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Lactobacillus Species Identification, H_2O_2 Production, and Antibiotic Resistance and Correlation with Human Clinical Status

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Lactobacilli recovered from the blood, cerebrospinal fluid, respiratory tract, and gut of 20 hospitalized immunocompromised septic patients were analyzed. Biochemical carbohydrate fermentation and total soluble cell protein profiles were used to identify the species. Hydrogen peroxide production was measured. Susceptibility to 19 antibiotics was tested by a diffusion method, and the MICs of benzylpenicillin, amoxicillin, imipenem, erythromycin, vancomycin, gentamicin, and levofloxacin were determined. A small number of species produced H_2O_2, and antibiotic susceptibilities were species related. Eighteen (90%) of the isolates were *L. rhamnosus*, one was *L. paracasei* subsp. *paracasei*, and one was *L. crispatus*. *L. rhamnosus*, *L. paracasei* subsp. *paracasei* isolates, and the type strains were neither H_2O_2 producers nor vancomycin susceptible (MICs, ≥256 μg/ml). *L. crispatus*, as well as most of the type strains of lactobacilli which belong to the *L. acidophilus* group, was an H_2O_2 producer and vancomycin susceptible (MICs, <4 μg/ml).

Lactobacilli are ubiquitous and widespread commensal bacteria in the human and animal microflora. They are widely used by humans: as adjuvants against gastrointestinal disorders, as dietary supplements, and as biological food processors in view of their fermentative properties (1, 15).

Severe lactobacillus infections occur as endocarditis in patients with valve defects and as local or disseminated infections in neutropenic patients with sepsis receiving broad-spectrum antibiotics. The conditions of the patients in the latter group are usually leukemia with chemotherapy, an immunocompromised state due to organ transplantation, or AIDS (1, 2, 5, 13, 15, 22, 23, 25, 31). The current widespread use of glycopeptides and broad-spectrum cephalosporins for the management of sepsis may increase the rate of occurrence of lactobacillus infections among immunocompromised patients (5, 13).

Recent phylogenetic analyses have resulted in taxonomic changes in this genus (6, 26, 30, 34, 36). For example, in 1989, *Lactobacillus casei* subsp. *rhamnosus* was elevated to species status as *L. rhamnosus* sp. nov., and all other members of the *L. casei* subspecies except *L. casei* subsp. *casei* were grouped in a separate species, *L. paracasei* sp. nov. (7). Further changes are under way (9, 10, 30). Since 1980, the members of the *L. acidophilus* group have been divided into two subgroups, the *L. acidophilus* subgroup and the *L. gasseri* subgroup, and the two subgroups are divided into six species (14, 24).

Identification of *Lactobacillus* species to the species level is not possible on a routine basis. Commercially available carbohydrate fermentation tests fail to identify various *Lactobacillus* species. However, highly standardized whole-cell protein patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have proved useful for *Lactobacillus* species identification (27, 32). The potential value of other tests, such as hybridization with oligonucleotide probes, is under investigation (32).

Few studies of lactobacilli from normal and diseased vaginal and intestinal mucosae report the species involved (11, 29). In vitro H_2O_2 production by lactobacilli from the vaginal microflora has recently been surveyed. H_2O_2-producing lactobacilli predominate in the normal vagina but are seldom found in the vaginas of patients with bacterial vaginosis (11). H_2O_2 is known to inhibit the growth of some bacteria and may be involved in the control of the normal microflora.

Vancomycin and teicoplanin are active against most gram-positive bacteria. However, various species (*L. rhamnosus*, *L. casei*, and *L. plantarum*) are intrinsically resistant to glycopeptides (21, 35). In contrast, most of the lactobacilli from the vaginal flora that we have tested were susceptible to these antibiotics (unpublished data). Our aim was to characterize the lactobacilli isolated from 20 patients with sepsis. The clinical status, carbohydrate fermentation profiles, whole-cell protein patterns, ability to produce H_2O_2, and antibiotic susceptibilities of the strains were analyzed.

