Peritonitis Associated with Vancomycin-Resistant \textit{Lactobacillus rhamnosus} in a Continuous Ambulatory Peritoneal Dialysis Patient: Organism Identification, Antibiotic Therapy, and Case Report

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A case of \textit{Lactobacillus rhamnosus}-associated peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis is reported. The patient was treated with vancomycin after isolation of glycopeptide-susceptible coagulase-negative staphylococci. After a skin rash developed, vancomycin was discontinued and replaced with teicoplanin. Seven weeks after the glycopeptide therapy was discontinued, a \textit{Lactobacillus} strain was isolated in pure cultures. The isolate was identified first incorrectly as \textit{L. acidophilus} but later correctly as \textit{L. rhamnosus}. Antibiotic susceptibility testing showed that the isolate was resistant to glycopeptides but susceptible to several other antibiotics. The antibiotic treatment was then switched to imipenem and was successful.

Lactobacilli are gram-positive, nonmotile, non-spore-forming facultative anaerobes. They are usually catalase negative and require complex nutrients for optimum growth. Lactobacilli occur as commensals and are part of the indigenous microflora in the oral cavity, gastrointestinal tract, and vagina in humans and animals. They are isolated from plants or material of plant origin like silage and are used in the natural or artificial fermentation of milk and dairy products, meat and meat products, and fish and marinated fish. They also contribute to spoilage of food and are used for feed fermentation (6, 18). Lactobacilli are isolated from plants or material of plant origin like silage and are used in the natural or artificial fermentation of milk and dairy products, meat and meat products, and fish and marinated fish. They also contribute to spoilage of food and are used for feed fermentation (6, 18). Lactobacillus strains have been isolated from clinical material. However, the clinical relevance of these isolates is questionable (1–3, 8). Species isolated as facultative pathogens or opportunistic microorganisms in an immunocompromised host are \textit{Lactobacillus rhamnosus}, \textit{L. plantarum}, \textit{L. gasseri}, \textit{L. crispatus}, and, in some cases, \textit{L. acidophilus}. Because of their low significance and their special growth requirements, they are often overlooked or incorrectly identified as \textit{L. acidophilus}, whereas other species are involved (5, 8). One explanation is that \textit{L. acidophilus} is the best-known species within the genus \textit{Lactobacillus}. We therefore report a case in which antibiotic therapy led to overgrowth of an opportunistic \textit{Lactobacillus} strain in peritoneal fluid. The species was first incorrectly described as \textit{L. acidophilus}. This is, to our knowledge, the first reported case of such a complication of CAPD (continuous ambulatory peritoneal dialysis)—peritonitis—and incorrect identification of the pathogen as \textit{L. acidophilus}, whereas CAPD peritonitis associated with lactobacilli has been described previously (14, 16, 17). This complication, as well as the misidentification, can be considered typical for opportunistic infections with lactobacilli in general.

Case report. A 57-year-old man was referred for end stage renal disease. He had a history of diabetes mellitus since 1976 and had been treated with insulin since 1989. Peripheral vascular disease was treated with a femoropopliteal vascular graft in 1991; in September 1995, the right leg had to be amputated at the thigh. The patient also had chronic osteomyelitis after an injury in 1946; he had amyloidosis of the stomach and esophagus proven by biopsy. A peritoneal dialysis catheter was inserted in December 1995, CAPD was started without complications, and the patient was discharged.

In January 1996, 4 weeks after starting CAPD, he presented with abdominal pain, a cloudy peritoneal effluvate, and fever. The dialysate grew \textit{Candida glabrata}, and the patient was treated intraperitoneally (i.p.) with fluycytosine and fluconazole for 6 weeks. In April 1996, the patient again presented with clinical signs of peritonitis and was treated first with fluoxacillin while continuing the application of fluconazole. The dialysate was microbiologically analyzed on the first day and grew coagulase-negative staphylococci 4 days later that were resistant to oxacillin, ceftotan, and imipenem but susceptible to vancomycin and teicoplanin. The therapy was switched to i.p. administered vancomycin for 2 days, but after a skin rash developed, the vancomycin was discontinued and replaced with teicoplanin until the end of April. In June 1996, the dialysate became cloudy and abdominal discomfort developed. One week later, the patient had persistent abdominal discomfort and a temperature of 37.5°C. He was treated outside the hospital with metronidazole and ceftriaxone for 1 week. He was then readmitted to the hospital. The dialysate contained 570 leukocytes/µl, the cell count increased to 7,300/µl in the following days, and the dialysate grew gram-positive organisms that were later identified as \textit{L. rhamnosus}. The \textit{Lactobacillus} isolate was resistant to vancomycin and teicoplanin but susceptible to imipenem and ciprofloxacin. The patient was treated with i.p. administered imipenem, and the peritonitis gradually improved over the next 3 weeks.

