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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013928
Article Type:	Protocol
Date Submitted by the Author:	17-Aug-2016
Complete List of Authors:	Kołodziej, Maciej; The Medical University of Warsaw , Department of Paediatrics SZAJEWSKA, Hania; The Medical University of Warsaw, Dept of Paediatrics
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Paediatrics, Gastroenterology and hepatology
Keywords:	probiotics, antibiotics, diarrhea, Clostridium difficile, RCT

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

Maciej Kołodziej, Hania Szajewska

Department of Paediatrics, The Medical University of Warsaw, Poland

Corresponding author:
Prof. Hania Szajewska, MD
Department of Paediatrics, The Medical University of Warsaw
Żwirki i Wigury 63A, 02-091 Warsaw, Poland,
email: hania@ipgate.pl

Date and protocol version identifier: 12/08/2016

Key words: probiotics, antibiotics, diarrhea, *Clostridium difficile,* RCT

Number of tables: 2
Number of references: 23

Competing interests statement: HS served as a speaker for BioGaia, the manufacturer of *L reuteri* DSM 17938. MK declares no conflicts of interest.

Contributorship statement: HS conceptualized 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.

Funding statement: This study will be fully funded by The Medical University of Warsaw.

ABSTRACT

Introduction: Administration of some probiotics appears to reduce the risk of antibiotic-associated diarrhea (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 diarrhea 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, randomized, placebo-controlled trial in which they will additionally receive *L reuteri* DSM 17938 at a dose 2 x 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 diarrhea and AAD. Diarrhea will be defined according to one of 3 definitions: (a) ≥3 loose or watery stools per day for a minimum of 48 hours during antibiotic treatment; (b) ≥3 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment; or (c) ≥2 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment. AAD will be diagnosed in cases of diarrhea, defined clinically as above, caused by *Clostridium difficile* or for otherwise unexplained diarrhea (i.e., 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 pediatric journal. Abstracts will be submitted to relevant national and international conferences.

Trial registration number: The study protocol is in the process of being registered at ClinicalTrials.gov. [Number will be added]

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The study design (randomized 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 diarrhea (AAD) in children.
- The findings of this RCT, whether positive or negative, will contribute to the formulation of recommendations on the use of *Lactobacillus 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 diarrhea (AAD) is defined as unexplained diarrhea 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 diarrhea 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 diarrhea remain unclear. The clinical presentation of AAD varies from mild diarrhea 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, the effects of probiotics are strain specific, thus, the efficacy and safety of each probiotic strain should be established separately.[8]

Lactobacillus 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 RCT evaluated the efficacy of *L reuteri* DSM 17938 at a dose of 10^8 colony-forming units (CFU) for the prevention of AAD in 97 hospitalized 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 diarrhea was surprisingly low (1 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 diarrhea and AAD in children. We hypothesize 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 (will be added) and any important changes in the protocol will be implemented there.

Trial design

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

Settings and participants

The recruitment will take place in 2 hospitals in Poland (pediatric academic hospital in Warsaw and community hospital in Łuków). However, other recruiting 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 September 2016 and should be completed within the following 2 years.

Eligibility criteria

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

Children will be excluded for the following reasons: pre-existing acute or chronic diarrhea, history of chronic gastrointestinal disease (e.g., inflammatory bowel disease, cystic fibrosis, celiac disease, food allergy) or other severe chronic disease

(e.g., neoplastic diseases), immunodeficiency, use of probiotics within 2 weeks prior to enrollment, use of antibiotics within 4 weeks prior to enrollment, prematurity, and exclusive breastfeeding.

Interventions

The intervention under investigation will be administration of *L reuteri* DSM 17938. The placebo will be maltodextrin and silicon dioxide in oil suspension. 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 by obtained by physicians involved in the study. Participants will be randomized after admission to the hospital and administration of antibiotic treatment. Eligible patients will receive either *L reuteri* DSM 17938 at a dose of 2 x 10^8 CFU or placebo, orally, twice daily, in drops (i.e., 2 x 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 to 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. *Yersinia* spp.). *Clostridium 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

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 milliliter equals 20 drops. Based on previously published trials, it seems to be appropriate to consider those subjects receiving less than 75% of the recommended doses as noncompliant.

