

## Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations

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### ABSTRACT

**Objective** To compare the efficacy of five probiotic preparations recommended to parents in the treatment of acute diarrhoea in children.

**Design** Randomised controlled clinical trial in collaboration with family paediatricians over 12 months.

**Setting** Primary care.

**Participants** Children aged 3-36 months visiting a family paediatrician for acute diarrhoea.

**Intervention** Children's parents were randomly assigned to receive written instructions to purchase a specific probiotic product: oral rehydration solution (control group); *Lactobacillus rhamnosus* strain GG; *Saccharomyces boulardii*; *Bacillus clausii*; mix of *L delbrueckii* var *bulgaricus*, *Streptococcus thermophilus*, *L acidophilus*, and *Bifidobacterium bifidum*; or *Enterococcus faecium* SF68.

**Main outcome measures** Primary outcomes were duration of diarrhoea and daily number and consistency of stools. Secondary outcomes were duration of vomiting and fever and rate of admission to hospital. Safety and tolerance were also recorded.

**Results** 571 children were allocated to intervention. Median duration of diarrhoea was significantly shorter ( $P<0.001$ ) in children who received *L rhamnosus* strain GG (78.5 hours) and the mix of four bacterial strains (70.0 hours) than in children who received oral rehydration solution alone (115.0 hours). One day after the first probiotic administration, the daily number of stools was significantly lower ( $P<0.001$ ) in children who received *L rhamnosus* strain GG and in those who received the probiotic mix than in the other groups. The remaining preparations did not affect primary outcomes. Secondary outcomes were similar in all groups.

**Conclusions** Not all commercially available probiotic preparations are effective in children with acute diarrhoea. Paediatricians should choose bacterial preparations based on effectiveness data.

