MAJOR ARTICLE

Lactobacillus Bacteremia during a Rapid Increase in Probiotic Use of *Lactobacillus rhamnosus* GG in Finland

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Lactobacilli supposedly have low pathogenicity; they are seldom detected in blood culture. *Lactobacillus rhamnosus* GG, which originates indigenously in the human intestine, became available for use as a probiotic in 1990 in Finland. We evaluated the possible effects of the increased probiotic use of *L. rhamnosus* GG on the occurrence of bacteremia due to lactobacilli. Lactobacilli were isolated in 0.02% of all blood cultures and 0.2% of all blood cultures with positive results in Helsinki University Central Hospital and in Finland as a whole, and no trends were seen that suggested an increase in *Lactobacillus* bacteremia. The average incidence was 0.3 cases/100,000 inhabitants/year in 1995–2000 in Finland. Identification to the species level was done for 66 cases of *Lactobacillus* bacteremia, and 48 isolates were confirmed to be *Lactobacillus* strains. Twenty-six of these strains were *L. rhamnosus,* and 11 isolates were identical to *L. rhamnosus* GG. The results indicate that increased probiotic use of *L. rhamnosus* GG has not led to an increase in *Lactobacillus* bacteremia.

Lactobacilli are gram-positive rods found in the normal gastrointestinal and genitourinary flora [1]. They are often regarded as contaminants with no clinical significance or as opportunistic pathogens that might cause infections in immunocompromised individuals [1–3]. In 2 surveys, lactobacilli represented only 0.1%–0.2% of all isolates found in blood cultures [4, 5]. Despite the presumed low virulence of these pathogens, cases of pneumonia, endocarditis, and local suppurative deep abscesses have been described in association with *Lactobacillus* infection, even in patients with normal immunity [1]. The importance of lactobacilli as pathogens might be growing, because many case reports on *Lactobacillus* bacteremia in immunocompromised patients have recently been published [6–10].

Live lactic acid bacteria traditionally have been widely consumed in fermented dairy food products in many countries. In addition, selected *Lactobacillus* strains that tolerate intestinal conditions have increasingly been used in human food because of their putative health effects. These strains, called "probiotics," are described as "live microbial feed supplements that beneficially affect the host animal by improving its microbial balance" [11]. One of the most studied probiotic strains is *Lactobacillus rhamnosus* GG (ATCC 53103), which was originally isolated from human intestinal flora. *L. rhamnosus* GG has been shown to shorten the duration and ameliorate the symptoms of infantile rotavirus diarrhea, to have some effect on preventing atopic diseases among infants, and to modulate immune responses [12–16].

The safety of the widespread use of probiotics has not been thoroughly investigated, although no hazard-

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ous adverse events were found in a review of 143 clinical trials of probiotics [17]. Concern has been raised by reports of transfer of antibiotic resistance by a probiotic bacterium and of fungemia caused by a probiotic yeast and by a case report of an *L. rhamnosus* GG–like isolate in a liver abscess [18–20].

In 1990, *L. rhamnosus* GG was introduced into dairy products in Finland. After a slow start, its annual consumption increased rapidly and reached 6 L/person (e.g., 3×10^{11} cfu/person/year) in 1999. Since 1990, we have collected all *Lactobacillus* isolates from blood culture specimens in our survey laboratory at Helsinki University Central Hospital (HUCH; Helsinki). In addition, national surveillance of all bloodstream infections, including those caused by lactobacilli, was started in late 1994 by Finland's National Public Health Institute. We studied the possible impact of increased consumption of *L. rhamnosus* GG on the occurrence of bloodstream infections caused by lactobacilli, including species characterization and comparison by molecular epidemiological methods with the probiotic *L. rhamnosus* GG strain.

MATERIALS AND METHODS

Collection of* Lactobacillus *isolates, HUCH, 1990–2000. Blood and CSF culture isolates characterized as lactobacilli were collected at our survey laboratory in HUCH during 1990–2000. For 1990–1993, blood culture isolates were retrieved from routinely stored (−70°C) blood culture isolates; since 1993, all *Lactobacillus* isolates have been stored separately when first identified. In 1997, another laboratory was merged into our survey laboratory, which expanded the catchment area and increased the number of blood culture isolates available; after that, the area covered an average population of 1.3 million, approximately one-fourth of the population of Finland.