MATERIALS AND METHODS

Patients and controls. The 20 patients were immunocompromised adults and children hospitalized in St-Louis Hospital between 1993 and 1995 (Table 1).

Strains. Lactobacilli were isolated from diverse samples taken in the course of the biological management of sepsis and were then stored at −80°C in the laboratory and freeze-dried in the Bacterial Strains Collection at the Institute Pasteur (CIP). No strain was an obligate anaerobe.

Type strains. The following CIP type strains were tested in parallel as references: *L. rhamnosus* CIP A 157^T, *L. paracasei* subsp. *paracasei* CIP 103918^T, and *L. jensenii* CIP 69.17^T. The following six strains of the *L. acidophilus* group were tested in parallel as references: four strains from the *L. acidophilus* subgroup (*L. acidophilus* CIP 76.13^T, *L. crispatus* CIP 102990^T, *L. gallinarum* CIP 103611^T, and *L. amylovorus* CIP 102989^T) and two strains from the *L. gasseri* subgroup (*L. gasseri* CIP 102991^T and *L. johnsonii* CIP 103620^T).

Fermentation profile. The API 50 CH test kit and API CHL medium (bioMérieux, La Balme les Grottes, France) were used to test the abilities of the strains to ferment 49 carbohydrates. An 18-h culture in de Man-Rogosa-Sharpe (MRS) broth was centrifuged, and 200 μl of the sediment was introduced into API CHL medium. These samples were then tested with the API strips according to the manufacturer's instructions, the top of the cupule was covered with mineral oil, and the results were read after 24 h of incubation at 37°C under aerobic conditions.

The species identifications obtained from the biochemical profiles were confirmed with identification software (bioMérieux). Carbohydrate fermentations gave either the species identification (noted as good or doubtful) or inconclusive results.

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TABLE 1. Characteristics of 20 immunocompromised patients infected with a *Lactobacillus*

Strain no.[a]	Age (yr)/ sex[b]	Underlying condition[c]	Anti-mitotic drug	Duration (wk) of granulo-cytopenia	Other sign(s)	Antibiotic(s) administered		Origin of isolates (no. of samples)	Time of death (days) after isolation of *Lacto-bacillus*
						Glyco-peptide	Others[d]		
1132	35/M	Lymphoma, HBMT		8	GVH[e]		IMP, PEF, MTR	Stools	
1199	12/M	AA		150		+	CAZ, CIP	Blood (4), catheter, throat	7
1200	50/F	AML	+	4	Diarrhea	+	CAZ, CIP, RIF, POL (p.o.[f])	Stools	
1201	55/M	PCM, ABMT			Diarrhea			Sputum	
1202	11/F	AA, CBT		2		+	PIP, AM, OFL (p.o.)	Blood (14), CSF	
1273	7/M	AML, ABMT		2		+	CAZ, AMX, CIP, POL (p.o.)	Blood (4; relapsed)	
1274	24/M	AML	+	4		+	CAZ, OFLO, AM, POL (p.o.)	Throat	
1284	4/F	ALL	+	2		+ (p.o.)	AM, FOS	Stools	
1285	9/M	Relasped AML	+	1		+	CAZ, AM, TMP-SMZ	Blood (1)	
1286	35/F	AML	+	1	Diarrhea		CAZ, NET	Stools	
1287	65/M	Gut carcinoma, surgery		<1	Septic shock		CTX, AM, MTR	Blood (1)	1
1288	16/M	Relapsed ALL	+	2	Mucositis	+ (po)	CAZ, NET, POL (p.o.)	Blood (2)	
1290	29/M	Kidney transplantation		8	Hepatitis, diarrhea	+ (po)	CAZ, TMP-SMZ, CIP	Blood (2)	1
1292	6/M	AL, CBT		2	GVH, diarrhea	+ (po)	IMP, MTR (p.o.), TMP-SMZ	Stools	
1293	2/F	AML, HBMT		3	GVH, mucositis	+	CAZ, AM, POL (p.o.)	Blood (5)	
1294	80/M	Diabetes, liver failure						Blood (1)	
1295	34/M	AIDS		8			TMP-SMZ	Blood (1)	
1296	10/M	AML	+	1		+	CAZ, AM	Blood (1)	
1297	52/M	PCM, ABMT		8	Septic shock	+	PIP, CIP, GM	Lower respiratory tract	
1298	29/M	AIDS		1	Shock, diarrhea		TMP-SMZ	Blood (3)	1