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 diarrhea and AAD.[18 19] Three different definitions of diarrhea will be used, as the definitions of diarrhea/AAD in published studies vary. These will include diarrhea defined as: (a) ≥3 loose or watery stools per day for a minimum of 48 hours during antibiotic treatment; (b) ≥3 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment, and (c) ≥2 loose or watery stools

per day for a minimum of 24 hours during antibiotic treatment. AAD will be diagnosed in cases of diarrhea, defined clinically as above, caused by *C. difficile* or for otherwise unexplained diarrhea (i.e., negative laboratory stool tests for infectious agents). In all cases, loose or watery stools will correspond to scores of 5 to 7 on the BSF scale or A-consistency on the AISS.

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

Participant timeline

For the time schedule for enrollment, interventions, assessment, and visits for the participants, see **Table 1.**

Table 1. Timetable of activities planned during the study.

	STUDY PERIOD							
	Enrollment	**Allocation**	**Post-allocation Antibiotic therapy**					**Close-out (After the end of follow-up period)**
TIMEPOINT**	*Day 1*	**Day 1**	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Every day*	
ENROLLMENT:								
Eligibility screen	X							
Informed consent	X							
Randomization of the subject	X							
Study product distribution	X							
Diary of symptoms	X							
INTERVENTIONS:								

L reuteri DSM 17938			←	—	—	—	→	
Placebo			←	—	—	—	→	
ASSESSMENTS:								
Adverse events			X	X	X	X	X	X
Stool analysis in case of diarrhea/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

Sample size

The primary outcome of the study is the frequency of diarrhea. 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% reduction, 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.[20 21 22] Table 2 summarizes sample size calculations depending on the definition used.

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	28.3%	11.8%	104 + 104	250

per day for a minimum of 24 hours during antibiotic treatment[22]				
≥2 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment[20]	26%	8%	79 + 79	190

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 center, and the trial design, will be evaluated.

Sequence generation

A computer-generated randomization list prepared by a person unrelated to the trial will be used to allocate subjects to the study groups in blocks of eight. Consecutive randomization numbers will be given to participants at enrollment. 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 randomization list.

Allocation concealment

An independent person will dispense the numbered study products according to a computer generated randomization list.

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 & management

All study participants will be assigned a study identification number. Case report forms (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 for the involved researchers only.

Statistical analysis

All analysis will be conducted on an intention-to-treat (ITT) basis, including all participants in the groups to which they are randomized 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 summarize baseline characteristics. The Student 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 $\chi 2$ test or Fisher 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% confidence interval, 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 (MD) 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. There will be no interim analysis, no stopping rules, and no data monitoring committee. This is because *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.[23]

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

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

FUNDING STATEMENT

This trial will be funded by The Medical University of Warsaw. At the time of submission of this protocol for publication, no specific grant from any funding agency in the public, commercial, or not-for-profit sectors has been awarded to this project.

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www.fda.gov/downloads/food/ingredientspackaginglabeling/gras/noticeinventory/ucm287952.pdf (accessed 02 Jan 2016).

