Randomised clinical trial: efficacy of a new synbiotic formulation containing *Lactobacillus paracasei* B21060 plus arabinogalactan and xilooligosaccharides in children with acute diarrhoea

A. Passariello*,†, G. Terrin‡, G. Cecere*, M. Micillo*, G. De Marco*, M. Di Costanzo*, L. Cosenza*, L. Leone*, R. Nocerino* & R. Berni Canani*,§

*Department of Paediatrics, University of Naples "Federico II", Naples, Italy.
†Neonatal Unit "V. Monaldi" Hospital, Naples, Italy.
‡Department of Woman's Health and Territorial Medicine, University of Rome "La Sapienza", Rome, Italy.
§European Laboratory for the Investigation of Food Induced Diseases (ELFID), University of Naples "Federico II", Naples, Italy.

Correspondence to:
Dr R. Berni Canani, Department of Pediatrics, University of Naples "Federico II", Via S. Pansini 5, 80131 Naples, Italy.
E-mail: berni@unina.it

Publication data
Submitted 8 September 2011
First decision 21 September 2011
Resubmitted 14 January 2012
Accepted 19 January 2012
EV Pub Online 13 February 2012

SUMMARY

Background
Acute diarrhoea is a frequent problem in children with heavy economic burden for families and society.

Aim
To test the efficacy of a new synbiotic formulation containing *Lactobacillus paracasei* B21060, arabinogalactan and xilooligosaccharides in children with acute diarrhoea.

Methods
Double-blind, randomised, placebo-controlled trial, including children (age 3–36 m) with acute diarrhoea who were allocated to placebo or synbiotic group. Major outcome was resolution rate of diarrhoea at 72 h. Total duration of diarrhoea, daily stool outputs, stool consistency, working days lost by parents, adjunctive medications, and hospitalisation were also assessed.

Results
We enrolled 55 children in placebo group and 52 in synbiotic group. The two groups were similar for demographic and clinical characteristics. Resolution rate of diarrhoea at 72 h was significantly higher in synbiotic group (67%) compared to placebo group (40%, $P = 0.005$). Children in synbiotic group showed a significant reduction in the duration of diarrhoea (90.5 h, 78.1–102.9 vs. 109.8 h, 96.0–123.5, $P = 0.040$), daily stool outputs (3.3, 2.8–3.8 vs. 2.4, 1.9–2.8, $P = 0.005$) and stool consistency (1.3, 0.9–1.6 vs. 0.6, 0.4–0.9, $P = 0.002$) compared to placebo group (data expressed as mean, 95% CI). Rate of parents that missed at least one working day (41.8% vs. 15.4%, $P = 0.003$), rate of children that needed adjunctive medications (25.5% vs. 5.8%, $P = 0.005$) or hospitalisation (10.9% vs. 0%, $P = 0.014$) after the first 72 h of treatment, were reduced in synbiotic group.

Conclusion
The synbiotic formulation studied is effective in children with acute diarrhoea. Australian New Zealand Clinical Trials Registry (ACTRN12611000641998).

Aliment Pharmacol Ther 2012; **35:** 782–788

doi:10.1111/j.1365-2036.2012.05015.x

INTRODUCTION

Acute diarrhoea is frequent in infants and children, representing a heavy economic burden for families and society. The standard treatment of acute diarrhoea remains oral rehydration solution (ORS). Probiotics have gained an important role as adjuvant therapy, so they were included in recent guidelines on the management of acute diarrhoea of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Pediatric Infectious Disease (ESPID).[1] The guidelines state that 'probiotics may be an effective adjunct to the management of diarrhea' but that 'only the use of probiotic strains with proven efficacy and in appropriate doses is suggested'. A large number of studies, including randomised and controlled trials and metanalyses reported an anti-diarrhoeal effects of probiotics, particularly in children,[2–12] although limited data were available on the efficacy of products containing probiotics associated with prebiotics namely synbiotics.[13] It has been demonstrated that prebiotics improve probiotics strain survival, by making specific substrates readily available for their fermentation, with a clear advantage to the host.[13] Synbiotics may be given as separate supplements or may exist in functional food as additives.[13] The aim of this study was to investigate the efficacy of a synbiotic formulation containing *Lactobacillus paracasei* B21060 plus arabinogalactan and xilooligosaccharides in the treatment of children with acute diarrhoea.

