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Complete List of Authors:	Peeters, Daphne; Haga Hospital Juliana Childrens Hospital, Department of paediatrics van Geloven, Nan; Leiden University Medical Center, Department of Biomedical Data Sciences, Medical Statistics Visser, Loes; Haga Teaching Hospital, Department of Hospital Pharmacy Bogaert, Debby; University of Edinburgh, Center for Inflammation Research. Queen Medical Research Institute.; Wilhelmina Children's Hospital University Medical Centre, Department of Paediatric Infectious Diseases and Immunology van Rossum, AM; Erasmus MC Sophia, Department of paediatrics Driessen, Gertjan; Haga Hospital Juliana Childrens Hospital, Department of paediatrics; Maastricht UMC+, Department of paediatrics Verhagen, Lilly; Wilhelmina Children's Hospital University Medical Centre, Department of Paediatric Infectious Diseases and Immunology
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Study protocol for a randomised controlled trial evaluating the clinical effects of antibiotic prophylaxis in children with recurrent respiratory tract infections: the APPROACH study

Trial acronym: APPROaCH study (Antibiotic ProPhylaxis for recurrent RespiratOry infections in CHildren).

Mrs. Daphne Peeters, MD.
Department of paediatrics, Juliana Children's Hospital, The Hague, The Netherlands.
D.peeters@hagaziekenhuis.nl. https://orcid.org/0000-0002-3110-9434

Dr. Nan van Geloven, PhD.
Department of Biomedical Data Sciences, Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands. N.van_Geloven@lumc.nl https://orcid.org/0000-0002-5600-9093

Dr. Loes E. Visser, PharmD, PhD.
Department of Hospital Pharmacy, Haga Teaching Hospital, The Hague, The Netherlands.
l.e.visser@hagaziekenhuis.nl https://orcid.org/0000-0002-1421-6246

Prof. Debby Bogaert, MD, PhD.

1. Center for Inflammation Research. Queen Medical Research Institute. University of Edinburgh, UK. D.Bogaert@ed.ac.uk. https://orcid.org/0000-0003-2651-7000

2. Department of Paediatric Infectious Diseases and Immunology, University Medical Center Utrecht, Utrecht, The Netherlands.

Prof. Annemarie M.C. van Rossum, MD, PhD.
Department of paediatrics, Erasmus MC-Sophia, Rotterdam, The Netherlands.
a.vanrossum@erasmusmc.nl . https://orcid.org/0000-0002-1259-477X

Dr. Gertjan J.A. Driessen*, MD, PhD.
1. Department of paediatrics, Juliana Children's Hospital, The Hague, The Netherlands.
G.driessen@hagaziekenhuis.nl https://orcid.org/0000-0002-4566-9547
2. Department of Paediatrics, Maastricht University Medical Center+ (MUMC+), Maastricht, the Netherlands

Dr. Lilly M. Verhagen*#, MD, M.H.Sc. Epidemiologist, PhD.
Department of Paediatric Infectious Diseases and Immunology, Wilhelmina Children's Hospital, Lundlaan 6, 3584 EA Utrecht, The Netherlands. L.M.Verhagen-3@umcutrecht.nl
https://orcid.org/0000-0002-3451-9201

*These authors contributed equally

Corresponding author

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ABSTRACT

Introduction

Respiratory tract infections (RTIs) affect children all over the world and are associated with significant morbidity and mortality. In particular recurrent RTIs cause a high burden of disease and lead to frequent doctor visits. Children with recurrent RTIs generally have no significant alterations or deficits in systemic immunity. In an attempt to treat the assumed bacterial component involved, they are often treated with prolonged courses of prophylactic antibiotics taken on a daily basis. Despite its common use, there is no evidence that this is beneficial. Studies assessing the clinical effectiveness of antibiotic prophylaxis as well as potential adverse effects, such as antibiotic resistance development, are therefore urgently needed.

Methods and analysis

We present a protocol for a randomized double-blind placebo-controlled trial comparing co-trimoxazole with placebo treatment in children with recurrent RTIs. A total of 158 children (aged six months – five years) with recurrent RTIs without significant comorbidity will be enrolled from a minimum of 10 Dutch hospitals. One group receives co-trimoxazole 18mg/kg twice daily (36mg/kg/day) and the other group receives a placebo twice daily for a period of three months. The main objective is to determine whether antibiotic prophylaxis is more effective than placebo to prevent/reduce respiratory symptoms in children with recurrent RTIs. Respiratory symptoms will be scored by parents on a daily basis in both study arms by use of a mobile phone application. Our primary outcome will be the number of days with at least two respiratory symptoms during treatment.

Ethics and dissemination

Ethics approval was obtained. A manuscript with the study results will be submitted to a peer-reviewed journal. All participants will be informed about the study results. The results of the study will inform clinical guidelines regarding the treatment of children with recurrent RTIs.

Trial registration number

NL7044 (NTR); Pre-results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Apart from studies focusing exclusively on otitis media, this is the first randomized controlled trial that examines whether co-trimoxazole prophylaxis is effective for recurrent respiratory tract infections (RTIs) in children.

- We will examine the clinical response to prophylactic antibiotic treatment not only during the period in which treatment is taken but also in the three months following that period, because of an extended follow-up duration of six months in total.
- We will examine predictors of treatment effect, such as clinical characteristics, microbiota parameters, and immunological characteristics.
- All children receive the same dose per kg bodyweight of co-trimoxazole and this study does not measure pharmacokinetic parameters. Therefore, we won't be able to determine the most optimal dosage of co-trimoxazol when prescribed for a prophylactic indication in children with recurrent RTIs.
- We will not enrol children with underlying chronic illnesses, such as cardiorespiratory or neuromuscular conditions, immune deficiency and congenital abnormalities, so the results of our study cannot be extrapolated to these groups of patients.

Keywords: PAEDIATRICS, paediatric infectious disease & immunisation, Clinical trials, Respiratory infections

INTRODUCTION

Lack of evidence-based guidelines for a common clinical problem

Young children (up to two years of age) experience symptoms of a respiratory tract infection (RTI) for a median of 44 days per year. The median number of infectious episodes is almost double in children with recurrent RTIs when compared to healthy peers.[1] Even in the absence of high-risk conditions such as major immune deficiencies or congenital malformations, some children develop many more RTIs than their peers.[2] Recurrent RTIs in children are among the leading reasons for primary care consultations and for referral to paediatricians and ear, nose and throat (ENT) specialists.[3] In developed countries, recurrent RTIs, defined as a minimum of six to eight episodes per year, affect 15-20% of children under five years of age.[4] Most children suffer from recurrent RTIs of the upper airways, but in approximately 10-30% the lower respiratory tract is also affected.[5] The high disease burden can lead to failure to thrive and developmental delays in children, as well as parental productivity losses associated with children's illness, leading to high costs for the community.[6, 7] There is no international consensus about the best treatment for children with recurrent RTIs. Previous studies suggest that antibiotic prophylaxis, treatment with the immunomodulator OM-85, active immunization and/or parental education on risk factors (passive smoking) may be at least of some benefit.[4, 8-11] In general, antibiotics are frequently used for the treatment of acute RTIs in children. In an attempt to treat the assumed bacterial component involved in recurrent RTIs, prolonged antibiotic regimens are often prescribed. Previous studies in children with recurrent acute otitis media (AOM) showed that antibiotic prophylaxis prevented one and a half episode for every 12 months of treatment per child.[12] Studies that examined the clinical effectiveness of antibiotic prophylaxis in children suffering from recurrent RTIs are scarce and mainly focus on high risk groups with recurrent lower RTIs.[8] For example, prolonged treatment with co-trimoxazole reduced mortality and hospital admissions in children with HIV.[13] Children with cancer who were treated with co-trimoxazol prophylaxis showed a decrease in pneumonia, upper respiratory tract infections and acute otitis media.[14] In children with an IgA and/or IgG subclass deficiency, the number of infections and antibiotic courses decreased after prophylactic treatment with benzathine penicillin G.[15] Additionally, penicillin prophylaxis reduced the risk of pneumococcal infections in young children with sickle cell disease[16] and azitromycin prophylaxis reduced the number of pulmonary exacerbations in children with cystic fibrosis (CF).[17, 18] For most of these high risk groups, outcomes were evaluated after at least 18 months of treatment. No studies have been performed on clinical effectiveness of antibiotic prophylaxis in otherwise healthy children who suffer from recurrent RTIs.

The antibiotic regimen that is most commonly prescribed in children with recurrent RTIs is trimethoprim/sulfamethoxazole (co-trimoxazole). Both trimethoprim and sulfamethoxazole are bacteriostatic if used alone. Combining trimethoprim and sulfamethoxazole elicits a synergistic effect and makes the antibiotic regimen bactericidal. Co-trimoxazole is a fixed antibiotic combination of trimethoprim and sulfamethoxazole (1:5) which covers most Gram-positive and Gram-negative potential pathogens as well as Pneumocystis jiroveci.

In this study we will compare the clinical effectiveness of co-trimoxazole prophylaxis with placebo in children with recurrent RTIs.

Role of microbiota in respiratory infection and disease

Prolonged antibiotic treatment is of major concern, since antimicrobial resistance (AMR) development increases with duration of the antimicrobial course.[19] Probably the most important route of AMR gene selection in humans is antibiotic-induced changes to our protective microbial communities, also called the microbiota.[20] Whereas microbial disturbances elicited by antibiotic treatment in adults are mostly temporary, exposure to antibiotic treatment early in life may have a lasting impact on the composition of the microbiota leading to permanent replacement by resistant organisms.[21, 22] While the microbiota of the gastro-intestinal tract has been studied most extensively[23, 24], the human nasopharynx is considered the niche from which both upper and lower RTIs originate and resistance can also emerge in commensals or pathogens colonizing this body site.[25] During the past decade, high-throughput pipelines have become available to also characterize the complete nasopharyngeal microbiota.[26]

In this study we will examine short-term and long-term effects of co-trimoxazole prophylaxis on the microbiota composition and antibiotic resistance in children who suffer from recurrent RTIs.

OBJECTIVES AND STUDY PARAMETERS

Primarily, we aim to investigate the clinical efficacy of co-trimoxazole prophylaxis in Dutch children (aged six months up to five years) with recurrent RTIs. Children will be randomized to co-trimoxazole or placebo for a treatment period of three months, since this is the treatment period after which a beneficial effect was achieved in children with recurrent acute or chronic suppurative otitis media.[12]

Primary objective

To determine whether three months of prophylactic treatment with co-trimoxazole causes a reduction in the number of days a child experiences at least two RTI symptoms in children aged 6 months to ≤5 years with recurrent RTIs, when compared to placebo.

Secondary Objectives

1. To determine whether co-trimoxazole prophylactic therapy reduces:

- Time to resolution of symptoms;
- The severity of symptoms defined by the number and type of different infectious symptoms;
- Use of analgesics / antipyretics;
- Use of antibiotic treatment courses;
- Absenteeism from day care or school and/or parental absenteeism from work;
- Alterations in nutritional status.

2. To examine predictors (e.g. demographic, environmental, family history, microbiological and immunological characteristics) for the (absence of) prophylactic treatment effect.