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym √
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry √
	2b	All items from the World Health Organization Trial Registration Data Set X
Protocol version	3	Date and version identifier √
Funding	4	Sources and types of financial, material, and other support √
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors √
	5b	Name and contact information for the ~~trial sponsor~~ √
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities √
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) X
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention √
	6b	Explanation for choice of comparators √
Objectives	7	Specific objectives or hypotheses √
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) √

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained √
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) √
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered √
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) √
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) √
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial √
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended √
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) √
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations √
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size √

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions √

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned √
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions √
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how √
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial X
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol √
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols X
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol √
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol √
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) √
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) √
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed √

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial √
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct √
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor √
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval √
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) √
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) √
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable X
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial √
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site √
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators √
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation X
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions √
	31b	Authorship eligibility guidelines and any intended use of professional writers √
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code √

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates √
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable √

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013928.R1
Article Type:	Protocol
Date Submitted by the Author:	04-Nov-2016
Complete List of Authors:	Kołodziej, Maciej; The Medical University of Warsaw , Department of Paediatrics SZAJEWSKA, Hania; The Medical University of Warsaw, Dept of Paediatrics
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Paediatrics, Gastroenterology and hepatology
Keywords:	probiotics, antibiotics, diarrhea, Clostridium difficile, RCT

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Maciej Kołodziej, Hania Szajewska

Department of Paediatrics, The Medical University of Warsaw, Poland

Corresponding author:
Prof. Hania Szajewska, MD
Department of Paediatrics, The Medical University of Warsaw
Żwirki i Wigury 63A, 02-091 Warsaw, Poland,
email: hania@ipgate.pl

Date and protocol version identifier: 12/08/2016

Key words: probiotics, antibiotics, diarrhea, *Clostridium difficile,* RCT

Number of tables: 2
Number of references: 23

Competing interests statement: HS served as a speaker for BioGaia, the manufacturer of *L reuteri* DSM 17938. MK declares no conflicts of interest.

Contributorship statement: HS conceptualized 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.

Funding statement: This study will be fully funded by The Medical University of Warsaw.

ABSTRACT

Introduction: Administration of some probiotics appears to reduce the risk of antibiotic-associated diarrhea (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 diarrhea 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, randomized, placebo-controlled trial in which they will additionally receive *L reuteri* DSM 17938 at a dose 2 x 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 diarrhea and AAD. Diarrhea will be defined according to one of 3 definitions: (a) ≥3 loose or watery stools per day for a minimum of 48 hours during antibiotic treatment; (b) ≥3 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment; or (c) ≥2 loose or watery stools per day for a minimum of 24 hours during antibiotic treatment. AAD will be diagnosed in cases of diarrhea, defined clinically as above, caused by *Clostridium difficile* or for otherwise unexplained diarrhea (i.e., 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 pediatric journal. Abstracts will be submitted to relevant national and international conferences.

Trial registration number: The study protocol is in the process of being registered at ClinicalTrials.gov. (NCT02871908).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The study design (randomized 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 diarrhea (AAD) in children.
- The findings of this RCT, whether positive or negative, will contribute to the formulation of recommendations on the use of *Lactobacillus 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 diarrhea (AAD) is defined as unexplained diarrhea 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 diarrhea 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 diarrhea remain unclear. The clinical presentation of AAD varies from mild diarrhea 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]

Lactobacillus 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 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 3 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 hospitalized 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 diarrhea was surprisingly low (1 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 diarrhea and AAD in children. We hypothesize 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 randomized, double-blind, placebo-controlled trial, with allocation of 1:1.

Settings and participants

The recruitment will take place in 2 hospitals in Poland (pediatric academic hospital in Warsaw and community hospital in Łuków). We aim to recruit hospitalized children in general pediatric 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 fulfill all of the following criteria: age younger than 18 years; oral or intravenous antibiotic therapy which started within 24 hours of enrollment; signed informed consent.

Children will be excluded for the following reasons: pre-existing acute or chronic diarrhea, history of chronic gastrointestinal disease (e.g., inflammatory bowel disease, cystic fibrosis, celiac disease, food allergy) or other severe chronic disease (e.g., neoplastic diseases), immunodeficiency, use of probiotics within 2 weeks prior to enrollment, use of antibiotics within 4 weeks prior to enrollment, prematurity, and exclusive breastfeeding.

