Lactobacillus Sepsis and Probiotic Therapy in Newborns: Two New Cases and Literature Review

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Abstract

Many term and preterm infants are commonly supplemented with probiotics to prevent adverse effects of antibiotic administration and necrotizing enterocolitis and they are believed to be safe. However, the supplementation with *Lactobacillus rhamnosus* GG has been associated with the development of sepsis with a cause–effect relationship in six newborns and children. In this study, we report two further cases and discuss the emerging issue of probiotic supplementation safety in neonates. We conclude that physicians must be aware that supplementation with *L. rhamnosus* GG can cause sepsis in high-risk patients on rare occasions.

Keywords
- lactobacillus
- probiotics
- infection
- sepsis
- infant

It is well known that necrotizing enterocolitis (NEC)[1] and nosocomial infections[2] increase morbidity and mortality in preterm infants and, therefore, their prevention is of crucial importance for improving outcome in these patients. Probiotic supplementation has been widely studied as one of the proposed interventions for the prophylaxis of NEC and nosocomial infections. Probiotics can enhance the enteric microbiota composition and counteract the loss of gut commensals such as *Bifidobacterium* and *Lactobacillus* species, as occurs in preterm infants undergoing prolonged antibiotic treatment, delayed enteral feeding, and lack of human milk, which can favor the proliferation of pathogenic microflora and abnormal gut colonization.[3] Thus, probiotics may help to decrease translocations of pathogens from the gut and ultimately the development of NEC and nosocomial infections.[3]

A recent meta-analysis of 24 randomized controlled studies showed that probiotics are effective in significantly decreasing NEC occurrence and mortality, but not nosocomial infections, and concludes that these findings support a change in the current practice and they should be widely used.[4] It is noteworthy that this review specifies that none of the included studies report systemic infections due to administered probiotic organisms, thus supporting the safety of probiotic supplementations in preterm infants.[4]

On this basis, every day thousands of extremely and very preterm infants have been and are supplemented with probiotics. However, some cases of sepsis attributable to *Lactobacillus* species have been documented in patients supplemented with probiotics, such as two preterm infants with short-gut syndrome,[5] one child with short-gut syndrome,[5] one infant with congenital heart disease,[6] one child with cerebral palsy,[6] and one term infant with intrauterine growth restriction.[7] Moreover, these reports confirm previous concerns regarding the risk of infections due to

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Lactobacillus species previously documented in adult human beings.[8,9]

Thus, the purpose of this report is to document two further cases of sepsis caused by *Lactobacillus rhamnosus* that occurred in our neonatal intensive care in a term infant affected by multiple chromosomal disorders and in an extremely preterm infant, respectively, and to discuss the emerging issue of probiotic supplementation safety in neonates.

Data Sources

The National Library of Medicine (MEDLINE) database was searched from 1995 to 2014. Search criteria included the following MESH: (1) *Lactobacillus* or probiotic; (2) sepsis, bacteremia, or short-gut syndrome; and (3) infant, newborn, preterm, or premature.

Case 1

A Caucasian female was born at 39 weeks of gestation by vaginal delivery and was affected by trisomy 18 and triple-X syndromes. Her birthweight was 1,660 g and Apgar scores were 5 and 8 at 1 and 5 minutes, respectively. Heart ultrasound demonstrated atrial and ventricular septal defects, bicuspid aortic valve, and patent ductus arteriosus (PDA). Furthermore, her postnatal course was complicated by status epilepticus, relapsing systemic infections (sepsis caused by *Staphylococcus aureus*, pneumonia caused by *Stenotrophomonas maltophilia* and *Staphylococcus aureus*), respiratory failure requiring mechanical ventilation (MV), need of pulmonary arterial banding, and surgical closure of the PDA for hemodynamic worsening. On day 97 of life, during MV and with central venous catheter (CVC) in place, the patient had a temperature of 38.7°C and pulse of 120 beats/min, without other signs and symptoms. Blood sample for culture was drawn from CVC and a peripheral vein, bronchoalveolar lavage for culture was performed, and empiric antibiotic treatment with daptomycin (6 mg/kg, dose every 24 hours) and ceftazidime (30 mg/kg, dose every 6 hours) was started. Laboratory analyses evidenced a white blood cell count (WBC) of 8,040 cells/mL, platelet count of 80,000 cells/mL, serum C-reactive protein (CRP) level of 135.3 mg/L, and serum procalcitonin (PCT) level of 10.12 ng/mL. Blood culture from the peripheral vein was positive for *L. rhamnosus* species. Since the 9th day of life our patient was given oral drop supplementation with 5×10^9 colony-forming unit (CFU) of *L. rhamnosus* GG (Dicoflor, Dicofarm, Rome, Italy) twice daily, through the orogastric tube, for the prevention antibiotic-associated diarrhea. After the results of a positive blood culture the probiotic supplementation was discontinued. The isolate *Lactobacillus* isolate was susceptible to penicillin G, erythromycin, ampicillin, gentamicin, clindamycin, linezolid, and was resistant to vancomycin. Therefore, we discontinued ceftazidime and started clindamycin (5 mg/kg, dose every 6 hours). Our patient's clinical conditions remained stable and after 10 days of therapy with clindamycin, her WBC, CRP, and PCT normalized and antibiotic therapy was discontinued. Ultimately, our patient was discharged at 300 days of life with gastrostomy for enteral nutrition and tracheotomy for respiratory support due to her syndromes.

