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TITLE:
Bronchiectasis exacerbation study on azithromycin and amoxycillin-clavulanate for respiratory exacerbations in children (BEST-2): study protocol for a randomized controlled trial

ABSTRACT:
BACKGROUND: Bronchiectasis unrelated to cystic fibrosis (CF) is being increasingly recognized in children and adults globally, both in resource-poor and in affluent countries. However, high-quality evidence to inform management is scarce. Oral amoxycillin-clavulanate is often the first antibiotic chosen for non-severe respiratory exacerbations, because of the antibiotic-susceptibility patterns detected in the respiratory pathogens commonly associated with bronchiectasis. Azithromycin has a prolonged half-life, and with its unique anti-bacterial, immunomodulatory, and anti-inflammatory properties, presents an attractive alternative. Our proposed study will test the hypothesis that oral azithromycin is non-inferior (within a 20% margin) to amoxycillin-clavulanate at achieving resolution of non-severe respiratory exacerbations by day 21 of treatment in children with non-CF bronchiectasis. METHODS: This will be a multicenter, randomized, double-blind, double-dummy, placebo-controlled, parallel group trial involving six Australian and New Zealand centers. In total, 170 eligible children will be stratified by site and bronchiectasis etiology, and randomized (allocation concealed) to receive: 1) azithromycin (5 mg/kg daily) with placebo amoxycillin-clavulanate or 2) amoxycillin-clavulanate (22.5 mg/kg twice daily) with placebo azithromycin for 21 days as treatment for non-severe respiratory exacerbations. Clinical data and a parent-proxy cough-specific quality of life (PC-QOL) score will be obtained at baseline, at the start and resolution of exacerbations, and on day 21. In most children, blood and deep-nasal swabs will also be collected at the same time points. The primary outcome is the proportion of children whose exacerbations have resolved at day 21. The main secondary outcome is the PC-QOL score. Other outcomes are: time to next exacerbation; requirement for hospitalization; duration of exacerbation, and spirometry data. Descriptive viral and bacteriological data from nasal samples and blood inflammatory markers will be reported where available. DISCUSSION: Currently, there are no published randomized controlled trials (RCT) to underpin effective, evidence-based management of acute respiratory exacerbations in children with non-CF bronchiectasis. To help address this information gap, we are conducting two RCTs. The first (bronchiectasis exacerbation study; BEST-1) evaluates the efficacy of azithromycin and amoxycillin-clavulanate compared with placebo, and the second RCT (BEST-2), described here, is designed to determine if azithromycin is non-inferior to amoxycillin-clavulanate in achieving symptom resolution by day 21 of treatment in children with acute respiratory exacerbations.
TRIAL REGISTRATION: Australia and New Zealand Clinical Trials Register (ANZCTR) number http://ACTRN12612000010897. http://www.anzctr.org.au/trial_view.aspx?id=347879

Aims of the study ::: Background:
In the second phase of BEST (BEST-2) the primary question will be: ‘Is daily oral azithromycin non-inferior (within a 20% margin) to oral amoxycillin-clavulanate at achieving resolution of exacerbations by day 21 of treatment?’
The secondary aims are similar to those in BEST-1 [32], and are to: 1) determine the effect of azithromycin or amoxycillin-clavulanate on QOL, systemic inflammation, time to next respiratory exacerbation, and duration of exacerbations; 2) examine factors that predict response to the two antibiotics, including respiratory pathogens (viruses, bacteria, macrolide-resistant bacteria) present in respiratory secretions, and systemic markers of inflammation; and 3) describe, by using sensitive molecular detection techniques, the point prevalence and diversity of respiratory viruses and Mycoplasma pneumoniae and Chlamydiales species during exacerbations, compared with the findings at enrolment when the children are clinically stable.
The study will test the primary hypothesis that oral azithromycin is non-inferior (within a 20% margin) to oral amoxycillin-clavulanate at achieving resolution of respiratory exacerbations by day 21 of treatment in children with non-CF bronchiectasis.

Study design ::: Methods:
We are conducting a multicenter, parallel group, double-dummy, double-blind placebo RCT (with concealed allocation) to assess whether oral azithromycin is non-inferior to oral amoxycillin-clavulanate at treating children with a non-severe exacerbation of bronchiectasis. Our study plan is summarized in Figure 1.