3. To examine whether cessation of antibiotic prophylactic treatment affects the presence of RTI symptoms and how this correlates with clinical, microbiological and immunological characteristics of the patients.

4. To record and evaluate adverse events:

- The occurrence of mild adverse effects as described in the Summary of Product Characteristics (SPC), such as skin rash, gastro-intestinal complaints, pruritus or mild headache;
- The occurrence of severe adverse reactions.

5. To examine short-term and long-term effects of co-trimoxazole prophylaxis on microbiota deviation, AMR and immunological outcomes.

METHODS AND ANALYSIS

Study design

We will conduct a randomized placebo-controlled double-blind clinical trial in which we compare co-trimoxazole with a placebo in 158 children with recurrent upper and/or lower RTIs. The inclusion and exclusion criteria are listed in table 1. We will enrol children aged six months up to five years. Children younger than six months won't participate in this study, because at this age the presence of recurrent RTIs cannot be established yet. For age-specific definitions of recurrent RTIs, we took the twofold standard deviation of the mean number of upper RTIs per year in a cohort of 1314 German children. This means yearly at least 11 parental-reported upper RTIs for children younger than two years and eight parental-reported upper RTIs for children aged two to five years.[27] Recurrent lower RTIs (i.e. pneumonia, bronchopneumonia or acute bronchitis) are defined as at least two episodes per year or three or more episodes during the child's life regardless of age. These definitions of recurrent upper and lower RTIs were also used in the Dutch national guideline for diagnostic strategies in children with recurrent RTIs.[28] If an underlying immune deficiency or contra-indication for co-trimoxazole [29] hasn't been ruled out yet, these will be tested in the blood sample taken in all participants before randomization. Children will be randomized to one of two oral suspension regimens for three months: co-trimoxazole 36 mg/kg/day (2 x 18 mg/kg) or placebo twice daily. The dose of co-trimoxazole is in accordance with the therapeutic dose (for acute infections) as described in the Dutch paediatric drug formulary. Doses and duration of treatment are also based on studies of antibiotic prophylaxis in paediatric populations with recurrent acute or chronic suppurative otitis media.[12] In case of a new RTI episode occurring during follow-up for which the child has a clinical indication to receive (additional) antibiotic treatment, the child will receive antibiotics conform national guidelines and the study medication will be discontinued for the duration of this antibiotic treatment. Medication

compliance will be measured in two ways. During the T3 visit to the hospital, parents will be asked to bring the bottles of trial medication and to answer questions about compliance. By doing so, we will be able to compare the self-reported compliance will the number of empty and (partly) full bottles that have been returned.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	- Presenting to one of the participating clinics; - Age 6 months – 5 years; - Suffering from recurrent respiratory tract infections (RTIs)*; - Informed consent from parent(s)/caregiver(s) with legal custody.
Exclusion criteria	- Current prophylactic antibiotic use or prophylactic antibiotic use during the previous month; - Underlying immune deficiency other than for IgA or IgG subclasses; - Congenital abnormalities (including but not limited to cleft palate, neuromuscular or cardiac disorders and syndromes); - Suffering from chronic respiratory disease, such as cystic fibrosis (CF), primary ciliary dyskinesia (PCD) or anatomical abnormalities; - Only experiencing recurrent AOM or chronic suppurative otitis media without other recurrent RTIs; - Known allergy to co-trimoxazole; - Known contra-indication for co-trimoxazole, e.g. liver failure or impaired kidney function and/or haematologic disorders.

* Recurrent upper RTIs: for children aged <2 years yearly at least 11 and for children aged 2-5 years yearly at least 8 parental-reported upper RTIs including, but not limited to, otitis media. Recurrent lower RTIs (i.e. pneumonia, bronchopneumonia or acute bronchitis) are defined as at least 2 episodes per year or 3 or more episodes during the child's life regardless of age.[27, 28]

Randomization

The randomization procedures are performed by a member of the trial pharmacy. Randomization is computer-generated and subjects will be allocated in a 1:1 ratio with random block sizes of two, four or six subjects to prevent predictability of the allocation. Study medication is blinded for the subjects, his/her parent(s), physicians, the monitor and the study team. Only the members of the trial pharmacy have access to information on the allocation for each subject, because they are responsible for the preparation and delivery of the study medication, and for emergency de-blinding, if needed.

Measurements

The schedule of study enrolment, interventions and measurements of endpoints is shown in table 2. For the parent-reported occurrence of symptoms of RTIs, a mobile application will be used. Previous studies used paper diaries to detect parent-reported symptoms. These are prone to non-compliance and hamper real-time detection of disease occurrence. The use of this Diary-App for symptom

recording costs parents less than one minute per day and has shown to improve case-finding and questionnaire completeness from 60% to ≥90%.[30] Also, parents will be asked to answer extra questions once a month. These include questions about the use of other antibiotics (see online supplementary file 1 for the monthly questionnaire).

At inclusion and after one, three and six months (+/- two weeks for each sample point) non-invasive respiratory (nasopharyngeal swab and saliva) and faecal samples for microbiota composition, immunologic analysis and AMR gene detection will be collected. Before inclusion, blood samples will be taken to test for possible contra-indications (e.g. kidney and/or liver dysfunction). After treatment we will also collect blood samples to monitor for possible kidney, liver and haematological side effects of co-trimoxazole use. Extra blood samples will be collected for additional immunological analyses at both time points. Before the start of treatment parents will be asked to fill in a questionnaire. The study will be started in a minimum of 10 hospitals in the Netherlands. Inclusion will take place during both the winter and summer period to account for seasonal differences in microbiota composition in our analyses. If necessary, the number of study locations can be extended during the study, depending on the speed of subject enrolment.

Table 2. Schedule of enrolment, intervention, and measurement of outcomes

	Enrolment		Post-allocation					
TIMEPOINT	t_0	t_r	t_1	t_2	t_3	t_4	t_5	t_6
ENROLMENT	X							
Eligibility screen	X							
Informed consent	X							
Screening for exclusion criteria	X							
Randomization		X						
INTERVENTION								
Co-trimoxazol or placebo		←	—	—	→			
MEASUREMENTS								
Baseline questionnaire	X							
Digital diary InfectionApp	←	—	—	—	—	—	—	→
Questionnaire on infectious episodes			X	X	X	X	X	X
Physical examination	X				X			X
Blood sample	X				X			
Nasopharynx, saliva and faecal sample	X		X		X			X

Sample size

We assumed 90% compliance with symptom monitoring via the app, i.e. app data available for 0.90 x 3 months (90 days) = 81 days per participant. There is limited literature available about the number of

days with RTI symptoms per time period in children with recurrent RTIs. Toivonen et al. published a large prospective cohort study from Finland including 1089 children followed up from birth to two years of age for respiratory infections by a daily symptom diary.[1] In this study, children with recurrent RTIs (defined as number of days with symptoms >90% percentile limit) had a median of 31 days per 100 days with at least one respiratory symptom. In a pilot study performed in the winter season in one of the participating centres (UMC Utrecht) including 18 children with recurrent RTIs we observed a median of 76 days with at least one respiratory symptom and a median of 42 days with at least two respiratory symptoms per 100 days (this would be 34 per 81 days). We estimated that the median number of days with at least two symptoms in the Finnish study would be (42/76)*31 = 17 days per 100 = 14 days per 81 days. Following, we took the average of the Finnish study and our own study to end up with an estimated median number of symptomatic days of (14+34)/2 = 24 days per 81 days in the placebo group.

The IQR in the Finnish study was 104 - 136, translating into a SD of 23.7, which was comparable to the SD in our pilot study (20.5); as such we took the SD of our study for our sample size calculation since the period in which symptoms were measured in the pilot study (mean 115 days) better reflected the follow-up period of the trial (90 days) than the period in which symptoms were measured in the Toivonen study (at least one year).

Assuming 24 days with at least two RTI symptoms in the placebo group and taking our aim to detect a clinically meaningful 40% reduction (i.e. a reduction of 0.40 x 24 = 10 days with RTI symptoms) in the antibiotic treatment arm, we need to include 71 children per arm, so a total number of 71 x 2 = 142 children, according to the Wilcoxon-Mann-Whitney test for two groups (two-tailed) with alfa 0.05 and power 80%. Including a dropout rate of 10%, this brings us to a number of 71 / 0.9 = 79 children per arm, so a total number of 158 children.

Outcomes and data analysis

Table 2 summarizes the assessment and sampling schedule.

Assessment of RTI symptoms

Descriptive statistics for demographic and clinical characteristics for both treatment allocation groups will be described. These include but are not limited to age, height, weight, ethnicity, co-morbidity, previous ENT-interventions, use of medication, the number of RTI episodes before inclusion, the severity of the infections (e.g. hospital admissions), and type of RTI (e.g. bronchiolitis, otitis, pneumonia). Also, we will examine the frequency distribution of risk factors for the development of RTIs, which include for example smoking in the household, number of siblings and daycare attendance.

Analyses will be performed on the basis of the 'intention-to-treat principle', comparing the treatment arm with the placebo arm, defining the treatment group based on the treatment allocation. In the

intention-to-treat analysis every randomized subject will be included according to treatment assignment, thus ignoring potential non-adherence, protocol deviations, withdrawal, and anything that happens after randomization, therefore maintaining prognostic balance generated from the original random treatment allocation. The analyses will only include subjects of whom at least 80% of the symptom diary data is available. An inventory of missing data will be made and if over 5% of 142 subjects have less available data than 80% of the symptom diary days, an imputation strategy will be used. We will also conduct a 'per-protocol analysis' in which we will only include the days that patients adhered to the protocolled treatment allocation. R and IBM SPSS Statistics will be used for statistical analyses.[31]

For our primary objective, we will compare the number of days with at least two RTI symptoms during 90 days of receiving antibiotic treatment / placebo. Since the actual number of monitored days may vary per patient, we will analyse the incidence rate (number of days with at least two 2 RTI symptoms divided by the total number of days monitored). We will use a negative binomial regression analysis with outcome the number of days with at least two respiratory symptoms and use the number of days monitored as offset. Our target parameter is the incidence rate ratio for treatment. We will include main effects of strong predictors of RTI symptoms in the model.

To determine which set of available determinants predicts heterogeneity of treatment effect, we will first develop a prediction model including well-known risk factors for the development of RTI complaints in children. These include for example age of the child, smoke exposure in the household, day-care attendance and number of siblings. In this prediction model, we will adjust for treatment allocation.[32] From this model, a summary score will be derived that is used as a risk score in the final model. The interaction between this risk score and the treatment allocation will be added as a covariate in a model for the primary outcome to investigate to what extent these host factors affect the effect of treatment on our primary outcome.

As a secondary analysis, we will also perform a negative binominal regression analysis with number of days with at least two RTI symptoms as the dependent variable and number of monitored days as offset, during the total 0-6 month period in order to assess the outcome both during and after cessation of co-trimoxazole versus placebo. In addition, we will also apply a mixed effect model to estimate whether the pattern of RTI symptoms over time changes according to treatment allocation.