Case 2

A white male, was born at 23 weeks of gestation by vaginal delivery. His birth weight was 660 g and Apgar scores were 4 and 7 at 1 and 5 minutes, respectively. The postnatal course was complicated by the development of respiratory distress syndrome, pharmacological closure of PDA, and sepsis caused by *Staphylococcus haemolyticus*. On the 18th day of life, during noninvasive respiratory support (nasal intermittent MV) and with CVC in place, the patient developed episodes of mixed apnea associated with metabolic acidosis without other signs and symptoms of infection. Blood samples for culture were made from the CVC and a peripheral vein, and empiric antibiotic treatment with linezolid (10 mg/kg, dose every 8 hours) and gentamicin (4 mg/kg, dose every 36 hours) was started. Laboratory analyses evidenced a WBC of 20,500 cells/mL, CRP of 25.7 mg/L, and PCT of 2.90 ng/mL. The blood culture from the peripheral vein was positive for *L. rhamnosus*. Since the 2nd day of life our patient was given daily oral drop supplementation with 5×10^9 CFU of *L. rhamnosus* GG (Dicoflor), through the orogastric tube, to prevent NEC. After positive blood culture appeared, probiotic supplementation was discontinued. The isolate had the same antibiotic susceptibility and resistance of the previous case. Therefore, we discontinued gentamicin and started clindamycin (5 mg/kg, dose every 6 hours) that was given for 10 days.

On the 26th day of life, the infant developed severe respiratory failure requiring MV and 100% oxygen, caused by a chest X-ray confirmed pneumonia. Laboratory analyses evidenced a WBC of 6,950 cells/mL, platelet count of 119,000 cells/mL, CRP of 84.7 mg/L, and PCT of 3.80 ng/mL. Blood samples for culture were made from the CVC and a peripheral vein, and daptomycin (6 mg/kg, dose every 24 hours) and meropenem (20 mg/kg, dose every 8 hours) were administered empirically. Also, the second peripheral blood culture was positive for *L. rhamnosus* with the same antimicrobial susceptibility profile as the first positive blood culture and therefore meropenem was stopped and gentamicin (4 mg/kg, dose every 24 hours) was given. Persistence of *L. rhamnosus* bacteremia was documented in a third blood sample, obtained from a peripheral vein after 6 days (34th day of life). However, after 10 days of therapy with gentamicin, WBC, CRP, PCT, and chest X-ray normalized, and his clinical condition progressively improved. Ultimately, our patient was discharged at 117 days of life in good health.

Characterization of the *Lactobacillus* Isolates

The *Lactobacillus* isolates from the two blood cultures were identified by MALDI-TOF mass spectrometry (VITEKMS, bioMérieux, Marcy L'Etoil, France) as *L. rhamnosus*, suggesting a correlation with the probiotic preparation given to the infants. To compare the two cultures with the probiotic strain, genotyping by pulsed field gel electrophoresis (PFGE) profiling of the genomic DNA digested with the NotI and SfiI restriction endonucleases was performed.