Interventions

The intervention under investigation will be administration of *L reuteri* DSM 17938. The placebo drops consists 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 by obtained by physicians involved in the study. Participants will be randomized after admission to the hospital and administration of antibiotic treatment. Eligible patients will receive either *L reuteri* DSM 17938 at a dose of 2 x 10^8 CFU or placebo, orally, twice daily, in drops (i.e., 2 x 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 to 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. *Yersinia* spp.). *Clostridium 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 milliliter equals 20 drops. Based on previously published trials, it seems to be appropriate to consider those subjects receiving less than 75% of the recommended doses as noncompliant.

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

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

Participant timeline

For the time schedule for enrollment, interventions, assessment, and visits for the participants, see **Table 1.**

Table 1. Timetable of activities planned during the study.

	STUDY PERIOD							
	Enrollment	**Allocation**	**Post-allocation** **Antibiotic therapy**					**Close-out (After the end of follow-up period)**
TIMEPOINT**	***Day 1***	**Day 1**	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Every day***	

ENROLLMENT:								
Eligibility screen	X							
Informed consent	X							
Randomization of the subject	X							
Study product distribution	X							
Diary of symptoms	X							
INTERVENTIONS:								
L reuteri DSM 17938			—	—	—	—	—	
Placebo			—	—	—	—	—	
ASSESSMENTS:								
Adverse events			X	X	X	X	X	X
Stool analysis in case of diarrhea/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

Sample size

The primary outcome of the study is the frequency of diarrhea. 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.[20] [21] [22] Table 2 summarizes sample size calculations depending on the definition used.

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

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 center, and the trial design, will be evaluated.

Sequence generation

A computer-generated randomization list prepared by a person unrelated to the trial will be used to allocate subjects to the study groups in variable blocks of eight. Consecutive randomization numbers will be given to participants at enrollment. 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 randomization list.

Allocation concealment

An independent person will dispense the numbered study products according to a computer generated randomization 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 & 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 for the involved researchers only.

Statistical analysis

All analysis will be conducted on an intention-to-treat (ITT) basis, including all participants in the groups to which they are randomized 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 summarize baseline characteristics. The Student 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 $\chi 2$ test or Fisher 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%

confidence interval, 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 (MD) 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.[23] 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% subjects 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

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

FUNDING STATEMENT

This trial will be funded by The Medical University of Warsaw. At the time of submission of this protocol for publication, no specific grant from any funding agency in the public, commercial, or not-for-profit sectors has been awarded to this project.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym PAGE: 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry PAGE: 2
	2b	All items from the World Health Organization Trial Registration Data Set X
Protocol version	3	Date and version identifier PAGE: 1
Funding	4	Sources and types of financial, material, and other support PAGE: 1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors PAGE: 1
	5b	Name and contact information for the ~~trial sponsor~~ PAGE: 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities PAGE: 1,6
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) X
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention PAGE: 4
	6b	Explanation for choice of comparators PAGE: 4
Objectives	7	Specific objectives or hypotheses PAGE: 5

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) PAGE: 5
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained PAGE: 5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) PAGE: 5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered PAGE: 6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) PAGE: 7
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) PAGE: 7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial PAGE: 7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended PAGE: 8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) PAGE: 8-9
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations PAGE: 9-10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size PAGE: 10
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions PAGE: 10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned PAGE: 10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions PAGE: 10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how PAGE: 11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial X
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol PAGE: 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols X
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol PAGE: 11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol PAGE: 11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) PAGE: 11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) PAGE: 11

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed PAGE: 12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial PAGE: 12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct PAGE: 12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor PAGE: 12

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval PAGE: 12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) PAGE: 12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) √
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable X
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial PAGE: 11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site PAGE: 12-13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators PAGE: 11
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation X

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions PAGE: 12
	31b	Authorship eligibility guidelines and any intended use of professional writers X
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code PAGE: 12
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates - Available on request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable PAGE:8

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.