Respiratory and gut microbiota

For the secondary objective to detect shifts in microbiota composition in the group that received antibiotic prophylaxis compared to the placebo group, we will collect nasopharyngeal swabs (paediatric Copan e-swab with flocked nylon tip) and faecal samples from which bacterial DNA will be extracted according to previously validated methods. Swabs and faecal samples are frozen at -80°C until further analyses. Metagenomic sequencing will be conducted in order to identify the microbiota

composition and AMR genes of the faecal samples. For nasopharyngeal samples, 16S-based sequencing will be used to examine the microbiota composition.[23, 33]

Saliva and blood samples

Blood samples (peripheral blood mononuclear cells and plasma) and saliva samples will be collected at pre-determined intervals (Table 2) for immunological analyses aimed at the identification of markers associated with clinical outcome. Techniques include flow cytometry, proliferative studies, cytokine release assays and RNA expression profiling. Saliva samples will also be used for the determination of antimicrobial peptides, inflammatory cytokines and mucosal antibodies.

Data management

Clinical data will be collected from the Electronic Medical Record by the research staff. All research data will be stored in the data management program Castor EDC. The handling of personal data will comply with the Dutch General Data Protection Regulation (in Dutch: Algemene Verordening Gegevensbescherming (AVG)). Data will be handled confidentially. The research team has access to coded data. To be able to trace data to an individual subject, a subject identification code list will be used to link the research data to the subject, which will be safeguarded by the local investigators and the trial pharmacy. This trial is monitored in accordance with the Good Clinical Practice guidelines.

PATIENT AND PUBLIC INVOLVEMENT

Because we are supported by two patient involvement groups, we feel confident that not only physicians but also patients will be open to our study results. The patient involvement groups were actively involved in the development of our study protocol. During the yearly conference day of the Foundation for Primary Immune Deficiencies ('Stichting voor Afweerstoornissen'), one of our group members (L.M. Verhagen) has discussed the use of antibiotic prophylactic treatment for mild immune deficiencies in children with patients and parents. Many patients and parents expressed their worries about prolonged antibiotic use, mainly related to its adverse effects and the possibility of future infections with resistant bacteria that can no longer be treated with antibiotics. Following this discussion, we started discussion sessions with small groups of parents of children visiting the airway clinic in the Wilhelmina Children's Hospital, UMC Utrecht, at several time points. The results showed that parents felt that more research was needed to determine whether antibiotic prophylaxis is an effective treatment. The involvement of both patient groups facilitates acceptance and implementation of our study results in the patient community. We will provide these patient support groups with our study results by publication of the outcomes in 'Paraplu', the monthly journal of the Foundation for Primary Immune Deficiencies, and by discussion of the results during meetings of the patient support groups. Also, we will send a half-yearly newsletter and an information bulletin containing the final results to the study participants.

ETHICS AND DISSEMINATION

This study will be conducted according to the principles of the Declaration of Helsinki (2013, Brazil, version 64) and in accordance with the *Research Involving Human Subjects Act (WMO).* The Medical Research Ethics committee LDD (Leiden, The Netherlands) has approved the study protocol. Approval of the local board of each trial site will be obtained before enrolment of the first subject in that specific hospital. This study is registered in The Netherlands National Trial Register (NTR) as Trial NL7044. After completion of this study, results will be submitted to a peer-reviewed journal.

CURRENT TRIAL STATUS

The first subject was enrolled in January 2019. All the local boards of the first 10 trial sites have given their approval to start enrolling patients in this study, the latest approval was obtained in February 2020. If enrolment is slower than expected, a request for the addition of extra trial sites will be submitted to the Medical Research Ethics committee.

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Authors' contributions

All authors helped with the design of this study and approved the final manuscript. DP, LMV and GD wrote the first draft of the study protocol and manuscript. NG was consulted for the statistical analysis plan for this study. LV helped designing the trial medication and advised on improving safety measures in relation to the trial medication. DB was consulted for the secondary outcomes on microbiota composition and AMR genes. AvR was consulted for the design of the study.

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Competing interests statement

None declared.

Ethics approval

This study has been approved by the Medical Research Ethics committee LDD (Leiden, The Netherlands) and is registered in the Netherlands National Trial Register (NTR) as Trial NL7044.

Supplementary file 1

Monthly questionnaire for participants of the Approach study

1. Did you consult a physician for your child because of a respiratory tract infection in the past month?

☐ Yes, the paediatrician of our own hospital
☐ Yes, the general practitioner
☐ Yes, the otolaryngologist
☐ Yes, another physician
☐ No

1b. If yes, how many times did your child visit a physician because of a respiratory tract infection in the past month?

2. Did you consult a physician for your child because of a stomach infection or gastro-enteritis in the past month?

☐ Yes, the paediatrician of our own hospital
☐ Yes, the general practitioner
☐ Yes, another physician
☐ No

2b. If yes, how many times did your child visit a physician because of a stomach infection or gastro-enteritis in the past month?

3. Did your child take any antibiotics in the past month?

☐ Yes, prescribed by the paediatrician of our own hospital
☐ Yes, prescribed by the general practitioner
☐ Yes, prescribed by the otolaryngologist
☐ Yes, prescribed by another physician
☐ No

Question 4 and 5 are only applicable if question 3 is answered 'Yes'.

4. How many antibiotic regimens did your child use in the past month?

The (sub)questions of question 5 are asked for every antibiotic regimen separately.

5a. For what infection was your child treated with antibiotics? (multiple choice)

☐Rhinitis
☐Otitis
☐Tonsillitis
☐Bronchitis or pulmonary infection
☐Stomach infection or gastroenteritis
☐Other infection (other than respiratory or gastro-intestinal infection)

5b. Which date did your child start with the antibiotic treatment?
DD/MM/YYYY

5c. Which date did your child stop with the antibiotic treatment?
DD/MM/YYYY

5d. What is the name of the antibiotic regimen?

6. Did your child get any vaccines in the past month? (multiple choice)
☐No
☐DKTP-Hib-HepB
☐Pneumococcal
☐MMR
☐Meningococcal C
☐Other vaccine

7. Has your child been admitted to the hospital because of an (suspected) infection in the past month?
☐Yes
☐No

7b. If yes, for what infection was your child admitted to the hospital? (multiple choice)
☐Rhinitis
☐Otitis
☐Throat infection / tonsillitis
☐Bronchitis or pulmonary infection
☐Stomach infection or gastroenteritis
☐Other infection (other than respiratory or gastro-intestinal infection)

7c. If yes, how many days has your child been admitted to the hospital in the past month?

8. Did your child visit any form of day-care in the past month?
☐Yes
☐No

8b. If yes, how many half days* did your child visit day-care in the past month?
8c. If yes, how many half days* did your child miss from day-care because of an infection in the past month?
** A half day is a morning or an afternoon.*

9. Did you or your partner miss work due to your child having an infection during the past three months?
☐Yes
☐No

9b. If yes, how many half days* did you and your partner miss combined?
** A half day is a morning or an afternoon.*

10. Do you have any additional comments about the past month?

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5-6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	7 (table 1)
	4b	Settings and locations where the data were collected	7-8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5-6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7-8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9-11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9-11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	NA
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	1-2
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	-
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	1
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Study protocol for a randomised controlled trial evaluating the clinical effects of antibiotic prophylaxis in children with recurrent respiratory tract infections: the APPROACH study

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Study protocol for a randomised controlled trial evaluating the clinical effects of antibiotic prophylaxis in children with recurrent respiratory tract infections: the APPROACH study

Trial acronym: APPROaCH study (Antibiotic ProPhylaxis for recurrent RespiratOry infections in CHildren).

Mrs. Daphne Peeters, MD.
Department of paediatrics, Juliana Children's Hospital, The Hague, The Netherlands.
D.peeters@hagaziekenhuis.nl. https://orcid.org/0000-0002-3110-9434

Dr. Nan van Geloven, PhD.
Department of Biomedical Data Sciences, Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands. N.van_Geloven@lumc.nl https://orcid.org/0000-0002-5600-9093

Dr. Loes E. Visser, PharmD, PhD.
1. Department of Hospital Pharmacy, Haga Teaching Hospital, The Hague, The Netherlands.
l.e.visser@hagaziekenhuis.nl https://orcid.org/0000-0002-1421-6246
2. Department of Hospital Pharmacy, Erasmus MC, Rotterdam, The Netherlands
3. Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands

Prof. Debby Bogaert, MD, PhD.

1. Center for Inflammation Research. Queen Medical Research Institute. University of Edinburgh, UK.
D.Bogaert@ed.ac.uk. https://orcid.org/0000-0003-2651-7000

2. Department of Paediatric Infectious Diseases and Immunology, University Medical Center Utrecht, Utrecht, The Netherlands.

Prof. Annemarie M.C. van Rossum, MD, PhD.
Department of paediatrics, Erasmus MC-Sophia, Rotterdam, The Netherlands.
a.vanrossum@erasmusmc.nl . https://orcid.org/0000-0002-1259-477X

Prof. Dr. Gertjan J.A. Driessen*, MD, PhD.
1. Department of paediatrics, Juliana Children's Hospital, The Hague, The Netherlands.
G.driessen@hagaziekenhuis.nl https://orcid.org/0000-0002-4566-9547
2. Department of Paediatrics, Maastricht University Medical Center+ (MUMC+), Maastricht, the Netherlands

Dr. Lilly M. Verhagen*#, MD, M.H.Sc. Epidemiologist, PhD.
Department of Paediatric Infectious Diseases and Immunology, Wilhelmina Children's Hospital, Lundlaan 6, 3584 EA Utrecht, The Netherlands. L.M.Verhagen-3@umcutrecht.nl
https://orcid.org/0000-0002-3451-9201

*These authors contributed equally
Corresponding author

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ABSTRACT

Introduction

Respiratory tract infections (RTIs) affect children all over the world and are associated with significant morbidity and mortality. In particular recurrent RTIs cause a high burden of disease and lead to frequent doctor visits. Children with recurrent RTIs generally have no significant alterations or deficits in systemic immunity. In an attempt to treat the assumed bacterial component involved, they are often treated with prolonged courses of prophylactic antibiotics taken on a daily basis. Despite its common use, there is no evidence that this is beneficial. Studies assessing the clinical effectiveness of antibiotic prophylaxis as well as potential adverse effects and antibiotic resistance development, are therefore urgently needed.

Methods and analysis

We present a protocol for a randomized double-blind placebo-controlled trial comparing co-trimoxazole with placebo treatment in children with recurrent RTIs. A total of 158 children (aged six months – ten years) with recurrent RTIs without significant comorbidity will be enrolled from a minimum of 10 Dutch hospitals. One group receives co-trimoxazole 18mg/kg twice daily (36mg/kg/day) and the other group receives a placebo twice daily for a period of three months. The main objective is to determine whether antibiotic prophylaxis is more effective than placebo to prevent/reduce respiratory symptoms in children with recurrent RTIs. Respiratory symptoms will be scored by parents on a daily basis in both study arms by use of a mobile phone application. Our primary outcome will be the number of days with at least two respiratory symptoms during treatment.

Ethics and dissemination

Ethics approval was obtained. A manuscript with the study results will be submitted to a peer-reviewed journal. All participants will be informed about the study results. The results of the study will inform clinical guidelines regarding the treatment of children with recurrent RTIs.

