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Randomised clinical trial: *Lactobacillus reuteri* DSM 17938 vs. placebo in children with acute diarrhoea - a double-blind study

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SUMMARY

Background
Probiotics may be of help for the management of acute diarrhoea, however, the effect is strain specific and efficacy needs to be proven.

Aim
To test the efficacy and safety of *Lactobacillus reuteri* DSM 17938 derived from *L. reuteri* ATCC 55730 in children with acute diarrhoea. Primary outcomes were the rate of unresolved diarrhoea after 3 days of treatment and duration of diarrhoea.

Methods
Children (6–36 months), hospitalised in three paediatric hospitals in Southern Italy for acute diarrhoea with clinical signs of dehydration were randomised to receive in a double-blind fashion either *L. reuteri* (dose of 4×10^8 colony-forming units/die) or placebo.

Results
Out of 96 eligible children, 74 were enrolled, five patients were withdrawn; 35 in the *L. reuteri* group and 34 in the placebo group. *Lactobacillus reuteri* significantly reduced the duration of watery diarrhoea as compared with placebo (2.1 ± 1.7 days vs. 3.3 ± 2.1 days; $P < 0.03$); on day two and three of treatment watery diarrhoea persisted in 82% and 74% of the placebo and 55% and 45% of the *L. reuteri* recipients respectively ($P < 0.01$; $P < 0.03$). Finally, children receiving *L. reuteri* had a significantly lower relapse rate of diarrhoea (15% vs. 42%; $P < 0.03$). There was not a significant difference in hospital stay between the groups. No adverse events were recorded.

Conclusion
Our study shows that *L. reuteri* DSM 17938 as an adjunct to rehydration therapy is efficacious in the treatment of acute diarrhoea reducing the frequency, duration and recrudescence rate of the disease.

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INTRODUCTION

Acute gastroenteritis is generally defined as a decrease in the consistency of stools (loose or liquid) and/or an increase in the frequency of evacuations (typically more than 3 in 24 h), with or without fever or vomiting. Diarrhoea typically lasts less than 7 days and not longer than 14 days.[1]

The incidence of acute diarrhoea ranges from 0.5 to 1.9 episodes per child per year in children younger than 3 years old in Europe, with Rotavirus being the most frequent agent.[1]

Oral rehydration therapies (ORT) are the mainstay of management of acute diarrhoea.[1, 2] Although its composition continues to improve, the oral glucose-electrolyte rehydration solution (ORS) recommended by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and United Nations Children's Fund neither shortens the duration of the illness nor reduces the stool loss and may in fact increase the stool volume at least during the first hours in children with acute diarrhoea.[3]

Probiotics may be an effective adjunct to the management of acute diarrhoea and a recent meta-analysis has shown that used alongside rehydration therapy, probiotics appear to be safe and have clear beneficial effects in shortening the duration and reducing stool frequency in acute infectious diarrhoea.[4] However, more research is needed to guide the use of particular probiotic regimens and strain[1, 2, 4] and as there is still no evidence of efficacy for many preparations, the ESPGHAN and the National Institute for Health and Clinical Excellence have suggested the use of probiotic strains with proven efficacy and in appropriate doses for the management of children with acute gastroenteritis as an adjunct to rehydration therapy.[1, 2]

Previously, in two separate prospective randomised trials *Lactobacillus reuteri* (*L. reuteri*) ATCC 55730 was shown to effectively colonise the gastrointestinal tract and to significantly shorten the duration of watery diarrhoea associated with rotavirus infection.[5, 6] Recently, this strain was found to carry specific, unusual, potentially transferable resistance traits for tetracycline and lincomycin, which led to the development of a new daughter strain, *L. reuteri* DSM 17938 derived from *L. reuteri* ATCC 55730 by the natural removal of these unwanted plasmid-borne resistances.[7] The daughter strain retained the probiotic properties and its safety and tolerance in adults.[7]

The aim of the present study was to assess the efficacy of this new strain of *L. reuteri* DSM 17938 as an adjunct to rehydration therapy in the treatment of children hospitalised with acute diarrhoea.

