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Clinical significance of *Lactobacillus* bacteremia, with special focus on probiotic *L. rhamnosus* GG*

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Introduction

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). There is no reliable epidemiological data on the occurrence of *Lactobacillus* bacteremia. In France they have estimated that the frequency of lactobacilli bloodstream infections was 0.1 % of all bacteremias between 1988–1990 (4). Despite the presumed low virulence, pneumonia, endocarditis and local suppurative deep abscesses even in patients with normal immunity have been described (1). During 55 years there have been published 53 patients with *Lactobacillus* endocarditis in the medical literature (4). This suggests an extremely low frequency of the disease. The majority (89 %) of these patients had structural underlying heart disease before the emergence of *Lactobacillus* endocarditis (4). The importance of lactobacilli as pathogens might be growing, as many case reports on *Lactobacillus* bacteremia in immunocompromised patients have recently been published (5–9).

One of the most studied probiotic strains is *Lactobacillus rhamnosus* GG (ATCC 53103, LGG), originally isolated from human intestinal flora. *L. rhamnosus* GG has been shown to shorten the duration and ameliorate the symptoms of infantile rotavirus diarrhoea, to have some effect on preventing atopic diseases among infants and on modulating immune responses (10–14). The safety of widespread use of probiotics has not been thoroughly investigated. Concern has been raised by transferred antibiotic resistance by a probiotic bacterium, and a case report of *L. rhamnosus* GG-like isolate in a liver abscess (15).

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Incidence of *Lactobacillus* bacteremia

In 1990 probiotic *L. rhamnosus* GG was introduced into dairy products in Finland. After a slow start its consumption increased rapidly in 1996, and reached six litres per capita in 1999. We wanted to study the possible impact of the increased consumption of *L. rhamnosus* GG on the occurrence of bloodstream infections caused by lactobacilli.

Collection of Lactobacillus isolates and cases. Blood culture isolates characterised as lactobacilli were collected from two sources. From our survey laboratory in Helsinki University Central Hospital between 1990–2000 (consisting of population of 1.3 million) and since late 1994 by the National Public Health Institute the national surveillance of all bloodstream infections, including bacteremias caused by lactobacilli covering the whole country (16).

Identification and the growth of the isolates, their preliminary biochemical characterization, *L. rhamnosus* species specific PCR and typing by PFGE have been described elsewhere (16). Partial 16S rDNA sequencing (17) or species specific PCR for *L. casei* (18), *L. fermentum* (19), *L. gasseri* and *L. zeae* (20) were applied to identify the strains (16).

There were 90 blood culture findings originally reported as lactobacilli during 1995–2000 in whole Finland and 43 in southern Finland between 1990–2000 (16). The relative proportion of annually reported lactobacilli was in an average 0.2 % of all positive blood cultures. The annual incidence rate during 1995–2000 was in an average 0.3/100000 inhabitants in the whole Finland. Neither the relative proportion nor the annual incidence rate did show any significant trend during the observation years (Figure 1).

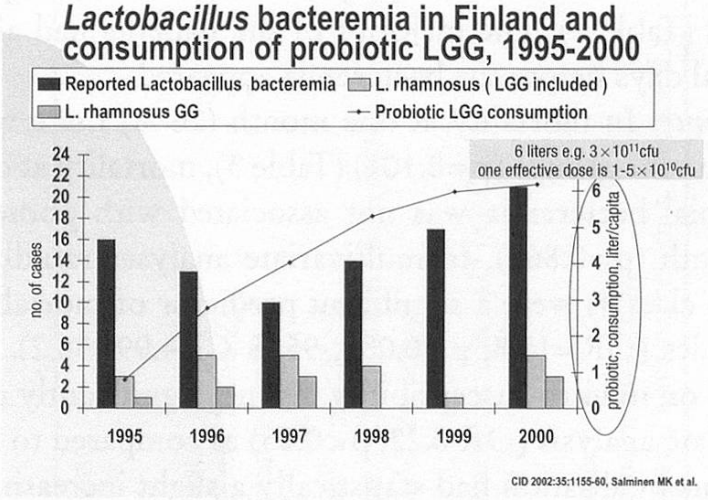

Figure 1 **Annual no. of cases in which blood culture isolates were reported to the National Infectious Disease Registry in Finland, 1995–2000, and the annual no. of cases in which isolates were confirmed to be *Lactobacillus rhamnosus* or *L. rhamnosus* GG. The annual probiotic consumption of *L. rhamnosus* GG products in food is also shown**

From the overall 109 reported findings identification to the species level was performed in 66 cases, and a total of 48 *Lactobacillus* strains were found; 26 strains were *L. rhamnosus* of which 11 isolates were identical to the probiotic *L. rhamnosus* GG by PFGE-analysis. The results suggest that probiotic use of *L. rhamnosus* GG is generally safe as the relative proportion of lactobacilli among blood culture findings was unaffected despite increased consumption and only occasional *L. rhamnosus* GG isolates in blood culture were observed.