Trial registration number

NL7044 (NTR); Pre-results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Apart from studies focusing exclusively on otitis media, this is the first randomized controlled trial that examines whether co-trimoxazole prophylaxis is effective for recurrent respiratory tract infections (RTIs) in children.
- We will examine the clinical response to prophylactic antibiotic treatment not only during the period in which treatment is taken but also in the three months following that period, because of an extended follow-up duration of six months in total.
- We will examine predictors of treatment effect, such as clinical characteristics, microbiota parameters, and immunological characteristics.
- All children receive the same dose per kg bodyweight of co-trimoxazole and this study does not measure pharmacokinetic parameters. Therefore, we won't be able to determine the most optimal dosage of co-trimoxazol when prescribed for a prophylactic indication in children with recurrent RTIs.
- We will not enrol children with underlying chronic illnesses, such as cardiorespiratory or neuromuscular conditions, immune deficiency and congenital abnormalities, so the results of our study cannot be extrapolated to these groups of patients.

Keywords: PAEDIATRICS, paediatric infectious disease & immunisation, Clinical trials, Respiratory infections

INTRODUCTION

Lack of evidence-based guidelines for a common clinical problem

Young children (up to two years of age) experience symptoms of a respiratory tract infection (RTI) for a median of 44 days per year. The median number of infectious episodes is almost double in children with recurrent RTIs when compared to healthy peers.[1] Even in the absence of high-risk conditions such as major immune deficiencies or congenital malformations, some children develop many more RTIs than their peers.[2] Recurrent RTIs in children are among the leading reasons for primary care consultations and for referral to paediatricians and ear, nose and throat (ENT) specialists.[3] In developed countries, recurrent RTIs, defined as a minimum of six to eight episodes per year, affect 15-20% of children under five years of age.[4] Most children suffer from recurrent RTIs of the upper airways, but in approximately 10-30% the lower respiratory tract is also affected.[5] Compared to their healthy peers, children with recurrent RTIs often visit the outpatient clinic and they more often need hospitalization. In addition, these children are treated more frequently with medication, e.g. inhaled bronchodilators and corticosteroids as well as antibiotics.[1] The high disease burden can also lead to failure to thrive and developmental delays in children, as well as parental productivity losses associated with children's illness and absenteeism from work, leading to high costs for the community.[1, 6, 7] Lower RTIs in childhood can also lead to pulmonary sequelae such as bronchiectasis and irreversible lung damage [8-10], putting children with recurrent RTIs even more at risk for long-term damage. There is no international consensus about the best treatment for children with recurrent RTIs. Previous studies suggest that antibiotic prophylaxis, treatment with the immunomodulator OM-85, active immunization and/or parental education on risk factors (passive smoking) may be at least of some benefit.[4, 11-14] In general, antibiotics are frequently used for the treatment of acute RTIs in children. In an attempt to treat the assumed bacterial component involved in recurrent RTIs, prolonged antibiotic regimens are often prescribed. Previous studies in children with recurrent acute otitis media (AOM) showed that antibiotic prophylaxis prevented one and a half episode for every 12 months of treatment per child.[15] Studies that examined the clinical effectiveness of antibiotic prophylaxis in children suffering from recurrent RTIs are scarce and mainly focus on high risk groups with recurrent lower RTIs.[11, 16-21] No studies have been performed on clinical effectiveness of antibiotic prophylaxis in otherwise healthy children who suffer from recurrent RTIs.

The antibiotic regimen that is most commonly prescribed in children with recurrent RTIs is trimethoprim/sulfamethoxazole (co-trimoxazole). Both trimethoprim and sulfamethoxazole are bacteriostatic if used alone. Combining trimethoprim and sulfamethoxazole elicits a synergistic effect and makes the antibiotic regimen bactericidal. Co-trimoxazole is a fixed antibiotic combination of trimethoprim and sulfamethoxazole (1:5) which covers most Gram-positive and Gram-negative potential pathogens as well as Pneumocystis jiroveci. In addition, several studies suggest that co-trimoxazol has an immunomodulatory effect which could enhance the immune response.[22-25] The combination of antimicrobial and immunomodulatory properties could provide an additional beneficial effect in the prevention or reduction of RTIs.

In this study we will compare the clinical effectiveness of co-trimoxazole prophylaxis with placebo in children with recurrent RTIs.

Role of microbiota in respiratory infection and disease

Prolonged antibiotic treatment is of major concern, since antimicrobial resistance (AMR) development increases with duration of the antimicrobial course.[26] Probably the most important route of AMR gene selection in humans is antibiotic-induced changes to our protective microbial communities, also called the microbiota.[27] Whereas microbial disturbances elicited by antibiotic treatment in adults are mostly temporary, exposure to antibiotic treatment early in life may have a lasting impact on the composition of the microbiota leading to permanent replacement by resistant organisms.[28, 29] While the microbiota of the gastro-intestinal tract has been studied most extensively[30, 31], the human nasopharynx is considered the niche from which both upper and lower RTIs originate and resistance can also emerge in commensals or pathogens colonizing this body site.[32] During the past decade, high-throughput pipelines have become available to also characterize the complete nasopharyngeal microbiota.[33]

In this study we will examine short-term and long-term effects of co-trimoxazole prophylaxis on the microbiota composition and antibiotic resistance in children who suffer from recurrent RTIs.

METHODS AND ANALYSIS

Objectives and study parameters

Primarily, we aim to investigate the clinical efficacy of co-trimoxazole prophylaxis in Dutch children (aged six months up to ten years) with recurrent RTIs. Children will be randomized to co-trimoxazole or placebo for a treatment period of three months, since this is the treatment period after which a beneficial effect was achieved in children with recurrent acute or chronic suppurative otitis media.[15]

Primary objective

To determine whether three months of prophylactic treatment with co-trimoxazole causes a reduction in the number of days a child experiences at least two RTI symptoms in children aged 6 months to ≤10 years with recurrent RTIs, when compared to placebo.

Secondary Objectives

1. To determine whether co-trimoxazole prophylactic therapy reduces:

- Time to resolution of symptoms;
- The severity of symptoms defined by the number and type of different infectious symptoms;
- Use of analgesics / antipyretics;
- Use of antibiotic treatment courses;
- Absenteeism from day care or school and/or parental absenteeism from work;
- Alterations in nutritional status.

2. To examine predictors (e.g. demographic, environmental, family history, mucosal, microbiological and immunological characteristics) for the (absence of) prophylactic treatment effect

3. To examine whether cessation of antibiotic prophylactic treatment affects the presence of RTI symptoms and how this correlates with clinical, microbiological and immunological characteristics of the patients.

4. To record and evaluate adverse events:

- The occurrence of mild adverse effects as described in the Summary of Product Characteristics (SPC), such as skin rash, gastro-intestinal complaints, pruritus or mild headache;
- The occurrence of severe adverse reactions.

5. To examine short-term and long-term effects of co-trimoxazole prophylaxis on microbiota deviation, AMR and (mucosal and systemic) immunological outcomes.

Study design

We will conduct a randomized placebo-controlled double-blind clinical trial in which we compare co-trimoxazole with a placebo in 158 children with recurrent upper and/or lower RTIs. The inclusion and exclusion criteria are listed in table 1. We will enrol children aged six months up to ten years. Children younger than six months won't participate in this study, because at this age the presence of recurrent RTIs cannot be established yet. For age-specific definitions of recurrent RTIs, we took the twofold standard deviation of the mean number of upper RTIs per year in a cohort of 1314 German children, except for children aged 5-10 years, in whom we used the same definition as younger children (2-5 years). This means that we define recurrent RTIs as at least 11 parental-reported upper RTIs for children younger than two years and eight parental-reported upper RTIs for children aged two to ten years per year.[34] Recurrent lower RTIs (i.e. pneumonia, bronchopneumonia or acute bronchitis) are defined as at least two episodes per year or three or more episodes during the child's life regardless of age. These definitions of recurrent upper and lower RTIs were also used in the Dutch national guideline for diagnostic strategies in children with recurrent RTIs.[35] If an underlying immune deficiency or contra-indication for co-trimoxazole [36] hasn't been ruled out yet, these will be tested in the blood sample taken in all participants before randomization. Children will be randomized to one of two oral suspension regimens for three months: co-trimoxazole 36 mg/kg/day (2 x 18 mg/kg) or placebo twice daily. The dose of co-trimoxazole is in accordance with the therapeutic dose (for acute infections) as described in the Dutch paediatric drug formulary. Doses and duration of treatment are also based on studies of antibiotic prophylaxis in paediatric populations with recurrent acute or chronic suppurative otitis media.[15] In case of a new RTI episode occurring during follow-up for which the child has a clinical indication to receive (additional) antibiotic treatment, the child will receive antibiotics

that conform to national guidelines and the study medication will be discontinued for the duration of this antibiotic treatment. Medication compliance will be measured in two ways. During the T3 visit to the hospital, parents will be asked to bring the bottles of trial medication and to answer questions about compliance. By doing so, we will be able to compare the self-reported compliance will the number of empty and (partly) full bottles that have been returned.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	- Presenting to one of the participating clinics; - Age 6 months – 10 years; - Suffering from recurrent respiratory tract infections (RTIs)*; - Informed consent from parent(s)/caregiver(s) with legal custody.
Exclusion criteria	- Current prophylactic antibiotic use or prophylactic antibiotic use during the previous month; - Underlying immune deficiency other than for IgA or IgG subclasses; - Congenital abnormalities (including but not limited to cleft palate, neuromuscular or cardiac disorders and syndromes); - Suffering from chronic respiratory disease, such as cystic fibrosis (CF), primary ciliary dyskinesia (PCD) or anatomical abnormalities; - Only experiencing recurrent AOM or chronic suppurative otitis media without other recurrent RTIs; - Known allergy to co-trimoxazole; - Known contra-indication for co-trimoxazole, e.g. liver failure or impaired kidney function and/or haematologic disorders.

* Recurrent upper RTIs: for children aged <2 years yearly at least 11 and for children aged 2-10 years yearly at least 8 parental-reported upper RTIs including, but not limited to, otitis media. Recurrent lower RTIs (i.e. pneumonia, bronchopneumonia or acute bronchitis) are defined as at least 2 episodes per year or 3 or more episodes during the child's life regardless of age.[34, 35]

Randomization

The randomization procedures are performed by a member of the trial pharmacy. Randomization is computer-generated and subjects will be allocated in a 1:1 ratio with random block sizes of two, four or six subjects to prevent predictability of the allocation. Study medication is blinded for the subjects, his/her parent(s), physicians, the monitor and the study team. Only the members of the trial pharmacy have access to information on the allocation for each subject, because they are responsible for the preparation and delivery of the study medication, and for emergency de-blinding, if needed.