Eligibility ::: Methods:
The inclusion criteria are: age less than 19 years at time of enrolment; diagnosis of bronchiectasis by a respiratory physician following high-resolution computed tomography in the 5 years immediately prior to study entry, or if diagnosed earlier, evidence of regular follow-up by a respiratory physician for treatment of bronchiectasis; and more than two respiratory exacerbations in the 18 months prior to study entry. Children who have participated in BEST-1 [32] may participate subsequently in BEST-2. These children will be re-randomized for BEST-2.
Exclusion criteria are: current or recent severe exacerbation of bronchiectasis (dyspnea, hemoglobin desaturation <90% in air or hospitalization) in the 8 weeks immediately prior to study entry; presence of CF or liver dysfunction; hypersensitivity to beta-lactam or macrolide antibiotics; current or recent (in the 4-months before study enrolment) lower-airway infection by a member of the Pseudomonas genus of gram-negative bacteria; receipt of beta-lactam or macrolide antibiotics within the 3 weeks preceding study entry; or current treatment for cancer.

Recruitment ::: Methods:
Eligible children will be identified from clinics at one of six sites (Brisbane, Darwin, Melbourne Perth and Sydney in Australia, and Auckland in New Zealand). Parents will be approached, and informed consent obtained. Baseline pre-exacerbation data will be collected (Figure 1), parents will be contacted monthly, and children will be reviewed every 3 months. Parents will be educated specifically on how to recognize the symptoms of an acute respiratory exacerbations, and asked to contact the research nurse at the onset of an exacerbation event.

Intervention and follow-up ::: Methods:
A double-dummy design is planned. If eligibility is fulfilled, and after informed consent has been obtained, the child will be randomized to one of two arms. At the start of the exacerbation, the child will receive orally: 1) azithromycin with placebo amoxycillin-clavulanate or 2) amoxycillin-clavulanate with placebo azithromycin. The azithromycin dose is 5 mg/kg/day (to a maximum of 200 mg daily, and for amoxycillin-clavulanate it is 22.5 mg/kg/dose twice daily (maximum 900 mg/dose). Equivalent volumes of placebo will be given for both trial medications. All treatments will continue for 21 days.
An exacerbation is defined as an increase in sputum volume or purulence, or change in cough (>20% increase in cough score [46] or type (dry to wet) [47]) for more than 3 days. We validated this definition in our prospective study, and found that the kappa values (between clinicians) of these symptoms and signs were excellent (>0.75) [48]. Daily diaries will also be collected during exacerbations until the scores for 2 or more days reflect the child's baseline state, which for each child will be established at enrolment, prior to any exacerbations. This assessment consists of a combination of symptoms (daily cough (yes/no), cough quality (wet/dry/none), cough score [46] averaged over two consecutive days) and signs (sputum color (if any present) using a color chart card (BronkoTest Ltd, London, UK), and crackles on chest auscultation). Children will be reviewed on days 14 and 21, and at resolution of the exacerbation. The exacerbation is considered resolved when symptoms and signs are the same as the baseline state. Post-exacerbation, the children will be followed up and evaluated clinically every 3 months for a period of 18 months or until their next exacerbation (whichever is sooner). The time to next exacerbation will be determined by duration of days from the resolution of the current exacerbation to the beginning of the next exacerbation.

Randomization, allocation, and blinding ::: Methods:
Upon enrolment, the child will be assigned to the next unique number on the appropriate stratified list. The allocation will be performed by the trial pharmacist at the Royal Children's Hospital (Brisbane, Australia). Randomization is stratified by site (Brisbane, Darwin, Melbourne, Perth, Sydney, Auckland), age (≤5 or >5 years) and underlying etiology (idiopathic/post-pneumonia or all other causes). The randomization sequence is computer-generated and uses permuted blocks. The allocation sequence will be concealed at all times throughout the study. The computer-generated allocation sequence was prepared by a statistician external to the study team.
The specially manufactured placebo medications (Institute of Drug Technology Australia Ltd, Melbourne, Victoria, Australia), have a similar taste and color to their respective antibiotics. Both active medications (azithromycin and amoxycillin-clavulanate) will be repackaged and relabeled so that both antibiotics and their respective placebos are provided in identical opaque bottles. For all trial medications, equal volumes of water are added using a syringe and needle by punching the seal. Adherence will be assessed by parent report and return of empty bottles.