Microbiology. Peritoneal dialysate cultures were examined between 22 June and 2 July 1996. Peritoneal dialysate was
collected either in blood culture bottles (BACTEC) or in sterile bottles without transport medium. Direct Gram staining of CAPD fluid showed only epithelial cells and granulocytes but not bacteria. Pure cultures of a Lactobacillus species later identified as L. rhamnosus were isolated from both aerobic and anaerobic blood cultures (incubated for 4 days at 37°C) collected on 4 separate days. The isolate was first incorrectly identified as L. acidophilus by API 20E and API 20NE (bio-Mérieux, Marcy l’Etoile, France) based on the recommendations of Kandler and Weiss (6). The organism was catalase negative and oxidase negative, hemolysis on blood agar did not occur, and Gram staining revealed short, gram-positive rods in chains. We then tested the physiological and biochemical characteristics on the basis of more detailed recommendations (10, 12). These tests were macrotube tests. Twenty-one carbohydrates were tested for acidification reactions in a solid medium (12) after 6 days; growth at a defined temperature was read after 3 days at 15 ± 0.1°C, after 2 days at 20 ± 0.1°C, and after 1 day at 45 ± 0.1°C. The carbohydrates tested were L-(+)-arabinose (Merck 1492), D-(+)-glucose (Merck 8342), lactose (Merck 7657), D-(+)-sucrose (Merck 7651), D-(+)-maltose (Merck 5910), D-(+)-trehalose (Merck 8353), D-(+)-melibiose (Merck 12240), D-(+)-cellobiose (Merck 2352), D-(+)-raffinose (meltose) (Merck 7549), D-(−)-mannitol (Merck 5982), D-(+)-salicin [2-O-(β-D-glucopyranoside)-benzylalcohol] (Merck 7665), L-(+)-rhamnose (Merck 4736), D-(+)-xylose (Merck 8689), D-(+)-mannose (Merck 5984), D-(+)-melizitose (Serva 28550), myo-inositol (Merck 4728), D-(+)-sorbitol (Merck 7758), D-(+)-melibiose (Merck 4733), dextrin (Merck 3006), D-(+)-galactose (Merck 4062), and D-(−)-fructose (Merck 5325).

The organism could then easily be identified as L. rhamnosus. The reactions crucial for the differentiation of L. rhamnosus from L. acidophilus and L. gasseri are growth at 15°C and fermentation of rhamnose. As very few L. acidophilus strains are able to grow at 20°C, testing for growth at 15°C is more reliable. Growth at both 15 and 45°C can be considered a characteristic of L. rhamnosus. Mannitol fermentation may be another differentiation criterion, because L. rhamnosus is able to ferment mannitol while L. acidophilus is not. However, some strains of L. acidophilus, especially clinical isolates, are able to ferment mannitol. Therefore, rhamnose fermentation is a more reliable criterion for the differentiation of these two species than is mannitol fermentation (9). Another criterion may be that L. acidophilus strains are almost always susceptible to vancomycin (7, 9, 20, 21), whereas L. rhamnosus is naturally resistant to glycopeptides (7, 20). Because of their rod shape, with a tendency to coccoïd growth only on certain media, L. rhamnosus strains cannot be confused with vancomycin-resistant enterococci, as reported in other cases (15). If there are doubts concerning the morphology of the organism, a subculture on a solid medium (for instance, MRS agar [6]) and a Gram stain from this medium are recommended.