Measurements

The schedule of study enrolment, interventions and measurements of endpoints is shown in table 2. For the parent-reported occurrence of symptoms of RTIs, a mobile application will be used. Previous studies used paper diaries to detect parent-reported symptoms. These are prone to non-compliance

and hamper real-time detection of disease occurrence. The use of this Diary-App for symptom recording costs parents less than one minute per day and has shown to improve case-finding and questionnaire completeness from 60% to ≥90%.[37] Also, parents will be asked to answer extra questions once a month. These include questions about the use of other antibiotics (see online supplementary file 1 for the monthly questionnaire).

At inclusion and after one, three and six months (+/- two weeks for each sample point) non-invasive respiratory (nasopharyngeal swab and saliva) and faecal samples for microbiota composition, immunologic analysis and AMR gene detection will be collected. Also, these samples could be used for viral analysis. In addition, mucosal lining fluid will be collected at two time points in at least 50 subjects. Before inclusion, blood samples will be taken to test for possible contra-indications (e.g. kidney and/or liver dysfunction). After treatment we will also collect blood samples to monitor for possible kidney, liver and haematological side effects of co-trimoxazole use. Extra blood samples will be collected for additional immunological analyses at both time points. Before the start of treatment parents will be asked to fill in a questionnaire. The study will be started in a minimum of 10 hospitals in the Netherlands. Inclusion will take place during both the winter and summer period to account for seasonal differences in microbiota composition in our analyses. If necessary, the number of study locations can be extended during the study, depending on the speed of subject enrolment.

Table 2. Schedule of enrolment, intervention, and measurement of outcomes

	Enrolment		Post-allocation					
TIMEPOINT	t_0	t_r	t_1	t_2	t_3	t_4	t_5	t_6
ENROLMENT	X							
Eligibility screen	X							
Informed consent	X							
Screening for exclusion criteria	X							
Randomization		X						
INTERVENTION								
Co-trimoxazol or placebo		←	—	—	→			
MEASUREMENTS								
Baseline questionnaire	X							
Digital diary InfectionApp	←	—	—	—	—	—	—	→
Questionnaire on infectious episodes			X	X	X	X	X	X
Physical examination	X				X			X
Blood sample	X				X			
Nasopharynx, saliva and faecal sample	X		X		X			X
Mucosal lining fluid sample*	X		X		(X)			(X)

*Sampling at 2/4 time points, preferably T0 and T1.

Sample size

We assumed 90% compliance with symptom monitoring via the app, i.e. app data available for 0.90 x 3 months (90 days) = 81 days per participant. There is limited literature available about the number of days with RTI symptoms per time period in children with recurrent RTIs. Toivonen et al. published a large prospective cohort study from Finland including 1089 children followed up from birth to two years of age for respiratory infections by a daily symptom diary.[1] In this study, children with recurrent RTIs (defined as number of days with symptoms >90% percentile limit) had a median of 31 days per 100 days with at least one respiratory symptom. In a pilot study performed in the winter season in one of the participating centres (UMC Utrecht) including 18 children with recurrent RTIs we observed a median of 76 days with at least one respiratory symptom and a median of 42 days with at least two respiratory symptoms per 100 days (this would be 34 per 81 days). We estimated that the median number of days with at least two symptoms in the Finnish study would be (42/76)*31 = 17 days per 100 = 14 days per 81 days. Following, we took the average of the Finnish study and our own study to end up with an estimated median number of symptomatic days of (14+34)/2 = 24 days per 81 days in the placebo group.

The IQR in the Finnish study was 104 - 136, translating into a SD of 23.7, which was comparable to the SD in our pilot study (20.5); as such we took the SD of our study for our sample size calculation since the period in which symptoms were measured in the pilot study (mean 115 days) better reflected the follow-up period of the trial (90 days) than the period in which symptoms were measured in the Toivonen study (at least one year).

Assuming 24 days with at least two RTI symptoms in the placebo group and taking our aim to detect a clinically meaningful 40% reduction (i.e. a reduction of 0.40 x 24 = 10 days with RTI symptoms) in the antibiotic treatment arm, we need to include 71 children per arm, so a total number of 71 x 2 = 142 children, according to the Wilcoxon-Mann-Whitney test for two groups (two-tailed) with alfa 0.05 and power 80%. Including a dropout rate of 10%, this brings us to a number of 71 / 0.9 = 79 children per arm, so a total number of 158 children.

Outcomes and data analysis

Table 2 summarizes the assessment and sampling schedule.

Assessment of RTI symptoms

Descriptive statistics for demographic and clinical characteristics for both treatment allocation groups will be described. These include but are not limited to age, height, weight, ethnicity, co-morbidity, previous ENT-interventions, use of medication, the number of RTI episodes before inclusion, the severity of the infections (e.g. hospital admissions), and type of RTI (e.g. bronchiolitis, otitis, pneumonia). Also, we will examine the frequency distribution of risk factors for the development of

RTIs, which include for example smoking in the household, number of siblings and daycare attendance.

Analyses will be performed on the basis of the 'intention-to-treat principle', comparing the treatment arm with the placebo arm, defining the treatment group based on the treatment allocation. In the intention-to-treat analysis every randomized subject will be included according to treatment assignment, thus ignoring potential non-adherence, protocol deviations, withdrawal, and anything that happens after randomization, therefore maintaining prognostic balance generated from the original random treatment allocation. The analyses will only include subjects of whom at least 80% of the symptom diary data is available. An inventory of missing data will be made and if over 5% of 142 subjects have less available data than 80% of the symptom diary days, an imputation strategy will be used. We will also conduct a 'per-protocol analysis' in which we will only include the days that patients adhered to the protocolled treatment allocation. R and IBM SPSS Statistics will be used for statistical analyses.[38]

For our primary objective, we will compare the number of days with at least two RTI symptoms during 90 days of receiving antibiotic treatment / placebo. Since the actual number of monitored days may vary per patient, we will analyse the incidence rate (number of days with at least two 2 RTI symptoms divided by the total number of days monitored). We will use a negative binomial regression analysis with outcome the number of days with at least two respiratory symptoms and use the number of days monitored as offset. Our target parameter is the incidence rate ratio for treatment. We will include main effects of strong predictors of RTI symptoms in the model.

To determine which set of available determinants predicts heterogeneity of treatment effect, we will first develop a prediction model including well-known risk factors for the development of RTI complaints in children. These include for example age of the child, smoke exposure in the household, day-care attendance and number of siblings. In this prediction model, we will adjust for treatment allocation.[39] From this model, a summary score will be derived that is used as a risk score in the final model. The interaction between this risk score and the treatment allocation will be added as a covariate in a model for the primary outcome to investigate to what extent these host factors affect the effect of treatment on our primary outcome.

As a secondary analysis, we will also perform a negative binominal regression analysis with number of days with at least two RTI symptoms as the dependent variable and number of monitored days as offset, during the total 0-6 month period in order to assess the outcome both during and after cessation of co-trimoxazole versus placebo. In addition, we will also apply a mixed effect model to estimate whether the pattern of RTI symptoms over time changes according to treatment allocation.

Respiratory and gut microbiota

For the secondary objective to detect shifts in microbiota composition in the group that received antibiotic prophylaxis compared to the placebo group, we will collect nasopharyngeal swabs

(paediatric Copan e-swab with flocked nylon tip) and faecal samples from which bacterial DNA will be extracted according to previously validated methods. Swabs and faecal samples are frozen at -80°C until further analyses. Metagenomic sequencing will be conducted in order to identify the microbiota composition and AMR genes of the faecal samples. For nasopharyngeal samples, 16S-based sequencing will be used to examine the microbiota composition.[30, 40]

Saliva, blood and mucosal lining fluid samples

Blood samples (peripheral blood mononuclear cells and plasma), saliva samples and mucosal lining fluid will be collected at pre-determined intervals (Table 2) for immunological analyses aimed at the identification of markers associated with clinical outcome. Techniques include flow cytometry, proteomics, proliferative studies, cytokine release assays and RNA expression profiling. Saliva samples will also be used for the determination of antimicrobial peptides, inflammatory cytokines, proteomics and mucosal antibodies. Mucosal lining fluid will be used to measure (protein) immune markers such as antibodies, chemokines and cytokines.

Data management

Clinical data will be collected from the Electronic Medical Record by the research staff. All research data will be stored in the data management program Castor EDC. The handling of personal data will comply with the Dutch General Data Protection Regulation (in Dutch: Algemene Verordening Gegevensbescherming (AVG)). Data will be handled confidentially. The research team has access to coded data. To be able to trace data to an individual subject, a subject identification code list will be used to link the research data to the subject, which will be safeguarded by the local investigators and the trial pharmacy. This trial is monitored in accordance with the Good Clinical Practice guidelines.

PATIENT AND PUBLIC INVOLVEMENT

Because we are supported by two patient involvement groups, we feel confident that not only physicians but also patients will be open to our study results. The patient involvement groups were actively involved in the development of our study protocol. During the yearly conference day of the Foundation for Primary Immune Deficiencies (‘Stichting voor Afweerstoornissen’), one of our group members (L.M. Verhagen) has discussed the use of antibiotic prophylactic treatment for mild immune deficiencies in children with patients and parents. Many patients and parents expressed their worries about prolonged antibiotic use, mainly related to its adverse effects and the possibility of future infections with resistant bacteria that can no longer be treated with antibiotics. Following this discussion, we started discussion sessions with small groups of parents of children visiting the airway clinic in the Wilhelmina Children’s Hospital, UMC Utrecht, at several time points. The results showed that parents felt that more research was needed to determine whether antibiotic prophylaxis is an effective treatment. The involvement of both patient groups facilitates acceptance and implementation

of our study results in the patient community. We will provide these patient support groups with our study results by publication of the outcomes in 'Paraplu', the monthly journal of the Foundation for Primary Immune Deficiencies, and by discussion of the results during meetings of the patient support groups. Also, we will send a half-yearly newsletter and an information bulletin containing the final results to the study participants.

ETHICS AND DISSEMINATION

This study will be conducted according to the principles of the Declaration of Helsinki (2013, Brazil, version 64) and in accordance with the *Research Involving Human Subjects Act (WMO).* The Medical Research Ethics committee LDD (Leiden, The Netherlands) has approved the study protocol. Approval of the local board of each trial site will be obtained before enrolment of the first subject in that specific hospital. This study is registered in The Netherlands National Trial Register (NTR) as Trial NL7044. After completion of this study, results will be submitted to a peer-reviewed journal.

CURRENT TRIAL STATUS

The first subject was enrolled in January 2019. All the local boards of the first 10 trial sites have given their approval to start enrolling patients in this study, the latest approval was obtained in February 2020. If enrolment is slower than expected, a request for the addition of extra trial sites will be submitted to the Medical Research Ethics committee.

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Authors' contributions

All authors helped with the design of this study and approved the final manuscript. DP, LMV and GD wrote the first draft of the study protocol and manuscript. NG was consulted for the statistical analysis plan for this study. LV helped designing the trial medication and advised on improving safety measures in relation to the trial medication. DB was consulted for the secondary outcomes on microbiota composition and AMR genes. AvR was consulted for the design of the study.

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Competing interests statement

None declared.

Ethics approval

This study has been approved by the Medical Research Ethics committee LDD (Leiden, The Netherlands) and is registered in the Netherlands National Trial Register (NTR) as Trial NL7044.