Data collection ::: Methods:
All data will be recorded on standardized forms. On enrolment, demographic information (including age, gender, ethnicity, and household size), birth history, breast-feeding history, prior illness, and in utero and household smoke exposure, will be recorded, and a physical examination will be performed by a study physician. The primary and secondary outcome measures will be collected at the time points specified above. Serious and non-serious adverse effects (AEs: nausea, vomiting, diarrhea, rash) will also be documented and monitored. Safety exit points are discussed under 'End points' below.

Specimen collection ::: Methods:
At enrolment (baseline) all children will have a deep-nasal swab (NS) specimen collected. In a subset, additional specimens will be collected at baseline and during exacerbations, depending upon feasibility (some children may be unable to attend the study center at the onset of the exacerbation) and willingness of parents to allow additional NS collections and venepuncture procedures to be performed. These specimens are:
A deep NS specimen for respiratory viruses, respiratory bacterial pathogens (including antibiotic susceptibility testing), and other potentially important respiratory pathogens (M. pneumoniae, Chlamydiales spp) at baseline, and at the beginning and resolution of an exacerbation. The techniques used are identical to previous studies [49-51], in which the specimens were described as nasopharyngeal swabs. In this study, we have elected to call these specimens 'deep-nasal swabs' as it is anatomically accurate to do so. The NS specimens will be handled in accordance with our research laboratory protocol (see below).
Blood samples will be taken at baseline and at the beginning and end of each exacerbation for determination of C-reactive protein (CRP), interleukin (IL)-6 (a neutrophilic marker of inflammation [52]), serum amyloid A (SAA) [48], and markers of viral infection (IL-10, interferon γ-inducible protein (IP)-10) [53].
Sputum samples will also be taken at baseline and at the beginning and end of each exacerbation (when possible) for lower-airway microbiology cultures and antibiotic sensitivity tests.

Cough score ::: Further description of scores and laboratory methods ::: Methods:
The verbal categorical descriptive score is a validated daily diary score of cough rated on a six-point scale (0 (no cough) to 5 (severe cough and cannot perform usual activities)) with increasing scores reflecting greater interference with usual activities. This rating was validated against an objective cough-meter measure [46], and changes in cough scores have been shown to reflect changes in objective cough counts [54].

Parent-proxy cough-specific quality of life score ::: Further description of scores and laboratory methods ::: Methods:
The parent-proxy cough-specific quality of life (PC-QOL) score is a 27-item questionnaire designed to assess the level of frequency of feelings (15 items) and worry (12 items) related to their child's cough. It uses a seven-point Likert-type scale, with higher scores reflecting less frequency and fewer worry concerns (that is, greater QOL) [55,56]. The minimal important difference is 0.62 as determined by the distribution method, and 0.9 as determined by the anchor method [57].

Bacteriology of nasal swab ::: Further description of scores and laboratory methods ::: Methods:
Compared with NS, oropharyngeal sampling underestimates Streptococcus pneumoniae carriage by approximately 50% [58], Thus NS is the preferred method when evaluating the presence of antibiotic-resistant bacteria. Culturing, identifying, and when appropriate, serotyping common respiratory bacteria are established techniques in our research laboratory [51,59]. Swabs are stored in skim-milk tryptone-glucose-glycerol broth medium at −80°C ,before being batch-processed for typical respiratory bacterial pathogens, notably S. pneumoniae, H. influenzae (including strains of non-typeable H. influenzae) and Moraxella catarrhalis. Batches of swabs are thawed, and 10 μL aliquots cultured overnight on selective media at 37°C in 5% CO2. Growth of S. pneumoniae, H. influenzae and M. catarrhalis is recorded and confirmed by standard techniques [51,60]. Two isolates each of S. pneumoniaH. influenzae and M. catarrhalis per positive swab are tested for anti-microbial resistance and stored [51,60]. S. pneumoniae isolates are serotyped using the Quellung method (antisera from Statens Serum Institute, Copenhagen, Denmark). Routine susceptibility testing using the calibrated dichotomous susceptibility disk-

