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Supplement: Guidance for Assessing Probiotics Beneficial Effects—How to Fill the GAP

Guidance for Substantiating the Evidence for Beneficial Effects of Probiotics: Prevention and Management of Infections by Probiotics[1–3]

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Abstract

The rationale for the use of probiotics in the management of infectious diseases is supported by their potential to influence and stabilize the composition of gut microbiota, enhance colonization resistance, and modulate immune function parameters. A literature review was conducted to determine the efficacy of using probiotics in selected infections: *1*) infectious diarrhea in infants and children, *2*) traveler's diarrhea, *3*) necrotizing enterocolitis in infants, *4*) *Helicobacter pylori* infection, *5*) respiratory tract infections in adults and children, *6*) ear, nose, and throat infections, and *7*) infectious complications in surgical and critically ill patients. The different types of infections that have been subject to clinical studies with different probiotics obviously prevent any generic conclusions. Furthermore, the lack of consistency among studies focusing on 1 specific infection, in study design, applied probiotic strains, outcome parameters, and study population, along with the still limited number of studies, preclude clear and definite conclusions on the efficacy of probiotics and illustrate the need for better-aligned study designs and methodology. Exceptions were the management of infectious diarrhea in infants and traveler's diarrhea, antibiotic-associated diarrhea, and necrotizing enterocolitis. Sufficient consistent data exist for these applications to conclude that certain probiotics, under certain conditions, and in certain target populations, are beneficial in reducing the risk of infection. In addition, some evidence exists, although conclusions are premature, for the management of *Helicobacter pylori* infection and possible reduction of treatment side effects. Certain probiotics may also reduce the risk of various symptoms of respiratory tract infections in adults and children, including ear, nose, and throat infections, although data are currently far too limited to distill any clinical recommendations in this area. Positive but also negative results have been obtained in prevention of infectious complications in surgical and critically ill patients. For future studies it is recommended that researchers provide adequate power, identify pathogens, and report both clinical outcomes and immune biomarkers relating to putative underlying mechanisms. J. Nutr. 140: 698S–712S, 2010.

Risk reduction and management of infections by probiotics

In 1916, Nissle demonstrated that transferring members of the human gut microbiota to healthy typhoid carriers resulted in *Salmonella* being cleansed from their intestines (1). This finding pointed for the first time to one of the most important

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physiological functions of the gut microbiota, i.e., to defend the gut against the colonization by exogenous microorganisms, now termed colonization resistance. Since that time, it has been demonstrated that different aerobic as well as anaerobic species of the gut microbiota are involved in this function (2).

Our understanding of the role of the microbiota in the resistance against infections has further evolved, and the importance of the interaction of the microbiota with the immune system and intestinal epithelial cells providing additional barriers to infectious agents is increasingly being recognized (3).

The potential impact that probiotics may have at the level of gut microbiota, on gut epithelium and its associated mucosal immune system, as well as systemically (4) provides a rationale of why probiotics are promising food components for the reduction of risk or management of infectious diseases. Most notably these relate to infections of the gastrointestinal tract, but infections at other sites have also been targeted recently.

For our review of the existing literature we defined a number of categories of infections, based on the localization of the infection (respiratory tract, gastrointestinal, systemic) and target populations (children, adults, elderly; at-risk groups or patients). Studies dealing with the effect of probiotics on urinary tract, vaginal infections, and skin infections were not analyzed. Furthermore, although there are many indications that various immune function parameters may be affected by probiotics, we chose to focus on studies investigating a clinical outcome and did not incorporate studies that solely describe effects of probiotics on biomarkers of the immune system.

Based on the available information, the subcategories are the following:

1. Infectious diarrhea in infants and children including acute infectious diarrhea and antibiotic-associated diarrhea (AAD).[10]
2. Traveler's diarrhea (TD).
3. Necrotizing enterocolitis (NEC) in infants.
4. *Helicobacter pylori* infection.
5. Respiratory tract infections in adults and children.
6. Ear, nose, and throat (ENT) infections.
7. Infectious complications in surgical and critically ill patients.

Infectious diarrhea

Acute infectious diarrhea. Oral rehydration is the mainstay of therapy for acute gastroenteritis and should continue to be fostered, encouraged, and supported. However, despite its proven efficacy, oral rehydration therapy remains underused (5). The main reasons for this are that an oral rehydration solution does not reduce the frequency of bowel movements and fluid loss, nor does it shorten the duration of the illness, which reasons both decrease acceptance. As a result, there is an interest on the part of patients, caregivers, and practitioners in simple, safe, and effective measures that will visibly reduce the rate of stool loss and/or the duration of diarrhea.

The evidence from several meta-analyses of randomized controlled trials (RCT) (6–9) [**Table 1** (6–74)] consistently shows significant effects and moderate clinical benefits of some probiotic strains in the management of acute watery diarrhea, often rotaviral, primarily in infants and young children. So far, the beneficial effects of probiotics in acute infectious diarrhea appear to be moderate (~1-d reduction in the duration of diarrhea), strain-dependent (75), dose-dependent [greater for doses $>10^{10}$–10^{11} colony-forming units (CFU)/d], significant in watery diarrhea and viral gastroenteritis but absent in invasive, bacterial diarrhea, more evident when treatment with probiotics is initiated early in the course of disease, and more evident in developed countries.

The use of probiotics for acute infectious diarrhea in children is an accepted therapy in Europe. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition and European Society of Paediatric Infectious Diseases Expert Working Group have stated that selected probiotics with proven clinical efficacy [e.g., *Lactobacillus rhamnosus* GG (76), *Saccharomyces boulardii* (77)] and in appropriate dosage, according to the strain and the population (Table 1) may be used as an adjunct for the management of children with acute gastroenteritis being given rehydration therapy (78). Other probiotic strains may also be used provided their efficacy is documented in high-quality RCTs (or in meta-analyses).

AAD and Clostridium difficile diarrhea

A common side effect of antibiotic treatment is AAD, defined as otherwise unexplained diarrhea occurring in association with antibiotics administration (79). In the pediatric population, AAD occurs in ~11–40% of children between the initiation of therapy and up to 2 mo after cessation of treatment (80,81).

Several systematic reviews with or without meta-analysis documented that most of the tested probiotics have been shown to be effective in reducing the risk of AAD in the general (mainly adult) population (28–31). Also, a few of the more recent trials in adults, not included yet in the meta-analyses, showed encouraging results in prevention of AAD (34,35). Evidence from 3 recent systematic reviews of RCTs also suggests that probiotics reduce the risk of AAD in children (25–27) (Table 1).

In contrast, there is only weak or inconclusive evidence from 2 systematic reviews for the effectiveness of probiotics for the prevention and treatment of *C. difficile*-associated diarrhea (Table 1). The authors of the first review concluded that available evidence (all in adults) does not support the administration of probiotics with antibiotics to prevent or treat *C. difficile* diarrhea (38). The conclusions from the second systematic review (with meta-analysis) do support probiotic use (82). However, the latter meta-analysis has been criticized for combining the results from 1 study on prevention of *C. difficile* diarrhea with results from 5 studies on treatment of *C. difficile* diarrhea and pooling data on different probiotics, different conditions, and different patient characteristics as well as some methodological issues calling for caution in interpreting the conclusions (82,83). In children, despite some anecdotal evidence of the efficacy of probiotics (84,85), no RCT investigating such a possibility has been conducted.