Supplementary file 1

Monthly questionnaire for participants of the Approach study

1. Did you consult a physician for your child because of a respiratory tract infection in the past month?
☐ Yes, the paediatrician of our own hospital
☐ Yes, the general practitioner
☐ Yes, the otolaryngologist
☐ Yes, another physician
☐ No

1b. If yes, how many times did your child visit a physician because of a respiratory tract infection in the past month?

2. Did you consult a physician for your child because of a stomach infection or gastro-enteritis in the past month?
☐ Yes, the paediatrician of our own hospital
☐ Yes, the general practitioner
☐ Yes, another physician
☐ No

2b. If yes, how many times did your child visit a physician because of a stomach infection or gastro-enteritis in the past month?

3. Did your child take any antibiotics in the past month?
☐ Yes, prescribed by the paediatrician of our own hospital
☐ Yes, prescribed by the general practitioner
☐ Yes, prescribed by the otolaryngologist
☐ Yes, prescribed by another physician
☐ No

Question 4 and 5 are only applicable if question 3 is answered 'Yes'.

4. How many antibiotic regimens did your child use in the past month?

The (sub)questions of question 5 are asked for every antibiotic regimen separately.

5a. For what infection was your child treated with antibiotics? (multiple choice)
☐Rhinitis
☐Otitis
☐Tonsillitis
☐Bronchitis or pulmonary infection
☐Stomach infection or gastroenteritis
☐Other infection (other than respiratory or gastro-intestinal infection)

5b. Which date did your child start with the antibiotic treatment?
DD/MM/YYYY

5c. Which date did your child stop with the antibiotic treatment?
DD/MM/YYYY

5d. What is the name of the antibiotic regimen?

6. Did your child get any vaccines in the past month? (multiple choice)
☐No
☐DKTP-Hib-HepB
☐Pneumococcal
☐MMR
☐Meningococcal C
☐Other vaccine

7. Has your child been admitted to the hospital because of an (suspected) infection in the past month?
☐Yes
☐No

7b. If yes, for what infection was your child admitted to the hospital? (multiple choice)
☐Rhinitis
☐Otitis
☐Throat infection / tonsillitis
☐Bronchitis or pulmonary infection
☐Stomach infection or gastroenteritis
☐Other infection (other than respiratory or gastro-intestinal infection)

7c. If yes, how many days has your child been admitted to the hospital in the past month?

8. Did your child visit any form of day-care in the past month?
☐Yes
☐No

8b. If yes, how many half days* did your child visit day-care in the past month?
8c. If yes, how many half days* did your child miss from day-care because of an infection in the past month?
** A half day is a morning or an afternoon.*

9. Did you or your partner miss work due to your child having an infection during the past three months?
☐Yes
☐No

9b. If yes, how many half days* did you and your partner miss combined?
** A half day is a morning or an afternoon.*

10. Do you have any additional comments about the past month?

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5-6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	7 (table 1)
	4b	Settings and locations where the data were collected	7-8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5-6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7-8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9-11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9-11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	NA
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	1-2
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	-
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	1
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Study protocol for a randomised controlled trial evaluating the clinical effects of antibiotic prophylaxis in children with recurrent respiratory tract infections: the APPROACH study

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Complete List of Authors:	Peeters, Daphne; Haga Hospital Juliana Childrens Hospital, Department of paediatrics van Geloven, Nan; Leiden University Medical Center, Department of Biomedical Data Sciences, Medical Statistics Visser, Loes; Haga Teaching Hospital, Department of Hospital Pharmacy; Erasmus MC, Department of Hospital Pharmacy and Department of Epidemiology Bogaert, Debby; University of Edinburgh, Center for Inflammation Research. Queen Medical Research Institute.; Wilhelmina Children's Hospital University Medical Centre, Department of Paediatric Infectious Diseases and Immunology van Rossum, AM; Erasmus MC Sophia, Department of paediatrics Driessen, Gertjan; Haga Hospital Juliana Childrens Hospital, Department of paediatrics; Maastricht UMC+, Department of paediatrics Verhagen, Lilly; Wilhelmina Children's Hospital University Medical Centre, Department of Paediatric Infectious Diseases and Immunology
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Study protocol for a randomised controlled trial evaluating the clinical effects of antibiotic prophylaxis in children with recurrent respiratory tract infections: the APPROACH study

Trial acronym: APPROaCH study (Antibiotic ProPhylaxis for recurrent RespiratOry infections in CHildren).

Mrs. Daphne Peeters, MD.
Department of paediatrics, Juliana Children's Hospital, The Hague, The Netherlands.
D.peeters@hagaziekenhuis.nl. https://orcid.org/0000-0002-3110-9434

Dr. Nan van Geloven, PhD.
Department of Biomedical Data Sciences, Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands. N.van_Geloven@lumc.nl https://orcid.org/0000-0002-5600-9093

Dr. Loes E. Visser, PharmD, PhD.
1. Department of Hospital Pharmacy, Haga Teaching Hospital, The Hague, The Netherlands.
l.e.visser@hagaziekenhuis.nl https://orcid.org/0000-0002-1421-6246
2. Department of Hospital Pharmacy, Erasmus MC, Rotterdam, The Netherlands
3. Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands

Prof. Debby Bogaert, MD, PhD.

1. Center for Inflammation Research. Queen Medical Research Institute. University of Edinburgh, UK.
D.Bogaert@ed.ac.uk. https://orcid.org/0000-0003-2651-7000

2. Department of Paediatric Infectious Diseases and Immunology, University Medical Center Utrecht, Utrecht, The Netherlands.

Prof. Annemarie M.C. van Rossum, MD, PhD.
Department of paediatrics, Erasmus MC-Sophia, Rotterdam, The Netherlands.
a.vanrossum@erasmusmc.nl . https://orcid.org/0000-0002-1259-477X

Prof. Dr. Gertjan J.A. Driessen*, MD, PhD.
1. Department of paediatrics, Juliana Children's Hospital, The Hague, The Netherlands.
G.driessen@hagaziekenhuis.nl https://orcid.org/0000-0002-4566-9547
2. Department of Paediatrics, Maastricht University Medical Center+ (MUMC+), Maastricht, the Netherlands

Dr. Lilly M. Verhagen*#, MD, M.H.Sc. Epidemiologist, PhD.
Department of Paediatric Infectious Diseases and Immunology, Wilhelmina Children's Hospital, Lundlaan 6, 3584 EA Utrecht, The Netherlands. L.M.Verhagen-3@umcutrecht.nl
https://orcid.org/0000-0002-3451-9201

*These authors contributed equally

Corresponding author

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ABSTRACT

Introduction

Respiratory tract infections (RTIs) affect children all over the world and are associated with significant morbidity and mortality. In particular recurrent RTIs cause a high burden of disease and lead to frequent doctor visits. Children with recurrent RTIs generally have no significant alterations or deficits in systemic immunity. In an attempt to treat the assumed bacterial component involved, they are often treated with prolonged courses of prophylactic antibiotics taken on a daily basis. Despite its common use, there is no evidence that this is beneficial. Studies assessing the clinical effectiveness of antibiotic prophylaxis as well as potential adverse effects and antibiotic resistance development, are therefore urgently needed.

Methods and analysis

We present a protocol for a randomized double-blind placebo-controlled trial comparing co-trimoxazole with placebo treatment in children with recurrent RTIs. A total of 158 children (aged six months – ten years) with recurrent RTIs without significant comorbidity will be enrolled from a minimum of 10 Dutch hospitals. One group receives co-trimoxazole 18mg/kg twice daily (36mg/kg/day) and the other group receives a placebo twice daily for a period of three months. The main objective is to determine whether antibiotic prophylaxis is more effective than placebo to prevent/reduce respiratory symptoms in children with recurrent RTIs. Respiratory symptoms will be scored by parents on a daily basis in both study arms by use of a mobile phone application. Our primary outcome will be the number of days with at least two respiratory symptoms during treatment.

Ethics and dissemination

Ethics approval was obtained from the Medical Ethics Research Committee Zuidwest Holland/LDD. A manuscript with the study results will be submitted to a peer-reviewed journal. All participants will be informed about the study results. The results of the study will inform clinical guidelines regarding the treatment of children with recurrent RTIs.

Trial registration number

NL7044 (NTR); Pre-results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Apart from studies focusing exclusively on otitis media, this is the first randomized controlled trial that examines whether co-trimoxazole prophylaxis is effective for recurrent respiratory tract infections (RTIs) in children.
- We will examine the clinical response to prophylactic antibiotic treatment not only during the period in which treatment is taken but also in the three months following that period, because of an extended follow-up duration of six months in total.
- We will examine predictors of treatment effect, such as clinical characteristics, microbiota parameters, and immunological characteristics.
- All children receive the same dose per kg bodyweight of co-trimoxazole and this study does not measure pharmacokinetic parameters. Therefore, we won't be able to determine the most optimal dosage of co-trimoxazol when prescribed for a prophylactic indication in children with recurrent RTIs.
- We will not enrol children with underlying chronic illnesses, such as cardiorespiratory or neuromuscular conditions, immune deficiency and congenital abnormalities, so the results of our study cannot be extrapolated to these groups of patients.

Keywords: PAEDIATRICS, paediatric infectious disease & immunisation, Clinical trials, Respiratory infections

INTRODUCTION

Lack of evidence-based guidelines for a common clinical problem

Young children (up to two years of age) experience symptoms of a respiratory tract infection (RTI) for a median of 44 days per year. The median number of infectious episodes is almost double in children with recurrent RTIs when compared to healthy peers.[1] Even in the absence of high-risk conditions such as major immune deficiencies or congenital malformations, some children develop many more RTIs than their peers.[2] Recurrent RTIs in children are among the leading reasons for primary care consultations and for referral to paediatricians and ear, nose and throat (ENT) specialists.[3] In developed countries, recurrent RTIs, defined as a minimum of six to eight episodes per year, affect 15-20% of children under five years of age.[4] Most children suffer from recurrent RTIs of the upper airways, but in approximately 10-30% the lower respiratory tract is also affected.[5] Compared to their healthy peers, children with recurrent RTIs often visit the outpatient clinic and they more often need hospitalization. In addition, these children are treated more frequently with medication, e.g. inhaled bronchodilators and corticosteroids as well as antibiotics.[1] The high disease burden can also lead to failure to thrive and developmental delays in children, as well as parental productivity losses associated with children's illness and absenteeism from work, leading to high costs for the community.[1, 6, 7] Lower RTIs in childhood can also lead to pulmonary sequelae such as bronchiectasis and irreversible lung damage [8-10], putting children with recurrent RTIs even more at risk for long-term damage. In our experience, children who visit the outpatient clinics because of recurrent RTIs are generally <10 years of age and most are treated on a 'trial and error' base because there is no international consensus about the best treatment for children with recurrent RTIs. Previous studies suggest that antibiotic prophylaxis, treatment with the immunomodulator OM-85, active immunization and/or parental education on risk factors (passive smoking) may be at least of some benefit.[4, 11-14] In general, antibiotics are frequently used for the treatment of acute RTIs in children. In an attempt to treat the assumed bacterial component involved in recurrent RTIs, prolonged antibiotic regimens are often prescribed. Previous studies in children with recurrent acute otitis media (AOM) showed that antibiotic prophylaxis prevented one and a half episode for every 12 months of treatment per child.[15] Studies that examined the clinical effectiveness of antibiotic prophylaxis in children suffering from recurrent RTIs are scarce and mainly focus on high risk groups with recurrent lower RTIs.[11, 16-21] No studies have been performed on clinical effectiveness of antibiotic prophylaxis in otherwise healthy children who suffer from recurrent RTIs.

