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Lactobacillus reuteri DSM 17938 in the prevention of antibiotic-associated diarrhoea in children: protocol of a randomised controlled trial

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ABSTRACT

Introduction: Administration of some probiotics appears to reduce the risk of antibiotic-associated diarrhoea (AAD). The effects of probiotics are strain-specific, thus, the efficacy and safety of each probiotic strain should be established separately. We aim to assess the effects of *Lactobacillus reuteri* DSM 17938 administration for the prevention of diarrhoea and AAD in children.

Methods and analysis: A total of 250 children younger than 18 years treated with antibiotics will be enrolled in a double-blind, randomised, placebo-controlled trial in which they will additionally receive *L. reuteri* DSM 17938 at a dose 10^8 colony-forming units or an identically appearing placebo, orally, twice daily, for the entire duration of antibiotic treatment. The primary outcome measures will be the frequencies of diarrhoea and AAD. Diarrhoea will be defined according to 1 of 3 definitions: (1) ≥3 loose or watery stools per day for a minimum of 48 hours during antibiotic treatment; (2) ≥3 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment; or (3) ≥2 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment. AAD will be diagnosed in cases of diarrhoea, defined clinically as above, caused by *Clostridium difficile* or for otherwise unexplained diarrhoea (ie, negative laboratory stool tests for infectious agents).

Ethics and dissemination: The Bioethics Committee approved the study protocol. The findings of this trial will be submitted to a peer-reviewed paediatric journal. Abstracts will be submitted to relevant national and international conferences.

Trial registration number: NCT02871908.

Strengths and limitations of this study

- The study design (randomised controlled trial, RCT) is the gold standard research design to assess the effectiveness of healthcare interventions.
- A precise clinical question has been posed to fill a gap in knowledge as to whether administration of *Lactobacillus reuteri* DSM 17938 is effective in the prevention of antibiotic-associated diarrhoea (AAD) in children.
- The findings of this RCT, whether positive or negative, will contribute to the formulation of recommendations on the use of *L. reuteri* DSM 17938 during antibiotic treatment.
- The frequency of AAD may be lower than expected.
- There is no single, generally accepted definition of AAD.

INTRODUCTION

Antibiotic-associated diarrhoea (AAD) is defined as unexplained diarrhoea that occurs in association with antibiotic therapy.[1] The prevalence of AAD varies depending on the criteria used to diagnose it; however, it is estimated at 5–30%.[2 3] AAD may occur just a few hours after antibiotic administration or up to several months after its discontinuation,[4] and it is associated with increased costs and hospital length of stay.[5] One of the potential mechanisms by which antibiotics cause diarrhoea is a direct effect of the antibiotics on the intestinal mucosa. As a consequence, alterations in the gut microbiota composition and overgrowth of pathogens, primarily by *Clostridium difficile*, but also *Staphylococcus*, *Candida*, *Enterobacteriaceae* and *Klebsiella* may occur.[6] However, often the mechanism(s) by which antibiotics cause diarrhoea remain unclear. The clinical presentation of AAD varies from mild diarrhoea to colitis or fulminant pseudomembranous colitis.[7] Preventive measures to reduce the risk of AAD include the use of probiotics.[8]

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'.[9] The rationale for the use of probiotics is based on the assumption that AAD results from the disruption of the commensal gut microbiota caused by antibiotic therapy.[10] Available evidence documents that the

administration of some probiotics significantly reduces the risk of AAD.[8] Examples of probiotics with proven efficacy include *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii*.[11 12] However, in line with the position of the Working Group on Probiotics of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, the effects of probiotics are strain-specific, thus, the efficacy and safety of each probiotic strain should be established separately.[8]

L. reuteri DSM 17938 is a Gram-positive bacterium that naturally inhabits the gut of mammals. First described in the early 1980s, it has been safely used in infants and adults.[13] One randomised controlled trial (RCT) evaluated the efficacy of *L. reuteri* DSM 17938 at a dose of 10^8 colony-forming units (CFU) for the prevention of AAD (defined as at least three loose or watery stools per day in a 48-hour period that occurred during or up to 21 days after cessation of antibiotic treatment) in 97 hospitalised children.[14] No significant difference in the risk of AAD was found between the placebo group and the group receiving *L. reuteri* DSM 17938. However, the overall frequency of diarrhoea was surprisingly low (one case in each study group). Thus, the efficacy of *L. reuteri* DSM 17938 for preventing AAD remains unclear.