METHODS

Study design

We performed a prospective, randomised, double-blind, placebo-controlled trial in collaboration with family paediatricians, who care for children up to 14 years of age in the Italian Public Health System. The study protocol was reviewed and approved by the Ethics Committee of the University Federico II of Naples and registered in the Australian New Zealand Clinical Trials Registry (ACTRN12611000641998), and it was illustrated and discussed during three meetings with all physicians involved in the research.

Participants

From November 2010 to March 2011, all children 3–36 months of age consecutively observed in the paediatrician offices presenting with diarrhoea lasting less than 24 h with mild-moderate dehydration were considered eligible for the study. Diarrhoea was defined as three or more outputs of loose or liquid stools/day.[8] At the enrolment, after the initial assessment, the paediatricians were asked to estimated the degree of dehydration of each patient by using a seven-point Likert scale and to determine their capillary refill time by using standard clinical techniques (<2 s or >2 s) as used in a previous study.[14, 15] Exclusion criteria were diarrhoea lasting more than 24 h, malnutrition as judged by a body weight/height ratio below the fifth percentile, clinical signs of severe dehydration, clinical signs of a coexisting severe acute infection (meningitis, sepsis, pneumonia), immunodeficiency, underlying severe chronic diseases, cystic fibrosis, food allergy or other chronic gastrointestinal diseases, endocrinopathy, use of pre/probiotics or antibiotics or any anti-diarrhoeal medication in the previous 3 weeks. Written informed consent was obtained from parents of the enrolled children. Microbiologic and other laboratory investigations were performed only in the presence of specific clinical reasons.

Intervention

Enrolled patients were randomly allocated to placebo group (one sachet dissolved in 50 mL of water b.d. for 5 days) or synbiotic group (one sachet dissolved in 50 mL of water b.d. for 5 days containing *Lactobacillus paracasei* B21060, 2.5×10^9 CFU, plus arabinogalactan, 500 mg, and xilooligosaccharides, 700 mg b.d.; Flortec Bracco, Milan, Italy). The parents were instructed to rehydrate orally their children with hypotonic ORS in 3–4 h and then to administer ORS for dehydration prevention until end of symptoms, and to refeed the child with a normal appropriate-for-age diet including full strength lactose-containing formula or cow's milk (for guidelines see reference 1).

Outcome

Primary outcome was the rate of resolution of diarrhoea at 72 h of treatment. Diarrhoea was considered to have stopped after a patient had passed the last abnormal (loose or liquid) stools preceding a normal stool output, as applied in a previous study.[8]

Randomisation and blinding

Patients were allocated to each group according to a computer-generated randomisation list. The researcher responsible for enrolling patients allocated the next available number on entry in the trial. Each patient received white aluminium foil sachets contained in a blank paper box blind code labelled. The aspect of paper box, sachets and organoleptic characteristics of placebo or active

treatment were identical. The parents of enrolled children were instructed to record daily on a specific form: time, number and consistency (graded as 0: normal, 1: loose, 2: semi liquid, and 3: liquid), faecal outputs; missed working days, use of other medications, hospital admission; and adverse events. The investigators collecting the reporting forms were blind to the patient's treatment assignment, which was concealed until statistical analysis was completed.

Sample size

To obtain a power of the study of 85% (type 1 error = 0.05; two-tailed test), considering a difference of 30% (40% vs. 70%) in the rate of resolution of diarrhoea at 72 h between the two groups, 48 patients in each group were estimated. We enrolled 55 patients per group considering a possible drop out up rate as high as 15%.

Statistical analysis

Statistical analysis was performed by a statistician blind to preparations received by children in the two groups. Because of Gaussian distribution assessed by the Kolmorogov–Smirnov test, continuous variables were expressed as means and 95% CI. For categorical variables, the Pearson chi-square test or Fisher's exact test was performed as appropriate. The two groups were compared for continuous variables by *t*-test for equality of means. Kaplan–Meier method was used to estimate the probability of diarrhoea at 72 h in each study group, and the resulting functions were compared with the log-rank test. Analyses were conducted on an intention-to-treat basis. Patients allocated in each group were considered available for ITT analysis when received at least two doses of active treatment or placebo. All tests of significance were two sided. A P value of <0.05 was considered significant. The statistical analysis was performed using the SPSS software package for Windows (release 16.0.0; SPSS Inc., Chicago, IL, USA) and Starts Direct (release 2.6.6).