Collection of cases of* Lactobacillus *bacteremia, National Infectious Disease Register (NIDR), 1995–2000. National mandatory notification of all bacterial findings in blood and CSF by all the microbiological laboratories in Finland (population at the end of the surveillance period, 5.2 million) was instituted in late 1994 as the NIDR, which is maintained by the National Public Health Institute. All clinical microbiology laboratories that had notified the NIDR of *Lactobacillus* findings in blood or CSF were asked to send routinely stored *Lactobacillus* blood culture isolates from reported cases and possibly unreported cases (those that were not reported to the NIDR but should have been) from 1995–2000 to our survey laboratory. Isolates were stored at −70°C until analysis was done.

Characterization of* Lactobacillus *strains. Characterization of the isolates to the species level was done at Valio R&D (Helsinki). The isolates were grown anaerobically at 37°C on de Man–Rogosa–Sharpe (MRS) agar (LabM, International Diagnostics Group) or Trypcase Soy agar (BioMérieux) supplemented with 5% horse blood. Preliminary characterization of the *Lactobacillus* isolates was performed according to their carbohydrate fermentation profile, using API 50 CH strips and APILAB Plus software (version 4.0; BioMérieux).

The identification was confirmed by species-specific PCR. *L. rhamnosus*–specific PCR was performed as described elsewhere [21]. When necessary, some *Lactobacillus* isolates and all potentially non-*Lactobacillus* isolates that did not grow on MRS agar were identified by sequencing part of the 16S rDNA [22]. All *L. rhamnosus* strains were compared with *L. rhamnosus* GG by PFGE, as described elsewhere [23]. Four restriction enzymes, *Not*I, *Sfi*I, *Asc*I, and *Fse*I, were used separately to cut the genomic DNA of the strains. The genomic fragments were separated by agarose gel electrophoresis, using *L. rhamnosus* GG as a control in every gel. Electrophoresis was carried out on a CHEF DR II apparatus (Bio-Rad) in 1% PFGE Certified agarose (Bio-Rad), using 0.5× TBE buffer (10× TBE buffer is 89 m*M* Tris, 89 m*M* boric acid, and 25 m*M* EDTA [pH 8.0]). The running parameters were as follows: for *Asc*I and *Fse*I, pulse, 1–15 s; current, 7 V/cm; temperature, 12°C; time, 25 h; for *Not*I, 1–8 s, 6 V/cm, 14°C, and 27 h; and for *Sfi*I, 1–15 s, 5 V/cm, 14°C, and 22 h. A 120° angle was used in all runs. The agarose gel was stained with ethidium bromide (0.5 μg/mL) and visualized under UV light. The PFGE patterns of the strains were compared visually.

Definitions. *Lactobacillus* isolates found in blood or CSF samples from a particular patient up to 3 months after the first positive isolate was detected were handled as if they were from a single case of infection. Isolates for which PFGE patterns were identical to that of *L. rhamnosus* GG with all 4 restriction enzymes were regarded as *L. rhamnosus* GG strains. Only the first blood isolate from each case was used for typing. All postmortem *Lactobacillus* blood isolates originating from fetus mortus samples ($n = 9$) were excluded.

Statistical analysis. For annual incidence, the annual number of cases of *Lactobacillus* infection was compared with the annually registered Finnish population (according to official statistics from Statistics Finland). A linear trend test using Poisson regression was used to evaluate possible annual trends in the relative proportion of lactobacilli among all positive blood culture findings and all blood cultures [24]. This test was also used to evaluate possible trends in the annual number of positive blood cultures in relation to all blood cultures. The analyses were done with Egret for Windows (version 2.03; Cytel Software).

RESULTS

Lactobacillus *bacteremia in HUCH, 1990–2000.* Altogether, 43 blood culture isolates were originally reported as lactobacilli during the 11-year study period in our surveillance laboratory

Table 1. Results of blood cultures and *Lactobacillus* species identification done at Helsinki University Central Hospital, by year, 1990–2000.