Trial objectives and hypothesis

We aim to assess the effectiveness and safety of *L. reuteri* DSM 17938 administration for the prevention of diarrhoea and AAD in children. We hypothesise that children who receive *L. reuteri* DSM 17938 during the antibiotic therapy will have a lower risk of AAD than children receiving a placebo.

METHODS AND ANALYSIS

The trial is registered at ClinicalTrials.gov (NCT02871908) and any important changes in the protocol will be implemented there.

Trial design

This study is designed as a randomised, double-blind, placebo-controlled trial, with allocation of 1:1.

Settings and participants

The recruitment will take place in two hospitals in Poland (paediatric academic hospital in Warsaw and community hospital in Łuków). We aim to recruit hospitalised children in general paediatric wards. However, inclusion of outpatients and involvement of other recruiting wards and/or sites are under consideration provided that the personnel are adequately trained and competent in conducting clinical trials. The start of the recruitment is planned in December 2016 and should be completed within the following 2 years.

Eligibility criteria

Children eligible for the trial must fulfil all of the following criteria: age younger than 18 years; oral or intravenous antibiotic therapy which started within 24 hours of enrolment; signed informed consent.

Children will be excluded for the following reasons: pre-existing acute or chronic diarrhoea, history of chronic gastrointestinal disease (eg, inflammatory bowel disease, cystic fibrosis, coeliac disease, food allergy) or other severe chronic disease (eg, neoplastic diseases), immunodeficiency, use of probiotics within 2 weeks prior to enrolment, use of antibiotics within 4 weeks prior to enrolment, prematurity, and exclusive breast feeding.

Interventions

The intervention under investigation will be administration of *L. reuteri* DSM 17938. The placebo drops consist of a mixture of pharmaceutical grade medium chain triglycerides and sunflower oil together with pharmaceutical grade silicon dioxide to give the product the correct rheological properties. The formulation is identical with the active product but without *L. reuteri* DSM 17938. In our trial, we choose to use a placebo for a comparator, as it is widely regarded as the gold standard for testing the efficacy of new treatments.[15] The study products (*L. reuteri* DSM 17938 and placebo) will be manufactured and supplied by BioGaia (Lund, Sweden) free of charge. The manufacturer will have no role in the conception, protocol development, design or conduct of the study, or in the analysis or interpretation of the data.

Study procedure

Caregivers will receive oral and written information regarding the study. Written informed consent will be obtained by the physicians involved in the study. Participants will be randomised after admission to the hospital and administration of antibiotic treatment. Eligible patients will receive either *L. reuteri* DSM 17938 at a dose of 10^8 CFU or placebo, orally, twice daily, in drops (ie, 2×5 drops), during the entire period of antibiotic treatment. Throughout the study period, healthcare providers and/or caregivers will record the number and consistency of stools in a standard stool diary. To record stool consistency, in children younger than 1 year, the Amsterdam Infant Stool Scale (AISS) will be used, and loose or watery stools will correspond to A-consistency.[16] In children older than 1 year, the Bristol Stool Form (BSF) scale will be used, and loose or watery stools will correspond to scores of 5–7.[17] In the case of missing or incomplete data, data from hospital charts will be obtained. At any time, caregivers will have the right to withdraw the participating child from the study; they will be not obliged to give reasons for this decision, and there will be no effect on subsequent physician and/or institutional medical care.