RESULTS

Figure 1 shows the flow of children through the study. The demographic and clinical characteristics of the children enrolled in the two groups were similar at the baseline (Table 1). No infants were breastfed. No patients in the study had hematochezia. Resolution of diarrhoea at 72 h was higher in synbiotic group (67%) than in placebo group (40%; OR 0.324, 95% CI 0.147–0.715; $P = 0.005$). The number of daily stool outputs and consistency resulted significantly reduced in synbiotic group compared with placebo group at 72 h of treatment

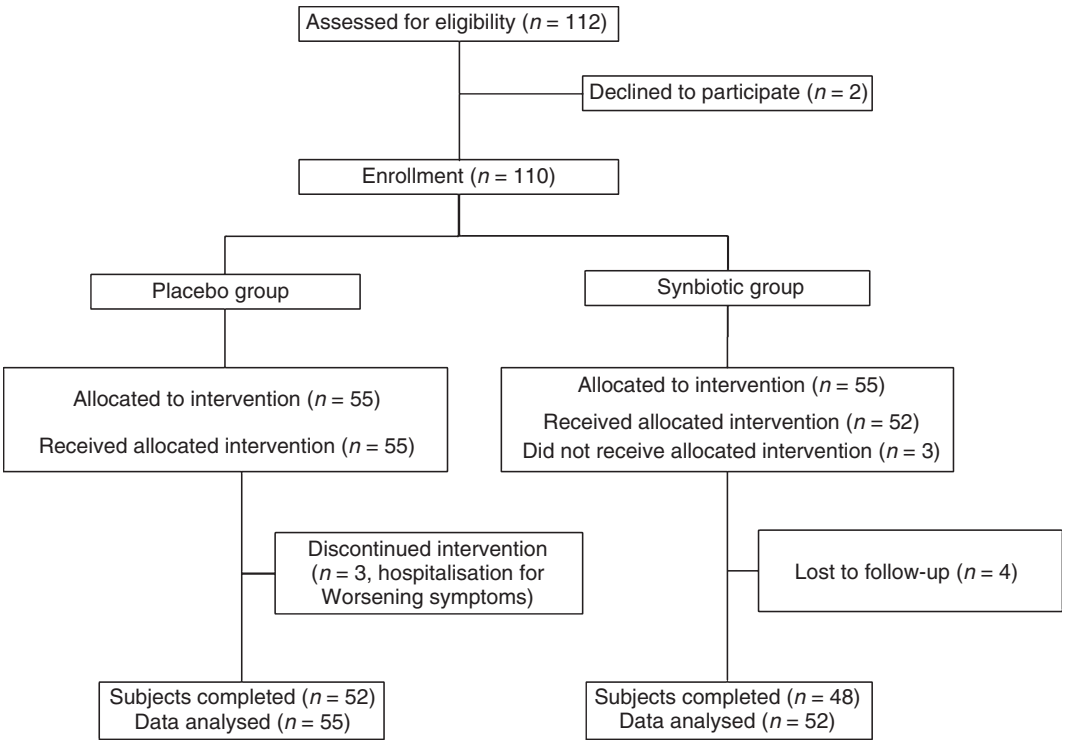

Figure 1 | Flow of children through the study.

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Table 1 | Baseline features of the children allocated to study treatments

	Placebo group (n = 55)	Synbiotic group (n = 52)	P
Age, m	20.9 (17.6–24.3)	20.0 (16.7–23.5)	0.711
Body weight, kg	11.9 (10.9–12.9)	12.2 (11.2–13.3)	0.704
Male, *n* (%)	31 (56.4)	27 (51.9)	0.645
Duration of symptoms before treatment, h	8.4 (8.0–8.7)	8.3 (8.0–8.7)	0.965
Presence of vomiting, *n* (%)	15 (27.3)	13 (25.0)	0.807
Degree of dehydration*			
Mild	23 (41.8)	25 (48.1)	0.515
Moderate	32 (58.2)	27 (51.9)	0.515

Data are expressed as mean (95% CI) when not specified.