The antibiotic regimen that is most commonly prescribed in children with recurrent RTIs is trimethoprim/sulfamethoxazole (co-trimoxazole). Both trimethoprim and sulfamethoxazole are bacteriostatic if used alone. Combining trimethoprim and sulfamethoxazole elicits a synergistic effect and makes the antibiotic regimen bactericidal. Co-trimoxazole is a fixed antibiotic combination of trimethoprim and sulfamethoxazole (1:5) which covers most Gram-positive and Gram-negative potential pathogens as well as Pneumocystis jiroveci. In addition, several studies suggest that co-trimoxazol has an immunomodulatory effect which could enhance the immune response.[22-25] The

combination of antimicrobial and immunomodulatory properties could provide an additional beneficial effect in the prevention or reduction of RTIs.

In this study we will compare the clinical effectiveness of co-trimoxazole prophylaxis with placebo in children with recurrent RTIs.

Role of microbiota in respiratory infection and disease

Prolonged antibiotic treatment is of major concern, since antimicrobial resistance (AMR) development increases with duration of the antimicrobial course.[26] Probably the most important route of AMR gene selection in humans is antibiotic-induced changes to our protective microbial communities, also called the microbiota.[27] Whereas microbial disturbances elicited by antibiotic treatment in adults are mostly temporary, exposure to antibiotic treatment early in life may have a lasting impact on the composition of the microbiota leading to permanent replacement by resistant organisms.[28, 29] While the microbiota of the gastro-intestinal tract has been studied most extensively[30, 31], the human nasopharynx is considered the niche from which both upper and lower RTIs originate and resistance can also emerge in commensals or pathogens colonizing this body site.[32] During the past decade, high-throughput pipelines have become available to also characterize the complete nasopharyngeal microbiota.[33]

In this study we will examine short-term and long-term effects of co-trimoxazole prophylaxis on the microbiota composition and antibiotic resistance in children who suffer from recurrent RTIs.

METHODS AND ANALYSIS

Objectives and study parameters

Primarily, we aim to investigate the clinical efficacy of co-trimoxazole prophylaxis in Dutch children (aged six months up to ten years) with recurrent RTIs. Children will be randomized to co-trimoxazole or placebo for a treatment period of three months, since this is the treatment period after which a beneficial effect was achieved in children with recurrent acute or chronic suppurative otitis media.[15]

Primary objective

To determine whether three months of prophylactic treatment with co-trimoxazole causes a reduction in the number of days a child experiences at least two RTI symptoms in children aged 6 months to ≤10 years with recurrent RTIs, when compared to placebo.

Secondary Objectives

1. To determine whether co-trimoxazole prophylactic therapy reduces:

- Time to resolution of symptoms;
- The severity of symptoms defined by the number and type of different infectious symptoms;
- Use of analgesics / antipyretics;

- Use of antibiotic treatment courses;
- Absenteeism from day care or school and/or parental absenteeism from work;
- Alterations in nutritional status.

2. To examine predictors (e.g. demographic, environmental, family history, mucosal, microbiological and immunological characteristics) for the (absence of) prophylactic treatment effect

3. To examine whether cessation of antibiotic prophylactic treatment affects the presence of RTI symptoms and how this correlates with clinical, microbiological and immunological characteristics of the patients.

4. To record and evaluate adverse events:
- The occurrence of mild adverse effects as described in the Summary of Product Characteristics (SPC), such as skin rash, gastro-intestinal complaints, pruritus or mild headache;
- The occurrence of severe adverse reactions.

5. To examine short-term and long-term effects of co-trimoxazole prophylaxis on microbiota deviation, AMR and (mucosal and systemic) immunological outcomes.

Study design

We will conduct a randomized placebo-controlled double-blind clinical trial in which we compare co-trimoxazole with a placebo in 158 children with recurrent upper and/or lower RTIs. The inclusion and exclusion criteria are listed in table 1. We will enrol children aged six months up to ten years. Children younger than six months won't participate in this study, because at this age the presence of recurrent RTIs cannot be established yet. For age-specific definitions of recurrent RTIs, we took the twofold standard deviation of the mean number of upper RTIs per year in a cohort of 1314 German children, except for children aged 5-10 years, in whom we used the same definition as younger children (2-5 years). This means that we define recurrent RTIs as at least 11 parental-reported upper RTIs for children younger than two years and eight parental-reported upper RTIs for children aged two to ten years per year.[34] Recurrent lower RTIs (i.e. pneumonia, bronchopneumonia or acute bronchitis) are defined as at least two episodes per year or three or more episodes during the child's life regardless of age. These definitions of recurrent upper and lower RTIs were also used in the Dutch national guideline for diagnostic strategies in children with recurrent RTIs.[35] If an underlying immune deficiency or contra-indication for co-trimoxazole [36] hasn't been ruled out yet, these will be tested in the blood sample taken in all participants before randomization. Children will be randomized to one of two oral suspension regimens for three months: co-trimoxazole 36 mg/kg/day (2 x 18 mg/kg) or placebo twice daily. The dose of co-trimoxazole is in accordance with the therapeutic dose (for acute infections) as described in the Dutch paediatric drug formulary. Doses and duration of treatment are

also based on studies of antibiotic prophylaxis in paediatric populations with recurrent acute or chronic suppurative otitis media.[15] In case of a new RTI episode occurring during follow-up for which the child has a clinical indication to receive (additional) antibiotic treatment, the child will receive antibiotics that conform to national guidelines and the study medication will be discontinued for the duration of this antibiotic treatment. Medication compliance will be measured in two ways. During the T3 visit to the hospital, parents will be asked to bring the bottles of trial medication and to answer questions about compliance. By doing so, we will be able to compare the self-reported compliance will the number of empty and (partly) full bottles that have been returned.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	- Presenting to one of the participating clinics; - Age 6 months – 10 years; - Suffering from recurrent respiratory tract infections (RTIs)*; - Informed consent from parent(s)/caregiver(s) with legal custody.
Exclusion criteria	- Current prophylactic antibiotic use or prophylactic antibiotic use during the previous month; - Underlying immune deficiency other than for IgA or IgG subclasses; - Congenital abnormalities (including but not limited to cleft palate, neuromuscular or cardiac disorders and syndromes); - Suffering from chronic respiratory disease, such as cystic fibrosis (CF), primary ciliary dyskinesia (PCD) or anatomical abnormalities; - Only experiencing recurrent AOM or chronic suppurative otitis media without other recurrent RTIs; - Known allergy to co-trimoxazole; - Known contra-indication for co-trimoxazole, e.g. liver failure or impaired kidney function and/or haematologic disorders.

* Recurrent upper RTIs: for children aged <2 years yearly at least 11 and for children aged 2-10 years yearly at least 8 parental-reported upper RTIs including, but not limited to, otitis media. Recurrent lower RTIs (i.e. pneumonia, bronchopneumonia or acute bronchitis) are defined as at least 2 episodes per year or 3 or more episodes during the child's life regardless of age.[34, 35]

Randomization

The randomization procedures are performed by a member of the trial pharmacy. Randomization is computer-generated and subjects will be allocated in a 1:1 ratio with random block sizes of two, four or six subjects to prevent predictability of the allocation. Study medication is blinded for the subjects, his/her parent(s), physicians, the monitor and the study team. Only the members of the trial pharmacy have access to information on the allocation for each subject, because they are responsible for the preparation and delivery of the study medication, and for emergency de-blinding, if needed.

Measurements

The schedule of study enrolment, interventions and measurements of endpoints is shown in table 2. For the parent-reported occurrence of symptoms of RTIs, a mobile application will be used. Previous studies used paper diaries to detect parent-reported symptoms. These are prone to non-compliance and hamper real-time detection of disease occurrence. The use of this Diary-App for symptom recording costs parents less than one minute per day and has shown to improve case-finding and questionnaire completeness from 60% to ≥90%.[37] Also, parents will be asked to answer extra questions once a month. These include questions about the use of other antibiotics (see online supplementary file 1 for the monthly questionnaire).

At inclusion and after one, three and six months (+/- two weeks for each sample point) non-invasive respiratory (nasopharyngeal swab and saliva) and faecal samples for microbiota composition, immunologic analysis and AMR gene detection will be collected. Also, these samples could be used for viral analysis. In addition, mucosal lining fluid will be collected at two time points in at least 50 subjects. Before inclusion, blood samples will be taken to test for possible contra-indications (e.g. kidney and/or liver dysfunction). After treatment we will also collect blood samples to monitor for possible kidney, liver and haematological side effects of co-trimoxazole use. Extra blood samples will be collected for additional immunological analyses at both time points. Before the start of treatment parents will be asked to fill in a questionnaire. The study will be started in a minimum of 10 hospitals in the Netherlands. Inclusion will take place during both the winter and summer period to account for seasonal differences in microbiota composition in our analyses. If necessary, the number of study locations can be extended during the study, depending on the speed of subject enrolment.

Table 2. Schedule of enrolment, intervention, and measurement of outcomes

	Enrolment		Post-allocation					
TIMEPOINT	t_0	t_r	t_1	t_2	t_3	t_4	t_5	t_6
ENROLMENT	X							
Eligibility screen	X							
Informed consent	X							
Screening for exclusion criteria	X							
Randomization		X						
INTERVENTION								
Co-trimoxazol or placebo		←	—	—	→			
MEASUREMENTS								
Baseline questionnaire	X							
Digital diary InfectionApp	←	—	—	—	—	—	—	→
Questionnaire on infectious episodes			X	X	X	X	X	X
Physical examination	X				X			X
Blood sample	X				X			
Nasopharynx, saliva and faecal sample	X		X		X			X

Mucosal lining fluid sample*	X		X		(X)			(X)

*Sampling at 2/4 time points, preferably T0 and T1.

Sample size

We assumed 90% compliance with symptom monitoring via the app, i.e. app data available for 0.90 x 3 months (90 days) = 81 days per participant. There is limited literature available about the number of days with RTI symptoms per time period in children with recurrent RTIs. Toivonen et al. published a large prospective cohort study from Finland including 1089 children followed up from birth to two years of age for respiratory infections by a daily symptom diary.[1] In this study, children with recurrent RTIs (defined as number of days with symptoms >90% percentile limit) had a median of 31 days per 100 days with at least one respiratory symptom. In a pilot study performed in the winter season in one of the participating centres (UMC Utrecht) including 18 children with recurrent RTIs we observed a median of 76 days with at least one respiratory symptom and a median of 42 days with at least two respiratory symptoms per 100 days (this would be 34 per 81 days). We estimated that the median number of days with at least two symptoms in the Finnish study would be (42/76)*31 = 17 days per 100 = 14 days per 81 days. Following, we took the average of the Finnish study and our own study to end up with an estimated median number of symptomatic days of (14+34)/2 = 24 days per 81 days in the placebo group.