Table 1 Comparison of MIC values (determined by broth microdilution method) of penicillin, erythromycin, ampicillin, gentamycin, clindamycin, linezolid, and vancomycin of the two *Lactobacillus rhamnosus* clinical isolates and *L. rhamnosus* GG. Results were interpreted according to CLSI M45-A2 document

	Isolate case 1	Interpretation	Isolate case 2	Interpretation	*Lactobacillus rhamnosus* GG	Interpretation
Penicillin	0.5	S	1	S	0.5	S
Erythromycin	≤ 0.12	S	≤ 0.12	S	≤ 0.12	S
Ampicillin	2	S	1	S	1	S
Gentamicin	4	S	2	S	4	S
Clindamycin	≤ 0.12	S	≤ 0.12	S	≤ 0.12	S
Linezolid	2	S	2	S	1	S
Vancomycin	> 256	R	> 256	R	> 256	R

Abbreviations: MIC, minimum inhibitory concentration, expressed in µg/mL; R, resistant; S, susceptible.

The genomic DNA in agarose blocks was prepared by the method of Tynkkynen et al.[10] Restriction enzyme digestion was performed overnight at 37°C. Electrophoresis was performed with a CHEF-DRIII apparatus (Bio-Rad, Hemel Hempstead, United Kingdom) in 1% PFGE certified agarose (Bio-Rad) with 0.5× TBE buffer. The pulse time was 1 to 15 seconds the current was 5 V/cm, the temperature was 14°C, and running time was 22 hours. After electrophoresis, the agarose gel was stained with ethidium bromide (0.5 µg/mL), visualized under ultraviolet light, and the PFGE profiles were compared.

The isolates from both the patients exhibited an identical PFGE profile to that of the probiotic strain *L. rhamnosus* GG (ATCC 53103) (data not shown). Antimicrobial susceptibility testing of the *L. rhamnosus* GG strain from the probiotic formulation revealed a profile identical to that of the two clinical isolates, with minimal inhibitory concentrations of penicillin G, erythromycin, ampicillin, gentamicin, clindamycin, linezolid, daptomycin, and vancomycin of 0.5, $\leq$ 0.12, 1, 4, $\leq$ 0.12, 1, 1, and $>$ 256 mg/L, respectively (►**Table 1**).

Discussion

In this study, we report two cases of sepsis caused by *L. rhamnosus* GG that developed during the patients' probiotic supplementation with the same strain, thus supporting a cause–effect relationship between supplementation and the development of sepsis. By reviewing the international literature we identified six other cases of sepsis due to *L. rhamnosus* GG occurring during probiotic supplementation with the same strain in infants[5,7,8] and children[6,7] (►**Table 2**). All these patients were supplemented with *L. rhamnosus* GG with the purpose of preventing or treating gastrointestinal complications, such as antibiotic-associated diarrhea or NEC.

These cases are in agreement with previous studies reporting the development of systemic infections caused by *Lactobacillus* species in infants and children who were not supplemented with probiotics.[11–17] Both supplemented and nonsupplemented patients had similar risk factors, such as immune-deficiency (including that associated with prematurity[18]), previous gastrointestinal or cardiac surgery, previous antibiotic therapy, particularly with vancomycin, NEC, ileostomy, malabsorption, and placement of CVC, but it is probable that supplementation may further enhance the risk of developing *L. rhamnosus* GG sepsis through the daily prolonged overload of microorganisms.

Thus, *L. rhamnosus* GG is considered a commensal microbe in human beings and part of the normal gut microbial flora,[6] is safe and nonpathogenic in most patients,[19] but can induce serious infections, including sepsis,[5–8] pneumonia, and meningitis[14,15] in compromised newborns and children. It is likely that similar considerations may be extended to other probiotics commonly given to preterm infants, such as *Bifidobacterium* species, since five cases of bacteremia/sepsis have already been documented in newborns.[20–22] However, it must be underlined that only a few cases of severe infection by probiotics have been reported in comparison to the thousands of preterm infants who have been or are supplemented for preventing NEC.