The current available evidence suggests a moderate beneficial effect of selected probiotics, such as *Saccharomyces boulardii* or *Lactobacillus rhamnosus* GG, in the prevention of AAD in children, or *Lactobacillus casei* DN 114 001 in the elderly. Because these probiotics have been shown to be capable of providing reasonable protection against the development of AAD, their use is probably warranted whenever preventing this usually self-limited complication is important. The available data provide evidence that selected probiotics significantly reduce the risk of diarrhea in patients treated with antibiotics in general. However, not all antibiotics are likely to be equal in causing AAD. Currently, conclusions about the efficacy of probiotics in preventing diarrhea attributable to any single antibiotic class cannot be made. Why some probiotics are

[10] Abbreviations used: AAD, antibiotic-associated diarrhea; AOM, acute otitis media; AP, acute pancreatitis; CFU, colony-forming units; ENT, ear, nose, and throat; NEC, necrotizing enterocolitis; RCT, randomized controlled trial; TD, traveler's diarrhea.

TABLE 1 Probiotics in the prevention and treatment of infections[1]

Disease/marker	Reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
1. Respiratory tract						
Common cold	De Vrese et al. 2005 (10)	479 adults	*L. gasseri*, *B. longum*, *B. bifidum* mixture	3–5.5 mo	R, DB, PC	Reduction duration (1.9 d) No effect on incidence Reduction days with fever (0.7 d) Reduction symptoms
Common cold	Winkler et al. 2005 (11)	477 adults	*L. gasseri*, *B. longum*, *B. bifidum* mixture	3–5.5 mo	R, DB, PC	Reduction symptoms Reduction days with fever (54%) No effect on duration Near sign reduction in incidence (14%)
RT and GI symptoms	Tubelius et al. 2005 (12)	262 adults	*L. reuteri*	80 d	R, DB, PC	Reduced incidence sickness (>50%)
RT, ENT, and GI symptoms	Turchet et al. 2003 (13)	360 elderly	*L. casei*	3 wk	R, C	Reduced duration (1.7 d) No effect on incidence
RT and GI symptoms	Kekkonen et al. 2007 (14)	119 trained adults	*L. rhamnosus* GG	3 mo	R, DB, PC	No effect on incidence during training (intervention) Reduced duration (1.3 d) of GI symptoms during recovery (wash-out)
RT symptoms	Tiollier et al. 2007 (15)	47 trained adults	*L. casei*	1 mo	R, DB, PC	No effect on incidence, duration, and symptoms
RT symptoms	Cox et al. 2008 (16)	20 trained adults	*L. fermentum*	30 d	PC, DB, cross over	Reduction in number of days (>50%) Near sign reduction in symptoms
RT and GI symptoms	Hatakka et al. 2001 (17)	571 children	*L. rhamnosus* GG	7 mo (5 d/wk)	R, DB, PC	Reduced absence from daycare (0.9 d) Reduced incidence of complications (8.6%) Size of effects diminished after age adjustment
Respiratory illness, diarrhea, fever	Weizman et al. 2005 (18)	201 infants	*B. animalis* or *L. reuteri*	12 wk	R, DB, PC	Reduced incidence fever Reduced incidence and duration diarrhea No effect on RT
RT symptoms	Marseglia et al. 2007 (19)	80 children	*B. clausii*	90 d	R, SB, C	Reduced duration during treatment (2.7 d) and follow up (4.3 d)
Otitis media	Hatakka et al. 2007 (20)	309 otitis-prone children and toddlers	Mixture of *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* 99, *P. freudenreichii* ssp. *shermanii* JS	6 mo	R, DB, PC	No effect on incidence and duration Increased presence *M. Catarrhalis*
Otitis media	Roos et al. 2001 (21)	108 otitis-prone children and toddlers	*Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus oralis* mixture (nasal spray)	10 d	R, DB, PC	Reduced incidence of recurrence (22% vs. 42%)
Tonsillitis	Roos et al. 1993 (22)	36 adults with recurrent tonsillitis	*S. sanguis* and *S. mitis* mixture (oral spray)	10 d	R, DB, PC	Reduced incidence of recurrence (0 vs. 7)
Tonsillitis	Roos et al. 1996 (23)	112 adults with recurrent tonsillitis	*S. sanguis* and *S. mitis* mixture (oral spray)	10 d	R, DB, PC	Reduced incidence of recurrence (2% vs. 23%)
Tonsillitis	Falck et al. 1999 (24)	342 adults and children with recurrent tonsillitis	*S. sanguis* and *S. mitis* mixture (oral spray)	10 d	R, DB, PC	No effect on early recurrence Reduction of late recurrence
2. Probiotics in treatment of acute infectious diarrhea						
Diarrhea lasting 3 d	Szajewska et al. 2001 (6)	731 children	*L. rhamnosus* GG; *L. reuteri; L. acidophilus* LB; *Streptococcus thermophilus lactis; L. acidophilus* and *L. bulgaricus; S. boulardii.*	NA	R, PC	Relative risk (95% CI): 0.4 (0.3 to 0.5) Number needed to treat (95% CI): 4 (3 to 9)

(*Continued*)