**Trial registration number** Current Controlled Trials ISRCTN56067537.

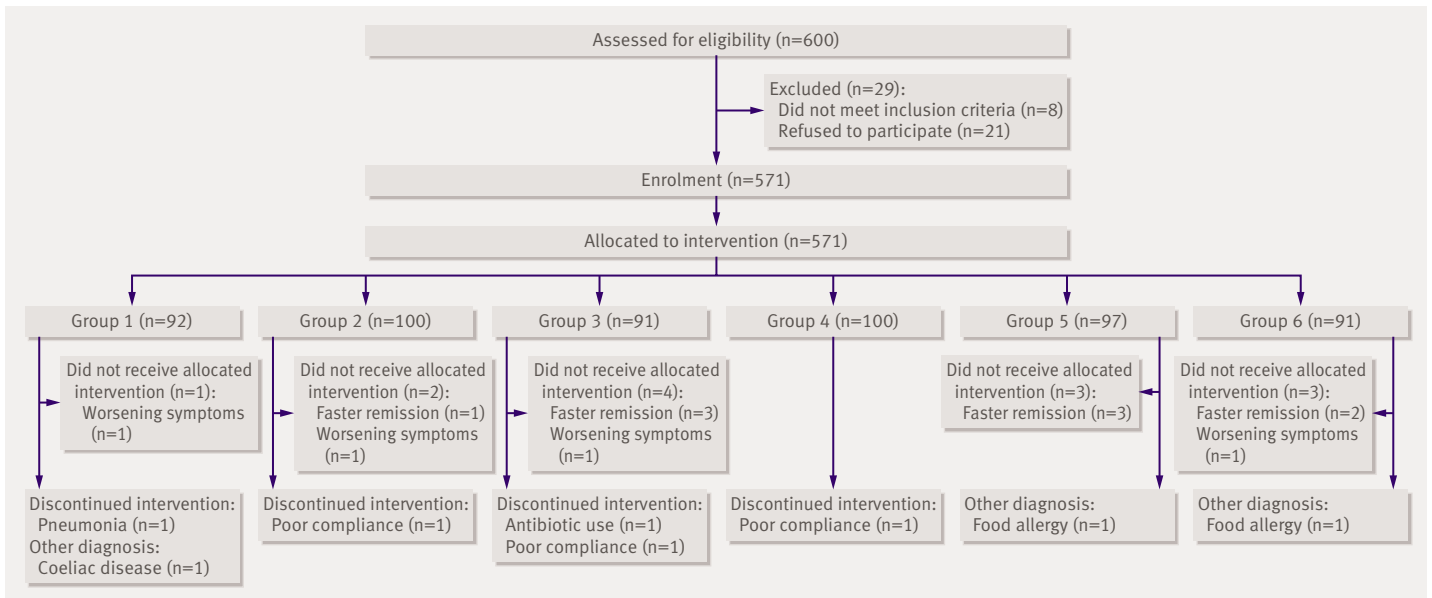
### INTRODUCTION

The management of acute diarrhoea consists of the replacement of lost fluid with glucose-electrolyte oral rehydration solution. This solution, however, reduces neither the severity nor the duration of diarrhoea. The search for such agents started over 20 years ago.<sup>1</sup> Probiotics, defined as micro-organisms that exert beneficial effects on human health when they colonise the bowel, have been proposed as adjunctive therapy in the treatment of acute diarrhoea.<sup>2</sup> Several micro-organisms are effective in reducing the severity and duration of acute diarrhoea in children: *Lactobacillus rhamnosus* (formerly "*Lactobacillus casei* strain GG" or "*Lactobacillus* GG"), *L plantarum*, several strains of bifidobacteria, *Enterococcus faecium* SF68, the yeast *Saccharomyces boulardii*, and preparations containing a mix of strains.<sup>2-7</sup> Several trials with probiotic preparations have been conducted in different settings and with different end points. Meta-analyses of probiotic efficacy, including a Cochrane review, are also available.<sup>2-8-10</sup> Few of these studies, however, meet the criteria of properly controlled trials.<sup>10</sup>

In a recent study of Italian children with diarrhoea, probiotics were the most commonly prescribed treatment.<sup>11</sup> With the increasing availability and widespread use of probiotics, it is important to identify the most effective preparations. We evaluated the efficacy of five probiotic preparations for the treatment of acute diarrhoea in children.

### METHODS

The study was a prospective single blind randomised controlled trial in which parents of children with acute diarrhoea received written instructions to purchase a specific brand of probiotic. The trial was performed in collaboration with family paediatricians, who in the Italian public health system care for children up to 12 years of age. We discussed the study design with six family paediatricians in three meetings. Diarrhoea was defined as three or more outputs of loose or liquid stools a day. Eligible children were those aged 3-36 months who were seen in paediatricians' offices from October 1999 to



Flow of participants through trial of probiotic preparations for treatment of childhood acute diarrhoea

September 2000 because of diarrhoea. We included in the study all children with diarrhoea lasting less than 48 hours for whom parents gave informed consent. Exclusion criteria were malnutrition as judged by the ratio of weight to height, clinical signs of severe dehydration, clinical signs of coexisting acute systemic illnesses (meningitis, sepsis, pneumonia), immunodeficiency, underlying severe chronic diseases, cystic fibrosis, food allergy or other chronic gastrointestinal diseases, use of probiotics in the previous three weeks, use of antibiotics or any antidiarrhoeal medication in the previous three weeks and during the study, and poor compliance (defined by administration of less than four doses of the study medication).

All children were given oral rehydration solution for three to six hours and then fed with full strength formula containing lactose or cows' milk, depending on age.<sup>12-14</sup> Microbiological investigation was performed only if required for specific clinical reasons. Children were also randomised to oral rehydration alone; *Lactobacillus* GG; *S. boulardii*; *Bacillus clausii*; mix of *L. delbrueckii* var *bulgaricus*, *Streptococcus thermophilus*,

*L. acidophilus*, and *Bifidobacterium bifidum*; or *E. faecium* strain SF68. Table 1 shows the main characteristics of the probiotic preparations.

Patients were allocated to each group according to a computer generated randomisation list. Random allocation was made in blocks of six to obtain groups of similar size. The sequence was concealed until treatments were assigned. The researchers responsible for enrolling the patients allocated the next available number on entry into the trial, and the parent of each child received written instructions to purchase the assigned probiotic product.

Probiotic preparations were prescribed for five days and administered orally in 20 ml water according to the manufacturers' instructions. All the probiotic products used in this study were available only in pharmacies and had a similar brand image and price. Each pack of probiotic preparation was sufficient for the entire therapeutic course. The group of children who received only oral rehydration served as controls.