The pathogenesis of *Lactobacillus* infection is not well known, but its adhesion to the intestinal mucosa and subsequent colonization are considered important steps because they can prolong persistence in the intestine.[23] This consideration seems to support our speculation that prolonged daily probiotic supplementation, as occurred in our and previous patients,[5–8] may represent a relevant risk factor for the development of related infections. When supplemented patients develop *L. rhamnosus* GG sepsis its only plausible portal of entry is through enteral administration that is probably followed by *Lactobacillus* access to the bloodstream through translocation across the epithelium. This event might be favored by local gut injuries, such as those potentially caused by decreased blood perfusion able to injure the gastrointestinal mucosa (i.e., systemic hypotension, gastrointestinal surgery, congenital heart disease, intrauterine growth restriction, treatment with nonsteroidal anti-inflammatory drugs for PDA closure, treatment with corticosteroids, etc.). Another uncommon possibility might be CVC contamination, either during the opening of the probiotic bottle or through hand-related transmission.[6]

Table 2 Summary of reported cases of sepsis by *Lactobacillus rhamnosus* GG during its supplementation in infants and children

	Age	Main risk factors	Dose (CFU)	Exposure before sepsis (d)	Outcome	Effective antibiotic therapy	Typing methods
Kunz et al[5]	3 mo	Prematurity, short-gut syndrome	Unknown	23	Unknown	Ampicillin	No confirmatory typing
	10 wk	Prematurity, gastroschisis, short-gut syndrome	Unknown	169	Unknown	Ceftriaxone, ampicillin	PFGE
De Groote et al[6]	11 mo	Prematurity, gastrostomy, short-gut syndrome, CVC	Not reported	35	Unknown	Ampicillin, gentamycin	rRNA sequencing
Land et al[7]	6 wk	CHD, antibiotic related diarrhea	10×10^9	20	Alive after 6 wk	Penicillin G, gentamycin	PCR DNA fingerprinting
	6 y	Cerebral palsy, jejunostomy feeding, CVC, antibiotic- associated diarrhea	10×10^9	44	Discharged after 86 d	Ampicillin	PCR DNA fingerprinting
Sadowska-Krawczenko et al[8]	6 d	IUGR	3×10^9	4	Discharged after 86 d	Ticarcillin plus clavulanic acid	PCR DNA fingerprinting
Present cases	3 mo	Trisomy 18, triple X syndrome, CHD, CVC	5×10^9	88	Discharged after 300 d	Clindamycin	PFGE
	18 d	Prematurity, PDA, CVC	5×10^9	16	Discharged after 117 d	Clindamycin, gentamycin	PFGE

Abbreviations: CHD, congenital heart disease; CVC, central venous catheter; IUGR, intrauterine growth restriction; PDA, patent ductus arteriosus; PFGE, pulsed field gel electrophoresis.

We evaluated the possible role of dose and duration of exposure to *L. rhamnosus* GG in our cases in comparison with previous reports,[4–7] and we observed a great heterogeneity. In fact, while some reports did not detail the supplementation dose,[4,5] we administered 10×10^9 CFU in the first case and 5×10^9 CFU in the second, Land et al[7] gave 10×10^9 CFU, and Sadowska-Krawczenko et al[7] gave 3×10^9 CFU. Moreover, the duration of supplementation with *L. rhamnosus* GG ranged from 4 to 95 days.[5,8] Such heterogeneity precludes the possibility of drawing conclusions regarding the possible effect of probiotic dose and exposure duration on the risk of developing related sepsis. However, after these two cases and due to the lack of evidence-based recommendations on these points,[24] we have decided to decrease the daily dose of *L. rhamnosus* GG in our patients to 3×10^9 CFU.

Antimicrobial susceptibility of the infecting *L. rhamnosus* GG strains has been reported in only some of the *L. rhamnosus* case reports reviewed in this article.[5,7,8] These data showed some variability, but these discrepancies could also be attributed to differences among susceptibility testing techniques and interpretative criteria adopted by different laboratories. However, a consistent finding among all the reports was the resistance of *L. rhamnosus* strains to vancomycin and their susceptibility to ampicillin.[5,7,8]

In summary, we report two cases of sepsis in neonates caused by *L. rhamnosus* GG during enteral supplementation in addition to the six cases previously reported.[5–8] Probiotic supplementation most likely caused the sepsis in these patients, although all of them had further documented risk factors for sepsis. In these few cases, the dose and duration of probiotic supplementation do not seem to be positively related to the risk of developing sepsis and the antibiotic susceptibility of isolated strains varied between patients. We conclude that, although none of the thousands of patients enrolled in previous studies[4] developed systemic infections due to administered probiotics, neonatologists must be aware that supplementation with *L. rhamnosus* GG can cause sepsis in high-risk patients on rare occasions. Further studies evaluating the most effective and safe dose and duration of each probiotic supplementation should be performed.

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