TABLE 1 Continued

Disease/marker	Reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Diarrhea lasting 3 d	Allen et al. 2004 (9)	1341 children and adults	*L. rhamnosus* GG, *L. reuteri, L. acidophilus LB, Strep. thermophilus lactis, L. acidophilus,* and *L. bulgaricus, Enterococcus* SF68, *L. acidophilus*, and *L. bifidus, L. casei, L. rhamnosus,* and *L. reuteri, S. boulardii.*	NA	R, PC	Relative risk (95% CI): 0.7 (0.6 to 0.8) Number needed to treat (95% CI): 5 (4 to 7)
Duration of diarrhea	Szajewska et al. 2001 (6)	731 children	*L. rhamnosus* GG, *L. reuteri, L. acidophilus* LB, *Strep. thermophilus lactis, L. acidophilus* and *L. bulgaricus, S. boulardii.*	NA	R, PC	WMD (95% CI): −18 h (−27 to −10)
Duration of diarrhea	Van Niel et al. 2002 (7)	*675 children*	*L. rhamnosus* GG, *L. reuteri, L. acidophilus,* and *L. bulgaricus.*	NA	R, PC	WMD (95% CI): −17 h (−29 to −7)
Duration of diarrhea	Huang et al. 2002 (8)	1917 children	*L. rhamnosus* GG, *L. acidophilus, L. bulgaricus, S. thermophilus, L. rhamnosus, Yalacta (L. rhamnosus, L. delbruckii, L. bulgaricus), L. reuteri, Enterococcus* SF68, *S. boulardii, S. subtilis, B. bifidum* and *B. infantis.*	NA	R, DB, PC, open label	WMD (95% CI): −19 h (−26 to −14)
Duration of diarrhea	Allen et al. 2004 (9)	970 children and adults	*L. rhamnosus* GG, *L. reuteri, L. acidophilus LB, Streptococcus thermophilus lactis, L. acidophilus and L. bulgaricus, Enterococcus* SF68, *L. acidophilus and L. bifidus, L. casei, L. rhamnosus, andL. reuteri, S. boulardii.*	NA	R, PC	WMD (95% CI): −30 h (−42 to −19)
3. Probiotics in prevention of AAD						
Prevention of AAD	Szajewska et al. 2006 (25)	766 children	*L. acidophilus and L. bulgaricus* (1 RCT); *L. acidophilus and B. infantis* (1 RCT); *B. lactis* Bb12 and *Str. thermophilus* (1 RCT); *L. rhamnosus* GG (2 RCTs); *S. boulardii* (1 RCT).	NA	R, PC	Relative risk (95% CI): 0.44 (0.25 to 0.77)
Prevention of AAD	Johnston et al. 2006 (26)	707 children	*L. acidophilus and L. bulgaricus* (1 RCT); *L. acidophilus and B. infantis* (1 RCT); *L. rhamnosus* GG (2 RCTs); *S. boulardii* (1 RCT); *L. sporogens* and fructooligosaccharides (1 RCT).	NA	R, PC	Relative risk (95% CI): 0.43 (0.25 to 0.75) Relative risk (95% CI): 1.01 (0.64 to 1.61)
Prevention of AAD	Johnston et al. 2007 (27)	1946 children	*L. rhamnosus* GG (2 RCTs); *S. boulardii* (3 RCTs); *B. lactis and Str. thermophilus* (1 RCT); *L. acidophilus and B. infantis* (1 RCT); *L. sporogenes* and fructooligosaccharides (1 RCT); *L. acidophilus and L. bulgaricus* (1 RCT).	NA	R, PC	Relative risk (95% CI): 0.49 (0.32 to 0.74)

(*Continued*)

TABLE 1 Continued

Disease/marker	Reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Prevention of AAD	D'Souza et al. 2002 (28)	830 children and adults	*S. boulardii*	NA	R, PC	OR (95% CI): 0.39 (0.25 to 0.62) Number needed to treat (95% CI): 11 (8 to 20)
		384 children and adults	*L. acidophilus and L. bulgaricus* (2 RCTs); *L. acidophilus and B. longum* (1 RCT); *L. rhamnosus* GG (1 RCT); *E. faecium* SF68 (1 RCT).			OR (95% CI): 0.34 (0.19 to 0.61) Number needed to treat (95% CI): 11 (8 to 18)
		Total: 1214	Total			OR (95% CI): 0.37 (0.26 to 0.53)
Prevention of AAD	Cremonini et al. 2002 (29)	446 children and adults	*L. rhamnosus* GG (3 RCTs), *Lactobacillus* spp. (1 RCT)	NA	R, PC	Relative risk (95% CI): 0.5 (0.4 to 0.7) Number needed to treat (95% CI): 9 (7 to 17)
		435 children and adults	*S. boulardii*			Relative risk (95% CI): 0.6 (0.4 to 0.9) Number needed to treat (95% CI): 14 (8 to 18)
		Total: 881				Relative risk (95% CI): 0.4 (0.3 to 0.6) Number needed to treat (95% CI): 9 (7 to 14)
Prevention of AAD	Szajewska et al. 2005 (30)	1076 children and adults	*S.boulardii*	NA	R, PC	Relative risk (95% CI): 0.4 (0.2 to 0.8) Number needed to treat (95% CI): 10 (7 to 16)
Prevention of AAD	Hawrelak et al. 2005 (31)	692 children and adults	*L. rhamnosus* GG	NA	R, PC	No statistical pooling
Prevention of AAD	Sazawal et al. 2006 (32)	No data	Various	NA	R, PC	Relative risk (95% CI): 0.52 (0.35 to 0.65)
Prevention of AAD RCT	McFarland et al. 2006 (33)	2810 N (Exp/Cont)	Various	NA	R, PC	Relative risk (95% CI): 0.43 (0.31 to 0.58)
Prevention of AAD	Wenus et al. 2008 (34)	87 children and adults (46/41) FU 63/87 (72%)	*L. rhamnosus* GG, La-5, Bb-12	14 d	R, PC	Relative risk (95% CI): 0.21 (0.05 to 0.93)
Prevention of AAD	Hickson et al. 2007 (35)	113 children and adults (57/56) FU 113/135 (83%)	*L. casei* DN-114001, *S. thermophilus, L. bulgaricus*	Ab + 7 d	R, PC	Relative risk (95% CI): 0.36 (0.2 to 0.76) Number needed to treat (95% CI): 5 (3 to 16)
Prevention of AAD	Ruszczynski et al. 2008 (36)	240 children and adults (120/120)	*L. rhamnosus* (strains E/N, Oxy and Pen)	Ab	R, PC	Relative risk (95% CI): 0.45 (0.2 to 0.95) Number needed to treat (95% CI): 11 (6 to 106)
Prevention of AAD	Szymánski et al. 2008 (37)	78 children and adults (40/30)	*B. longum* PL03, *L. rhamnosus* KL53A, *L. plantarum* PL02	Ab	R, PC	Relative risk (95% CI): 0.47 (0.04 to 5) Number needed to treat (95% CI): Not significant
4. Probiotics in prevention and management of *C. difficile* diarrhea						
Treatment of *C. difficile* diarrhea	Dendukuri et al. 2005 (38)	No data	*S. boulardii* (2 RCTs), *L. plantarum* 299v (1 RCT)	NA	R, PC	No statistical pooling
Treatment of *C. difficile* diarrhea	McFarland et al. 2006 (33)	354 adults	*S. boulardii* (2 RCTs), *Lactobacillus* GG (2 RCTs), *L. plantarum* 299v (1 RCT), *L. acidophilus and B. bifidum (1 RCT).*	NA	R, PC	Relative risk (95% CI): 0.59 (0.4 to 0.85) Number needed to treat (95% CI): 8 (6 to 22)
Prevention of *C. difficile* diarrhea	Dendukuri et al. 2005 (38)	No data	*S. boulardii* (3 RCT), *Lactobacillus* GG (1 RCT), *L. acidophilus and B. bifidum (1 RCT).*	NA	R, PC	No statistical pooling
Prevention of *C. difficile* diarrhea	McFarland et al. 2006 (33)	No data	*L. acidophilus* and *B. bifidum*	NA	R, PC	Relative risk (95% CI): 0.33 (0.07 to 1.59) Number needed to treat (95% CI): Not significant
5. Probiotics for prevention of NEC						
Prevention of NEC	Deshpande et al. 2007 (39)	1393 neonates	*B. breve; L. rhamnosus* GG*; S. boulardii; B. infantis and S. themophilus and B. bifidum; L. acidophilus and B. infantis; Lactobacillus casei; B. lactis.*	3 - > 6 wk	R, PC	Outcome measure: NEC stage 2 Relative risk (95% CI): 0.36 (0.2 to 0.65) Number needed to treat (95% CI): 30 (19 to 71)