The primary outcome measures were the total duration of diarrhoea and the number of stools a

Table 1 | Micro-organism load (according to product label when the study was performed), administration, and main characteristics of preparations analysed

Groups	Micro-organisms	Strains	Dose (twice daily)	Brand*	Price (€)
2	<i>Lactobacillus casei</i>	Rhamnosus GG	6×10 <sup>9</sup> CFU/dose	Dicoflor 60	10.50
3	<i>Saccharomyces boulardii</i>	<i>S. boulardii</i> lt	5×10 <sup>9</sup> live micro-organisms/dose	Codex†	8.50
4	<i>Bacillus clausii</i>	O/C84, N/R84, T84, SIN84	10 <sup>9</sup> CFU/dose	Enterogermina†	6.50
5	<i>L. delbrueckii</i> var <i>bulgaricus</i> , <i>L. acidophilus</i> , <i>Streptococcus thermophilus</i> , <i>B. bifidum</i>	LMG-P17550, LMG-P 17549, LMG-P 17503, LMG-P 17500	10 <sup>9</sup> CFU, 10 <sup>9</sup> CFU, 10 <sup>9</sup> CFU, 5×10 <sup>8</sup> CFU/dose	Lactogermina†	10.50
6	<i>Enterococcus faecium</i>	SF 68	7.5×10 <sup>7</sup> CFU/dose	Bioflorin†	10.50

CFU=colony forming units.

\*All sold in coloured cardboard boxes.

†Composition, brand name, and costs of these probiotic preparations have changed since study ended.

**Table 2 | Baseline features of children with diarrhoea allocated to study treatments**

	1 (n=92)	2 (n=100)	3 (n=91)	4 (n=100)	5 (n=97)	6 (n=91)
No (%) of boys	41 (44)	60 (60)	44 (48)	49 (49)	49 (50)	39 (42)
Median (IQR) age (months)	17 (11-28)	20 (13-25)	18 (10-27)	19 (10-24)	16 (10-28)	15 (8-22)
Median (IQR) weight (kg)	11.9 (9.9-12.7)	12.1 (9.1-13.2)	11.5 (9.2-14.0)	12.3 (9.4-13.4)	12.7 (9.5-14.2)	12.2 (10.3-13.5)
<b>Feeding (%)</b>						
Breast milk	13.5	11.7	13.3	10.0	13.4	17.9
Formula	55.8	55.0	55.6	60.2	46.2	43.6
Cows' milk	30.8	33.3	31.1	29.8	40.5	38.5
Median (IQR) duration of diarrhoea before treatment (hours)	9 (4-13)	10 (5-18)	11 (6-18)	10 (4-15)	9 (5-17)	10 (5-16)

IQR=interquartile range.

day and their consistency. Duration of diarrhoea was the time in hours from the first to the last abnormal (loose or liquid) stools preceding a normal stool output. Stool consistency was evaluated through a score system, as previously described,<sup>15</sup> and faeces were graded as 1 (normal), 2 (loose), 3 (semiliquid), and 4 (liquid). Secondary outcome measures were the incidence and median duration of vomiting, fever (>37.5°C), and the number of hospital admissions in each group. We also investigated safety and tolerability.

On enrolment we identified each child, determined the duration and severity of diarrhoea, assessed associated clinical features (fever, vomiting, dehydration), and established nutritional status and previous treatment. Parents received a coded reporting form on which to record clinical data. They were instructed to record daily the number of faecal outputs and their consistency, the type and doses of probiotic preparation taken by the child, the presence of vomiting and fever, any necessity for hospital admission, and all adverse events.

The study was performed according to a multicentre single blind and controlled design. Because of the problems of performing a double blind study of commercially available products in a large population, we used the third party blind observer method to assess efficacy. To ensure unbiased assessment, the family paediatricians, who were in charge of treatment allocation, gave written instructions to the parents to purchase a brand of probiotic and verified

compliance on the reporting form, whereas the investigators collecting the reporting forms were blinded to the assigned treatment. All reporting forms were delivered to the coordinating centre at the Department of Paediatrics for analysis. This procedure was applied in previous studies of the efficacy of anti-diarrhoeal treatments.<sup>15 16</sup> All parents of enrolled children gave informed consent.

#### ESTIMATE OF SAMPLE SIZE

To obtain the required power (95%, type I error=0.05, two tailed test) we needed 45 participants in each group. This estimate assumes a mean difference in duration of diarrhoea of 24 hours between the treated and control children (corresponding to means of 120 and 96 hours) with an SD of 30 hours within the group. This computation was based on the results of a preliminary open trial.<sup>15</sup> To investigate the secondary outcomes, we doubled the number of patients. Our estimation of sample size allowed for a drop out of up to 10%.