* Assessed according to standardised criteria, as previously described.[14, 15]

Table 2 | Secondary outcomes in the two groups of the study

	Placebo group (n = 55)	Synbiotic group (n = 52)	P
Total diarrhoea duration	109.8 (96.0–123.5)	90.5 (78.1–102.9)	0.040
Number of stool outputs (from 48 to 72 h after treatment)	3.3 (2.8–3.8)	2.4 (1.9–2.8)	0.005
Stool consistency score (from 48 to 72 h after treatment)	1.3 (1.0–1.6)	0.6 (0.4–0.9)	0.002
Adjunctive medication, *n* (%)	14 (25.5)	3 (5.8)	0.005
Rate of patients requiring hospitalization, *n* (%)	6 (10.9)	0 (0)	0.014
Rate of parents missed at least one working day, *n* (%)	23 (41.8)	8 (15.4)	0.003

Data as expressed as mean (95% CI) when not specified.

(Table 2). The total duration of diarrhoea was reduced in patients in synbiotic group compared to placebo group (Table 2). Probability of diarrhoea within 72 h of treatment was higher in placebo group compared with synbiotic group (Figure 2). The rate of parents missed at least one working day was significantly higher in placebo group (Table 2). Adjunctive medications within the 72 h were not used by any patient, whereas after the first 72 h the use of additional treatments were higher in subjects in placebo group than in children in synbiotic group (Table 2). In particular, the medications used were (no. of patients in placebo group vs. no. of patients in synbiotic group): probiotics (7 vs. 0), diosmectite (3 vs. 3), racecadotril (3 vs. 0), and domperidone (1 vs. 0). The rate of patients requiring hospitalisation because of worsening of symptoms was slightly but not significantly higher in placebo group (Table 2). No adverse event was observed in the two groups.

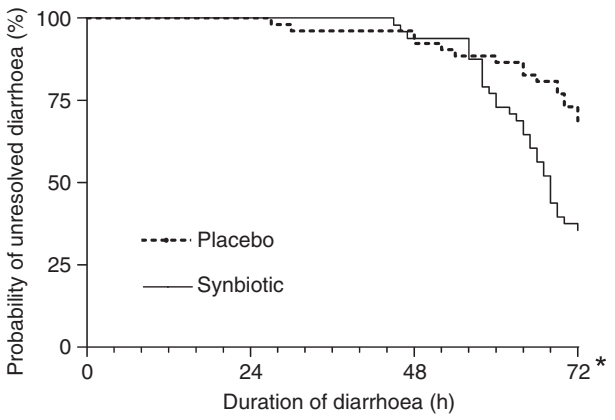

Figure 2 | Kaplan–Meyer curve showed a significant difference (log-rank test, P = 0.001) in the probability of unresolved diarrhoea at 72 h after starting treatment between placebo or synbiotic formulation (containing *Lactobacillus paracasei* B21060 plus arabinogalactan and xilooligosaccharides).

DISCUSSION

We demonstrated that a new synbiotic formulation containing *L. paracasei* B21060 plus arabinogalactan and xilooligosaccharides is effective in the treatment of acute diarrhoea in children. This synbiotic formulation resulted in the ability to reduce the duration and severity of diarrhoea. The results of the synbiotic tested in this trial are comparable with those reported in the recent Cochrane review, focused on the efficacy of probiotics in acute diarrhoea, showing a significant reduction of: diarrhoea duration of ~ 24 h (95% CI 15.9 + 33.6 h;

n = 4555, trials = 35) and diarrhoea lasting more than 4 days (risk ratio 0.41; 0.32–0.53; n = 2853, trials = 29).[2] In this Cochrane review and in a recent clinical report by the Committee of Nutrition Section Gastroenterology Hepatology and Nutrition of the American Academy of Pediatrics, *Lactobacillus* GG (LGG) resulted in the most effective probiotic in the treatment of acute diarrhoea.[2, 13] Interestingly, in a recent study Grossi *et al.* compared the therapeutic efficacy of the same synbiotic preparation investigated in our study with LGG, and the synbiotic proved to be more effective than LGG in the treatment of acute diarrhoea in adult treated at primary care setting.[16]

Numerous pre-pro-synbiotic preparations become available on the market every year and it could be difficult for a physician to select effective products for the treatment of different disorders. Because of strain, product and age specificities (especially for products containing mixtures of probiotic strains and prebiotics), and in order to be in agreement with recommendations of official and scientific organizations, it is recommended to perform randomised controlled trials with each commercialized product.[17] The results of our study support the clinical utility of this new synbiotic preparation in the treatment of ambulatory paediatric patients with acute diarrhoea.

