Molecular Diagnosis of Endocarditis Due to *Lactobacillus casei* subsp. *rhamnosus*

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We report a case of endocarditis due to *Lactobacillus casei* subsp. *rhamnosus*. The bacterium isolated from blood cultures and from valve tissue specimens was identified using both phenotypical analysis and DNA sequence analysis, which revealed that the rod profiles of the pathogens recovered from blood cultures and valve tissue specimens were the same.

*Lactobacillus* species are gram-positive rods that are considered nonpathogenic. Identification of *Lactobacillus* isolates to the species level is uncommon in routine laboratory practice for 2 reasons: (1) *Lactobacillus* species are ubiquitous in and commensal with the oral cavity, intestinal flora, and the female genital tract; and (2) identification is made by conventional (i.e., nonmolecular) means for only 30%–50% of the isolates; correct identification requires molecular analysis of the 16S rRNA gene [1, 2]. Few cases of endocarditis due to *Lactobacillus* species have been reported in the literature [3–5]. We report a case of endocarditis due to *Lactobacillus casei* subsp. *rhamnosus* that was isolated from blood cultures and from valve tissue specimens. The bacterium was identified by use of both phenotypical and molecular methods.

**Case report.**  In June 2001, a 73-year-old man was hospitalized in the cardiology ward because of progressive asthenia and fever (temperature, 39°C). Physical examination of the patient, who had a prosthetic Carpentier-Edwards valve in place for treatment of aortic stenosis, revealed a diastolic murmur (grade 5/6) at the cardiac apex, combined with an aortic murmur. The patient was known to have controlled arterial pressure. He showed no peripheral signs of endocarditis. The patient reported that he did not eat yogurt every day [6] and that he had not undergone dental manipulation before admission to the hospital [4, 7–10]. Laboratory tests revealed an erythrocyte sedimentation rate of 45 mm/h and a WBC count of 23 x 10⁹ cells/L (70% polymorphonuclear neutrophils). Transesophageal echocardiography revealed a pediculous vegetation (15 mm) on the large mitral cusp, which was perforated.

Before the initiation of antimicrobial treatment with vancomycin (160 mg q.d.) and gentamicin (160 mg q.d.), blood samples were obtained for 4 sets of aerobic and anaerobic cultures, the results of which were positive. Direct examination of a Gram-stained smear revealed a gram-positive pathogen that was possibly *Lactobacillus* species. The antimicrobial treatment regimen was modified to include amoxicillin (12 g q.d.) and rifampin (600 mg q.d.). After 1 month of treatment, a prosthetic mitral valve was implanted in the patient because of high-grade mitral insufficiency due to a cordal rupture, and the first prosthetic aortic valve was replaced. Cultures of specimens from the 2 valves, which were performed as described elsewhere [11], were sterile, whereas pathological microscopic examination of the valve specimens revealed typical features of definite infective endocarditis.

This patient with *Lactobacillus* endocarditis, who had not undergone digestive-tract surgery and had no history of cancer [4, 12, 13], had been hospitalized on 22 February 2000 because of meningitis due to *Streptococcus pneumoniae* with decreased susceptibility to penicillin G; the meningitis was treated with cefotaxime, ciprofloxacin, and vancomycin. On 15 March 2000, the patient presented with pneumonia due to meticillin-resistant *Staphylococcus aureus*, which was treated with teicoplanin and fosfomycin. This 2-month course of antibiotic treatment, inactive against *Lactobacillus* species [5, 13], might have selected for *Lactobacillus* and might explain the bowel flora imbalance. Because of a transitory, asymptomatic episode of bacteremia [14], a secondary vegetation (due to the aggregation of human platelets) developed on the mitral valve in June 2001, which was revealed by progressive asthenia [4, 5, 10, 15, 16].

With regard to the 4 positive blood cultures, a gram-positive rod grew on both blood agar and chocolate agar plates. Gram staining of the culture revealed a long, gram-positive bacillus. This nonmotile, non–spore forming, aerobic-facultative bacterium expressed a small degree of α-hemolysis (diameter, 1 mm) after 24 h of incubation. The bacterium was catalase negative and oxidase negative. Phenotypical identification was performed using an API 50CH test kit and API CHL medium (bioMérieux) in accordance with the manufacturer’s instruc-
ditions; the test identified *L. casei* subsp. *rhamnosus* (excellent identification [i.e., identification made with the kit], 99%; typicity, 0.93). Susceptibility testing was performed by the disk-diffusion method on Mueller-Hinton agar with 5% horse blood, which revealed that the bacterium was susceptible to ampicillin, piperacillin, rifampicin, pristinamycin, and gentamicin and that it was resistant to cefotaxime, trimethoprim-sulfamethoxazole, fluoroquinolones, and vancomycin.

To determine the involvement of *L. casei* subsp. *rhamnosus* in the endocarditic process and to confirm that the pathogen was correctly identified by conventional methods, the 16S rRNA gene was sequenced from colonies recovered from cultures and from bacteria recovered from the clinical mitral valve tissue specimen. By use of the Microseq 500 16S rDNA bacterial kit (PE Applied Biosystems), a 522-bp fragment was amplified from bacteria and sequenced with use of an automated sequencer (377 ABI Prism; PE Applied Biosystems). The fragment was then compared with National Center for Biotechnology Information (NCBI) GenBank entries, which indicated 100% homology with *L. rhamnosus* strain F11 (GenBank accession number AF243146). DNA was also extracted from a fragment of the mitral valve tissue with use of the QIAamp DNA minikit (Qiagen SA), in accordance with the manufacturer’s tissue protocol, with addition of lysozyme (20 mg/L) and protease K (20 μL/L). Amplification was then performed with the Microseq 500 16S rDNA bacterial kit, and the amplified fragment was checked via electrophoresis. After purification of the product with use of a SpinX kit (Costar), the 493-bp fragment obtained was sequenced and compared with NCBI GenBank entries by use of the Basic Local Alignment Search Tool (BLAST) algorithm, which indicated 99% homology with *L. rhamnosus* strain F11 (GenBank accession number AF243146). We used a negative PCR control that consisted of an aortic valve excised from a patient who had undergone valve replacement for mechanical etiology; no amplification was obtained with use of the same methodology, we proved that the *Lactobacillus* strain recovered from our patient was responsible for endocarditis.

By use of a simple extraction method with a commercial kit, a clinical laboratory with a molecular science department may easily diagnose infections due to fastidious or uncommon etiologic agents, such as *Lactobacillus* species, present in valve tissue and may confirm the real involvement of these bacteria found in blood cultures, as was described in this report. Other authors have confirmed this practice, such as Gauduchon et al. [21], who detected recurrent *Streptococcus mutans* endocarditis in the heart valve of a patient for whom 3 blood cultures yielded *Escherichia coli*. Because this method is effective for diagnosis of infective endocarditis, Millar et al. [19] suggested the inclusion of molecular detection as a major criterion in Duke’s classification scheme.

References