In the event of loose or watery stools, the presence of viral or bacterial pathogens in the stool samples will be investigated. The presence of viral pathogens will be checked by using a standard rapid, qualitative, chromatographic immunoassay that simultaneously detects

rotaviruses, adenoviruses and noroviruses. Standard microbiological techniques will be used to isolate and identify bacterial pathogens (*Salmonella* spp, *Shigella* spp, *Campylobacter* spp and *Yersinia* spp). *C. difficile* toxins A and B will be identified by standard enzyme immunoassay.

Follow-up

All study participants will be followed up for the duration of the intervention (antibiotic treatment) and then for up to 1 week after the intervention.

Compliance

In case of inpatients who will be discharged before the end of antibiotic therapy, and in outpatients, the caregivers will be asked to bring the remaining study product and diary to the study site at the end of the intervention period. Compliance with the study protocol will be assessed by direct interview with the patient and/or caregiver and by measuring the amount of the fluid left in the bottle, assuming that 1 mL equals 20 drops. Based on previously published trials, it seems to be appropriate to consider those participants receiving <75% of the recommended doses as non-compliant.

Concomitant medications

If needed, discontinuation or modification of the treatment may be considered at the discretion of the physician.

Outcome measures

As in previous studies carried out in our setting, the primary outcome measures will be the frequencies of diarrhoea and AAD.[18 19] Three different definitions of diarrhoea will be used, as the definitions of diarrhoea/AAD in published studies vary. These will include diarrhoea defined as: (1) ≥3 loose or watery stools per day for a minimum of 48 hours during antibiotic treatment; (2) ≥3 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment; and (3) ≥2 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment. AAD will be diagnosed in cases of diarrhoea, defined clinically as above, caused by *C. difficile* or for otherwise unexplained diarrhoea (ie, negative laboratory stool tests for infectious agents). In all cases, loose or watery stools will correspond to scores of 5–7 on the BSF scale or A-consistency on the AISS.

The secondary outcome measures will be as follows: infectious diarrhoea (rotavirus, adenovirus, norovirus, *Salmonella, Shigella, Campylobacter, Yersinia* and *C. difficile*), the need for discontinuation of the antibiotic treatment, the need for hospitalisation to manage the diarrhoea (in outpatients), the need for intravenous rehydration in any of the study groups, and adverse events.

Participant timeline

For the time schedule for enrolment, interventions, assessment and visits for the participants (table 1).

Sample size

The primary outcome of the study is the frequency of diarrhoea. Based on the data from studies previously conducted at Warsaw Medical University,[18] we assumed the frequency of AAD to be 23%. To detect a 15% difference between groups, with a power of 80% and a significance level of 5% and taking into account that 20% of the patients will be lost to follow-up, we have calculated that a total of 250 children will be needed. However, the frequency of AAD in earlier trials varied, depending on the definition of AAD used in the study.[19–21] Table 2 summarises sample size calculations depending on the definition used.

Recruitment

The recruitment rates will be monitored every month. In the case of poor or slow recruitment, the reasons at various levels, such as the patient, the recruiting clinician, the centre and the trial design, will be evaluated.

Sequence generation

A computer-generated randomisation list prepared by a person unrelated to the trial will be used to allocate participants to the study groups in variable blocks of eight. Consecutive randomisation numbers will be given to participants at enrolment. This procedure will be performed by a physician not involved in the study. The study products will be signed by consecutive numbers according to the randomisation list.

Allocation concealment

An independent person will dispense the numbered study products according to a computer-generated randomisation list. To ensure allocation concealment, allocation will be performed after getting informed consent and registering the basic demographic data to case report form (CRF).

Blinding

The active product and placebo will be packaged in identical bottles. Contents will look and taste the same. Researchers, caregivers, outcome assessors and a person responsible for the statistical analysis will be blinded to the intervention until the completion of the study. The information on intervention assignments will be stored in a sealed envelope in a safe in the administrative part of the department.

Data collection and management

All study participants will be assigned a study identification number. CRFs will be completed on paper forms. Data will then be entered and stored in a password-protected electronic database. The original paper copies of CRFs and all study data will be stored in a locker within the study site, accessible to the involved researchers only.