Clinical findings in *Lactobacillus* bacteremia

We wanted to evaluate what the *Lactobacillus* bacteremia means to the patient in practice. The patient records were retrieved, possible predisposing factors, clinical course, and treatment of the patients were evaluated.

Demographic characteristics and predisposing factors. The 89 *Lactobacillus* bacteremia cases were divided into three groups: *L. rhamnosus* GG isolates (**LGG;** n=11), *L. rhamnosus* isolates other than LGG (**LR;** n=14) and other confirmed *Lactobacillus* species (**OL;** n=22). The *Lactobacillus* cases with no confirmation to the species level, but defined previously as lactobacilli in clinical microbiology laboratories, were reviewed as the fourth group (**NCL;** n=42) (21). The age and the proportion of the male patients were similar in all these groups. Only a few cases had some other bacteria than lactobacilli in the blood culture. Characteristics between the groups were similar (p=0.092), and the majority of patients in all groups had ultimately or rapidly fatal underlying diseases, McCabe classes 3 or 4 (Table 1). The underlying diseases were mainly malignancies or serious gastrointestinal disorders. The majority of patients in all groups had undergone a surgical intervention with no significant differences between the groups (Table 2). There were no differences in the use of foreign devices or in the proportion of immunosuppression between these four groups (Table 2). The majority of the patients had already been in the hospital for several days before the bacteremia appeared.

Clinical outcome. In mortality at one month (26 %) there were no significant differences between the groups (p=0.101) (Table 3), mortality at one year rose up to 46 %. Polymicrobial bacteremia was not associated with poorer survival in any group at one month (p=0.864). In multivariate analyses rapidly fatal underlying diseases (McCabe class 4) were a significant predictor of mortality in cases due to *Lactobacillus* species (OR=15.8, p<0.001, 95 % CI 4.99–50.2). Therapy that was appropriate based on in vitro susceptibility testing, significantly reduced the risk of death in multivariate analysis (OR 0.22, p<0.05) as compared to in vitro ineffective treatment. Prior hospitalisation had statistically a slight increasing impact on mortality (OR=1.02, p=0.012, 95 % CI 1.01–1.04). After adjusting for these predicting variables in multivariate analyses, *L. rhamnosus* bacteremia (LGG and LR groups combined) was associated with higher mortality as compared to OL group (OR=3.11, p=0.012, 95 % CI 1.28–7.56) (21).

Table 1
General characteristics of patients with *Lactobacillus* bacteremia. Data is presented separately from patients that had bacteremia caused by *L. rhamnosus* GG (LGG), *L. rhamnosus* (LR), other specified *Lactobacillus* sp. (OL) and lactobacilli not available for species characterisation (NCL)

	L. rhamnosus		*Other Lactobacillus Species (OL)*	*Not species characterised lactobacilli (NCL)*
	L. rhamnosus GG	*L. rhamnosus*		
Number of patients	**11**	**14**	**22**	**42**
Age, mean±SD	60.9±22.6	64.3±18.0	60.1±24.0	53.5±23.3
Male (%)	5 (45%)	8 (57%)	11 (50%)	27 (64%)
Underlying diseases[1]				
non-fatal (classes 1 and 2)	1 (10%)	2 (14%)	4 (18%)	9 (22%)
Ultimately fatal (class 3)	5 (45%)	4 (29%)	7 (32%)	14 (33%)
rapidly fatal (class 4)	5 (45%)	8 (57%)	11 (50%)	19 (45%)
Temperature >38 °C or <36 °C	9 (82%)	11 (79%)	19 (86%)	30 (71%)
C-reactive protein (CRP), mean±SD (mg/l)				
at the onset of bacteremia	183±117	197±105	92±56***	115±99**
maximally[2]	226±104	236±104	118±66***	171±107*
Leucocytes, geometric mean±SD ($x10^9$/l)				
at the onset of bacteremia	4.58±19.75	5.65±8.42	9.12±19.83	5.37±7.62
maximally	18.55±19.65	14.37±13.79	15.57±3.25	14.18±7.60
Polymicrobial infection	2 (18%)	4 (29%)	10 (45%)	19 (45%)
Prior hospitalisations, mean±SD	5.1±17.1	7.2±22.7	5.3±22.8	2.7±8.4
Hospitalisation days, after bacteremia, mean±SD	9.2±11.8	11.8±15.9	12.0±9.7	12.5±9.7