METHODS

Study design and population

This is a randomised, double-blind study carried out in three paediatric disease wards in Southern Italy (Bari and Catania) during the viral epidemic season (Rotavirus, Adenovirus) from January to July 2009.

Children (6–36 months old), hospitalised with acute diarrhoea (2 or more consecutive watery stools during 24 h, with duration of no more than 7 days) with clinical signs of mild to moderate dehydration (prolonged capillary refill time, abnormal skin turgor and percentage loss of body weight)[1] but no clinical features of hypovolemic shock, were considered eligible for inclusion.

Exclusion criteria were: (i) underlying chronic disease, (ii) bloody stools at the moment of first examination, (iii) current use of antibiotic/probiotic/antidiarrhoeal medication, (iv) demonstration of a bacterial cause for diarrhoea and (v) need of parenteral rehydration. During the study period ingestion of fermented milk products containing live bacteria was not allowed. The institutional review board approved the study. Written informed consent was obtained from parents of children.

On admission children were weighed, examined clinically and the degree of dehydration assessed. All children were assigned consecutive numbers, starting with the lowest number available, and were randomly assigned with the use of a computer generated randomisation list, to receive orally either *L. reuteri* (dose of 4×10^8 colony-forming units per day) or placebo. Enrolled children were entered sequentially to receive the assigned treatment. The first dose was given immediately after informed consent had been obtained. The study preparation was administered for 7 days. Before start of treatment, one stool sample for the detection of bacterial pathogens, adenovirus and rotavirus, was collected.

Diarrhoea was defined as the presence of watery stools in any 24-h period, and conversely the end of episode was defined as the first 24-h period without watery stools. Information on diarrhoea, vomiting or other clinical symptoms was recorded on a diary for 1 week.

This trial was not registered in a publicly accessible registry. The Ethical Committee approved the study.

Rehydration

Dehydration was corrected using the oral rehydration solution (ORS) according to WHO recommended formulation.[6] Each child was given approximately 100 mL/kg of ORS during the first 4 h administered in frequent sips

using a spoon or by nasogastric tubes if not tolerated. After the first 4 h, faecal losses were replaced on a volume to volume basis until diarrhoea ceased.

Study preparations

The *L. reuteri* and placebo formulations were prepared, quality controlled and assured by distributor and manufacturer (NOOS, Rome, Italy; Biogaia, Stockholm, Sweden). Active study product consisted of a suspension of freeze-dried *L. reuteri* DSM 17938 in a mixture of sunflower oil and medium-chain triglyceride oil supplied in a 5-mL dark bottle fitted with a dropper cap. Both formulations were administered in five drops twice daily. Bottles containing placebo had the same shape, dimension, indication and appearance as those containing the viable *L. reuteri* but without the live bacteria and were provided by the probiotic producer, which ensured that the study was blinded for investigators and patients. All treatment preparations were kept at 4 °C until the day of use. Group assignment was concealed from participants and investigators. The code was revealed after the study and the statistical analyses were completed. Adherence was ensured by delivery of the product by nurses at the bed of the patients. Vomiting in the hour after product administration was considered noncompliancy.

Outcome measures

Primary outcomes were: (i) the rate of unresolved diarrhoea after 3 days of treatment (proportion of patients in each study group with continuing diarrhoea) and (ii) duration of diarrhoea (time in hours from admission until cessation of diarrhoea). Secondary outcomes were: (i) the duration of hospitalisation (time in hours from admission until discharge from hospital) and (ii) total intake of oral rehydration solution (volume of ORS taken from admission until cessation of diarrhoea expressed in millilitres per kilogram of body weight).

