

Molecular Analysis and Clinical Significance of *Lactobacillus* spp. Recovered from Clinical Specimens Presumptively Associated with Disease

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***Lactobacillus* spp. are part of the normal human flora and are generally assumed to be nonpathogenic. We determined the genotypic identification of > 100 *Lactobacillus* isolates from clinical specimens in the context of presumed pathogenic potential (e.g., recovered as the single/predominant isolate from a sterile site or at $\geq 10^5$ CFU/ml from urine). This study assessed the clinical significance and the frequency of occurrence of each *Lactobacillus* sp. We identified 16 species of *Lactobacillus* by 16S rRNA gene sequence analysis, 10 of which could not be associated with disease. While *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, and *Lactobacillus paracasei* were associated with infections, *L. gasseri* was also a common colonizing/contaminating species. *Lactobacillus casei*, *Lactobacillus johnsonii*, and *Lactobacillus delbrueckii* were associated with at least one infection. Species commonly used in probiotic products (e.g., *L. rhamnosus* and *L. casei*) were identical, by 16S rRNA gene sequencing, to our isolates associated with disease. Human isolates of *Lactobacillus* spp. have differing site associations and levels of clinical significance. Knowing the niche and pathogenic potential of each *Lactobacillus* sp. can be of importance to both clinical microbiology and the food and probiotic supplement industry.**

The genus *Lactobacillus* consists of a large and diverse spectrum of bacterial species, which in general are nonpathogenic and are considered part of the normal human microbiota. Lactobacilli are non-spore-forming Gram-positive rods that are microaerophilic and catalase negative. In the clinical microbiology laboratory, the proper identification of lactobacilli by conventional methods can be difficult (1). Biochemical characteristics are insufficient to allow reliable identification of lactobacilli to the species level, making molecular diagnostic approaches such as 16S rRNA gene sequencing necessary (2). Because lactobacilli range in size and shape, even presumptive genus identification can be difficult, as lactobacilli may be confused with other Gram-positive, catalase-negative organisms such as *Streptococcus* (3, 4) and *Leuconostoc* (5). Since lactobacilli rarely cause disease, many laboratories choose not to determine the species and simply report the isolated organism as "*Lactobacillus* sp." Apart from the known pathogenic association between lactobacilli and dental caries, several clinical case reports describe the opportunistic nature by which lactobacilli cause disease. Reported infections include endocarditis, bacteremia, peritonitis, and localized soft-tissue infections (1–3, 6–8). Most infections caused by *Lactobacillus* spp. are associated with underlying diseases (6).

Lactobacilli have been recognized as commensal flora that function to inhibit pathogenic bacteria by producing antimicrobial substances such as lactic acid, hydrogen peroxide, and bacteriocins (9–11). They are found as normal flora in the oral cavity, gastrointestinal (GI) tract, and female urogenital (UG) tract. At the time of birth, these lactic acid-producing bacteria are ingested and soon colonize the intestines. As individuals age, the lactobacillus profile changes over time (9, 12, 13). Moreover, changes in the species composition of commensal lactobacilli can suggest disease processes such as periodontitis (14) and bacterial vaginosis (11).

Exogenous sources of lactobacilli include fermented food products such as yogurt and cheeses, and lactobacilli are vital

components of probiotics (e.g., foods and dietary supplements containing live bacteria). The role of *Lactobacillus* spp. in food products dates back more than 100 years, and beneficial effects on health and longevity have long been proposed (13, 15). Probiotics, often containing members of the genera *Lactobacillus* and *Bifidobacterium*, have been accorded a number of beneficial effects in a variety of disorders, often with little or no scientific support. Recent studies have identified clinical applications in which probiotics are reported as being effective for UG infections (9), nosocomial rotavirus gastroenteritis in infants (16), antibiotic-associated diarrhea (17), acute infectious diarrhea, irritable bowel syndrome, and inflammatory bowel disease (13, 18).

With the increasing use of probiotic food products and dietary supplements by both healthy individuals and patients with various underlying diseases, it is important to distinguish the sites from which *Lactobacillus* may be recovered and the settings in which *Lactobacillus* spp. can behave as pathogens. The aim of this study was to determine the frequency of occurrence, in a routine clinical laboratory, of identified species of *Lactobacillus* in relation to the site of isolation and associated diseases.