(*Continued*)

TABLE 1 Continued

Disease/marker	Reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Prevention of NEC	AlFaleh et al. 2008 (40)	1264 neonates	*B. infantis and S. themophilus and B. bifidum; S. boulardii; L. rhamnosus* GG*; L. acidophilus and B. infantis; L. casei.*	14 d- discharge	R, PC	Outcome measure: NEC stage 2 Relative risk (95% CI): 0.32 (0.17 to 0.60) Number needed to treat (95% CI): 26 (17 to 57)
Prevention of necrotosing enterocolitis	Samanta et al., 2009 (41)	91+95 neonates	*B. infantis and B. bifidum and B. longum and L. acidophilus.*	Until discharge	R, PC	Outcome measure: NEC stage 2 Relative risk (95% CI): 0.48 (0.22 to 1.04) Number needed to treat (95% CI): 10 (5 to 63)
Prevention of NEC	Lin et al. 2008 (42)	217+217 neonates	*B. bifidum and L. acidophilus*	14 d- discharge	R, PC	Outcome measure: NEC stage 2 Relative risk (95% CI): 0.43 (0.19 to 1.00) Number needed to treat (95% CI): 22 (12 to 113)
6. Probiotics and TD						
TD	Hilton et al. 1997 (43)	245 adult travelers	*L. rhamnosus* GG	1–3 wk	R, DB, PC	Reduced incidence (3.9% vs. 7.4%)
TD	Oksanen et al. 1990 (44)	820 adult travelers	*L. rhamnosus* GG	1–2 wk	R, DB, PC	Nonsign reduction in incidence
TD	Pozo-Olano et al. 1978 (45)	50 adult travelers	*L. acidophilus* and *L. bulgaricus*	8 d	R, DB, PC	No effect on incidence
TD	Black et al. 1989 (46)	92 adult travelers	*L.acidophilus, L. bulgaricus, B. bifidum, S. thermophilus* mixture	2 wk		Reduced incidence (43% vs. 71%)
TD	Katelaris et al. 1995 (47)	282 adult travelers	*L. acidophilus* or *L. fermentum*	3 wk	R, DB, PC	No effect on incidence
TD	Kollaritsch et al. 1989 (48)	1231 adult travelers 319 adult travelers	*S. boulardii L.acidophilus*	3 wk	R, DB, PC	Reduced incidence (34% vs. 42%) No effect on incidence
Infectious diarrhea	Pereg et al. 2005 (49)	541 adults (military recruits)	L. casei	8 wk (6 d/wk)	R, SB, PC	No effect on incidence and duration
Repeated infections	Lodinova-Zadnikova 2003 (50)	1714 infants	E. coli	colonization at birth	R, C	Reduced incidence after 10 y
7. Probiotics and *H. pylori* infection						
H. pylori eradication treatment	Canducci et al. 2000 (51)	120 adults (dyspeptic)	*L. acidophilus* LB, dose: 1.5×10^{10}	10 d	O, R	Eradication rate ↑
H. pylori eradication treatment	Armuzzi et al. 2001 (52)	60 adults (asymptomatic)	*L. rhamnosus* GG, dose: 1.2×10^{10}	14 d	O, R	Adverse effects ↓
H. pylori eradication treatment	Armuzzi et al. 2001 (53)	120 adults (asymptomatic)	*L. rhamnosus* GG, dose: 1.2×10^{10}	14 d	DB, PC	Adverse effects ↓
H. pylori eradication treatment	Sheu et al. 2002 (54)	160 adults (dyspeptic)	*L. acidophilus* La5 and *B. lactis* Bb12, dose: 1×10^{10}	4 wk	O, R	Eradication rate ↑ Adverse effects ↓
H. pylori eradication treatment	Cremonini et al. 2002 (55)	85 adults (asymptomatic)	1. *L. rhamnosus* GG, 2. *Saccharomyces boulardii*, 3. *L. acidophilus* La5 and *B. lactis* Bb12, dose: 1–1.5×10^{10}	2 wk	DBPC	Adverse effects ↓ by all probiotic groups
H. pylori eradication treatment	Tursi et al. 2004 (98)	70 adults (dyspeptic with resistant *H. pylori)*	*L. casei* ssp. *casei* DG, dose: 1.6×10^{10}	10 d	O, R	Adverse effects ↓
H. pylori eradication treatment	Sykora et al. 2005 (57)	86 children (dyspeptic)	*L. casei* DN-114 001, dose: 1×10^{10}	2 wk	DB, PC	Eradication rate ↑
H. pylori eradication treatment	Myllyluoma et al. 2005 (58)	46 adults (asymptomatic)	Mixture of *L. rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freidenreichii* spp. *shermanii* JS and *B. breve* 99), dose 1.3×10^{11} first week then, dose: 6.5×10^{10}	4 wk	DB, PC	Adverse effects ↓

(*Continued*)