[a] All strains except strains 1294 and 1295 were *L. rhamnosus*.
[b] M, male; F, female.
[c] AA, aplastic aplasia; ABMT, autologous bone marrow transplantation; AL, acute leukemia (ALL, lymphoblastic; AML, myeloid); HBMT, homologous bone marrow transplantation; CBT, cord blood transplantation; PCM, plasma cell myeloma.
[d] AM, amikacin; AMX, amoxicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FOS, fosfomycin; GM, gentamicin; IMP, imipenem; MTR, metronidazole; NET, netilmicin; OFL, ofloxacin; PEF, pefloxacin; PIP, piperacillin; POL, polymyxin B; RIF, rifampin; TMP-SMZ, trimethoprim-sulfamethoxazole.
[e] GVH, graft-versus-host disease.
[f] p.o., per os.

Whole-cell protein profile. An 18-h culture on an MRS agar slope was harvested, and the proteins were extracted by disruption with glass microbeads. The samples were then subjected to gel electrophoresis (SDS-PAGE) and the proteins in the gel were stained with Coomassie blue.

Computerized normalization of densitometric scans of the gels and numerical analysis were done as described by Kersters and De Ley (27). Clusters were identified by unweighted average pair group method analysis with Gelcompar software (Applied Maths, Ghent, Belgium). The similarities between the protein patterns of the isolates and the type strains were scored as the Pearson product moment correlation coefficient. In agreement with previous assays, isolates and type strains with more than 82% similarity were clustered, and their assignment to the same species or the same *L. acidophilus* subgroup was considered (10, 28, 32).

H_2O_2 production. According to the qualitative method of Eschenbach et al. (11) lactobacilli were streaked onto a 20-ml MRS agar plate containing 5 mg of 3,3′,5,5′-tetramethylbenzidine (TMB; T2885; Sigma), a benzidine-like chromogenic substrate of peroxidase, and 0.20 mg of horseradish peroxidase (P6782; Sigma) (11). Peroxidase generates O_2 from any H_2O_2 produced by the lactobacilli, and the TMB stains the colonies blue in the presence of O_2. After 48 h of incubation under 5% CO_2 in air, colonies that produce H_2O_2 on MRS agar thus appear dark blue. Nonproducers are colorless. The media were used within 48 h after preparation. All strains were tested twice. The *Lactobacillus* type strains were used as quality controls.

Agar diffusion method for determination of antibiotic susceptibility patterns. Nineteen antibiotics were tested: benzylpenicillin, amoxicillin, cephalothin, streptomycin (10 IU), kanamycin (30 IU), gentamicin (10 IU), vancomycin, teicoplain, erythromycin, azithromycin, pristinamycin, lincomycin, rifampin, tetracycline, chloramphenicol, pefloxacin, sparfloxacin, fosfomycin, and fusidic acid (disks; Sanofi Diagnostics Pasteur, Marnes la Coquette, France). The instructions of the Comité Français de l'Antibiogramme related to streptococci were followed (34a).

Fifty microliters of the pellet of an overnight culture was diluted in MRS broth to about 10^7 CFU/ml. Mueller-Hinton agar plates containing 5% sheep blood (bioMérieux, Marcy l'Etoile, France) were flooded with this suspension in order to give confluent colonies and air dried for 15 min, and the disks impregnated with antibiotics were positioned on the plates. After 36 h of incubation at 37°C in air containing 5% CO_2, the diameters of the bacteria-free zones were measured.