diffusion method. If the if the azithromycin disk annulus is less than 6 mm the minimum inhibitory concentration (MIC) of azithromycin will also be determined (Etests; AB Biodisk, Solna, Sweden). For S. pneumoniae, the penicillin MIC will be determined for penicillin non-susceptible isolates (oxacillin and/or penicillin disk annulus < 6 mm) and for H. influenzae, the ampicillin MIC will be determined for ampicillin non-susceptible isolates (ampicillin disk annulus < 6mm). Interpretive criteria (Clinical and Laboratory Standards Institute breakpoints) used for S. pneumoniae are penicillin non-susceptible MIC greater 0.12 μg/ml and azithromycin resistance MIC 2 μg/ml or greater; and for H. influenzae, ampicillin resistance MIC 4 μg/ml or greater and azithromycin resistance MIC greater than 4 μg/ml. A nitrocephin-based test will identify beta-lactamase activity in H. influenzae and M. catarrhalis isolates.

Assessment for viruses and other bacteria ::: Further description of scores and laboratory methods ::: Methods:
We will use our previously described methods of assessment [61,62]. Nucleic acids will be extracted from the media (High Pure Viral Nucleic Acid Kit; Roche Diagnostics, Sydney, NSW, Australia), in accordance with the manufacturer's instructions. Real-time PCR assays will be used to detect respiratory syncytial viruses (A and B), adenoviruses, influenza viruses (A and B), parainfluenza, human metapneumovirus, human coronaviruses (OC43, HK1, 229E, and NL63), enteroviruses, rhinoviruses (including determining specific rhinovirus genotypes by sequencing the VP4-VP2 region [63]) and the more recently described human viruses (human bocavirus 1, parechoviruses, and human polyomaviruses K1 and WU) and M. pneumoniae and Chlamydiales species [64].

Blood markers ::: Further description of scores and laboratory methods ::: Methods:
CRP, threshold 5 mg/l) are standard tests that will be analyzed by the Diagnostic Laboratories of each participating center. SAA, IL-6 (threshold <3 pg/ml), IL-10 (threshold <0.5 pg/ml) and IP-10, (threshold 2.8 pg/ml) will be performed by commercial enzyme immunoassay kits (R&D Systems, Minneapolis, USA) at our research laboratory.
Spirometry (in children aged ≥5 years) will be performed using American Thoracic Society criteria and the recorded FEV1 % predicted. We elected not to use oscillatory measures, as we previously found no difference in airway resistance between steady and exacerbation states [48]. Thus, we will use conventional spirometry, although we do not expect to detect significant differences.

End points ::: Methods:
Participation will be complete when the child's clinical state returns to baseline and the time to next exacerbation has been obtained. Other exit points are: if the child deteriorates during treatment prior to day 21, or becomes sufficiently intolerant of the trial medications to require withdrawal from the study (as determined by the treating clinician).

Primary outcome ::: Outcome measures ::: Methods:
The primary outcome will be the proportion of children whose exacerbations have resolved by day 21 of treatment. Exacerbations will be considered resolved when symptoms and signs are the same as the baseline state. Children who are withdrawn from the study, or receive additional antibiotic treatment, will be categorized as non-resolved.

Secondary clinical outcomes ::: Outcome measures ::: Methods:
The main secondary outcome is the PC-QOL score. Other outcomes are 1) the time to next exacerbation; 2) requirement for hospitalization; 3) duration of exacerbation (persistence of symptoms till return to baseline state); and 4) FEV1 % predicted.

Secondary laboratory outcomes ::: Outcome measures ::: Methods:
Serum markers (CRP, SAA, IL-6, IL-10, IP-10) and data on viruses and respiratory bacterial pathogens, including their antibiotic susceptibility to penicillin and azithromycin.

Sample size ::: Methods:
We plan to enroll 170 children (85 per arm), providing 90% power ($\alpha = 0.05$, 1-sided) with 20% non-inferiority margin to detect 80% resolution rate by day 21. The margin selected is relatively large in statistical terms, but the physicians considered it clinically appropriate. As the primary outcome will be obtained in all enrolled children, retention fraction has not been factored in for the intention-to-treat analysis.