TABLE 1 Continued

Disease/marker	Reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
H. pylori eradication treatment	Lionetti et al. 2006 (59)	40 children (dyspeptic)	*L. reuteri* ATCC 55730, dose: 1 3 10^8	20 d	DB, PC	Adverse effects ↓
H. pylori eradication treatment	Sheu et al. 2006 (60)	138 adults (asymptomatic with resistant *H. pylori*)	*Lactobacillus* La5 and *B. lactis* Bb12, dose: 4 3 10^{10}	4 wk before treatment	O, R	Urease activity ↓ at pretreatment, Eradication rate ↑ Adverse effects ↓
H. pylori eradication treatment	Cindoruk et al. 2007 (61)	124 adults (dyspeptic)	*S. boulardii*	2 wk	DB, PC	Adverse effects ↓
H. pylori eradication treatment	de Bortoli et al. 2007 (62)	206 adults (asymptomatic)	*Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus thermophilus* with lactoferrin	7 d	DB, PC	Eradication rate ↑ Adverse effects ↓
Probiotic effects on *H.pylori* infection	Michetti et al. 1999 (63)	20 adults (asymptomatic)	*L. acidophilus (jonhsonii)* La1	14 d	DB, PC	Urease activity ↓
Probiotic effects on *H.pylori* infection	Felley et al. 2001 (64)	52 adults (asymptomatic)	*L. acidophilus (jonhsonii)* La1	3 wk	DB, PC	Urease activity ↓, *H. pylori* colonization ↓, Inflammation and gastritis ↓
Probiotic effects on *H.pylori* infection	Sakamoto et al. 2001 (65)	31 adults (asymptomatic)	*L. gasseri* OLL2716, dose: 1.8–2.5 3 10^9	8 wk	PC	Serum pepsinogen I / II ratio ↑, Serum pepsinogen ↓, Urease activity ↓
Probiotic effects on *H.pylori* infection	Wendakoon et al. 2002 (66)	27 adults (asymptomatic)	*L. casei* 03, *L. acidophilus* 2412 and *L. acidophilus* ACD1, dose: 2.8 3 10^{11}	30 d	O	Ineffective
Probiotic effects on *H.pylori* infection	Cruchet et al. 2003 (67)	236 children (asymptomatic)	Both living and heat-killed *L. acidophilus* La1 or *L. paracasei* ST1, dose: 1 10^{10}	4 wk	DB, PC	Urease activity ↓ by live La1
Probiotic effects on *H.pylori* infection	Pantoflickova et al. 2003 (68)	50 adults (asymptomatic)	*L. acidophilus (johnsonii)* La1, dose: 1.25 3 $10^{9–10}$	16 wk	DB, PC	*H. pylori* colonization ↓, Inflammation ↓
Probiotic effects on *H.pylori* infection	Cats et al. 2003 (69)	20 adults (asymptomatic), 6 adults in control group	*L. casei Shirota*, dose: 1.95 3 10^{10}	3 wk	O, C	Urease activity tended to ↓
Probiotic effects on *H.pylori* infection	Wang et al. 2004 (70)	70 dyspeptic adults, endoscopy for 14 participants	*L. acidophilus* La5 and *B. lactis* Bb12, dose: 1 3 10^{10}	4 wk	O, C	Urease activity ↓, Gastritis and *H. pylori* colonization ↓
Probiotic effects on *H.pylori* infection	Gotteland et al. 2005 (71)	254 children (asymptomatic)	*L.acidophilus* LB or *Saccharomyces boulardii (Sb)* with inulin, dose: LB 1 310^{10} *Sb* 500 mg + 10 g inulin	8 wk	O, R	Eradication ↑ *S. boulardii* with inulin more effective than *L. acidophilus* LB
Probiotic effects on *H.pylori* infection	Myllyluoma et al. 2007 (101)	16 adults referred for endoscopy	Mixture of *L. rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freidenreichii* spp. *shermanii* JS and *B. lactis* BB12), dose: 2 3 10^{10}	8 wk	O, C	Serum gastrin-17↓ Urease activity ↓
Probiotic effects on *H.pylori* infection	Gotteland et al. 2008 (73)	295 children (asymptomatic)	Both living and heat killed *L. acidophilus* La1 with and without cranberry juice, dose: 8 3 10^8	3 wk	DB, PC	Eradication ↑ with both La1 and granberry juice
Probiotic effects on *H.pylori* infection	Francavilla et al. 2008 (74)	40 adults (dyspeptic)	*L. reuteri* ATCC 55730	4 wk	DB, PC	Urease activity ↓ Feacal *H. pylori* antigen ↓ GSRS improved

[1] Ab, during antibiotic treatment; CI, confidence interval; CONT, control group; DB, double-blind; EXP, experimental group; NA, not available; O, open; PC, placebo-controlled; R, randomized; RCT, randomized control trial; WMD, weighted mean difference (negative values indicate that duration of diarrhea was shorter in the probiotic than control group); ↑, increase; ↓, decrease.

efficient in the reduction of risk and treatment of diarrhea associated with *C. difficile* infection remains unclear, particularly in the pediatric population.

Additional well-conducted clinical studies using validated clinical outcomes are recommended to further identify populations at high risk of AAD that would benefit most from probiotic intake and to select relevant probiotic candidates. Such validated outcomes should include a conservative definition of diarrhea to differentiate between clinically important cases and negligible changes in stool frequency and/or consistency. In addition, trials should evaluate potential important consequences of AAD such as the need for discontinuation of antibiotic treatment, hospitalization, or intravenous rehydration.

Traveler's diarrhea. TD is a common illness among travelers to developing countries and affects >50% of travelers. Major symptoms involve diarrhea, abdominal cramps, and nausea, and in most cases, bacterial pathogens are the causative agents. Six articles describe the efficacy of various probiotics (*Saccharomyces boulardii*, various lactobacilli, and 1 mix) in populations at high risk for TD.

Two double-blind placebo-controlled RCTs conclude that capsules or sachets containing *L. rhamnosus* GG (2×10^9 CFU/d for 1–2 wk) might prevent TD. Oksanen et al. (44) showed a near-significant reduction of 11.8% in the incidence of diarrhea in healthy Finnish adult travelers to Turkey compared with placebo, with a differential effect dependent on travel location. Hilton et al. (43) found that the incidence of diarrhea was significantly reduced in healthy adults traveling to developing countries when they were taking *L. rhamnosus* GG compared with a placebo capsule. Other studies applying various lactobacilli (Table 1) in doses ranging from 2×10^9 to 2×10^{11} did not show an effect on TD (47,48,86). Two studies applied *Saccharomyces boulardii* at various dose levels (5×10^9 CFU/d, 1×10^{10} CFU/d, 2×10^{10} CFU/d) and found a significant reduction in the incidence of TD among travelers to hot climates in a dose-dependent manner (48,87). Finally, a mixture of probiotic strains was successfully applied to tourists traveling to Egypt, significantly reducing incidence of TD from 71% in the placebo group to 43% in the treatment group (46).

In a recent meta-analysis, the pooled relative risk indicated that some probiotics can significantly prevent TD (88). However, the heterogeneity of the included studies with respect to probiotic strain, dose and duration of treatment, and travel destination and the unidentified causal agents of TD still hamper a more specific recommendation on the use of probiotics. In addition, very few studies report on the stability of the probiotic products, compliance to treatment, and/or recovery of the strain from fecal samples, and such factors may be ultimately important, especially during travel.

NEC in infants

The rationale for probiotic supplementation of preterms is based on data demonstrating differences in the establishment of the intestinal microbiota in preterm infants. The key features of intestinal microbiota in preterm infants, compared with healthy, full-term infants, are *1*) restricted number of species, with typically only 3 bacterial species found at 10 d of age; *2*) 3 groups including enterobacteria such as *E. coli* and *Klebsiella* spp., enterococci such as *E. faecalis*, and staphylococci such as *S. epidermidis*, *S. aureus*, and *S. haemolyticus* are the most frequently retrieved; *3*) all these facultative anaerobes persist at high levels in the fecal flora of preterm infants; and *4*) significantly delayed colonization with anaerobes, especially bifidobacteria (89–91). Additionally, preterm infants are often cared for at intensive care units and receive a broad-spectrum antibiotic, which further contributes to differences in colonization patterns. The direct consequences of disturbed gut microbiota for overall health are not known, but it has been speculated that abnormal pattern of colonization in preterm infants may contribute to the pathogenesis of NEC and to the increased susceptibility to infections. It has also been suggested that enteral administration of probiotics to preterm newborns could prevent infections and NEC and reduce the use of antibiotics (92).

Two systematic studies aimed at determining the effect of probiotics on the prevention of NEC in preterm infants were performed (Table 1). The first one, reported in 2007, identified 7 RCTs (39). This systematic review found that most of the investigated probiotics might reduce the risk of NEC in preterm neonates with <33 wk gestation. Risk of sepsis did not differ significantly among groups. Similarly, the Cochrane Review (40), published in 2008, found that enteral supplementation of certain probiotics reduced the risk of severe NEC and mortality in preterm infants born <1500 g. There was no evidence of significant reduction of nosocomial sepsis. However, it has been shown in both meta-analyses that not all probiotics tested were equally effective. Most effective were combinations used in the studies by Bin-Nun et al. (93) *(Bifidobacterium infantis* plus *Streptococcus thermophilus* plus *B. bifidus)* and by Lin et al. (94) *(Lactobacillus acidophilus* plus *B. infantis)*. One additional RCT documented the efficacy of *L. acidophilus* and *B. bifidum* (42).