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TABLE 1 Distribution of *Lactobacillus* species among different specimen types

<i>Lactobacillus</i> sp.	No. of isolates in ^a :						Total no. of isolates
	Urine/UG specimens	Blood	Respiratory tract specimens	Deep wounds	Superficial wounds	Fluids	
<i>L. gasseri</i>	19	3	3	7	4		36
<i>L. rhamnosus</i>	1	7	7	5	2	2	24
<i>L. crispatus</i>	10						10
<i>L. paracasei</i>		4		1		2	7
<i>L. fermentum</i>			4	2		1	7
<i>L. johnsonii</i>	1	1	1	2			5
<i>L. jensenii</i>	4						4
<i>L. casei</i>	1	1		1			3
<i>L. delbrueckii</i>	1	1					2
<i>L. salivarius</i>		1		1			2
<i>L. vaginalis</i>	1	1					2
<i>L. acidophilus</i>			1				1
<i>L. cantenaeformis</i>			1				1
<i>L. iners</i>	1						1
<i>L. plantarum</i>	1						1
<i>L. reuteri</i>						1	1
Total	40	19	17	19	6	6	107

^a The specimen types included urine and urogenital (UG) specimens (vaginal secretion and mucous membrane specimens), blood, respiratory tract specimens (lung tissue, pleural fluid, BAL fluid, bronchial brush, endotracheal aspirate, and sputum specimens), deep wounds (sterile tissue, abscesses, soft-tissue infections, and pericardial grafts), superficial wounds (ulcers and swabs), and sterile body fluids (bile, pericardial, peritoneal, and gallbladder fluids).

MATERIALS AND METHODS

Patients and bacterial isolates. In this retrospective study of laboratory records between 1998 and 2013 at the Veterans Affairs medical centers in Houston, TX, and Seattle, WA, we identified a total of 107 *Lactobacillus* isolates that were found in clinical specimens in the context of presumed pathogenic potential (e.g., recovered as the single/predominant isolate from a sterile site or at $\geq 10^5$ CFU/ml from urine) and could be assigned unambiguously to a species by 16S rRNA sequencing. Four strains were sent from other Veterans Affairs medical centers for identification (3). All of the available isolates were included in the study, with one exception; since isolates from male UG specimens were the most numerous, a convenience sample of about 30% of the total was chosen to match the number of female UG specimens. The patient group consisted of 22 women (age, 25 to 77 years [mean, 45 years]) and 59 men (age, 40 to 81 years [mean, 48 years]).

Assessment of clinical significance. For all strains, we determined the sites from which they were isolated. We were able to determine the age and gender for 95% of the patients. To assess the clinical significance of isolates from urine, we used urinalysis results, the concentrations of both *Lactobacillus* and other organisms that might have been present, and, if available, clinical notes documenting symptoms of a urinary tract infection (UTI). The significance of non-UG cultures was determined by the quantity of lactobacilli recovered (for blood cultures, the number of positive bottles), the presence of other organisms, the presence of inflammatory cells, and the description of the specimen. For the purposes of this study, we regarded localized infections in closed spaces as abscesses.

DNA preparation and strain identification by 16S rRNA sequencing. Strains were subcultured from freezer stocks onto blood agar plates and were incubated aerobically for 24 to 72 h. The DNA was extracted using Prepman reagent (PE Applied Biosystems, Foster City, CA), following the instructions of the manufacturer, from a loopful of bacteria either directly or by suspension in 500 μ l distilled H₂O and centrifugation at $15,000 \times g$ for 5 min, with the pellet being suspended in 300 μ l of TE buffer (10 mM Tris-HCl, 1 mM EDTA). The sequence for the first 500 bp of 16S rRNA was edited and compared with the reference database that is part of the MicroSEQ 16S rDNA bacterial identification system (Applied Biosystems). BIBI and GenBank databases were also used (19, 20). The

accession numbers for sequences from GenBank used in the dendrogram are listed in Table S1 in the supplemental material. Phylogenetic trees were constructed using sequences aligned with MUSCLE, a program for generating multiple alignments (21), with MEGA5 version 1 software (<http://www.megasoftware.net>).