Year	Total no. of blood cultures	No. of positive blood cultures	Isolates originally reported to be *Lactobacillus* species				
			Total no.	Percentage among all blood cultures	Percentage among positive blood cultures	No. available for species confirmation	No. confirmed to be *Lactobacillus*[a]
1990	15,321	1382	4	0.026	0.29	3	2
1991	16,850	1619	3	0.018	0.19	2	1
1992	14,636	1574	3	0.020	0.19	3	3
1993	16,563	1523	2	0.012	0.13	0	0
1994	14,737	1761	3	0.020	0.17	2	2
1995	16,941	1723	3	0.018	0.17	2	2
1996	17,050	1757	3	0.018	0.17	3	2
1997	20,087	3047	2	0.010	0.07	2	2
1998	20,827	2906	6	0.029	0.21	3	2
1999	22,575	3102	10	0.044	0.32	9	4
2000	33,910	2676	4	0.012	0.15	2	2
Total	209,497	23,070	43	—	—	—	—
Mean	—	—	—	0.021	0.19	31	22

[a] By API (bioMérieux) and species-specific PCR.

in HUCH (table 1). During this period, the total number of blood cultures done and the total number of positive blood culture findings increased by >2-fold. The largest increase in positive blood cultures coincided with the merging of a local microbiological laboratory and our survey laboratory in 1997.

Lactobacilli were reported in a mean of 0.02% of all blood cultures done and in 0.2% of positive blood cultures (table 1). No significant annual trend could be observed in the proportion of *Lactobacillus* isolates among all blood cultures ($P = .9702$) or the proportion of *Lactobacillus* isolates among all positive blood cultures ($P = .7282$). However, there was a significant increase in the proportion of positive blood cultures among all blood cultures ($P < .001$). The annual incidence of reported *Lactobacillus* bacteremia in the HUCH area ranged from 0.15 to 0.76 cases/100,000 inhabitants/year for the period 1997–2000, during which time the population of the catchment area was clearly defined.

Lactobacillus *bacteremia in Finland, 1995–2000.* A total of 90 cases of *Lactobacillus* bacteremia were identified in Finland during 1995–2000 (table 2). The NIDR was notified of 78 cases of *Lactobacillus* bacteremia by clinical microbiology laboratories, and 12 cases (13%) of which the NIDR was not notified

Table 2. Results of blood cultures and *Lactobacillus* species identification in Finland (population, 5.2 million), by year, 1995–2000.

Year	No. of positive blood cultures	Isolates originally reported to be *Lactobacillus* species		Reported incidence of *Lactobacillus* infection[a]	No. of isolates available for species confirmation	No. of isolates confirmed to be *Lactobacillus*[b]
		Total no.	Percentage among positive blood cultures			
1995	5291	16	0.30	0.31	11	7
1996	5717	13	0.23	0.25	9	6
1997	6113	10	0.16	0.19	6	6
1998	6166	13	0.21	0.25	6	5
1999	6566	17	0.26	0.32	11	5
2000	7067	21	0.30	0.40	12	10
Total	36,920	90	—	—	55	39
Mean	—	—	0.24	0.29	—	—

[a] Per 100,000 inhabitants.
[b] By API (bioMérieux) and species-specific PCR.

Table 3. Identification of *Lactobacillus* species isolated from blood cultures from Helsinki University Central Hospital, 1990–2000, and stored isolates collected nationwide in Finland, 1995–2000.

Lactobacillus species	No. (%) of isolates (n = 48)
L. rhamnosus	26 (54)
L. rhamnosus GG[a]	11 (23)
L. fermentum	9 (19)
L. casei	7 (15)
L. gasseri	2 (4)
L. jensenii	2 (4)
L. zeae	1 (2)
L. sake	1 (2)

[a] Included in *L. rhamnosus*.

(15% of the 78 reported cases) were identified when a request for stored *Lactobacillus* isolates was sent. Of these 90 cases, 28 had been diagnosed at HUCH.

The annual number of positive blood cultures had a clear rising trend during this 6-year period (table 2). The annual proportion of reported lactobacilli among all positive blood cultures was, on average, 0.2%, and did not change during the observation period ($P = .7100$). The annual incidence of *Lactobacillus* bacteremia in Finnish population was, on average, 0.29 cases/100,000 inhabitants/year.