#### STATISTICAL ANALYSIS

A statistician blind to allocation performed the statistical analyses. We used  $\chi^2$  test for categorical variables and Mann-Whitney U test for continuous variables by analysing differences between group 1 and groups 2-6. All analyses were conducted on an intention to treat basis with SPSS version 15.0.0 for Windows (SPSS, Chicago, IL).

**Table 3 | Primary outcome index: duration of diarrhoea (hours) in study groups**

Group	Treatment	Median (IQR) duration (hours)	Estimated difference (95% CI)*	P value†
1	Oral rehydration solution alone	115.5 (95.2-127)	—	—
2	<i>Lactobacillus casei</i> subsp <i>rhamnosus</i> GG	78.5 (56.5-104.5)	-32 (-41 to -23)	<0.001
3	<i>Saccharomyces boulardii</i>	105.0 (90-104.5)	-5 (-13 to 5)	0.38
4	<i>Bacillus clausii</i>	118.0 (95.2-128.7)	1 (-7 to 8)	0.76
5	<i>L delbrueckii</i> var <i>bulgaricus</i> , <i>L acidophilus</i> , <i>Streptococcus thermophilus</i> , <i>B bifidum</i>	70.0 (49-101)	-37 (-47 to -25)	<0.001
6	<i>Enterococcus faecium</i> SF 68	115.0 (89-144)	2 (-5 to 11)	0.61

IQR=interquartile range.

\*Compared with oral rehydration solution alone.

†Mann-Whitney U test. P value for comparison with oral rehydration solution alone.

**Table 4** | Primary outcome index: median (IQR) daily stool outputs from the first day of probiotic administration

Group	Treatment	Day						
		1	2	3	4	5	6	7
1	Oral rehydration solution alone	5 (4-6)	5 (4-7)	4 (4-6)	4 (3-5)	3 (2-4)	2 (2-3)	2 (1-3)
2	<i>Lactobacillus casei</i> subsp <i>rhamnosus</i> GG¶	6 (4-6)	4 (4-6)*	4 (3-5)†	3 (2-4)*	2 (2-3)‡	2 (2-3)	2 (1-2)
3	<i>Saccharomyces boulardii</i>	5 (4-6)	5 (4-7)	4 (3-6)	4 (3-5)	3 (2-4)	2 (2-3)	2 (1-3)
4	<i>Bacillus clausii</i>	6 (4-6)	5 (4-7)	4 (4-7)	4 (3-5)	3 (2-4)	2 (2-3)	2 (1-2)
5	<i>L delbrueckii</i> var <i>bulgaricus</i> , <i>L acidophilus</i> , <i>Streptococcus thermophilus</i> , <i>B bifidum</i> ¶	6 (4-6)	4 (4-6)*	3 (3-4.5)*	3 (2-4)*	2 (2-3)§	2 (2-3)	2 (1-2)
6	<i>Enterococcus faecium</i> SF 68	5 (4-6)	5 (4-7)	4 (4-6)	4 (3-5)	3 (2-4)	2 (2-3)	2 (1-2)

IQR=interquartile range.

\*P&lt;0.001, †P=0.001, ‡P=0.003, or §P=0.002 v oral rehydration solution alone at same time point by Mann-Whitney U test.

¶On days 2, 3, 4, and 5 estimated difference (95% CI) compared with oral rehydration solution alone was -1 (-1 to 0).

## RESULTS

The figure shows the flow of children through the study. A total of 600 children with acute diarrhoea were eligible for inclusion: 29 were excluded and 571 were randomised to receive intervention and contributed data to the intention to treat analysis.

The baseline features of the patients enrolled in the six groups were similar (table 2). The total duration of diarrhoea was significantly lower in children receiving *Lactobacillus* GG (group 2) and in those receiving the bacterial mix (group 5) than in patients receiving oral rehydration alone (group 1) (table 3). The three other probiotic preparations had no effect on diarrhoea, and the duration of diarrhoea in groups 3, 4, and 6 was similar to that in the group receiving only oral rehydration (table 3). Daily stool output was significantly lower (P<0.001) in groups 2 and 5 (table 4), starting the day after the first probiotic administration. Median stool outputs per day did not differ between groups 2 and 5 (table 4). Stool consistency, as judged by the scoring system, differed significantly (P<0.001) with preparations 2 and 5 versus the other groups (table 5). The median daily scores did not differ between groups 2 and 5 (table 5). Microbiological investigations were requested in only a few instances, and the results did not provide useful information.