[1] according to McCabe and Jackson classification (20)
[2] within 5 days after the onset of bacteremia

Compared to *L. rhamnosus* (=LGG+LR)
* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 2
Predisposing factors to *Lactobacillus* bacteremia caused by *L. rhamnosus* GG (LGG), *L. rhamnosus* (LR), other *Lactobacillus* species (OL) and lactobacilli that were not available for characterisation to species level (NCL). Data is shown as number of patients (%)

	L. rhamnosus GG	*L. rhamnosus*	*Other Lactobacillus species (OL)*	*Not species characterised lactobacilli (NCL)*
	n=11	*N=14*	*n=21*	*n=42*
Intra-venous catheter	7 (64)	8 (57)	12 (57)	12 (29)
Central venous catheter	6 (55)	5 (36)	8 (38)	14 (33)
Urinary catheter	3 (27)	4 (29)	3 (14)	6 (14)
Intubation	1 (9)	1 (7)	2 (10)	3 (7)
Ventilation support	5 (45)	4 (29)	3 (75)	5 (12)
Previous surgery or endoscopy	5 (45)	9 (64)	14 (67)	15 (36)
Prosthetic material	2 (18)	2 (14)	1 (5)	4 (10)
Neutropenia ($<0.5\times10^9$/l)	3 (27)	2 (14)	1 (5)	9 (21)
Prior antimicrobial therapy	7 (64)	10 (71)	10 (48)	19 (45)
Immune suppression	7 (64)	6 (43)	10 (48)	23 (55)
Corticosteroids	2 (18)	4 (29)	6 (29)	12 (29)
Cytotoxic therapy	4 (36)	3 (21)	5 (24)	14 (33)

Table 3
Cumulative survival in *Lactobacillus* bacteremia according to Kaplan-Meier analyses

Bacteria	*n*	*7 days*	*30 days*	*1 year*
LGG group				
L. rhamnosus GG	**11**	**8**	**8**	**6**
		73 %	73 %	55 %
LR-group				
L. rhamnosus	**14**	**11**	**7**	**4**
		79 %	50 %	29 %
OL-group[1]				
(Other *Lactobacillus* species)	**22**	**19**	**17**	**13**
		86 %	77 %	59 %
L. fermentum	**9**	**8**	**6**	**2**
		89 %	67 %	22 %
L. casei	**7**	**5**	**5**	**3**
		71 %	71 %	43 %
NCL-group				
(Not species characterized lactobacilli)	**42**	**40**	**34**	**25**
		95 %	81 %	60 %
Total	**89**	**78**	**66**	**46**
		88 %	74 %	52 %

[1] *L. jensenii* (2), *L. gasserii* (2), *L. sake* (1), *L. zeae* (1) not shown separately

Conclusions. The results indicate that lactobacilli in blood culture are of clinical significance and that their antimicrobial susceptibility should guide treatment decisions.

McCabe classification was the major forecast for death, which underlines the importance of the other diseases of the patient and not the *Lactobacillus* bacteremia

and if the patient was classified into McCabe group 4 the risk of death was 15 fold. *L. rhamnosus* was associated with somewhat higher risk of death as compared with other lactobacilli. If the patient had been longer in the hospital before the bacteremia it was a statistically significant denominator of death, but this risk increase was only 2 percent. *Lactobacillus* bacteremia seems to be a significant finding since mortality was clearly decreased if the antimicrobial treatment was adequate for *Lactobacillus.* Although we had the most comprehensive material of *Lactobacillus* bacteremia cases, it was still too small for detailed analysis between various species, and therefore the comparison of *L. rhamnosus* GG bacteremias to other *L. rhamnosus* and other species would not be reliable.