Isolation of *Lactobacillus* from food products and probiotic dietary capsules. We sequenced isolates from food products and probiotic dietary capsules to determine whether the food strains differed genetically from human strains of the same species. Two hundred microliters of yogurt and kefir was spread onto blood agar (Remel, Lenexa, KS) and Schaedler blood agar (Remel, Lenexa, KS) plates and incubated at 35°C both aerobically (with added CO₂) and anaerobically. One capsule of an acidophilus dietary supplement was mixed into 5 ml of brain heart infusion broth. Two hundred microliters was spread onto blood agar plates and incubated as previously mentioned. The plates were examined after 48 and 72 h. Multiple colonies of different morphological types were subcultured for isolation both aerobically (with added CO₂) and anaerobically. Gram-positive isolates (3 isolates from the yogurt, 4 bacterial isolates from the kefir, and 3 isolates from the probiotic capsules) were selected for 16S rRNA gene sequence analysis.

This study was completed with institutional review board approval (MIRB no. 01012).

RESULTS

The overall relationships between the *Lactobacillus* species and the site from which the species were recovered are shown in Table 1. The specimen types are categorized as UG (95% urine and 5% vaginal specimens), blood, respiratory (lung tissue, bronchoalveolar lavage [BAL] fluid, bronchial brush, pleural fluid, endotracheal aspirate, and sputum specimens), deep wounds (abscesses and tissue samples from normally sterile sites, including kidney, liver, and aortic graft), superficial wounds (swabs and ulcers), and body fluids (bile, gallbladder, peritoneal, and pericardial specimens). We found 16 species of *Lactobacillus*, of which *Lactobacillus gasseri* and *Lactobacillus rhamnosus* were most frequently isolated (Table 1). Fifty-three percent of the *L. gasseri* isolates were

TABLE 2 *Lactobacillus* spp. and deep wound associations

Patient no.	Organism	Specimen type ^a	Patient sex	Patient age (yr)	Quantity	Other significant organism(s)	Underlying disease(s)/condition(s) ^b
1	<i>L. gasseri</i>	Abscess	M	46	Few	None	Diverticulitis
2	<i>L. gasseri</i>	Wound, fistula after esophageal surgery	M	63	Moderate	Moderate <i>Candida albicans</i> , <i>Streptococcus mitis</i>	Small cell carcinoma, leak from fistula
3	<i>L. gasseri</i>	Abdominal abscess	M	61	NA	<i>C. albicans</i> and <i>Staphylococcus aureus</i>	Crohn's disease, bowel obstruction
4	<i>L. gasseri</i>	Pericolonic abscess, extension from duodenum	M	NA	NA	<i>Streptococcus constellatus</i>	DM, peptic ulcer disease
5a	<i>L. johnsonii</i>	Abscess on arm	M	55	Many	None	IVDA, DM, hepatitis C
5b	<i>L. johnsonii</i>	Abscess on arm 2 yr later	M	57	Many	None	IVDA, DM, hepatitis C
6	<i>L. paracasei</i>	Bile fluid	M	63	Moderate	None	Cholangiocarcinoma, obstruction
7	<i>L. paracasei</i>	Liver tissue	M	84	Moderate	Many <i>Escherichia coli</i>	Cholecystitis, gallbladder rupture
8	<i>L. reuteri</i>	Peritoneal fluid	M	64	Few	Rare <i>Streptococcus anginosus</i>	Perforated diverticulum, DM
9	<i>L. rhamnosus</i>	Abscess in spleen	M	NA	Many	Many <i>C. albicans</i>	Unknown
10	<i>L. rhamnosus</i>	Abscess at site of perforated viscus	M	70	Moderate	<i>Clostridium disporicum</i>	Perforated viscus
11	<i>L. rhamnosus</i> and <i>L. gasseri</i>	Abscess and cellulitis in hand	M	52	Many	None	IVDA, DM
12a	<i>L. rhamnosus</i>	Tissue from stump after lower limb removal	M	60	Moderate	None	IVDA, DM
12b	<i>L. rhamnosus</i> and <i>L. gasseri</i>	Abscess at injection site in upper extremity	M	60	Moderate	Moderate <i>S. aureus</i>	IVDA, DM

^a Specimens from deep wounds consisted of sterile tissue, abscess, soft tissue infection, and pericardial graft specimens.