None of the secondary outcome measures we evaluated was significantly modified in children receiving probiotic preparations or in the control group (table 6). All parents purchased the product indicated by the

paediatrician. The probiotic preparations included in the study were well received by nearly all the children, and no adverse events were observed.

## DISCUSSION

### Main study findings

In an evaluation of five probiotic preparations in children with diarrhoea we found substantial differences in efficacy. Two preparations reduced the duration and severity of diarrhoea, whereas the three others had no significant effect. A recent Cochrane meta-analysis of 23 randomised controlled trials found mild therapeutic benefit from probiotics that was generally reproducible regardless of organism.<sup>10</sup> In the only comparative trial reported previously, three preparations were tested in 46 children.<sup>17</sup>

Acute infectious diarrhoea is still a major cause of childhood morbidity. It is also a source of anxiety to families of affected children and represents a heavy economic burden for families and for society as a whole.<sup>18,19</sup> Drugs that affect intestinal motility, ion transport and adsorptive moieties, and living bacteria have been used in the attempt to reduce the duration of diarrhoea.<sup>18-20</sup> Probiotics have progressively gained in credibility for the treatment of diarrhoeal diseases.<sup>2,21,22</sup> In most countries, however, micro-organisms purported to have probiotic properties are considered to be food additives rather than drugs. Consequently, only safety features and not proof of efficacy are required for marketing.<sup>23</sup> In addition, the term

**Table 5** | Primary outcome index: median (IQR) daily stool consistency score from first day of probiotic administration

Group	Treatment	Day						
		1	2	3	4	5	6	7
1	Oral rehydration solution alone	3 (3-3)	3 (2-3)	2 (2-3)	2 (1-3)	2 (1-2)	1 (1-1)	1 (1-1)
2	<i>Lactobacillus casei</i> subsp <i>rhamnosus</i> GG‡	3 (3-3)	2 (2-3)†	1 (1-2)†	1 (1-2)†	1 (1-1)†	1 (1-1)	1 (1-1)
3	<i>Saccharomyces boulardii</i>	3 (3-3)	3 (2-3)	2 (2-3)	2 (2-3)	2 (1-2)	1 (1-2)	1 (1-1)
4	<i>Bacillus clausii</i>	3 (3-3)	3 (2-3)	2 (2-3)	2 (2-3)	2 (1-2)	1 (1-2)	1 (1-1)
5	<i>L delbrueckii</i> var <i>bulgaricus</i> , <i>L acidophilus</i> , <i>S thermophilus</i> , <i>B bifidum</i> ‡	3 (3-3)	2 (2-3)§	1 (1-2)†	1 (1-2)†	1 (1-1)†	1 (1-1)	1 (1-1)
6	<i>Enterococcus faecium</i> SF 68	3 (3-3)	3 (2-3)	2 (2-3)	2 (1-2)	2 (1-2)	1 (1-2)	1 (1-1)

IQR=interquartile range.

\*Stool consistency score system: 1=normal; 2=loose; 3=semiliquid; 4=liquid.<sup>15</sup>

†P&lt;0.001 or §P=0.001 v oral rehydration solution alone at same time point by Mann-Whitney U test.

‡On days 2 and 5 estimated difference (95% CI) compared with oral rehydration solution alone was 0 (-1 to 0); on days 3 and 4 it was -1 (-1 to 0).

**Table 6** | Secondary outcomes in children with diarrhoea according to treatment group

Group	1	2	3	4	5	6	P value
No (%) admitted to hospital	4 (4.3)	1 (1.0)	4 (4.4)	4 (4.0)	2 (2.1)	4 (4.4)	0.68*
<b>Fever:</b>							
No (%) of children	32 (34.8)	30 (30.0)	27 (29.7)	29 (29.0)	30 (30.9)	36 (39.6)	0.61*
Median (IQR) duration (days)	2 (1-2)	1 (1-2)	2 (1.7-2)	2 (1-3)	1 (1-3)	2 (1-3)	0.10†
<b>Vomiting:</b>							
No (%) of children	34 (37.0)	31 (31.0)	24 (26.4)	32 (32.0)	34 (35.1)	36 (39.6)	0.47*
Median (IQR) duration (days)	2 (1-2)	1 (1-2)	2 (1.0)	1.5 (1-2)	1 (1-2)	1.5 (1-2)	0.25†

IQR=interquartile range.