Summary

Lactobacillus bacteremia incidence is supposed to be low and its clinical significance is poorly defined. We have evaluated the possible effects of increasing probiotic use of *Lactobacillus rhamnosus* GG on the occurrence of bacteremia due to lactobacilli. Characterisation of the *Lactobacillus* isolates was carried out by species-specific PCR or 16S rDNA sequencing. Furthermore, all *L. rhamnosus* isolates were typed with pulsed-field gel electrophoresis (PFGE) and compared with the commercial probiotic *L. rhamnosus* GG strain. There were 90 blood culture findings originally reported as lactobacilli during 1995–2000 in whole Finland. The proportion of annually reported lactobacilli was in an average 0.2 % of all positive blood cultures. The annual incidence rate during 1995–2000 was in an average 0.3/100000 inhabitants in the whole Finland and no increasing trend could be detected. Species characterization was made in 53 % of the cases, 25 *L. rhamnosus* strains and 22 other *Lactobacillus* species were found. In eleven cases the strain was identical with the probiotic *L. rhamnosus* GG. In 82 % of the cases the patients had severe or fatal comorbidities. Predisposing factors to bacteremia were immunosuppression, prior prolonged hospitalization and prior surgical interventions. No significant differences were observed in these predisposing factors or clinical features between the various *Lactobacillus* species, except higher C-reactive protein values in patients with *L. rhamnosus* bacteremia. Mortality at one month was 26 % and at one year 48 %. In multivariate analysis severe underlying diseases were a significant predictor for mortality (OR 15.8), whereas in vitro effective antimicrobial treatment was associated with lower mortality (OR 0.22). It is concluded that lactobacilli in blood cultures are of clinical significance and their antimicrobial susceptibility should guide the treatment.

Zusammenfassung

Die Inzidenz bakterieller Infekte verursacht durch *Lactobacillus* wird als gering eingestuft und die klinische Signifikanz ist kaum bekannt. Wir haben die möglichen Effekte eines vermehrten probiotischen Einsatzes von *Lactobacillus rhamnosus* GG auf das Auftreten von bakteriellen Infekten ausgelöst durch *Lactobacilli* untersucht.

Die Charakterisierung der *Lactobacillus* Isolate geschah mittels spezies-spezifischem PCR oder der Sequenzierung der 16s rDNA. Darüber hinaus wurden alle *L. rhamnosus* Isolate mittels Pulsed-field Gel Elektrophorese (PFGE) typisiert und mit kommerziellen probiotischen *L. rhamnosus* GG Stämmen verglichen. Zwischen 1995 und 2000 wurden in ganz Finnland 90 Blut-Proben positiv auf *Lactobacilli* getestet. Dies entspricht einem jährlichen Durchschnitt von 0,2 % aller positiv getesteten Blutproben. Die jährliche Inzidenz zwischen 1995 und 2000 lag bei durchschnittlich 0.3/100000 Einwohner in ganz Finnland und es konnte keine Trenderhöhung festgestellt werden. Eine Spezies-Charakterisierung wurde in 53 % der Fälle vorgenommen, 25 *L. rhamnosus* und 22 andere *Lactobacillus* Arten wurden gefunden. In elf Fällen war der Stamm identisch mit dem probiotischen *L. rhamnosus* GG. In 82 % der Fälle hatten die Patienten bereits schwere oder gar tödliche Krankheiten. Prädisponierende Faktoren für einen bakteriellen Infekt waren Immunosuppression, vorgängige lange Hospitalisierung und vorgängige chirurgische Eingriffe. Keine signifikanten Unterschiede wurden beobachtet zwischen diesen prädisponierenden Faktoren oder den klinischen Merkmalen zwischen den verschiedenen *Lactobacillus* Arten ausser eines höheren C-reaktiven Protein-Wertes bei Patienten mit einem bakteriellen Infekt ausgelöst durch *L. rhamnosus.* Die Sterberate nach einem Monat lag bei 26 %, nach einem Jahr bei 48 %. Mittels multivariater Analyse konnte gezeigt werden, dass die Grundkrankheiten ein signifikanter Faktor für die Mortalität waren (OR 15,8) während mit antimikrobielle Behandlungen, die sich *in vitro* als wirksam erwiesen hatten, mit einer geringeren Todesrate (OR 0,22) assoziiert waren. Daraus kann geschlossen werden, dass *Lactobacilli* in Blutkulturen klinisch signifikant sind und dass deren antimikrobielle Empfindlichkeit die Behandlung bestimmen sollte.