The use of probiotics for the prevention of NEC in preterm infants is not a routine practice. Therefore, large, well-designed trials are needed to confirm the results. The efficacy and safety of probiotic supplementation in premature infants, <1000 g, need to be defined.

Helicobacter pylori infection

It has been established that *H. pylori* infection is a major cause of chronic gastritis and peptic ulcer disease and a first-class definite carcinogen for stomach cancer (95). Accordingly, there are still many issues to be solved for reducing *H. pylori* infection and improving outcomes of *H. pylori* infection. Those include improvement of *H. pylori* eradication treatment for prevention of gastritis progression and subsequent gastric cancer development. In the future, the treatment of infection will be made difficult by the rapid rate with which the bacteria acquire resistance to the drugs and also long-term changes in microbiota and undesirable side effects induced by the treatment (96,97). These facts provide the rationale for the use of probiotics in the management of *H. pylori* infection.

As a complement to antibiotics, some probiotics have the potential to improve the eradication rate and the overall tolerance of the treatment (Table 1). The early clinical study by Canducci and coworkers (51) provided evidence that *L. acidophilus* LB improved the eradication rate of *H. pylori* significantly. Since then, numerous studies have shown the ability of various probiotic formulations to decrease side effects of anti-*Helicobacter* treatments such as AAD, epigastric pain, discomfort, and flatulence. *L. rhamnosus* GG was able to reduce the occurrence of eradication treatment side effects in 2 separate studies (52,53). The results for *L. rhamnosus* GG were confirmed later in a study comparing *L. rhamnosus* GG, *Saccharomyces boulardii,* and a combination of *L. acidophilus* and *B. lactis* (55). All groups had beneficial effects during and following an anti-*Helicobacter* treatment. There is also a very recent study confirming the effect for *S. boulardii* (61). Furthermore, the probiotic multispecies combination consisting of *L.*

rhamnosus GG, *L. rhamnosus* LC705, *Propionibacterium freidenreichii* subspecies *shermanii* JS and *B. breve* 99 has also improved tolerance to the standard triple therapy and nonsignificantly increased the eradication rate (58). Also, the combination of *B. animalis* Bb12 and *L. acidophilus* LA5 has been beneficial in 2 different trials (54,60). Furthermore, both *L. casei* ssp. *casei* DG (98) and *L. casei* DN-114 001 (57) supplementation significantly increased the eradication rate, but only the *L. casei* DG was able to alleviate side effects.

There are limited numbers of studies on the ability of probiotics to attenuate changes in gastrointestinal microbiota followed by anti-*Helicobacter* triple treatment. In a pilot study a probiotic combination including 2 strains of *L. acidophilus* (CLT60 and CUL21) and 2 strains of *B. bifidum* (CUL17 and Rhodia) suppressed the rise in the numbers of facultative anaerobes seen in the placebo group (99). Later, the same probiotic product was shown to suppress an increase in antibiotic resistance among enterococci (100). Also, a multispecies probiotic combination (*L. rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freidenreichii* spp. *shermanii* JS, and *B. breve* 99) stabilized microbiota during and following the triple anti-*H. pylori* treatment (72). However, despite the probiotic supplementation, microbiota in all studies were susceptible to the effects of the antibiotics administered to eradicate *H. pylori*.

Probiotics as an alternative to antibiotics have also been the focus of several trials (Table 1). Administration of *L. acidophilus* La1 decreased *H. pylori* density in several different trials (63,64,67,68,73). Also, a decrease in gastric inflammation associated with *H. pylori* infection was evident in 2 of these studies (68,73). However, the regular intake of La1 did not permanently eradicate *H. pylori* in any of the studies. Also, *L. gasseri* OLL2716, *L. casei,* and *L. reuteri* ATCC 55730 were found to be effective in suppression of *H. pylori* and reduction in gastric mucosal inflammation (65,69,74). Similar effects of yogurt containing the combination of *L. acidophilus* La5 and *B. lactis* Bb12 have been reported (70). Recently, a probiotic combination consisting of *L. rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freidenreichii* subspecies *shermanii* JS, and *B. animalis* Bb12 revealed a decrease in gastrin-17 and ^{13}C-UBT values with *H. pylori*-infected patients (101). However, not all clinical trials have shown effectiveness. In 1 open study 3 *Lactobacillus* strains were ineffective on *H. pylori* infection (66). However, this study used strains that showed inhibition of *H. pylori* in vitro, but no other probiotic characteristics were documented.

To conclude, there are no consistent beneficial data available on the efficacy of probiotics in *H. pylori* infection. However, there are indeed fewer side effects from anti-*Helicobacter* treatment and the disturbance of the gut microbiota when certain probiotics are used. Also, regular consumption of probiotic products with a specified probiotic strain as an alternative to antibiotics may have some potential in suppression of *H. pylori* infection and gastric inflammation, but more clinical studies are needed to confirm their value.

Respiratory tract infections in adults and children

Thus far only a limited number of studies have addressed the potential effect of probiotics in reducing the risk of common upper respiratory tract infections. No studies were found dealing with management of existing respiratory tract infections. Ten human studies investigating the efficacy of probiotics against airway infections were evaluated, of which 3 were in healthy adults, 3 in athletes/soldiers, 1 in elderly, 1 in infants, and 2 in children (10–19). A number of these studies investigated the effect of probiotic consumption on both airway and gastrointestinal infections (12,13,17,18).

Each study used a different probiotic strain or mixture (Table 1).

Overall, each of the 10 individual studies reported significant improvements on specific sickness-related outcome parameters, but no overall consistency in the efficacy on particular parameters can be deduced. Some studies show an effect on the overall duration of disease and not on incidence (10,13,19), whereas others report an effect on incidence of the disease but not duration (12,17). Most studies also report effects on symptom scores, although different symptoms were affected in the various studies. The 1 study in infants did not report an improvement on respiratory tract infections.

The variety of probiotic strains that were used complicates the development of full insight into the effect of probiotics on airway infections. Two studies assessing the effect on the duration and severity of the common cold successfully used the same mix of strains (*L.gasseri* PA 16/8, *B. longum* SP 07/3, and *B. bifidum* MF 20/5), but in 1 of the studies they were combined with a vitamin supplement and dosed 10-fold higher (10,11), and some differences in effects on symptoms were noted. Two of the studies investigating probiotic efficacy on airway infections employed *L. rhamnosus* GG (14,17), 1 in marathon runners showing no effect on respiratory symptoms and 1 in children with some effect on respiratory infections. *L. reuteri* ATCC 55730 as well as *B. animalis* Bb-12 have shown no effect on respiratory illness in infants (18), but *L. reuteri* ATCC 55730 was effective in the reduction of absenteeism among workers because of respiratory tract or GI symptoms, although no analysis was performed to differentiate between respiratory tract and GI-related problems (12). Each remaining study employed different probiotic treatments, including *L. casei* DN-114 001, *L. fermentum* VRI 003, and a spore-forming *Bacillus clausii* strain, with varying degrees of success.