According to recent guidelines for the management of ambulatory children with acute diarrhoea, we did not investigate the aetiology of diarrhoea in our patients. However, considering epidemiologic data and that in Italy the national vaccination programme does not include *Rotavirus* universal vaccination, it is possible to speculate that the majority of patients presented acute diarrhoea induced by viral pathogens, in particular *Rotavirus*.[18–21] Probiotics exert a wide range of possible mechanisms of action against intestinal pathogens.[22] There are three general classes of anti-pathogenic mechanisms: direct antagonism, immune-modulation and exclusion.[23] The most recent research focused on the role of probiotic microorganisms and their secretion products in strengthening and modulating, through other mechanisms, the both congenital and adaptive immune response in the host. After the observation that immune and epithelial cells can discriminate among different microbial species through the activation of Toll-like receptors,[24] the hypothesis emerged that probiotics might exert protective effect by modulating immunologic activity and epithelial function, both in the small and large intestine.[25, 26] A recent study has highlighted the striking difference among different species and strains of lactobacilli modulating the immune and inflammatory response.[27] These authors compared the immunological properties *of L. plantarum NCIMB8826,* LGG and *L. paracasei* B21060, studying the stimulating effects of these different strains on dendritic cells either directly through a co-culture or indirectly through conditioning of an epithelial intermediary. In this study, the authors emphasised, at variance with LGG and *L. plantarum,* the immunomodulatory effect of *L. paracasei* B21060 that once in the intestine may act directly on intraepithelial dendritic cells limiting their ability to induce inflammation in the presence of potent inflammatory pathogens. Preliminary data suggested that some immunomodulatory effect of *L. paracasei* B 21060 could be exerted by soluble factors produced by the bacteria.[27]

Lactobacillus paracasei B 21060 is a novel strain of lactobacillus isolated from the faeces of breastfed babies and its non-occasional presence in the normal intestinal microflora was established after extensive monitoring by genetic identification methods[28] but future research is needed to better define the possible mechanisms of action of this probiotic strain in the treatment of acute diarrhoea.

Up to now, few data are available on the efficacy of synbiotics in the treatment of acute diarrhoea. Nondigestable carbohydrates seem to be unable to reduce the duration of diarrhoea[29] but they could confer additional benefits over a probiotic by normalising more rapidly intestinal microflora perturbation during enteric infection and stabilizing the effect of probiotics.[30–33] In a recent study, Drakoularakou *et al.* demonstrated the effectiveness of a prebiotic galacto-oligosaccharide mixture (B-GOS) on the severity and/or incidence of traveller's diarrhoea.[34] The results of a *in vitro* screening study that aimed identifying promising prebiotic and synbiotic candidates for the diarrhoea treatment, indicated that different types of microorganisms and microbial groups are able to ferment the tested oligosaccharides (xylo-oligosaccharides (XOS), xylo, galacto-oligosaccharides, fructo-oligosaccharide, polydextrose, lactitol, gentiobiose and pullan) in pure cultures, and that some of these compounds could be useful in the development of new product candidates as they promoted the growth of few, beneficial probiotic microbes in the competitive environment of the colon. XOS compounds enhanced the growth of a limited number of microbes, especially *B. lactis*.[35] Further prospective controlled trials should be planned to establish if the results obtained in this study are not due only to the probiotic component of the synbiotic. Our results suggest a positive cost-efficacy ratio in the use of this synbiotic preparation in the treatment of

acute diarrhoea in children. We observed a significant reduction in parents' working day loss and in medication use, the two most important parameters contributing up to 85% of the total cost of a single episode of acute diarrhoea.[36] One single therapeutic course using this new commercially available synbiotic product costs about 10 Euro. One single episode of acute diarrhoea in children in Italy costs about 137 Euro. Of this about 116 Euro are related to working day loss by the parents and additional medications use. The average cost of the commercially available probiotic products on the market is equal to the cost of this synbiotic product. In this light, the use of this synbiotic could be responsible for substantial reduction of the cost related to acute diarrhoea.