Table 1 Timetable of activities planned during the study

	Study period							
	Enrolment	Allocation	Postallocation Antibiotic therapy					Close-out (after the end of follow-up period)
Time point	Day 1	Day 1	Day 2	Day 3	Day 4	Day 5	Every day	
Enrolment								
Eligibility screen	X							
Informed consent	X							
Randomisation of the participant	X							
Study product distribution	X							
Diary of symptoms	X							
Interventions								
Lactobacillus reuteri DSM 17938								
Placebo								
Assessments								
Adverse events			X	X	X	X	X	X
Stool analysis in case of diarrhoea/AAD			X	X	X	X	X	X
Daily diary reporting			X	X	X	X	X	X
Telephone contact to check diary reporting and compliance in outpatients			X	X	X	X	X	X
Return of non-used study products								X

AAD, antibiotic-associated diarrhoea.

Table 2 Sample size calculations based on previously published studies

Definition of AAD	Control event rate (%)	Experimental event rate (%)	Sample size	Sample size including 20% lost to follow-up
≥3 loose or watery stools per day for a minimum of 48 hours during antibiotic treatment[18]	23	8	104+104	250
≥3 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment[22]	28.3	11.8	104+104	250
≥2 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment[20]	26	8	79+79	190

AAD, antibiotic-associated diarrhoea.

Statistical analysis

All analysis will be conducted on an intention-to-treat (ITT) basis, including all participants in the groups to which they are randomised for whom outcomes will be available (including dropouts and withdrawals). Additionally, per-protocol analysis will be performed, including all participants included in the ITT analysis, who participate in the study, without major protocol violations.

Descriptive statistics will be used to summarise baseline characteristics. The Student's t-test will be used to compare mean values of continuous variables approximating a normal distribution. For non-normally distributed variables, the Mann-Whitney U test will be used. The χ^2 test or Fisher's exact test will be used, as appropriate, to compare percentages. For continuous outcomes, differences in means or differences in medians (depending on the distribution of the data), and for dichotomous outcomes, the relative risk (RR) and number needed to treat, all with a 95% CI, will be calculated. The difference between study groups will be considered significant when the p value is <0.05, when the 95% CI for RR does not include 1.0 or when the 95% CI for mean difference does not include 0. All statistical tests will be two-tailed and performed at the 5% level of significance.

Monitoring

The study will be carried out in accordance with the approved protocol. *L. reuteri* DSM 17938 is being safely used worldwide for a number of indications, and the

Food and Drug Administration applied to it the Generally Recognized as Safe (GRAS) status.[22] Still, an independent Data and Safety Monitoring Board (DSMB) will be set up prior to the start of the study. The DSMB will review data after recruitment of 25%, 50% and 75% participants to review the study progress and all adverse events.

Harms

Although the occurrence of adverse events as a result of participation in the current trial is not expected, data on adverse events data will be collected. All serious adverse events will be immediately reported to the project leader who will be responsible for notifying the Ethics Committee, all participating investigators and the manufacturer of the study products.

Auditing

The Ethics Committee did not require auditing for this study.

ETHICS AND DISSEMINATION

Verbal and written information regarding informed consent will be presented to the caregivers. Any modifications to the protocol that may affect the conduct of the study will be presented to the Committee. The full protocol will be available freely due to open access publication. The findings of this RCT will be submitted to a peer-reviewed journal. Abstracts will be submitted to relevant national and international conferences. The standards from the guidelines of the Consolidated Standards of Reporting Trials (CONSORT) will be followed for this RCT.

Contributors HS conceptualised the study. MK developed the first draft of the manuscript. Both authors contributed to the development of the study protocol and approved the final draft of the manuscript. HS is the guarantor.

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Competing interests HS served as a speaker for BioGaia, the manufacturer of *Lactobacillus reuteri* DSM 17938.

Ethics approval The Ethics Committee of the Medical University of Warsaw approved the study before recruitment started.

Provenance and peer review Not commissioned; externally peer reviewed.

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