MICs. Benzylpenicillin, amoxicillin, imipenem, gentamicin, erythromycin, vancomycin, and levofloxacin MICs were tested by the E-test method (Biomedical Diagnostics, Marne La Vallée, France). The inoculum, agar plates, and incubation conditions for the MIC determinations were as described above for the susceptibility assay. A large, 12-cm square Mueller-Hinton agar plate was flooded with this suspension, air dried for 15 min, and overlaid with the seven E-test antibiotic strips. After 36 h of incubation at 37°C in air containing 5% CO_2, the elliptical zones of growth inhibition were examined and the MICs were interpreted as the value on the E-test strip scale where the inhibition zone intersected the edge of the strip. *Staphylococcus aureus* ATCC 25923 was tested simultaneously on Mueller-Hinton agar, with an overnight culture diluted in Mueller-Hinton broth.

Both MICs and susceptibility patterns were determined for benzylpenicillin, amoxicillin, vancomycin, gentamicin, and erythromycin. Only the MICs of imipenem and levofloxacin were measured.

RESULTS

Patients. The 20 patients (15 males and 5 females; age range, 2 to 80 years; median age, 27 years) had severe underlying conditions, as indicated in Table 1. Seven patients had undergone autologous, homologous bone marrow or cord blood transplantation, one had undergone a kidney transplantation, seven were receiving antimitotic chemotherapy, two had septic shock, and two had AIDS (Table 1). Eighteen patients were granulocytopenic. Sixteen patients were treated with several systemic antibiotics, and nine were treated with oral antibiotics.

Lactobacilli were isolated from the blood of 12 patients: 7 with persistent lactobacillus septicemia (a lactobacillus was also isolated from cerebrospinal fluid samples from one of these patients) and 5 for whom only one blood sample was cultured. Lactobacilli were isolated from the respiratory tracts of two patients and from the stools or throats of six patients during treatment for total bacterial decontamination.

Antibiotic regimens were designed according to lactobacillus susceptibility in vitro. Four patients died over the next few days, and one had a lactobacillemia relapse.

Carbohydrate fermentation. Table 2 summarizes the results of the API CH 50 test.

Eighteen isolates were *L. rhamnosus* (see Table 1 for strain

TABLE 2. Species of *Lactobacillus* isolates from 20 septic patients according to fermentation properties and performance by SDS-PAGE and result of respective H_2O_2 production

Isolate[a]	Total no. of isolates	Identification	No. of isolates identified by fermentation	SDS-PAGE		No. of isolates positive for H_2O_2 production
				No. of isolates identified	% Similarity[b]	
L. rhamnosus	18	*L. rhamnosus*	18	18	84–98	0
1295	1	*L. paracasei* subsp. *paracasei*	1	1	87	0
1294	1	*L. acidophilus* group	1[c]	1	84, 94[d]	1
Total	20		20	20		1

[a] See *L. rhamnosus* strain numbers in Table 1. All isolates are *L. rhamnosus* except isolates 1294 and 1295.
[b] Percent similarity of the protein pattern with that of the type strain of the species.
[c] *L. crispatus* according to fermentation pattern.
[d] Percent similarity with *L. crispatus*^{T} and *L. gasseri*^{T}, respectively.

numbers) one was *L. paracasei* subsp. *paracasei* (strain 1295), and one was *L. crispatus* (strain 1294).

Cluster analysis of whole-cell protein profiles. The results of cluster analysis of the protein profiles of the strains from patients and the type strains are presented in Fig. 1.

The 18 *L. rhamnosus* strains fell into one cluster; 17 had more than 90% similarity with *L. rhamnosus*^{T} and 1 (strain 1274) with 84% similarity with *L. rhamnosus*^{T}. The protein profile of *L. paracasei* subsp. *paracasei* shared 86% similarity with that of *L. paracasei* subsp. *paracasei*^{T}. The protein profile of the lactobacillus identified as *L. crispatus* by its fermentation profile (strain 1294) fell into a cluster that included all six type strains of the species from the *L. acidophilus* group (results for three members of this group, *L. acidophilus*^{T}, *L. crispatus*^{T}, and *L. gasseri*^{T}, are presented in Fig. 1) and shared 94% similarity with *L. gasseri*^{T} but only 84% similarity with *L. crispatus*^{T} (Table 2; Fig. 1).