^b DM, diabetes mellitus; IVDA, intravenous drug abuse; BKA, below-the-knee amputation; NA, not applicable.

isolated from urine, with superficial and deep isolates accounting for 11% and 19%, respectively, of the *L. gasseri* isolates. The second most commonly isolated species in this study, *L. rhamnosus*, was recovered from all sites, with significant proportions of isolates obtained from blood (29%), respiratory specimens (29%), and deep wounds (21%). All except one of the *Lactobacillus crispatus* strains were isolated from vaginal specimens or female urine samples. The majority (57%) of *Lactobacillus paracasei* strains were isolated from blood. In contrast, the majority (57%) of the *Lactobacillus fermentum* isolates were from respiratory sources. Only one strain each of *Lactobacillus acidophilus*, *Lactobacillus cantenaformis*, *Lactobacillus iners*, *Lactobacillus plantarum*, and *Lactobacillus reuteri* were isolated.

Lactobacilli were most frequently isolated from UG specimens; the proportion would have been higher, as only about one-third of all male UG specimens were included. *Lactobacillus jensenii*, *L. iners*, and *Lactobacillus vaginalis* isolates were all isolated from female UG specimens and were not associated with UTIs, which is not surprising since these species are reported to be common vaginal microbiota (22, 23). There were only two of 39 evaluable cases (all male subjects) in which lactobacilli were present as the only isolate at $\geq 10^5$ CFU/ml, and polymorphonuclear leukocytes (PMNs) were present. One isolate of *L. gasseri* and one of *Lactobacillus casei* were associated with urinary retention and dysuria with inguinal hernia and recurrent UTIs with renal carcinoma, respectively. *Lactobacillus delbrueckii* was isolated from the blood and urine from a patient, but clinical details were not available for these referred specimens.

Details of non-UG cases are shown in Tables 2, 3, and 4. Table 2 summarizes data for isolates recovered from deep wounds, abscesses, or sterile sites. Additional organisms were cultivated from eight of 14 specimens, often *Candida albicans*, *Staphylococcus au-*

reus, or viridans group streptococci. *L. rhamnosus*, *L. paracasei*, *L. gasseri*, and *Lactobacillus johnsonii* were all recovered as single isolates. The most common single-type specimen was an abscess at a drug injection site. We recovered *L. johnsonii* twice, 2 years apart, from the same patient, both times from an assumed injection site in his arm; in each case, PMNs and Gram-positive organisms were seen on Gram stains. *L. rhamnosus* and *L. gasseri* were both recovered from two injection sites. In addition, *L. gasseri* was recovered from two diabetic ulcers with other organisms and from an infected aortic graft.

Four of 10 cases of bacteremia were polymicrobial (Table 3). *L. rhamnosus* was the most common species, often associated with an underlying disease that had allowed bloodstream access to lower gastrointestinal flora. The white blood cell (WBC) count was rarely elevated; of the four cases in which there was a single species and the WBC count was above 10.5×10^3 cells/mm³, *L. rhamnosus* accounted for 3 cases and *L. paracasei* for one case. Also, a pure culture of *L. vaginalis* was recovered from a 70-year-old man at autopsy (data not shown).

Table 4 outlines cases in which *Lactobacillus* spp. were isolated from respiratory specimens such as lung tissue, sputum, pleural fluid, endotracheal aspirate, and bronchoalveolar lavage (BAL) fluid. Most respiratory cultures were polymicrobial, some with other organisms of high pathogenicity. As shown in the last column in Table 4, the *Lactobacillus* isolate was considered a contaminant or colonizing organism for most of the patients. *L. fermentum* and *L. rhamnosus* were the two most common respiratory isolates. The two examples of a *Lactobacillus* strain probably causing disease as a single agent involved *L. rhamnosus*. For example, for patient 7, *L. rhamnosus* was recovered from an endotracheal aspirate as a predominant isolate and was seen on Gram stains