The rationale for the choice of the particular probiotics has not been described in all studies. Immunomodulation, efficacy in animal studies, and efficacy in human studies with respect to other conditions such as GI infections seem to underlie choices for *L. rhamnosus* GG and *L. reuteri* ATCC 55730. The use of *B. clausii* spores was based on the observed Th-1–stimulating capacity of *B. clausii* in allergic children. However, this immunomodulating potential was not assessed during the study showing positive effect on the duration of recurrent respiratory tract infections in otherwise healthy children (19), making it impossible to directly correlate the positive clinical outcome to an underlying mechanism. The choice of the mix of *L. gasseri* PA 16/8, *B. longum* SP 07/3, and *B. bifidum* MF 20/5 that were used in 2 common cold studies (10,11) was based on their efficacy to protect mice from intestinal infections with pathogenic *E. coli*, but the relevance of this characteristic to their application in common cold studies is unclear.

Most studies that investigate the effect of probiotics on airway infections hypothesize that stimulation of the immune response may be the underlying mechanism. However, it is still rare that endpoint studies are combined with the investigation of biomarkers that may explain the underlying mechanism. In those studies where biomarkers were assessed alongside primary endpoints, at least some insight may have been obtained in the activity of the particular probiotic strain(s). The studies on common cold prevention with the probiotic mix of *L. gasseri* PA 16/8, *B. longum* SP 07/3, and *B. bifidum* MF 20/5 have shown an increase in the number of both CD4+ and CD8+ lymphocytes

in peripheral blood (10,11), but effects on other immune parameters such as lymphocyte activation and phagocytosis were not found. Furthermore, a study in athletes with *L. fermentum* VRI 003 showed a highly significant reduction in the number of days with respiratory symptoms next to a slight increase in blood-derived IFN-γ (16). These observations may provide some insight in relating immune-modulatory effects, but much more research is needed, especially from studies that integrate the measurements on endpoints with biomarkers of the immune system.

Overall, a promising outlook on the potential of probiotics in the combat of airway infections appears, although different probiotic strains may differ in their effects. The limited number of studies and lack of consistent interventions and populations mean a successful formula cannot yet be distilled.

ENT infections

Very few articles consider the efficacy of oral probiotic intake in ENT infections (Table 1). The effect of a mixture of probiotic strains containing *L. rhamnosus* GG, *L. rhamnosus* LC 705, *B. breve* 99, and *P. freudenreichii* JS on acute otitis media (AOM) was studied by Hatakka et al. (20), who reported that neither the duration nor the incidence of AOM was changed during an intervention period of 6 mo.

In contrast, an unexpected increase in the presence of the pathogen *M. catarrhalis* was found in the probiotic group. In another study by Hatakka et al. (17), the number of children with complications of airway infections (AOM, sinusitis, bronchitis, and pneumonia) was significantly reduced on intake of *L. rhamnosus* GG, although the size of the effect decreased after age adjustment.

An interesting alternative approach to the classic oral intake of probiotics for management of ENT infections is the use of airway commensals applied through nasal or oral sprays. A few multicenter studies showed that a 10-d course of spraying with Group A streptococci applied after standard antibiotic therapy significantly reduces the recurrence rate of pharyngo-tonsillitis in adults and children (22–24), as well as otitis media in children (21). This approach seems very promising, but all the studies were performed by the same research group, so more independent studies are needed.

The mechanisms underlying these types of probiotic interventions are thought to relate to colonization resistance. Studies in individuals prone to otitis, sinusitis, and tonsillitis have shown a microbial disbalance resulting in relatively more potential pathogens and fewer protective bacteria with interfering capacity such as Group A streptococci (102). In addition, antibiotic treatment often reinforces this disbalance. Bacterial interference with commensal Group A streptococci may restore balance in the airway microbiota, ensuring competition with potential pathogens. A factor contributing to success in the recolonization with commensal Group A streptococci may have been the preceding antibiotic treatment in patients suffering from ENT infections.

Infectious complications in surgical and critically ill patients

A category of patients who could potentially benefit most from the health-promoting potential of probiotics in terms of prevention of infections are critically ill patients, especially those admitted to the intensive care unit. In this category of patients the infection risk is very high, as is the associated mortality. A number of studies have been performed with critically ill patients in which positive results, but also negative results, have been reported. As with other applications, many factors may influence the outcome of intervention in this patient category including use of multi- or monospecies probiotics, amount and type of probiotics administered, methods of administration, the time point when instituted, administration of fibers, and type of tube feeding (103).

Several studies have investigated the potential beneficial effects of probiotics in patients undergoing major abdominal surgery. The first one is from the group of Rayes (104). Patients were divided into 3 groups: a conventional group, a placebo group, and a group that received *Lactobacilllus plantarum* 299v. Administration of live lactobacilli did not improve the infection rate in the entire study population but did show a trend toward fewer infections in patients with gastric and pancreas resections. The second study from the same study group, also performed in 2002 with *L. plantarum* 299v, involved patients scheduled for liver transplantation; patients were again divided into 3 groups. In liver transplantation patients this probiotic reduced the incidence of infections, the amount of antibiotics administered, and the length of hospital stay. A mixture of different probiotics (*Pediacoccus pentosaceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei* ssp. *paracasei* F19, and *L. plantarum* 2362) combined with 4 bioactive fibers was also effective in liver transplantation patients in prevention of postoperative bacterial infections (105). This same mixture of probiotics and bioactive fibers was also used in patients scheduled for pylorus-preserving pancreatoduodenectomy. This study (106) showed that early enteral nutrition with bioactive fibers combined with the mixture of probiotics was able to significantly reduce postoperative bacterial infections in patients following pylorus-preserving pancreatoduodenectomy.

In a study by McNaught et al. (107), patients undergoing major abdominal surgery were randomized to either a control group or a treatment group receiving *L. plantarum* 299v. The administration of the *L. plantarum* 299v did not influence the rate of bacterial translocation, gastric colonization, or incidence of postoperative septic morbidity.

Jain et al. (108) performed a study with patients who had been admitted to an intensive care unit, who received a placebo or a combination of probiotic bacteria (consisting of *L. acidophilus* La5, *Bifidobacterium lactic* Bb12, *Strep. thermophilus*, and *Lactobacillus bulgaricus*) with oligofructose. The administration of this mixture did alter the microbial composition of the upper gastrointestinal tract but had no effect on the intestinal permeability and was not associated with measurable clinical benefits.