Laboratory methods

Concentrations of serum sodium, potassium, blood acid-base analysis, Rotavirus and Adenovirus antigens were determined in the hospital laboratory using an immunochromatographic diagnostic system test (Combi–Strip; Coris BioConcept, Gembloux, Belgium).

Stool specimens were cultured by routine microbiological methods for bacterial pathogens that are endemic in our area, i.e. Salmonella spp., Shigella spp., *Yersinia enterocolitica*, Campylobacter spp. and *Escherichia coli* (enteropathogenic and enterotoxigenic).

Statistical methods

With the assumption of mean difference in duration of diarrhoea of 1 day (24 h) considering a difference of 30% (40% vs. 70%) in the rate of resolution of diarrhoea at day 3 between the treated and control children we calculated that a sample of 34 children per group would be required for the study to have 80% power with a type 1 error = 0.05 (two tailed test). This assumption is based on the results of similar trials. Statistical analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Variables were tested for normal distribution and compared using the Mann–Whitney *U*-test and χ^2 or Fisher's exact tests, as appropriate. Only an intention-to-treat analysis was performed. Statistical significance was accepted at $P < 0.05$.

RESULTS

Of 96 patients enrolled, nine (11%) had started a probiotic/antibiotic treatment before admission, seven (8%) received exclusively or mainly intravenous fluids for rehydration, in three (2%) a bacterial origin was identified (two patients Campylobacter, and in 1 Salmonella spp.) and three (2%) refused to participate therefore, a total of 74 patients were enrolled into the study, 37 in the *L. reuteri* group and 37 in the control group. Five patients, two from the *L. reuteri* group and three from the control group, were withdrawn from the study (Figure 1) because of either one of the following reasons: parental noncompliance (three patients); protocol deviations (two patients). Sixty-nine children, mean age 23.5 months, completed the study (Figure 1).

Microbiologic analyses

Data are available in 64 children; in 10 children with prompt recovery, additional test would have not been justified on the basis of routine clinical care. Rotavirus antigen was identified in 43 patients (62%) and adenovirus in 10 (14%) while in 11 (24%) no aetiology was found. No significant differences regarding age, gender or duration of diarrhoea before intervention were found between the study groups. The clinical characteristics and severity of gastroenteritis did not differ between treatment group and control group.

Baseline characteristics

The clinical characteristics of the treatment groups are presented in the Table 1. Both groups were similar in age, nutritional status, duration of diarrhoea before admission, stool output (first 4 h of the rehydration phase), percentage of dehydration and ethology. On