H_2O_2 production by lactobacilli. The findings from studies of H_2O_2 production by the lactobacilli tested are presented in Table 2.

Only one isolate, isolate 1294, either *L. crispatus* or *L. gasseri*, generated H_2O_2. Among the type strains studied, the only H_2O_2 producers were the members of the *L. acidophilus* group, but not *L. acidophilus*^{T} itself, and *L. jensenii*^{T} (data not shown).

Antibiotic susceptibility patterns determined by disc diffusion method. The mean diameters of the zones of inhibition (± standard deviations) were as follows: amoxicillin, 22.5 (± 6.4) mm; streptomycin, 12.1 (± 5.4) mm; kanamycin, 10.1 (±5.3) mm; gentamicin, 17.6 (±5.6) mm; erythromycin, 31.0 (±7.8) mm; azithromycin, 27.4 (±8.2) mm; pristinamycin, 27.7 (±5.5) mm; lincomycin, 22.6 (±10.5) mm; rifampin, 30.2 (±10) mm; tetracycline, 24.0 (±3) mm; and chloramphenicol, 23.0 (±4.8) mm. Fosfomycin and fusidic acid did not give inhibition zones for any strain tested. These results were similar to those for members of the normal vaginal flora studied simultaneously (data not shown).

For the strains from patients, the mean diameter of the zone of inhibition (±standard deviation) were as follows: benzylpenicillin, 22.5 (±6.4) mm; cephalothin, 13.4 (±9) mm; and vancomycin, no growth inhibition except for the strain from one patient (strain 1294). The diameters of the zones of inhibition for these antibiotics were significantly larger with normal vaginal lactobacillus. The findings for teicoplanin were similar to those for vancomycin. Among the quinolones, the mean (± standard deviation) diameter-for pefloxacin was 12.7 (±4) mm, and that for sparfloxacin was 24.3 (±5.2) mm. Both of these diameters are significantly larger than those found for normal vaginal lactobacilli (data not shown).

MIC results. The ranges of the MICs of benzylpenicillin, imipenem, and vancomycin were narrow: the MICs at which 50 and 90% of strains are inhibited were 0.5 and 2 μg/ml, respectively, for benzylpenicillin, 1 and 2 μg/ml, respectively, for imipenem, and ≥256 and ≥256 μg/ml, respectively, for vancomycin. For 19 (95%) isolates, all 18 *L. rhamnosus* strains and 1 *L. paracasei* strain), vancomycin MICs were ≥256 μg/ml.

MICs were generally similar for type strains and isolates from the same species. The range of MICs for *L. rhamnosus* and *L. paracasei* subsp. *paracasei* (19 isolates and 2 type strains) and for the *L. acidophilus* group (1 isolate and 6 type strains) are presented in Table 3. The results for vancomycin and levofloxacin are noteworthy. The MICs of vancomycin for *L. rhamnosus* and *L. paracasei* subsp. *paracasei* of all origins were ≥256 μg/ml, and the MICs for the *L. acidophilus* group