The IQR in the Finnish study was 104 - 136, translating into a SD of 23.7, which was comparable to the SD in our pilot study (20.5); as such we took the SD of our study for our sample size calculation since the period in which symptoms were measured in the pilot study (mean 115 days) better reflected the follow-up period of the trial (90 days) than the period in which symptoms were measured in the Toivonen study (at least one year).

Assuming 24 days with at least two RTI symptoms in the placebo group and taking our aim to detect a clinically meaningful 40% reduction (i.e. a reduction of 0.40 x 24 = 10 days with RTI symptoms) in the antibiotic treatment arm, we need to include 71 children per arm, so a total number of 71 x 2 = 142 children, according to the Wilcoxon-Mann-Whitney test for two groups (two-tailed) with alfa 0.05 and power 80%. Including a dropout rate of 10%, this brings us to a number of 71 / 0.9 = 79 children per arm, so a total number of 158 children.

Outcomes and data analysis

Table 2 summarizes the assessment and sampling schedule.

Assessment of RTI symptoms

Descriptive statistics for demographic and clinical characteristics for both treatment allocation groups will be described. These include but are not limited to age, height, weight, ethnicity, co-morbidity,

previous ENT-interventions, use of medication, the number of RTI episodes before inclusion, the severity of the infections (e.g. hospital admissions), and type of RTI (e.g. bronchiolitis, otitis, pneumonia). Also, we will examine the frequency distribution of risk factors for the development of RTIs, which include for example smoking in the household, number of siblings and daycare attendance.

Analyses will be performed on the basis of the 'intention-to-treat principle', comparing the treatment arm with the placebo arm, defining the treatment group based on the treatment allocation. In the intention-to-treat analysis every randomized subject will be included according to treatment assignment, thus ignoring potential non-adherence, protocol deviations, withdrawal, and anything that happens after randomization, therefore maintaining prognostic balance generated from the original random treatment allocation. The analyses will only include subjects of whom at least 80% of the symptom diary data is available. An inventory of missing data will be made and if over 5% of 142 subjects have less available data than 80% of the symptom diary days, an imputation strategy will be used. We will also conduct a 'per-protocol analysis' in which we will only include the days that patients adhered to the protocolled treatment allocation. R and IBM SPSS Statistics will be used for statistical analyses.[38]

For our primary objective, we will compare the number of days with at least two RTI symptoms during 90 days of receiving antibiotic treatment / placebo. Since the actual number of monitored days may vary per patient, we will analyse the incidence rate (number of days with at least two 2 RTI symptoms divided by the total number of days monitored). We will use a negative binomial regression analysis with outcome the number of days with at least two respiratory symptoms and use the number of days monitored as offset. Our target parameter is the incidence rate ratio for treatment. We will include main effects of strong predictors of RTI symptoms in the model.

To determine which set of available determinants predicts heterogeneity of treatment effect, we will first develop a prediction model including well-known risk factors for the development of RTI complaints in children. These include for example age of the child, smoke exposure in the household, day-care attendance and number of siblings. In this prediction model, we will adjust for treatment allocation.[39] From this model, a summary score will be derived that is used as a risk score in the final model. The interaction between this risk score and the treatment allocation will be added as a covariate in a model for the primary outcome to investigate to what extent these host factors affect the effect of treatment on our primary outcome.

As a secondary analysis, we will also perform a negative binominal regression analysis with number of days with at least two RTI symptoms as the dependent variable and number of monitored days as offset, during the total 0-6 month period in order to assess the outcome both during and after cessation of co-trimoxazole versus placebo. In addition, we will also apply a mixed effect model to estimate whether the pattern of RTI symptoms over time changes according to treatment allocation.

Respiratory and gut microbiota

For the secondary objective to detect shifts in microbiota composition in the group that received antibiotic prophylaxis compared to the placebo group, we will collect nasopharyngeal swabs (paediatric Copan e-swab with flocked nylon tip) and faecal samples from which bacterial DNA will be extracted according to previously validated methods. Swabs and faecal samples are frozen at -80°C until further analyses. Metagenomic sequencing will be conducted in order to identify the microbiota composition and AMR genes of the faecal samples. For nasopharyngeal samples, 16S-based sequencing will be used to examine the microbiota composition.[30, 40]

Saliva, blood and mucosal lining fluid samples

Blood samples (peripheral blood mononuclear cells and plasma), saliva samples and mucosal lining fluid will be collected at pre-determined intervals (Table 2) for immunological analyses aimed at the identification of markers associated with clinical outcome. Techniques include flow cytometry, proteomics, proliferative studies, cytokine release assays and RNA expression profiling. Saliva samples will also be used for the determination of antimicrobial peptides, inflammatory cytokines, proteomics and mucosal antibodies. Mucosal lining fluid will be used to measure (protein) immune markers such as antibodies, chemokines and cytokines.

Data management

Clinical data will be collected from the Electronic Medical Record by the research staff. All research data will be stored in the data management program Castor EDC. The handling of personal data will comply with the Dutch General Data Protection Regulation (in Dutch: Algemene Verordening Gegevensbescherming (AVG)). Data will be handled confidentially. The research team has access to coded data. To be able to trace data to an individual subject, a subject identification code list will be used to link the research data to the subject, which will be safeguarded by the local investigators and the trial pharmacy. This trial is monitored in accordance with the Good Clinical Practice guidelines.

PATIENT AND PUBLIC INVOLVEMENT

Because we are supported by two patient involvement groups, we feel confident that not only physicians but also patients will be open to our study results. The patient involvement groups were actively involved in the development of our study protocol. During the yearly conference day of the Foundation for Primary Immune Deficiencies (‘Stichting voor Afweerstoornissen’), one of our group members (L.M. Verhagen) has discussed the use of antibiotic prophylactic treatment for mild immune deficiencies in children with patients and parents. Many patients and parents expressed their worries about prolonged antibiotic use, mainly related to its adverse effects and the possibility of future infections with resistant bacteria that can no longer be treated with antibiotics. Following this

discussion, we started discussion sessions with small groups of parents of children visiting the airway clinic in the Wilhelmina Children's Hospital, UMC Utrecht, at several time points. The results showed that parents felt that more research was needed to determine whether antibiotic prophylaxis is an effective treatment. The involvement of both patient groups facilitates acceptance and implementation of our study results in the patient community. We will provide these patient support groups with our study results by publication of the outcomes in 'Paraplu', the monthly journal of the Foundation for Primary Immune Deficiencies, and by discussion of the results during meetings of the patient support groups. Also, we will send a half-yearly newsletter and an information bulletin containing the final results to the study participants.

ETHICS AND DISSEMINATION

This study will be conducted according to the principles of the Declaration of Helsinki (2013, Brazil, version 64) and in accordance with the *Research Involving Human Subjects Act (WMO).* The Medical Research Ethics committee Zuidwest Holland / LDD (The Hague / Leiden, The Netherlands) has approved the study protocol. Approval of the local board of each trial site will be obtained before enrolment of the first subject in that specific hospital. This study is registered in The Netherlands National Trial Register (NTR) as Trial NL7044. After completion of this study, results will be submitted to a peer-reviewed journal.

CURRENT TRIAL STATUS

The first subject was enrolled in January 2019. All the local boards of the first 10 trial sites have given their approval to start enrolling patients in this study, the latest approval was obtained in February 2020. If enrolment is slower than expected, a request for the addition of extra trial sites will be submitted to the Medical Research Ethics committee.

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Authors' contributions

All authors helped with the design of this study and approved the final manuscript. DP, LMV and GD wrote the first draft of the study protocol and manuscript. NG was consulted for the statistical analysis plan for this study. LV helped designing the trial medication and advised on improving safety measures in relation to the trial medication. DB was consulted for the secondary outcomes on microbiota composition and AMR genes. AvR was consulted for the design of the study.

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Competing interests statement

None declared.

Ethics approval

This study has been approved by the Medical Research Ethics committee LDD (Leiden, The Netherlands) and is registered in the Netherlands National Trial Register (NTR) as Trial NL7044.

Supplementary file 1

Monthly questionnaire for participants of the Approach study

1. Did you consult a physician for your child because of a respiratory tract infection in the past month?

☐ Yes, the paediatrician of our own hospital
☐ Yes, the general practitioner
☐ Yes, the otolaryngologist
☐ Yes, another physician
☐ No

1b. If yes, how many times did your child visit a physician because of a respiratory tract infection in the past month?

2. Did you consult a physician for your child because of a stomach infection or gastro-enteritis in the past month?

☐ Yes, the paediatrician of our own hospital
☐ Yes, the general practitioner
☐ Yes, another physician
☐ No

2b. If yes, how many times did your child visit a physician because of a stomach infection or gastro-enteritis in the past month?

3. Did your child take any antibiotics in the past month?

☐ Yes, prescribed by the paediatrician of our own hospital
☐ Yes, prescribed by the general practitioner
☐ Yes, prescribed by the otolaryngologist
☐ Yes, prescribed by another physician
☐ No

Question 4 and 5 are only applicable if question 3 is answered 'Yes'.

4. How many antibiotic regimens did your child use in the past month?

The (sub)questions of question 5 are asked for every antibiotic regimen separately.

5a. For what infection was your child treated with antibiotics? (multiple choice)

☐Rhinitis
☐Otitis
☐Tonsillitis
☐Bronchitis or pulmonary infection
☐Stomach infection or gastroenteritis
☐Other infection (other than respiratory or gastro-intestinal infection)

5b. Which date did your child start with the antibiotic treatment?
DD/MM/YYYY

5c. Which date did your child stop with the antibiotic treatment?
DD/MM/YYYY

5d. What is the name of the antibiotic regimen?

6. Did your child get any vaccines in the past month? (multiple choice)
☐No
☐DKTP-Hib-HepB
☐Pneumococcal
☐MMR
☐Meningococcal C
☐Other vaccine

7. Has your child been admitted to the hospital because of an (suspected) infection in the past month?
☐Yes
☐No

7b. If yes, for what infection was your child admitted to the hospital? (multiple choice)
☐Rhinitis
☐Otitis
☐Throat infection / tonsillitis
☐Bronchitis or pulmonary infection
☐Stomach infection or gastroenteritis
☐Other infection (other than respiratory or gastro-intestinal infection)

7c. If yes, how many days has your child been admitted to the hospital in the past month?

8. Did your child visit any form of day-care or school in the past month?
☐Yes
☐No

8b. If yes, how many half days* did your child visit day-care / school in the past month?
8c. If yes, how many half days* did your child miss from day-care / school because of an infection in the past month?
** A half day is a morning or an afternoon.*

9. Did you or your partner miss work due to your child having an infection during the past three months?
☐Yes
☐No

9b. If yes, how many half days* did you and your partner miss combined?
** A half day is a morning or an afternoon.*

10. Do you have any additional comments about the past month?

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5-6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	7 (table 1)
	4b	Settings and locations where the data were collected	7-8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5-6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7-8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9-11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9-11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	NA
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	1-2
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	-
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	1
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.