\* $\chi^2$  test.

†Mann-Whitney U test.

“probiotic” is often improperly used and information about specific probiotic properties of the strains contained in the products are not exhaustive.<sup>24</sup> We did not conduct a qualitative and quantitative study of the microbial content of the probiotic preparations in this trial because we wanted to carry out a field trial of the clinical effectiveness of commercially available probiotic products that had been prescribed by the paediatrician.

*Lactobacillus* GG was associated with a shorter duration of diarrhoea, which was not unexpected because proof of efficacy of this strain has been obtained in children in hospitals and outpatients in both industrialised and developing countries.<sup>2,15,25-27</sup> The results that we obtained with *Lactobacillus* GG closely resembled those obtained in a similar setting with the same strain.<sup>15</sup> The other effective preparation was a mix of four strains. A formula with *St thermophilus* and *B bifidum*, two of the four bacterial species in the effective preparation, protected against diarrhoea in chronically sick children aged below 24 months.<sup>5</sup> The three other preparations we evaluated had no or little clinical effect. This was unexpected in the case of *S boulardii* because a previous controlled trial showed it to be beneficial in children admitted to hospital for diarrhoea<sup>28</sup>—that is, with a more severe condition than the mild to moderate diarrhoea in the children in our trial—which could explain the different results obtained in the two studies. A previous trial with *Streptococcus faecium* strain SF68 resulted in clinical improvement in children with diarrhoea associated with respiratory infection and treated with parenteral antibiotics,<sup>29</sup> though it had no effect in adults with diarrhoea.<sup>30</sup> Finally, the *B clausii* preparation had no effect. None of the preparations had a significant effect on secondary outcomes, probably because of the relatively low incidence of fever, vomiting, and hospital admissions in our children. No side effects were recorded.

Diarrhoea in developed countries is usually self limiting, and active treatment is not generally recommended. Over the counter drugs or preparations, however, are widely used. We did not consider the cause of the diarrhoea. Probiotics are generally prescribed without a specific indication. All the children enrolled in our study were outpatients, and microbiological investigations were performed only in a few.

Based on the findings of a large study conducted in Italy,<sup>31</sup> it is reasonable to assume that most of the children were affected by viral acute gastroenteritis.

#### Possible confounding

We cannot exclude the possibility that expectations of parents confounded our results. The brands investigated were among the most widely used probiotic preparations in Italy when the study was conducted. These products were not advertised in the press or on television, there were no remarkable differences in their brand image, and they were available only in pharmacies at the time of the study. Preconceptions within the general public about their efficacy are therefore unlikely. Similarly, in Italy it is unlikely that the public would perceive one micro-organism to be more effective than another. The lack of preconceptions about the efficacy of treatment seems to be supported by our finding that only one of the two most widely used probiotic brands in Italy was effective (group 2) whereas the other was not (group 4). A high or low cost might have affected expectations either positively or negatively, but parents were probably unaware of the comparative costs of the products investigated. From these observations, it seems unlikely that parents advised to purchase one product would have higher or lower expectations than parents assigned to purchase another product, though we cannot exclude this.

In conclusion, the efficacy of probiotic preparations for the treatment of childhood acute diarrhoea is related to the individual strains of bacteria. We believe that probiotic preparations should be classified as drugs, and physicians should select preparations for which evidence of efficacy, in a given clinical condition, is supported by solid data.

#### WHAT IS ALREADY KNOWN ON THIS TOPIC

Several probiotic products are marketed in many European countries for the treatment of acute diarrhoea in children

#### WHAT THIS STUDY ADDS

The efficacy of probiotic preparations for the treatment of children with acute diarrhoea is related to the strain of bacteria



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**Contributors:** RBC and AG designed the study, coordinated the research team, and wrote the first draft of the report. PC, FA, LC, MIS, ADeV, and AP cared for the patients and contributed to the final version of the report. GDeM and GT performed the data analysis and FM contributed to the design of the study, developed the computer generated randomisation list, and performed statistical analyses. AG is guarantor.

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