The main secondary outcome (secondary aim 1) is PC-QOL. Based on a non-inferiority limit of 0.9 (minimum important difference [57]) and standard deviation of 0.9, our sample size provides a power of 99.9% ($\alpha = 0.05$, one-sided 95% CI) for data from at least 136 children (assuming at least 80% retention of children enrolled). For secondary aim 2 (see list under 'Aims of the study'), we will be examining eight main factors, and thus a sample size of 136 exceeds the recommended minimum (n = 10 per factor) [65]. The eight factors are: smoking, age, underlying etiology, detection of virus (any versus none, then single versus multiple viruses), presence of azithromycin resistance, and levels of various blood markers (IL-6, IL-10, IP-10).

Statistical analysis for secondary outcomes and aims ::: Statistical analysis and reporting ::: Methods:
For the clinical secondary outcome (secondary aim 1), the t-test or the Mann–Whitney test will be used for continuous variables (depending on normality of data distribution). A Kaplan-Meier curve will be constructed for each group for time to resolution and time to next exacerbation, as reported previously [67]. For secondary aim 2 (factors that predict response to antibiotics), univariate analyses will be used to examine several biologic factors (for example, smoking, age, ethnicity, underlying etiology, detection of virus (any versus none, then single versus multiple viruses), presence of azithromycin resistance, and levels of blood markers (IL-6, IL-10, IP-10)). Factors with $P<0.2$ will be included in a logistic regression model. Potential interactions (for example,. between viruses and bacteria) will be examined in the model. Descriptive data will be used for secondary aim 3 (point prevalence of respiratory pathogens).

Data safety monitoring committee ::: Methods:
A Data Safety Monitoring Committee has been established and met prior to commencement of this study.

Ethics approval ::: Methods:
The protocol has received ethics approval from the respective Human Research Ethics Committees of all the participating institutions (Brisbane: Children's Health Queensland Hospital and Health Service (Royal Children's Hospital) and University of Queensland; Darwin: Department of Health and Families and Menzies School of Health Research; Melbourne: Royal Children's Hospital; Perth: Princess Margaret Hospital; Sydney: Sydney Children's Hospital Network Human Research Ethics Committee; and Auckland: Northern Ethics Committee, Ministry of Health and Starship Children's Health local ethics committee). The study is being conducted under Australia's Therapeutic Goods Administration Clinical Trial Notification (CTN) scheme.

Rationale for our chosen outcome measures and timeframe ::: Discussion:
In BEST-1, we chose day 14 as the time point for this RCT, based on available data from our retrospective data of 115 respiratory exacerbations [77], and on parental and healthcare professional concerns over using placebo for an extended period [32]. For BEST-2, presented here, we chose day 21 as the crucial time point because hospitalization is usually recommended if there is no symptomatic improvement after 3 to 5 weeks of oral-antibiotic therapy.
Adult bronchiectasis studies show that QOL measures, particularly cough-specific QOL indices, are valid and important outcome measures [79,80]. Likewise, we have previously shown the utility of the PC-QOL score in children with bronchiectasis [27].

Trial status ::: Conclusion:
Recruitment for BEST-1 [32] started in mid-March 2012 in Darwin, and in June 2012 in Brisbane. Randomization for the BEST-2 component commenced in October 2012 in Brisbane and Darwin.

Abbreviations:
BEST: Bronchiectasis Exacerbation Study; CF: Cystic fibrosis; COPD: Chronic obstructive pulmonary disease; CRP: C-reactive protein; FEV1: Forced expiratory volume in 1 second; IL: Interleukin; IP: Interferon γ-inducible protein; MIC: Minimum inhibitory concentration; NS: Nasal swab; PC-QOL: Parent-proxy cough-specific quality of life; PCR: Polymerase chain reaction; QOL: Quality of life; RCT: Randomized controlled trial; SAA: Serum amyloid A.

Competing interests:
The authors declare that they have no financial competing interests related to this study.

Authors' contributions:
AC conceived the study, participated in its design and coordination, and drafted the manuscript. PM, CR, KG, PvA, AW, KO, PT, TS participated in study design and submission to the National Health and Medical Research Council (NHMRC). GM participated in initiating the project, and TS and IMM participated in the viral analysis plan. IBM, CB, and HB will assist in recruitment and assessment of the children. JU will participate in the biochemical analysis of the blood samples. All authors have read and approved the final manuscript.