Isolate V7418 was tested against 21 antibiotics and chemotherapeutics. MIC determination was performed in accordance with the recommendations of the National Committee for Clinical Laboratory Standards (13), by using the broth microdilution method. Microtiter panels containing the test substances in cation-adjusted Mueller-Hinton broth with 3% lysed horse blood (PML Microbiologistics, Portland, Oreg.) were used. The test results were read after incubation for 16 to 20 h at 37 ± 0.5°C under aerobic conditions. The MIC ranges of the National Committee for Clinical Laboratory Standards for enterococci (13) were suitable for lactobacilli in most cases. When no specific ranges for enterococci were mentioned, the ranges for gram-positive bacteria were used. Strain V7418 showed only a limited number of resistances; the most important one was glycopeptide resistance. Compared to other L. rhamnosus strains from clinical material and to the type strain, there were no major differences. The MICs (in micrograms per milliliter) and interpretations (susceptible [S], intermediate [I], and resistant [R]), respectively, for strain V7418 were as follows: penicillin G, 0.25 and S; ampicillin, 0.5 and S; methicillin, 16 and R; cephalothin, 32 and R; ceftriaxone, 8 and S; amoxicillin/clavulanic acid (2:1), 0.25/0.125 and S; erythromycin, <0.125 and S; tylosin, <0.5 and S; virginiamycin, <0.5 and S; clindamycin, <0.25 and S; gentamicin, <2 and S; imipenem, 2 and S; chloramphenicol, <2 and S; rifampin, 0.125 and S; ciprofloxacin, 0.25 and S; trimethoprim/sulfamethoxazole (1:2), 2/38 and S; tetracycline, <1 and S; vancomycin, >1,024 and R; teicoplanin, 16 and I; LY333328, 16 and I, avoparcin, >1,024 and R; LY333328 is a newly developed glycopeptide which is currently available only for research. Avoparcin (a glycopeptide), virginiamycin, and tylosin (both macrolides) are used as feed additives. For interpretation of the results obtained with the three feed additives and the new antibiotic LY333328, the interpretative guideline for related substances was used, i.e., the vancomycin guideline for avoparcin and LY333328 and the erythromycin guideline for the macrolides tylosin and virginiamycin. Pseudomonas aeruginosa ATCC 27853, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 29213, and Escherichia coli ATCC 25922 were used as reference strains.

Discussion. The API 20E and API 20NE biochemical test kits are not recommended for the identification of lactobacilli, but the reactions of these kits were combined and an identification scheme for lactobacilli described by Kandler and Weiss (6) was used. Nevertheless, they were only useful for identification of the genus Lactobacillus and not for species identification. For species identification, the API 50CHL test kit, which was specifically designed for lactobacilli, is more suitable. However, for reliable identification, no commercial test kit can be recommended. Macrodilution tube tests including physiological parameters like growth temperatures are necessary (9). Molecular techniques like protein fingerprinting or gene probes could be used as well (9, 18). Species identification is important for determination of the epidemiology of Lactobacillus-associated infections.

As far as we could ascertain, only three cases of CAPD peritonitis caused by Lactobacillus spp. have been reported. Two of them were associated with vancomycin-resistant L. rhamnosus strains (14–16), and one was associated with a vancomycin-resistant L. acidophilus strain (17). In these cases, the Lactobacillus strain appeared as an isolate in the peritoneal dialysate from the beginning of the infection. In the case reported here, the Lactobacillus appeared only after intensive treatment with vancomycin and teicoplanin directed against coagulase-negative, oxacillin-resistant staphylococci. Species identification is not necessary to avoid ineffective antibiotic therapy. Routine screening for glycopeptide resistance (e.g., on agar plates containing vancomycin at 4 μg/ml) would be more useful. The importance of screening for glycopeptide-resistant isolates has been recently underscored by the isolation of the first vancomycin-resistant clinical strain of methicillin-resistant Staphylococcus aureus. This strain was described by a Japanese group (4, 19), and the first such European strain has yet to be confirmed (11).

The general role of lactic acid bacteria (LAB) in clinical infections has been recently evaluated by a working group consisting of food microbiologists and clinical microbiologists (1). It was stated that LAB, including lactobacilli, can be considered safe, although some strains have been involved in op-
portunistic infections. No case has been described in which lactobacilli from food or fermented products were the causative agents of an infection. Superinfection with LAB from a patient’s own microflora (the clinical strains did not differ from strains of the patient’s flora) is possible only in an immunocompromised host (1).

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REFERENCES