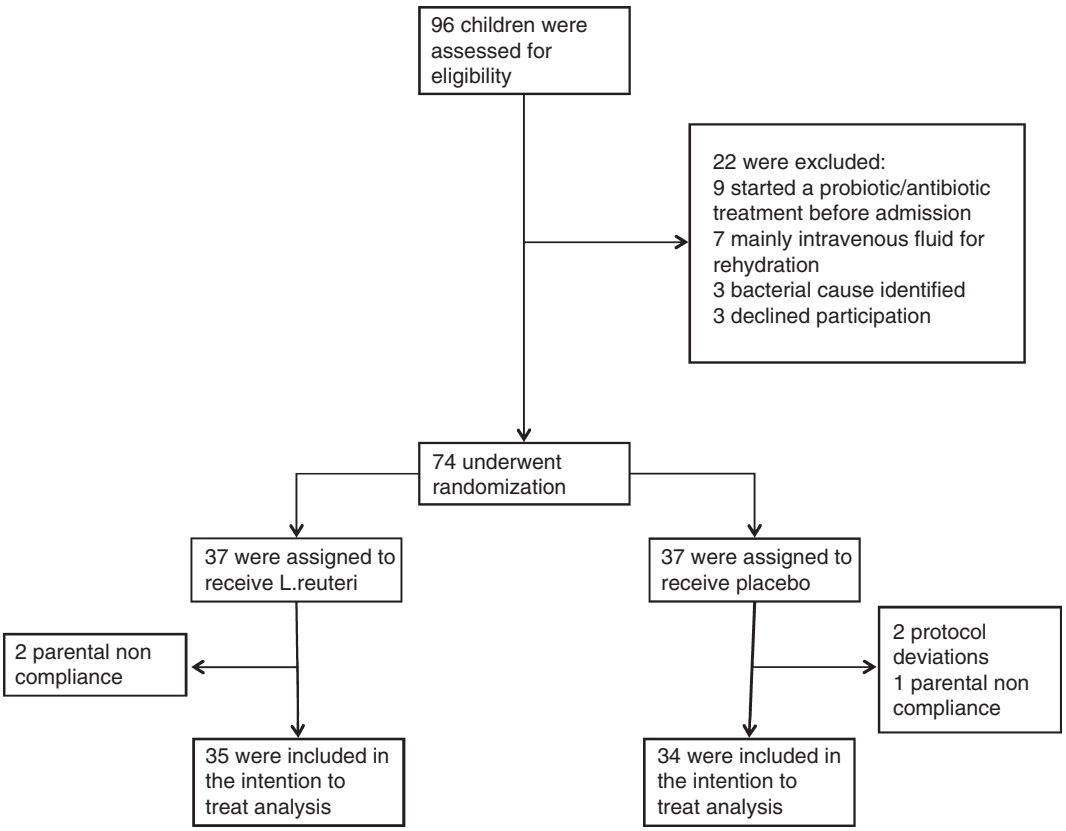

Figure 1 | Enrolment, assigned, intervention and follow-up.

admission most patients had mild dehydration. The serum sodium was between 130 and 144 mmol/L, with a mean of 138 mmol/L. The degree of dehydration in rotavirus-positive children was not significantly more severe than in negative patients.

No patient was lost in follow-up before stool consistency had normalised; no patient was excluded from the analysis.

Outcomes

The clinical outcome of the two treatment arms was similar for weight gain (190 ± 152 g), consumption of ORS solution (310.5 ± 215.3 mL/kg) correction of acidosis (base deficit -0.9 ± 1.5 mmol/L) and serum Na (138 ± 2 mmol/L).

Days 0–7 were calculated as 24-h periods after the initiation of treatment. *L. reuteri* significantly reduced the duration of watery diarrhoea as compared with placebo (2.1 ± 1.7 vs. 3.3 ± 2.1 days; $P < 0.03$) and the effect of *L. reuteri* was mostly seen in the second/third day of treatment (Figure 2). The intention-to-treat analysis shows that on the first day of treatment, watery diarrhoea persisted in 100% of those receiving placebo and in 87% of those receiving *L. reuteri*; on the second day of treatment watery diarrhoea persisted in 81% of the placebo and 55% of the *L. reuteri* recipients ($P < 0.02$). The frequency of watery diarrhoea per 24-h period was still significantly reduced on the third day of treatment in the *L. reuteri* recipients as compared with placebo recipients (73% vs. 46%; $P < 0.03$). Therefore, the number of children with a normalised stool consistency was significantly higher on day 2 and day 3 in the *L. reuteri* group. The per protocol analysis is reported in Table 2.

The mean (±s.d.) frequencies of watery diarrhoea in placebo and *L. reuteri* groups at day 1, 2, 3 and 4 were 7.2 (2.4) vs. 6.9 (3.2), 6.3 (2.1) vs. 4.3 (1.7), 4.3 (2.3) vs. 2.1 (1.8) and 1.8 (1.3) vs. 1.5 (0.9) (P = N.S.; $P < 0.02$; $P < 0.03$; P = N.S. respectively).

Fewer patients receiving *L. reuteri*, compared with those receiving placebo, had vomiting, starting from the second day of treatment (35% vs. 55%; $P = 0.16$).