Two relatively small and 1 large multicenter trial have addressed the potential for probiotics in preventing infectious complications in acute pancreatitis (AP). AP is an acute inflammatory disease caused by either gallstones or excessive alcohol abuse. AP usually runs a mild, self-limiting course, but ~1 in 5 patients will develop necrotizing pancreatitis, which is associated with a 10–30% mortality rate. Mortality is mostly attributed to infectious complications and infection of (peri) pancreatic necrotic tissue in particular. The infections are thought to be the sequelae of a cascade of events that starts with small-bowel bacterial overgrowth, mucosal barrier failure, and a proinflammatory response leading to translocation of intestinal bacteria. Antibiotic prophylaxis has been shown to be ineffective in preventing these infections. In a first study by Olah et al. (109), fewer patients in the group that received *L. plantarum* 299v developed infections of pancreas necrosis than in the placebo group (1 of 22 vs. 7 of 23). A later study from the same study group, now using a combination of 4 lactobacilli

(*Pediacoccus pentosaceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei* ssp. *paracasei* F19, and *L. plantarum* 2362) combined with 4 bioactive fibers in a total study group of 62 patients did not reduce the number of infections (110).

The Dutch Acute Pancreatitis Study Group has initiated a multicenter study (PROPATRIA) on the effectiveness of a multispecies mixture of microorganisms on prevention of infectious complications in AP (111). The placebo group was treated with high-fiber, high-energy enteral nutrition administered through a nasogastric tube. The intervention group received the enteral nutrition as well as a daily dose of 10^{10} bacteria: *L. acidophilus*, *Lactobacillus casei*, *Lactobacillus salivarius*, *Lactococcus lactis*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* for a maximum period of 28 d.

This trial in patients with predicted severe AP, in which a total of 296 patients were included, showed no beneficial effect of the treatment on the occurrence of infectious complications (30% in the intervention group vs. 28% in the placebo group). However, mortality in the intervention group was >2 times higher than that in the placebo group [24 (16%) and 9 (6%), respectively, $P < 0.01$]. In 9 patients in the intervention group (none in the placebo group), bowel ischemia was the cause of death (111).

Animal models: what can be learned and what are the drawbacks?

The immunomodulatory effects of probiotics have been studied extensively in vitro and in animal models. These studies have been of paramount importance in elucidating mechanisms of interaction between probiotics and the immune system. However, for many of the (infectious) diseases for which probiotics are being used or considered, animal models are not available or have little relevance for the human disease.

Animal models are very useful for screening potential candidate probiotic strains before these are used in humans. It is difficult to extrapolate from animal studies to the human target population. This especially holds true for consumer probiotics targeted at risk reduction for infectious diseases in the general population and also for allergic diseases and IBD.

Recommendations and gaps

To maximize learning and to generate data to arrive at more consistent evidence, future investigators designing trials on the effect of probiotics on infectious diseases are recommended to consider the following, in addition to the recommendations as outlined in the accompanying general paper (4).

Include biomarker measurements in clinical endpoint studies. Most human studies addressing the effect of a probiotic intervention on resistance against infectious diseases assess either endpoint parameters, such as episodes of diarrhea or severity of infection-related symptoms, or mechanistic parameters and biomarkers such as microbiota changes or changes in epithelial barrier function or immune function markers. Such designs unfortunately preclude the generation of more insight into underlying mechanisms of probiotic action.

In the broad area of infectious disease, many biomarkers could be measured, and the choice for a particular biomarker depends on several factors. Although general recommendations exist on measurement of biomarkers related to the immune system and intestinal health (112–114), the presumed biological activity of the probiotic (why it was selected) and the physiological mechanisms of the host that are involved in dealing with the infection are additional aspects to take into consideration. For instance, when infectious diarrhea is the focus, it seems more appropriate to include measures related to colonization resistance such as microbial shifts and fecal pH than when dealing with airway infections. In the latter case, when viruses are involved, it seems appropriate to include CD8+ T lymphocyte and natural-killer cell responses among biomarker measurements. Although general recommendations on which biomarkers to include can not be made, as they depend on the nature of the infection and the biological activity of the probiotic, researchers should take the point into consideration and make efforts to include biomarker measurements in clinical endpoint studies to gain more insight into probiotic mechanisms. This would generate data that may help to assess the usefulness of certain biomarkers in the prediction of endpoint outcomes.

Identification of the infectious agent. As the body's defenses against different types of pathogens are differentiated and specialized, the impact of probiotics on the resistance against various pathogens may depend importantly on the type of probiotic strain used and its specific impact on host defense. The efficacy of a probiotic in the management of a given infection may depend on the 1 hand on its ability to counteract pathogenic mechanisms used by pathogens to invade the host and on the other hand on its capacity to modulate a particular immune function or to compensate for some host risk factors (such as age). The identification of the infectious agent in studies addressing, e.g., airway or gastrointestinal infections may help decipher how different probiotics contribute to the management of infections by the host and why certain probiotic strains may be successful whereas others are not. Ultimately, such knowledge will contribute to the rationale for the selection of a particular probiotic in the combat against a particular infection.

Expected incidence and power calculations. Sample size of the study groups should be based on the primary parameters of the study, and if these are incidence, duration, or severity of an infection, the number of participants should be based on the expected incidence, duration, or severity in that target population. Retrospectively, the incidence or duration of the infection in the (control) study population should be compared with the expected incidence, as this may elucidate study outcomes, especially no-effect findings. In addition, reporting changes in incidences and duration should preferably include absolute differences, and the interpretation of such data should be done in context of what is considered clinically relevant to allow judgment of the impact of the effect.

Confounding factors. Use of medication such as antiviral agents, antibiotics, and immunosuppressants should be carefully reported, as should other possible confounding factors such as breast-feeding, and the stability, compliance, and conditions of use of the probiotic under specific circumstances such as travel. To deal with confounding factors, study treatments should, where possible, be stratified over relevant subgroups. Apart from the above recommendations on design of studies on risk reduction for infections, a number of gaps were identified that could be addressed specifically.

- Studies should address the applicability of human models of suboptimal health states [e.g., (physical) stress] that may increase the susceptibility to certain infections, to determine relevance for the general population.
- Studies should address the appropriate timing for probiotic intervention in the management of infections, also in relation to long-term probiotic ingestion and possible host adaptation.

Conclusions

The potential of probiotics to contribute to colonization resistance and to modulate immune function parameters offers a clear rationale for the use of probiotics in the management of infectious diseases. The different natures of the infections that have been subject to clinical studies with probiotics obviously prevent any generic conclusions in this area. Furthermore, the lack of consistency among studies focusing on 1 specific infection, in study design, applied probiotic strains, outcome parameters, and study population, along with the still-limited number of studies, often preclude a clear conclusion on the efficacy of probiotics in a specific infectious disease. Moreover, the lack of confirmative studies currently makes it impossible to identify the most promising probiotic strains. Positive exceptions are the management of infectious diarrhea (infants and TD), AAD, and NEC.

To advance our knowledge of the possible working mechanisms of probiotics in infectious diseases and to increase our understanding of the role of various biomarkers in risk reduction of infections, it is essential that more probiotic studies incorporate both clinical outcomes and measurement of biomarkers putatively related to the clinical effect such as immune markers and changes in the microbiota and gut barrier function. Furthermore, study designs should be improved so that the identification of infectious agents when relevant, the choice of the probiotic strain, a retrospective comparison of the actual incidence of infections with the predicted incidence, and a more careful reporting of possible confounders may help to understand the outcome, either positive or negative, of trials assessing the effect of probiotics on infections.

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