Lactobacillus *species.* Overall, 66 isolates were available for species-level identification. Of these, 48 were shown to be *Lactobacillus* isolates, and 7 different species were observed (table 3). *L. rhamnosus* was the most common species, constituting 54% of all *Lactobacillus* species. *Lactobacillus fermentum* contributed 19% and *Lactobacillus casei* 15% of the isolates. *Lactobacillus gasseri* and *Lactobacillus jensenii* were found in ≤2 cases each. In 35 cases, >1 addition bacterial species (other than *Lactobacillus*) was found, and in 28 cases, lactobacilli were found on >1 occasion in blood cultures for a single patient.

Of the 66 isolates that were analyzed for species identification, 27% (18) proved to be other microorganisms; among them, 4 were *Actinomyces* species and 4 were *Clostridium* species, 3 were *Bifidobacterium* species, 1 was *Weissella confusa,* and 1 was a *Carnobacterium* species. Of 26 *L. rhamnosus* strains, 11 *L. rhamnosus* GG isolates were detected that were not distinguishable by PFGE from the probiotic *L. rhamnosus* GG strain. The annually reported and analyzed *L. rhamnosus* and *L. rhamnosus* GG isolates for HUCH and for Finland are shown, in relation to the amounts of probiotic *L. rhamnosus* GG consumed in Finland, in figures 1 and 2, respectively. The annual consumption of *L. rhamnosus* GG probiotics increased rapidly after 1995 in Finland, reached an average of 3 L/person/year in 1996, and continued to increase, to 6 L/person/year (i.e., 3×10^{11} cfu/person/year) (data from Valio).

DISCUSSION

We used data from a large university hospital laboratory and from a mandatory national notification system for all findings of bacteria in blood to investigate whether there was an increase in bacteremia caused by *Lactobacillus* species in Finland during the extensive increase in the probiotic use of *L. rhamnosus* GG in food. No trends were observed that suggested an increase in the incidence of *Lactobacillus* bacteremia or in the proportion of *Lactobacillus* bacteremia among all cases of bacteremia. The relative proportion of lactobacilli among all isolates from blood cultures was, on average, 0.2% for the HUCH and 0.24% for

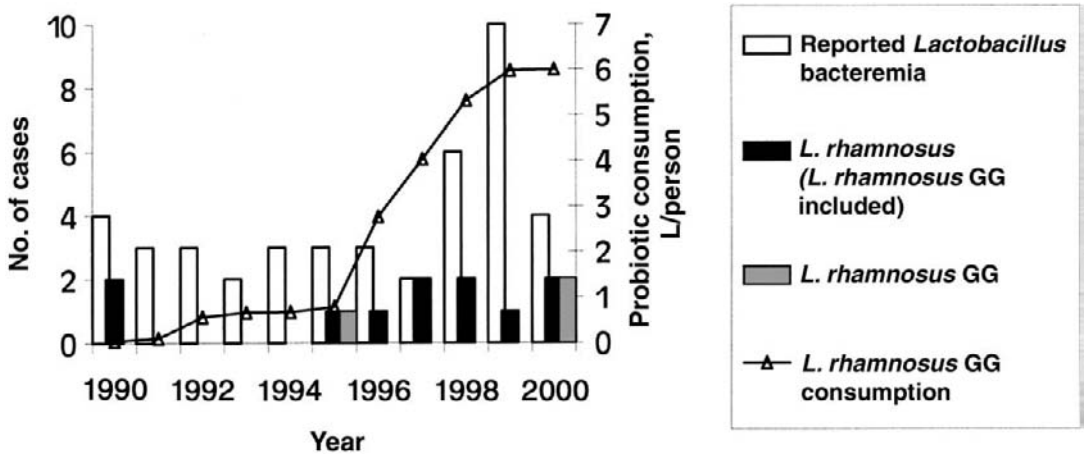

Figure 1. Annual no. of cases in which blood culture isolates were reported to be lactobacilli by Helsinki University Central Hospital, 1990–2000, and the annual no. of cases in which the isolate was confirmed to be *Lactobacillus rhamnosus* or *L. rhamnosus* GG. The annual consumption in Finland of probiotic *Lactobacillus* GG products in food is also shown.

