbillorum.*

The organism was susceptible to penicillin, second-generation cephalosporins, amoxicillin, tobramycin, vancomycin, erythromycin, rifampin, and clindamycin and resistant to chloramphenicol as tested with the ATB system (BioMérieux).

Because of the uncertain identification, the organism was sent for further investigation to two different university microbiology laboratories (laboratories 1 and 4).

Laboratory 1 is a university laboratory which applied biochemical identification and amplified rRNA gene (rDNA) restriction analysis (ARDRA) (9–11).

Because of the microscopic morphology—i.e., that of coryneform bacteria—and a positive catalase test, API Coryne (BioMérieux) was used, without addition of Tween 80 to the gallery cups. Identification code 3 000 004 was obtained after both 24 and 48 h, and this yielded an 80% sure identification of the organism as *Corynebacterium* ANF (absolute nonfermenter, not specified any further).

ARDRA comprises amplification of the 16S rDNA and subsequent restriction analysis of the amplified gene, independently and with different restriction enzymes. Identification is achieved by comparison of the obtained DNA fingerprints with those obtained from a collection of reference strains (11). Restriction analysis of the 16S rDNA with the enzymes *Alu*I, *Cfo*I, and *Rsa*I showed that the patterns observed were identical to those described in a previous report (11) for two *C. accolens* reference strains (Fig. 1).

Laboratory 3 is a *Corynebacterium* reference laboratory which carried out biochemical identification and DNA-DNA hybridization to verify the identification of the organism obtained by ARDRA.

Hybridization between labeled DNA and fragmented DNA preparations was carried out at 60°C for 16 h in 0.42 M NaCl by the S1 nuclease-trichloroacetic acid method (5). DNA-DNA hybridization experiments revealed that the isolate is 76% related to the *C. accolens* type strain CCUG 28779^T.

Acid production from sugars was tested by using a basal heart infusion (BioMérieux) at 25 g/liter, adjusted to pH 7.8 and containing 1% (wt/vol) sugar, 0.1% Tween 80 (Merck, Darmstadt, Germany), and phenol red as an indicator. Alkaline phosphatase activity was tested by using Diatabs (Rosco, Taatstrup, Denmark). The findings of this laboratory differed from those of laboratory 2, since the organism was found to be a pleomorphic gram-positive rod and since biochemically the organism was catalase positive. Additionally, it was found that nitrate was reduced to nitrite but alkaline phosphatase, gelatinase, and urease activities and esculin hydrolysis were lacking. With a medium supplemented with Tween 80, acidification from glucose, ribose, and mannitol, but not from sucrose and maltose or galactose, was observed. Laboratory 4—a university laboratory with special interest in the identification of gram-positive organisms—carried out biochemical identification according basically to the methods of Krech and Hollis (2) and Sierra (8), while alkaline phosphatase and pyrrolidonyl aminopeptidase production were studied by using Diatabs (Rosco) and confirmed independently the morphological and biochemical findings of laboratory 3.

Laboratories 3 and 4 showed that the organism formed small colonies on tryptic soy agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% horse blood after 24 h of incubation, that scant growth on tryptic soy agar was markedly enhanced when 1% Tween 80 was added, indicative of lipid-requiring organisms, and that weak Tween-esterase activity was observed after several days of incubation. After 3 days of incubation on tryptic soy agar, obvious satellitism was observed around *Staphylococcus aureus* streaks. These are typical features of *C. accolens* according to previous studies (3, 6).

Four laboratories carried out biochemical identification independently. Poor API-based biochemical identification may be explained by the difficulties in interpreting the Gram stain as representative for a coryneform organism, by the lack of addition of Tween 80 to the API gallery cups, and/or by the incubation time, which was 24 h instead of 48 h. In the original description of this species (3) it was observed that freshly isolated strains very often resemble ovoid coccobacilli and thus do not appear as typical coryneform rods. This—together with the false-negative catalase reaction—may explain why the organism initially was considered *Streptococcus*-like, leading to a presumptive identification as *G. morbillorum*. Furthermore, API guidelines do not mention the need for prolonged incubation or for addition of Tween 80 for the identification of lipid-requiring corynebacteria, while fermentation of sugars can be absent if the medium used is not supplemented by lipid such as serum or Tween 80.