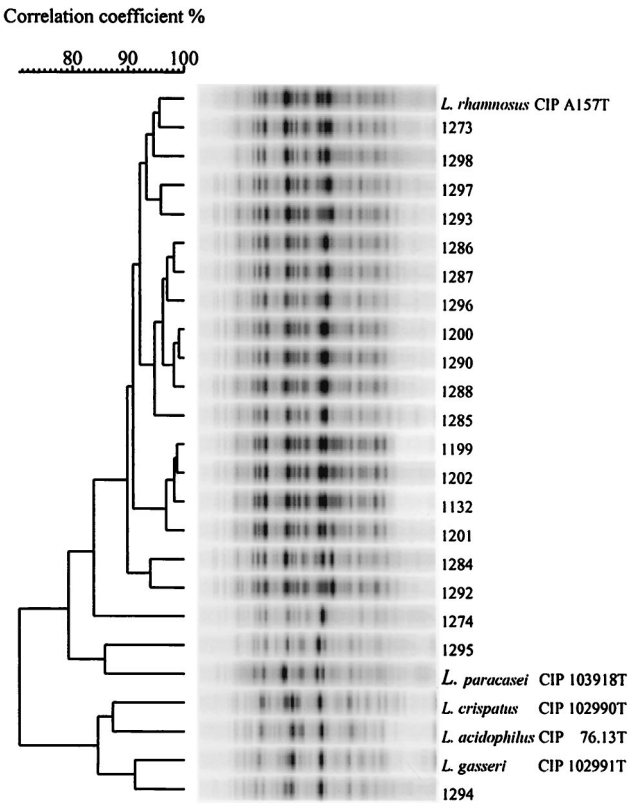

FIG. 1. Identification of *Lactobacillus* isolates from patients according to whole-cell protein patterns. Point traces were used for the calculation of the correlation coefficient (percent similarity) between individual traces. The clinical origin of each numbered strain is given in Table 1. The dendrogram was built by the unweighted average pair group method.

TABLE 3. Distribution of MICs according to *Lactobacillus* species[a]

Antibiotic	Species	No. of isolates for which the MIC (mg/liter) was as follows:									
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥32
Benzylpenicillin	*L. acidophilus* group			2	2	2			1			
	L. rhamnosus and *L. paracasei*				5	9	1	5	1			
Amoxicillin	*L. acidophilus* group					7						
	L. rhamnosus and *L. paracasei*					5	8	6	2			
Imipenem	*L. acidophilus* group			3	3	1						
	L. rhamnosus and *L. paracasei*				1	2	7	9	2			
Vancomycin	*L. acidophilus* group						5	2				
	L. rhamnosus and *L. paracasei*											21[b]
Gentamicin	*L. acidophilus* group							1	6			
	L. rhamnosus and *L. paracasei*								6	9	3	3
Erythromycin	*L. acidophilus* group	1	6									
	L. rhamnosus and *L. paracasei*	4	12	4	1							
Levofloxacin	*L. acidophilus* group											7
	L. rhamnosus and *L. paracasei*					3	14	1	3			

[a] Seven strains were from the *L. acidophilus* group and 21 strains were from the *L. rhamnosus* and *L. paracasei* subsp. *paracasei* groups (19 and 2 strains respectively).
[b] MICs, ≥256 μg/ml.

were ≤2 μg/ml. The MICs of levofloxacin for *L. rhamnosus* and *L. paracasei* subsp. *paracasei* were ≤4 μg/ml, and those for the *L. acidophilus* group were all ≥32 μg/ml.

DISCUSSION

The prevalence of *L. rhamnosus* in patients (up to 90%) is consistent with recent reports of disseminated lactobacillus infections in immunocompromised hosts (5, 15, 22, 25, 33). Our patients had severe underlying conditions (acute leukemia, neoplasia, organ transplantation, or AIDS) and received various systemic and oral antibiotics, including glycopeptides for 13 of them (65%) (Table 1).

Both fermentation profiles and protein profiles were reliable for the identification to the species level of all the isolates except the isolate belonging to the *L. acidophilus* group (Table 2; Fig. 1).

H_2O_2 was produced by one isolate, either *L. crispatus* or *L. gasseri*, by five of the six type strains of the *L. acidophilus* group, and by *L. jensenii*^{T} (Table 2). It was also produced by 80% of the normal vaginal isolates that we studied simultaneously (data not shown), in agreement with previous data (11).

The disk diffusion method and the E test gave concordant results. However, previous studies of cephalosporins determined that the MICs were much higher by the E test than by the dilution method with agar (8, 20).