In conclusion, our results showing that the new synbiotic preparation composed by *L. paracasei* B21060 plus arabinogalactan and xilooligosaccharides significantly reduces the duration and severity of diarrhoea of likely infection origin suggest a new possible effective and cost saving therapeutic strategy for the treatment of ambulatory children with this very common disease.

ACKNOWLEDGEMENTS

The authors acknowledge with gratitude the commitment of the Mother and Child Health Association (M.A.C.H.A.) to the research efforts. The authors thank the Bracco Pharmaceutical Company (Milan, Italy) for providing the active and placebo treatments. The Bracco Pharmaceutical Company had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. *Declaration of personal and funding interests*: None.

REFERENCES

1. Guarino A, Albano F, Ashkenazi S, *et al.* European Society for Paediatric Gastroenterology, Hepatology, and Nutrition/European Society for Paediatric Infectious Diseases Evidence-based guidelines for management of acute gastroenteritis in children in Europe. *J Pediatr Gastroenterol Nutr* 2008; **46**: S81–184.
2. Allen SJ, Martinez E, Gregorio GV, Dans LF. Probiotics for treating infectious diarrhea. *Cochrane Database Syst Rev* 2010; **11**: CD003048.
3. Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: a systematic review of published randomized, double-blind, placebo-controlled trials. *J Pediatr Gastroenterol Nutr* 2001; **33**(Suppl 2): S17–25.
4. Van Niel CW, Feudtner C, Garrison MM, Christakis DA. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* 2002; **109**: 678–84.
5. Huang JS, Bousvaros A, Lee JW, Diaz A, Davidson EJ. Efficacy of probiotic use in acute diarrhea in children: a meta-analysis. *Dig Dis Sci* 2002; **47**: 2625–34.
6. Szajewska H, Skorka A, Dylag M. Meta-analysis: saccharomyces boulardii for treating acute diarrhea in children. *Aliment Pharmacol Ther* 2007; **25**: 257–64.
7. Szajewska H, Skorka A, Ruszczynski M, Gieruszczak-Bialek D. Meta-analysis: Lactobacillus GG for treating acute diarrhea in children. *Aliment Pharmacol Ther* 2007; **25**: 871–81.
8. Berni Canani R, Cirillo P, Terrin G, *et al.* Probiotics for treatment of acute diarrhea in children: randomised clinical trial of five different preparations. *BMJ* 2007; **335**: 340–6.
9. Hummel AS, Hertel C, Holzapfel WH, Franz CM. Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. *Appl Environ Microbiol* 2007; **73**: 730–9.
10. Kastner S, Perreten V, Bleuler H, Hugenschmidt G, Lacroix C, Meile L. Antibiotic susceptibility patterns and resistance genes of starter cultures and probiotic bacteria used in food. *Syst Appl Microbiol* 2006; **29**: 145–55.
11. Egervarn M, Danielsen M, Roos S, Lindmark H, Lindgren S. Antibiotic susceptibility profiles of Lactobacillus reuteri and Lactobacillus fermentum. *J Food Prot* 2007; **70**: 412–8.
12. Kayser FH. Safety aspects of enterococci from the medical point of view. *Int J Food Microbiol* 2003; **88**: 255–62.
13. Thomas DW, Greer FR, Committee on Nutrition, Section on Gastroenterology Hepatology, and Nutrition. Clinical report. Probiotics and prebiotics in pediatrics. *Pediatrics* 2010; **126**: 1217–31.
14. Shavit I, Brant R, Nijssen-Jordan C, Galbraith R, Johnson DW. A novel imaging technique to measure capillary-refill time: improving diagnostic accuracy for dehydration in young children with gastroenteritis. *Pediatrics* 2006; **118**: 2402–8.
15. Friedman JN, Goldman RD, Srivastava R, Parkin PC. Development of a clinical dehydration scale for use in children between 1 and 36 months of age. *J Pediatr* 2004; **145**: 201–7.
16. Grossi E, Buresta R, Abbiati R, Cerutti R. Clinical trial on the efficacy of a new symbiotic formulation, Flortec, in patients with acute diarrhea: a multicenter, randomized study in primary care. *J Clin Gastroenterol* 2010; **44**: S35–41.
17. FAO/WHO. Expert consultation on evaluation of health and nutritional properties of probiotics. FAO/WHO: Cordoba, 2001.
18. Caprioli A, Pezzella C, Morelli R, *et al.* Enteropathogens associated with childhood diarrhea in Italy. The Italian study group on gastrointestinal infections. *Pediatr Infect Dis J* 1996; **15**: 876–83.
19. Giaquinto C, Dominiak-Felden G, Van Damme P, *et al.* Summary of effectiveness and impact of rotavirus vaccination with the oral pentavalent rotavirus vaccine: a systematic review of the experience in industrialized countries. *Hum Vaccin* 2011; **7**: 734–48.
20. Vesikari T, Karvonen A, Prymula R, *et al.* Immunogenicity and safety of the human rotavirus vaccine Rotarix co-administered with routine infant vaccines following the vaccination schedules in Europe. *Vaccine* 2010; **28**: 5272–9.