C. accolens or lipophilic diphtheroid genomospecies II (6) was described as an inhabitant of the upper respiratory tract (3, 6) and has been isolated in pure culture in multiple cases of otitis media ($n = 8$), sepsis ($n = 6$), keratoconjunctivitis ($n = 6$), and sinusitis maxillaris ($n = 2$) and in single cases of various other diseases, such as tonsillitis, ulcus corneae, endocervicitis, meningitis, and tongue abscess (3). The first probable isolate of this species came from autopsy material from a patient who died of subacute bacterial endocarditis (12), but the isolate was not preserved (3). In a recent review of *Corynebacterium*-re-

lated endocarditis no cases of *C. accolens*-related endocarditis were included (4).

An important portion of the reported *Corynebacterium*-related cases of endocarditis are due to lipophilic organisms—such as *Corynebacterium bovis* and *Corynebacterium jeikeium* (4), *Corynebacterium afermentans* subsp. *lipophilum* (7), and the organism reported here. Probably this is still an underestimation of the clinical importance of lipophilic coryneforms, since many cases may have been overlooked because of the need for lipid-containing media and for prolonged incubation for the isolation and correct biochemical identification of these organisms.

This could mean that when coryneform bacterium-related endocarditis is suspected, both types of media (lipid free and lipid containing) should be used to ensure a reliable biochemical identification.

C. accolens was originally described on the basis of its property of exhibiting satellitism near *S. aureus* (3). Taxonomic studies indicated a close relationship with the lipid-requiring *Corynebacterium macginleyi* (lipophilic diphtheroid genomospcies III) and strains forming lipophilic diphtheroid genomospecies I, named coryneform group G (6). Subsequently, lipid requirement could also be shown for *C. accolens* and satellitism around *S. aureus* cultures could be shown for *C. macginleyi* and coryneform group G (6). Only the species *C. macginleyi* and *C. accolens* can be clearly differentiated phenotypically from other lipid-requiring strains. *C. accolens* resembles best *C. macginleyi* in that both species are maltose fermentation and urease production negative and glucose fermentation and nitrate reductase production positive, but it can be differentiated from *C. macginleyi* since it is alkaline phosphatase negative.

The fastest identification—i.e., within 2 days after receipt of the isolate—was obtained with ARDRA (11). The identification as *C. accolens* by ARDRA could be confirmed by biochemical testing in reference laboratories and by DNA-DNA hybridization—which is considered the "gold standard" for assessing species identity. ARDRA and other PCR-based DNA fingerprinting techniques applicable for species identification—like tRNA PCR (13), 16S rDNA single-strand conformation polymorphism analysis (14), and rDNA intracistronic spacer length polymorphism analysis (1)—may have several advantages over phenotypic identification as well as PCR applied for direct detection of pathogens from clinical samples. Since a single small colony is sufficient to proceed, a final identification can be achieved within 8 to 12 h starting from culture, and identification by these approaches is less dependent on culture conditions. In contrast to PCR applications, which aim for specific detection of organisms directly from clinical samples and which necessarily rely on the use of species-specific primers and/or probes, the above-mentioned techniques make use of universal primers, i.e., primers complementary to well-conserved regions of the bacterial genome, and therefore can be applied for the identification of most bacterial species.

To our knowledge this is the first well-documented report of *C. accolens*-related endocarditis. Most cases of endocarditis due to coryneform bacteria are caused by skin commensals, while *C. accolens* is considered an inhabitant of the upper respiratory tract; most *Corynebacterium*-related cases of endocarditis occur with prosthetic valves, while this was a native valve endocarditis; and in most cases patients have predisposing factors, while here the 73-year-old patient had no known preexisting heart disease and was apparently not immunosuppressed. The possible source of infection remains unknown.

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