Résumé

L'incidence des bactérémies dues aux lactobacilles est supposée être faible et leur importance clinique est mal définie. Nous avons évalué les effets possibles de l'utilisation croissante de la souche probiotique, *Lactobacillus rhamnosus* GG, sur la fréquence des bactérémies associées aux lactobacilles. La caractérisation des isolats de *Lactobacillus* a été effectuée par PCR spécifique à l'espèce ou par séquencage de l'ADNr 16S. De plus, tous les isolats de *L. rhamnosus* ont été typés par électrophorèse sur gel en champ pulsé (PFGE) et comparés avec la souche probiotique commerciale, *L. rhamnosus* GG. Durant la période 1995–2000, il y a eu 90 résultats de culture de sang initialement rapportés aux lactobacilles ce qui représente une proportion annuelle moyenne de 0,2 % de toutes les cultures de sang positives. Le taux d'incidence annuel durant la même période s'est situé en moyenne à 0,3 pour 100000 habitants pour toute la Finlande et aucune tendance d'augmentation n'a été décelée. La caractérisation à l'espèce a pu être réalisée dans 53 % des cas, avec 25 souches de *L. rhamnosus* et 22 souches d'autres espèces de lactobacilles. Dans 11 cas, la souche était identique à la souche probiotique *L. rhamnosus* GG et dans 82 % de

ces cas, les patients avaient des morbidités associées sévères ou fatales. Le facteur prédisposant aux bactérémies était l'immunosuppression avant une hospitalisation prolongée ou une intervention chirurgicale. Aucune différence significative n'a été trouvée entre les différentes espèces de *Lactobacillus* pour ce facteur prédisposant ou les caractéristiques cliniques, à l'exception de valeurs plus élevées pour les protéines C-réactives chez les patients avec une bactérémie due à *L. rhamnosus.* Les mortalités après un mois et un an ont été respectivement de 26 et 48 %. Lors de l'analyse multivariée, la présence de maladies sévères sous-jacentes a constitué un facteur prédictif significatif de la mortalité (OR 15,8) alors qu'un traitement *in vitro* antimicrobien efficace a été associé à une mortalité plus faible (OR 0,22). Il est conclu de cette étude que les lactobacilles dans les cultures de sang sont d'importance clinique et leur sensibilité aux antimicrobiens devrait être utilisée comme guide pour le traitement.

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Key words

Lactobacillus, bacteremia, endocarditis, probiotic

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Veranstaltungskalender 2005

Datum	*Veranstaltung*	*Ort*	*Kontaktpersonen*
9. März	SGLH-Generalversammlung	CREMO, Villars-sur-Glâne mit Betriebsbesichtigung	leo.meile@ilw.agrl.ethz.ch
April	Workshop: Schnellnachweis, DNA-Sonden	Universität Zürich-Irchel	stephanr@fsafety.unizh.ch
Mai	Workshop: Schweizer Lebensmittelrecht im Umbruch: Visionen, Realität und EU-Druck	Bern	christina.gut@bag.admin.ch
29./30. Juni	Mikrobiologie Grundkurs: Modul 1: Mikrobiologische Arbeitstechniken	Hochschule Wädenswil HSW, Wädenswil	c.gantenbein@hsw.ch
4.–8. Juli	Weiterbildungskurs Toxikologie	ETH Zürich	renato.amado@ilw.agrl.ethz.ch
5.–8. Juli	Mikrobiologie Grundkurs: Modul 2: Klassische Methoden Lebensmittelbuch Kap. 56	Hochschule Wädenswil HSW, Wädenswil	c.gantenbein@hsw.ch
Sommer	Mikrobiologischer Grundkurs (Sprache: Französisch)	Hochschule Wallis HEVS, Sion	rudolf.schmitt@eiv.ch
6./7./8. September	Workshop: Lebensmittelvirologie: Prävention und Management von viralen, lebensmittelbürtigen Ausbrüchen	Hochschule Wädenswil HSW, IQFS, Wädenswil	t.luethi@hsw.ch
8./9. September	Jahresversammlung SGLUC: Mykotoxine	Kanton Thurgau	renato.amado@ilw.agrl.ethz.ch
September	Mikroskopiekurs II (1–2 Tage)	ETH Zürich	gdasen@vetclinics.unizh.ch
16. September	38. Arbeitstagung der SGLH Neuaufkommende pathogene Bakterien und Viren in Lebensmitteln	ETH Zürich	stephanr@fsafety.unizh.ch

Datum	*Veranstaltung*	*Ort*	*Kontaktpersonen*
21.–23. September	EURO FOOD CHME XIII: Macromolecules and their Degradation Products in Food – Physiological, Analytical and Technological Aspects	Chemische Institute der Universität Hamburg; Deutschland	hans.steinhart@chemie.uni-hamburg.de
November	Diskussionsnachmittag: Die neue HyV/La nouvelle OHyg	Zürich und Lausanne	christina.gut@bag.admin.ch