TABLE 3 *Lactobacillus* spp. and blood associations^a

Patient no.	Organism	Patient sex	Patient age (yr)	No. positive/no. of bottles	Other significant organism(s)	WBC count (CFU/ml)	Underlying disease(s)/condition(s) ^b
1	<i>L. johnsonii</i>	M	79	1/3	None	7.6	Bowel obstruction
2	<i>L. gasseri</i>	M	68	1/2	<i>Enterococcus faecalis</i>	10.1	Neurogenic bladder
3	<i>L. paracasei</i>	M	59	3/3	None	11.5	Perforated diverticulum
4	<i>L. rhamnosus</i>	M	60	1/3	None	22.3	Esophageal adenocarcinoma, pleural effusions
5	<i>L. rhamnosus</i>	M	38	1/4	None	11.4	Pancreatitis, hepatitis B, DM
6	<i>L. rhamnosus</i>	M	57	1/2	<i>Staphylococcus</i> sp.	5.5	HIV, IVDA, hepatitis C, recurrent abscesses
7	<i>L. rhamnosus</i>	M	57	8/8	None	10.9	Crohn's disease
8	<i>L. rhamnosus</i>	M	49	4/6	α -Hemolytic streptococci, <i>E. coli</i>	23.7	Necrotizing pancreatitis
9	<i>L. rhamnosus</i>	M	57	2/4	<i>C. albicans</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus</i> sp.	9.8	Total hip arthroplasty, infection of hardware
10	<i>L. casei</i>	M	65	2/2	None	NA	Pneumonectomy, lung cancer, sepsis

^a Significance was determined by (i) the number of positive cultures, (ii) whether other organisms were present (polymicrobial or monomicrobial), and (iii) the patient's WBC count at the time of blood drawing.

^b DM, diabetes mellitus; HIV, human immunodeficiency virus; IVDA, intravenous drug abuse; NA, not applicable.

with many inflammatory cells. Additionally, *L. rhamnosus* was associated with pneumonia in patient 9, who is described below.

L. rhamnosus was isolated from one patient from two sites (patient 4 in Table 3 and patient 9 in Table 4). The patient had un-

dergone an esophagectomy to remove an adenocarcinoma 12 days previously. The patient presented with severe acute shortness of breath and demonstrated pleural effusions, and the gastric conduit was distended with retained food. A pleural fluid sample was

TABLE 4 *Lactobacillus* spp. and respiratory associations

Patient no.	Organism	Patient sex	Patient age (yr)	Specimen type ^a	Other significant organism(s)	Underlying disease(s)/condition(s) ^b	Assessment
1	<i>L. fermentum</i>	F	50	Bronchial biopsy	None, in broth only	Eosinophilic pneumonia, acute or chronic	Contaminant
2	<i>L. fermentum</i> (few)	M	58	Sputum	<i>Aspergillus fumigatus</i>	<i>Aspergillus</i> pneumonia, myelodysplastic syndrome with myeloproliferative disease	Probable contaminant
3	<i>L. gasseri</i>	M	70	Lung tissue	<i>Staphylococcus epidermidis</i>	<i>S. epidermidis</i> sepsis	Probable contaminant
4	<i>L. gasseri</i> (moderate)	M	58	Pleural fluid	Many α -hemolytic streptococci, not <i>S. pneumoniae</i> , many PMNs	IR drainage of empyema, Crohn's disease	Contributed to infection
5	<i>L. acidophilus</i> and <i>L. johnsonii</i> (few)	M	54	Lung tissue	<i>Candida glabrata</i> , <i>Enterobacter cloacae</i> , <i>Corynebacterium</i> sp. (all few)	Pulmonary infiltrates with no histological evidence of infection or neoplastic etiology, patient has non-Hodgkin's lymphoma and is planning to undergo BMT	Contamination, tissue was handled in a nonsterile manner in histology department before being cultured
6	<i>L. rhamnosus</i>	M	74	Pleural fluid	<i>C. albicans</i> , <i>E. faecalis</i> , <i>S. epidermidis</i>	Gastrectomy, surgical leak	Leak from upper GI tract surgical site
7	<i>L. rhamnosus</i> (many)	M	87	Endotracheal aspirate	Few mixed normal flora, many PMNs	Pneumonia, COPD, gastrostomy tube, new multilobar infiltrates concerning for aspiration vs HAP	Infection or colonization
8	<i>L. rhamnosus</i> (25,000 CFU/ml)	M	80	BAL fluid	<i>Klebsiella oxytoca</i> (5,000 CFU/ml)	Endotracheal tube, MRSA septicemia, died	Infection or colonization
9	<i>L. rhamnosus</i>	M	60	Pleural fluid	None	Esophageal adenocarcinoma, pleural effusions	Infection or colonization
10	<i>L. fermentum</i> and <i>L. gasseri</i>	M	54	Bronchial brush	None	Chronic liver disease	Unknown

^a Specimens from respiratory sites included lung tissue, pleural fluid, endotracheal aspirate, sputum, and bronchoalveolar lavage (BAL) fluid specimens.