Side effects were not reported by the parents or physicians and none was excluded for low compliance. There was not a significant difference in hospital stay between the groups.

Table 1 | Demographic characteristics of children who completed the study

	L. reuteri (*n*: 37)	Placebo (*n*: 37)	*P*
Age (months)	26.1 ± 4.1	25.4 ± 2.1	N.S.
Male/Female	23/14	22/15	N.S.
Duration diarrhoea before admission (days)	1.5 (1–4)	1.5 (1–5)	N.S.
No. of stool motions 24 h before admission	5.6 ± 4.5	6.4 ± 5.1	N.S.
Rotavirus/Adenovirus/no aetiology found	22/6/9	22/5/10	N.S.
Vomiting	24 (65%)	24 (65%)	N.S.
Fever (>38.5 °C)	19 (51%)	18 (49%)	N.S.
Watery diarrhoea on admission	37 (100%)	37 (100%)	N.S.
Dehydration (mild/moderate/severe)	25/12/0	26/11/0	N.S.

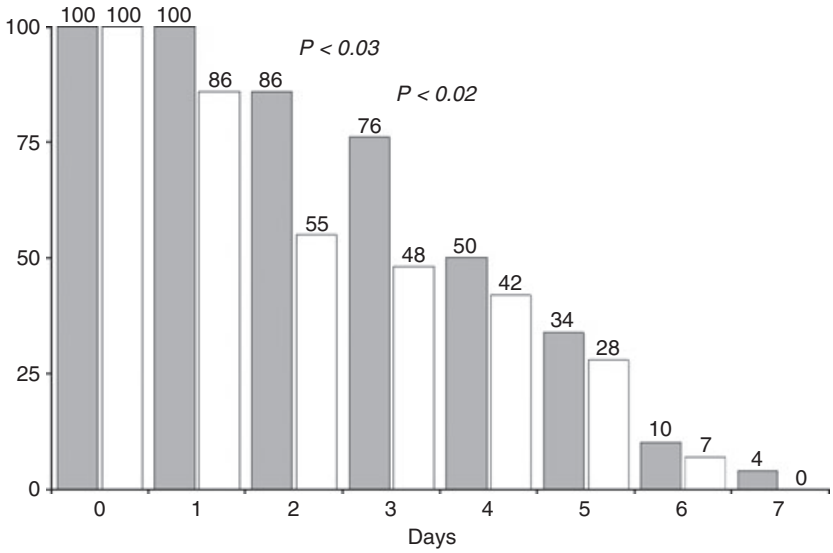

Figure 2 | Percentage of patients with persisting watery diarrhoea in the groups receiving placebo (grey) and *Lactobacillus reuteri* (white).

DISCUSSION

Our study shows that *L. reuteri* DSM 17938 as an adjunct to rehydration therapy is effective in the treatment of acute diarrhoea reducing the frequency and duration, of the disease.

Viewed from a global perspective, gastroenteritis in children is of enormous public health importance.[2] In the 1970s, there were almost 5 million childhood deaths worldwide from gastroenteritis each year. The use of ORT, contributed to a marked reduction in this death rate.[2] Nevertheless, gastroenteritis still causes between 1.6 and 2.6 million deaths in children younger than 5 years each year[8] and even in developed countries, at least 30% of hospital admissions for acute gastroenteritis in young children are the result of Rotavirus infection.[9, 10]

The research of new therapeutic strategies for the treatment of acute diarrhoea suggested the hypothesis that probiotics may play a role.[1, 2, 4, 5] According to the currently adopted definition by FAO/WHO, probiotics are 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'.[11] Microorganisms most commonly used in clinical practice are lactic acid-producing bacteria such as Lactobacillus spp., and Bifidobacteria.[11]