The median MICs of benzylpenicillin and amoxicillin for *L. rhamnosus* and *L. paracasei* subsp. *paracasei* were two times higher than those for the *L. acidophilus* group, and those of imipenem were four times higher (Table 3).

The MICs at which 90% of strains are inhibited for erythromycin and gentamicin were similar for all lactobacilli (0.06 and 4 μg/ml, respectively) (Table 3). A synergistic bactericidal effect between the penicillins and gentamicin has been demonstrated previously (3, 16).

The MICs of vancomycin are species related: the MICs for *L. rhamnosus* and *L. paracasei* subsp. *paracasei* were ≥256 μg/ml, whereas those for the *L. acidophilus* group were ≤2 μg/ml (Table 3). *L. casei*^{T} and *L. rhamnosus*^{T} have cell wall peptigoglycan precursors that end in a depsipeptide D-alanine–D-lactate instead of the dipeptide D-alanine–D-alanine, the target for vancomycin activity (4, 18).

The activities of the oral antibiotics used for the treatment of urinary tract infections were assessed with lactobacilli. The high levels of resistance to fosfomycin, norfloxacin, ofloxacin, and ciprofloxacin have been described before (17, 20). *L. rhamnosus* had low-level resistance to pefloxacin and sparfloxacin and was susceptible to levofloxacin. These are the first tests of the in vitro susceptibility of *L. rhamnosus* to levofloxacin to be published: the low levofloxacin MICs for *L. rhamnosus* are in contrast to the high-level resistance of the *L. acidophilus* group (Table 3). Most studies of the antibiotic susceptibilities of lactobacilli do not report on those for the *Lactobacillus* species. Thus, our results might help in the determination of the antibiotic susceptibilities of the *Lactobacillus* species.

Sixty-five percent of our patients were on oral or parenteral glycopeptides (Table 1). In studies of oral glycopeptide treatment human volunteers had increased levels of fecal carriage of vancomycin-resistant enterococci and lactobacilli (37). The pathogenicity of *L. rhamnosus* selected by the use of glycopeptides may be due to many factors such as its exploitation of mucosal defects and its colonization properties. Seventy-five percent of our patients had digestive tract mucosal alterations as a consequence of various conditions: mucositis following antimitotic chemotherapy, digestive graft-versus-host disease after bone marrow transplantation, protracted diarrhea with AIDS, septic shock, or gut carcinoma (Table 1). In liver transplant patients, biliary anastomosis is an independent risk factor for lactobacillus bacteremia (31). Platelet aggregation and endothelial cell binding have been demonstrated with *L. rhamnosus* strains responsible for endocarditis (19).

The *L. rhamnosus* isolates from patients with clinical infections and milk products studied by Klein et al. (28) were unrelated according to their total soluble protein patterns. The comparison of *L. rhamnosus* isolates from patients with bacteremia and of the probiotic strain of *L. rhamnosus* GG performed by Saxelin et al. (33) showed various fermentation

patterns. Our *L. rhamnosus* isolates belonged to various clusters according to their randomly amplified polymorphic DNA patterns (12).

Every lactobacillus isolate from immunocompromised patients with sepsis was identified to the species level according to its fermentation profile (Table 2). However, in a simultaneous study of normal vaginal isolates, fermentation profiles gave no identification for 40% of the isolates, and determination of the whole-cell protein pattern was necessary for their identification (data not shown).

The prevalence of the various *Lactobacillus* species is significantly different in immunocompromised patients with disseminated infections and the normal vaginal microflora (data not shown). H_2O_2 production and glycopeptide MICs are species related: H_2O_2 production and susceptibility to glycopeptides are common characteristics of the *L. acidophilus* group. These characteristics are not found in *L. rhamnosus* or *L. paracasei* subsp. *paracasei*. Identification of *Lactobacillus* species to the species level will help to elucidate the conditions for the emergence of infections by different species and will prompt study into species-related properties.

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