^b BMT, bone marrow transplantation; IR, interventional radiological; COPD, chronic obstructive pulmonary disease; HAP, hospital-acquired pneumonia; MRSA, methicillin-resistant *S. aureus*.

TABLE 5 *Lactobacillus* spp. and probiotic associations

Probiotic	Ingredients listed	Isolated organisms
Kefir	<i>Streptococcus lactis</i>	<i>L. rhamnosus</i>
	<i>Streptococcus cremoris</i>	<i>Streptococcus thermophilus</i>
	<i>S. lactis</i> subsp. <i>diacetylactis</i>	
	<i>L. casei</i>	
	<i>L. plantarum</i>	
	<i>Saccharomyces fragilis</i> <i>Leuconostoc cremoris</i>	
Yogurt	<i>L. acidophilus</i>	<i>Bifidobacterium animalis</i>
	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	<i>S. thermophilus</i>
	<i>Bifidobacterium bifidum</i>	
Capsule	<i>L. acidophilus</i>	<i>L. paracasei</i>
	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	<i>S. thermophilus</i>
	<i>L. paracasei</i>	
	<i>Bifidobacterium lactis</i>	

sent for culture. Direct Gram staining demonstrated 4+ WBCs and a rare Gram-positive organism; 16S rRNA gene sequencing identified the only organism from the pleural fluid as *L. rhamnosus*. *L. rhamnosus* was also isolated from blood cultures.

Table 5 summarizes the organisms listed as ingredients of three probiotics and the organisms actually isolated. Of the 5 possible *Lactobacillus* spp. (*L. casei*, *L. plantarum*, *L. acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *L. paracasei*) listed, we were able to isolate only *L. paracasei*. The kefir preparation claimed to contain *L. casei*, but we recovered only *L. rhamnosus*. Importantly, *L. casei* and *L. rhamnosus* are difficult to distinguish from each other, as they are similar by both 16S rRNA gene sequence analysis (Fig. 1) and phenotyping. We hypothesize that the strain used in the probiotic may actually be *L. rhamnosus*, which may have been incorrectly identified. The 16S rRNA gene sequences of some of the clinical *L. rhamnosus* isolates are identical to that of the probiotic isolate (Fig. 1).

DISCUSSION

In this study, we obtained isolates in the context of presumed pathogenic potential and found associations of *Lactobacilli* spp. with both the anatomical site and the clinical condition. Of the 16 species of lactobacilli identified, *L. gasseri* and *L. rhamnosus* were the most commonly isolated. Ten species could not be associated with disease. While *L. rhamnosus*, *L. gasseri*, and *L. paracasei* were associated with infections, *L. gasseri* was also a common colonizing/contaminating species. *L. casei*, *L. johnsonii*, and *L. delbrueckii* were associated with infections.

The problem of accurate identification of the *Lactobacillus* species affects the interpretation of their clinical significance; however, identification by reliable molecular methods such as 16S rRNA gene sequence analysis was not common until after 2005 (1, 24). In addition, changes in the taxonomy of *Lactobacillus* have expanded the number of species to more than 90 and changed the nomenclature for some species. For example, *L. canteniformis* has been found to be more closely related to *Erysipelothrix* (24). The use of matrix-assisted laser desorption ionization–time of flight mass spectrometry for *Lactobacillus* identification holds promise (25, 26).