Much research has been directed towards examining the potential benefit of a variety of probiotics in the treatment of infectious gastroenteritis. A recent Cochrane review including 56 trials in children concluded that specific probiotics are able to reduce the duration of diarrhoea of about 24 h and the frequency of stools was decreased on the second day 2.[4] There is evidence suggesting that lactobacilli [*Lactobacillus rhamnosus* GG, *L. reuteri* (ATCC 55730), *Lactobacillus acidophilus* LB], *Saccharomyces boulardii* and a mixture of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* have the best therapeutic effect for this particular indication.[12–15]

Shornikova evaluated *L. reuteri* in two separate trials and showed a significantly shortened duration of watery diarrhoea associated with its use with higher efficacy for higher doses (10^{10}–10^{11} CFU/day).[5, 6] In our study we decided to use the new strain of *L. reuteri* DSM 17938 to assess if it still retains the same therapeutic activity of the parental strain ATCC 55730. The present study

Table 2 | Number and percent (%) of patients with watery diarrhoea at days of treatment (per protocol analysis)

Day	*L. reuteri* (*n*: 35)	Placebo (*n*: 34)	*P*
0	35 (100)	34 (100)	N.S.
1	31 (91)	34 (100)	N.S.
2	19 (54)	28 (82)	<0.01
3	16 (46)	25 (74)	<0.03
4	14 (40)	17 (50)	N.S.
5	9 (26)	11 (32)	N.S.
6	2 (6)	3 (9)	N.S.
7	0	1 (3)	N.S.

demonstrates that children receiving *L. reuteri* DSM 17938 on days 2 and 3 were statistically significantly more likely to be diarrhoea-free and passed significantly fewer stools compared with those receiving placebo.

The possible mechanisms by which *L. reuteri* DSM 17938 may exert its beneficial effect include (i) the production of a broad-spectrum antimicrobial substance, called *reuterin*,[16] (which may be responsible for the inhibition of pathogenic microorganisms in the gastrointestinal tract); (ii) competition with pathogens for binding sites and substrates; (iii) stabilisation of the mucosal barrier with a decrease in intestinal permeability and (iv) stimulation of intestinal immune responses.[12, 16–18]

We are aware of some limits of the present study such as the small sample size, that, however, was appropriate for the statistical power and the fact that children were admitted in hospital and *L. reuteri* therapy was started at a relatively late stage of diarrhoea. Indeed it has been suggested[5] that probiotics might be more effective when given early when patients might be less ill, however, if this is the case, the efficacy of *L. reuteri* might have been even more pronounced if administered as early as possible.

Strengths of our study are the demonstration that *L. reuteri* DSM 17938 derived from ATCC 55730 is efficacious and safe for the sake of all physicians and parents who will prescribe and use this probiotic and the fact that children included in this study (6 months to 3 years) probably represent the most severe type of patients with diarrheal disease presenting with acute watery diarrhoea and signs of mild to moderate dehydration, thus, we can argue that the results of this study can be applied to patients with less severe diarrhoea.

Lactobacillus reuteri has been extensively studied and is widely used as a food additive to improve human gastrointestinal health. Oral administration delivers *L. reuteri* to the gastrointestinal tract, leading to shedding of live bacteria in the faeces.[19, 20] Clinical trials have shown that *L. reuteri* administration is safe both in adults and children[5, 6, 21–24] and we recently demonstrated its efficacy on the prevention of antibiotic-associated diarrhoea during anti-*Helicobacter pylori* treatment.[22] The bioequivalence of *L. reuteri* DSM 17938 with parent strain *L. reuteri* ATCC 55730 has been demonstrated in recent clinical trials on faecal recovery of live bacteria,[7] infantile colic[19] and gastrointestinal function in infants.[20] The present study provides an evidence of equivalence in acute diarrhoea in comparison with earlier studies.

In conclusion, the present study confirms that *L. reuteri* DSM 17938, as the parental strain ATCC 55730, is efficacious and safe alongside with rehydration therapy, shortening the duration and reducing stool frequency in acute infectious diarrhoea in young children.

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