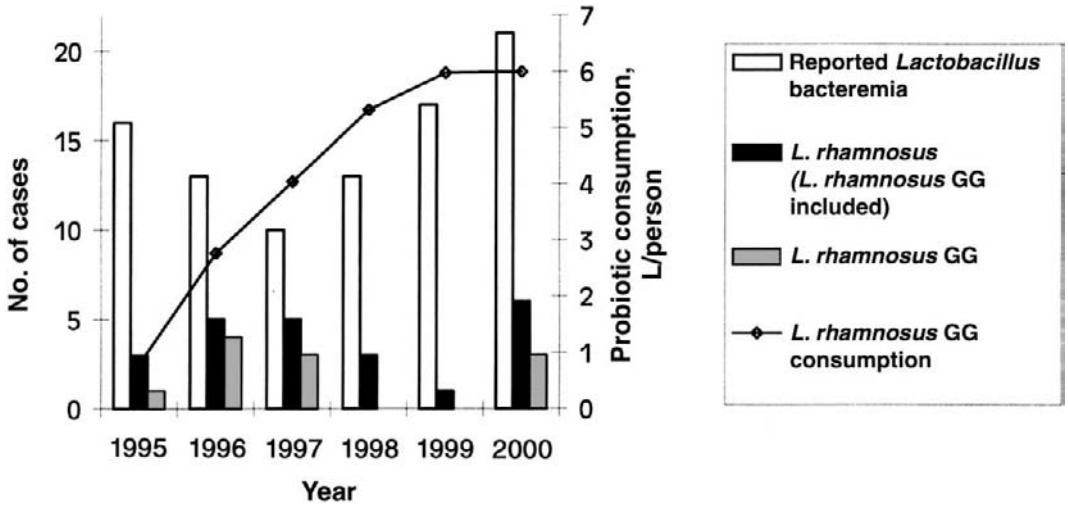

Figure 2. Annual no. of cases in which blood culture isolates were reported to the National Infectious Disease Registry or identified by an additional query as lactobacilli in Finland, 1995–2000, and the annual no. of cases in which isolates were confirmed to be *Lactobacillus rhamnosus* or *L. rhamnosus* GG. The annual probiotic consumption of *L. rhamnosus* GG products in food is also shown.

Finland, which is in line with the proportions (0.1%–0.2%) that have been published elsewhere [4, 5]. In addition, in our study, the population-based incidence of *Lactobacillus* bacteremia could be estimated nationally and was found to be, on average, 0.29 cases/100,000 inhabitants/year.

Only 61% of all the isolates originally reported by the clinical microbiology laboratories as lactobacilli were available for analysis. Approximately three-fourths of these were confirmed to be lactobacilli, which indicates that the actual occurrence of *Lactobacillus* bacteremia, taking into account the 15% of cases found through a separate request for stored strains, is probably lower than the incidence calculated on the basis of notifications to the national surveillance program.

In the present study, *L. rhamnosus* was the *Lactobacillus* species most commonly found in blood cultures. Eleven *L. rhamnosus* GG cases were found, without any temporally increasing trend that would suggest an association with the increase in the probiotic use. *L. rhamnosus* GG is acid and bile stable, and it attaches to and temporarily and effectively colonizes the human intestine [25]. These characteristics not only could be important to the indigenous nature and clinical efficacy of *L. rhamnosus* as a probiotic but also could increase the potential tendency of this species to cause infections. In 2 surveys, which included 43 and 45 *Lactobacillus* bacteremia cases, lactobacilli were primarily associated with an underlying disease or with immunosuppression [2, 3]. Thus, an increase in the number of immunosuppressed patients might also increase the impact of lactobacilli in clinical infections. Therefore, it would be essential, in the future, to analyze the actual relationship of different *Lactobacillus* findings to patient outcomes. In conclusion, the occurrence of *Lactobacillus* in blood culture, especially *L. rhamnosus* and *L. rhamnosus* GG, was evaluated in Finland, and no increase related to the increasing probiotic use of *L. rhamnosus* GG could be observed.

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