21. Saia M, Giliberti A, Callegaro G. Hospitalisation for rotavirus gastroenteritis in the paediatric population in the Veneto Region, Italy. *BMC Public Health* 2010; **10**: 636–40.
22. Guarino A, Lo Vecchio A, Berni Canani R. Probiotics as prevention and treatment for diarrhea. *Curr Opinion Gastroenterol* 2009; **25**: 18–23.
23. Preidis GA, Hill C, Guerrant RL, Ramakrishna BS, Tannock GW, Versalovic J. Probiotics, enteric and diarrheal diseases, and global health. *Gastroenterology* 2011; **140**: 8–14.
24. Kadowaki N, Ho S, Antonenko S, *et al.* Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001; **194**: 863–9.
25. Blum S, Schiffrin EJ. Intestinal microflora and homeostasis of the mucosal immune response: implications for probiotic bacteria? *Curr Issues Intest Microbiol* 2003; **4**: 53–60.
26. Sekirov I, Finlay BB. The role of the intestinal microbiota in enteric infection. *J Physiol* 2009; **587**: 4159–67.
27. Militi E, Matteoli G, Iliev ID, Rescigno M. Comparison of the immunomodulatory properties of three probiotic strains of Lactobacilli using complex culture systems: prediction for in vivo efficacy. *PLoS ONE* 2009; **4**: e7056.
28. Morelli L, Zonenschain D, Callegari ML, *et al.* Assessment of a new synbiotic preparation in healthy volunteers: survival, persistence of probiotic strains and its effect on the indigenous flora. *Nutr J* 2003; **2**: 11–7.
29. Vandenplas Y, Brunser O, Szajewska H. Saccharomyces boulardii in childhood. *Eur J Pediatr* 2009; **168**: 253–65.
30. Sekirov I, Finlay BB. The role of the intestinal microbiota in enteric infection. *J Physiol* 2009; **17**: 4159–67.
31. Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Van der Meer R. Dietary fructooligosaccharides dose-dependently increase translocation of salmonella in rats. *J Nutr* 2003; **133**: 2313–8.
32. Cummings JH, Christie S, Cole TJ. A study of fructo oligosaccharides in the prevention of travellers diarrhoea. *Aliment Pharmacol Ther* 2001; **15**: 1139–45.
33. Macfarlane GT, Steed H, Macfarlene S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J Appl Microbiol* 2008; **104**: 305–44.
34. Drakoularakou A, Tzortzis G, Rastall RA, Gibson GR. A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing traveller's diarrhea. *Eur J Clin Nutr* 2010; **64**: 146–52.
35. Makelainen H, Saarinen M, Stowell J, Rautonen N, Ouwehand AC. Xylo-oligosaccharides and lactiotol promote the growth of *Bifidobaterium lactis* and *Lactobacillus* species in pure cultures. *Benef Microbes* 2010; **1**: 139–48.
36. Fontana M, Zuin G, Pancheri P, Fusco FC, Lambertini A, Berni Canani R, SIGEP Working Group on Intestinal Infections. Cost associated with outpatient diarrhea in infants and toddlers: a nationwide study of the Italian Society of Paediatric Gastroenterology and Hepatology (SIGEP). *Dig Liver Dis* 2004; **36**: 523–7.