The problem of correct identification of isolates might have affected the large literature review of 200 cases, which included cases from as far back as 1950 (6). Misidentification of isolates is suggested by the high rate of vancomycin sensitivity and the frequent association with endocarditis, which is more commonly associated with viridians streptococci (which, when the cocci are elongated, can be confused with lactobacilli). None of the blood culture isolates in the present study was noted to be associated with endocarditis. The review by Cannon et al. (6) suggested that the two most common *Lactobacillus* species associated with disease were *L. casei* and *L. rhamnosus*. However, these species cannot be reliably distinguished by phenotypic methods (the methods used in most of the papers those authors reviewed) from each other (24). Similarly, *L. acidophilus* (their third most common strain) and *L. gasseri* are difficult to distinguish. It is thus possible that these organisms were misidentified and that, similar to what was observed in our study, *L. rhamnosus* and *L. gasseri* were more commonly isolated. The pathogenicity of *L. rhamnosus* has been confirmed by other reports (2, 7, 8, 27), particularly in association with respiratory infections (28). In addition, the Gram staining of lactobacilli from the original specimen can be confusing; several of our specimens were interpreted as Gram-positive cocci in chains and possible rods. For example, we initially considered several isolates to be *Streptococcus* based on the Gram staining, catalase test, and colony morphology results. However, 16S rRNA gene sequencing provided the correct identification.

Of 23 specimens from female patients, 22 isolates and six species (*L. crispatus*, *L. gasseri*, *L. jensenii*, *L. iners*, *L. plantarum*, and *L. vaginalis*) were obtained from UG sources and none was associated with disease. *L. crispatus* was the most common isolate, which confirms the site-specific propensity of *L. crispatus* and *L. jensenii* for the female UG tract. The most common lactobacilli in male

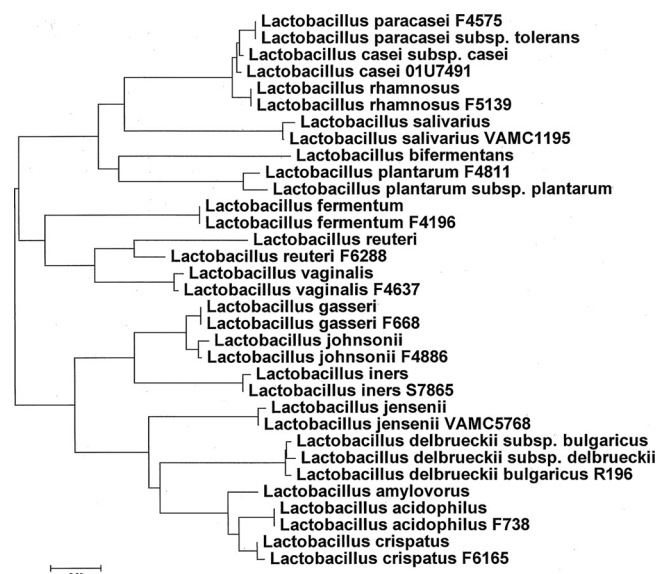


FIG 1 Dendrogram showing the relationships of the first ~500 bp of the 16S rRNA gene sequences. Our strains are indicated with alphanumeric designations following their names; the other strains are type or reference strains and are listed in Table S1 in the supplemental material. The evolutionary distances were computed using the maximum composite likelihood method, and the units are the numbers of base substitutions per site (MEGA software, version 5.1 [http://www.megasoftware.net]).

urine have been reported as *L. iners* (29), whereas we found *L. gasseri* to be the predominant species isolated from male urine. Recent studies showed that the urine microbiota consists mainly of lactobacilli (29, 30); therefore, the organisms isolated from UG samples in this study likely represent commensal organisms. However, *Lactobacillus* has been associated with urinary infections in the literature (31) and was associated with a UTI with obstruction in one of our patients (*L. rhamnosus* in the urine of a male patient). A limitation of our study is that it was conducted within a primarily adult male population.

Our observed site-species relationship for lactobacilli suggests that probiotic efficacy may vary depending on the species and the specific niche targeted. For example, in order to reestablish normal vaginal flora, a preparation containing *L. casei* may not be appropriate. While our investigation of organisms isolated from probiotics obtained from a health food store and identified using 16S rRNA gene sequencing was limited, the isolation of *L. rhamnosus*, which was not listed as an ingredient in the probiotic preparation, is a significant finding highlighting the fact that, since there is no regulation of these products, the actual strains in commercial preparations may be different than claimed (15, 18).

This is the largest study, to our knowledge, involving *Lactobacillus* spp. identified by 16S rRNA gene sequencing in association with both the site of isolation and the clinical significance. Knowing the niche and pathogenic potential of each *Lactobacillus* sp. is of importance to both the field of clinical microbiology and the food industry.

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