Statistical analysis plan (SAP)

Use of probiotics to reduce infections and death and prevent colonization with extended-spectrum beta-lactamase (ESBL)-producing bacteria among newborn infants in Tanzania (ProRIDE Trial): study protocol for a randomized controlled clinical trial.

Version 1.1
Date: 8th of May 2023

Table of content

Statistical analysis plan (SAP) 1
Table of content 2
Administrative information 4
1. Title and trial registration number 4
2. SAP version and document date 4
3. Protocol version 4
4. SAP revisions 4
5. Roles and responsibilities 4
6. Signatures 5
Introduction 6
7. Background and rationale 6
8. Objectives 6
Study methods 7
9. Trial design 7
10. Randomization 7
11. Sample size 7
12. Framework 8
13. Statistical interim analysis and stopping guidance 8
14. Timing of final analysis 8
15. Timing of outcome assessments 8
Statistical principles 9
16. Levels of significance, P values 9
17. Rationale for any adjustment for multiplicity, and, if so how the type 1 error is to be controlled 9
18. Confidence intervals to be reported 9
19. Adherence and protocol deviations 9
20. Analysis populations 9
Trial population 10
21. Screening data 10
22. Eligibility 10
23. Recruitment 10
24. Withdrawal / follow-up 10
25. Baseline patient characteristics 11

Analysis 11
26. Outcome definitions 11
27. Analysis methods 11
28. Missing data 12
29. Statistical software 12
Publication plan 12
30. Primary outcome 12
31. Secondary outcomes 12
32. Cost-efficacy and ethical issues 13
References 13

Administrative information

1. Title and trial registration number

Title: *Use of Probiotics to Reduce Infections and Death and Prevent Colonization with Extended-spectrum beta-lactamase (ESBL) producing bacteria among newborn infants in Tanzania (ProRIDE Trial): a randomized controlled clinical trial*

Trial registration number: *ClinicalTrials.gov, NCT04172012*

2. SAP version and document date

This is SAP version 1.1
Document date: 8th of May 2023

3. Protocol version

Kuwelker K, et al. Use of probiotics to reduce infections and death and prevent colonization with extended-spectrum beta-lactamase (ESBL)-producing bacteria among newborn infants in Tanzania (ProRIDE Trial): study protocol for a randomized controlled clinical trial. Trials 2021 Apr 29; 22(1): 312. doi: 10.1186/s13063-021-05251-3

4. SAP revisions

SAP revision history: First version (1.0) was included in the publication published April 29, 2021.
Justification for each revision: This is a more extensive revision specifically dealing more in details with statistical analyses. This revised SAP will be published at Clin. Trials.gov before 6 months follow up is completed and before unblinding of study results.
Timing of SAP revisions in relation to interim analyses: Interim analyses have been performed, and no changes to the protocol have been suggested.

5. Roles and responsibilities

Coordinating Investigator and trial leader
- Nina Langeland, UiB /Haukeland University Hospital

Co-Principal Investigators, Co-trial lead:
- Claus Klingenberg, UiT-The Arctic University of Norway, Tromsø, Norway
- Joshua Gideon, Haydom Lutheran Hospital, Mbulu district, Tanzania
- Bjørn Blomberg K2, UiB/ Haukeland University Hospital, Norway

Other investigators:
- Sabrina Moyo, UiB /Haukeland University Hospital, Norway
- Museveni Justine, Haydom Lutheran Hospital, Mbulu district, Tanzania
- Iren Løhr, Stavanger University Hospital, Norway

- Veronika Pettersen, UiT-The Arctic University of Norway, Tromsø, Norway

6. Signatures

Persons writing the SAP:

Claus Klingenberg

Bjørn Blomberg

Senior statistician responsible

Bjørn Blomberg

Chief investigator, clinical lead:

Nina Langeland

Introduction

7. Background and rationale

Synopsis of trial background (from published study protocol)
Infections continue to be a considerable cause of death and disease among infants in low- and middle-income countries. In Sub-Saharan Africa, infections contribute to 3/4 of under-five mortality. In Tanzania, the under-five mortality was 55 per 1000 live births in 2016, whereof 42 deaths per 1000 live births occurred before the age of one year.
An increasing prevalence of antimicrobial resistance (AMR) is among the most urgent global threats. Previous studies of hospitalized children in Tanzania showed that blood-stream infections caused by extended-spectrum beta-lactamase producing Enterobacteriaceae caused a mortality rate of more than 70%, compared to 20% or less for malaria infections and BSI with susceptible bacteria. WHOs SDG3 call for an effort to end preventable newborn and child deaths. New strategies to achieve this goal are needed. Probiotics have reduced mortality in preterm infants in high-income countries, and may reduce AMR development.

Research question
Can routine probiotic supplementation reduce mortality and morbidity among infants during first 6 months of life, in a low-income country with high infant mortality and high rates of severe infections?

Justification for trial
In a large study in India by Panigrahi et al (1), probiotics combined with a prebiotic administered to healthy newborn infants reduced severe infections and/or death by 40% compared to placebo. This approach has not been tested or reported in Sub-Saharan Africa. We therefore embarked on a study with inclusion of 2000 infants randomly assigned 1:1 to probiotic or placebo in order to evaluate whether such a low-cost and simple intervention can reduce infant mortality and morbidity. Inclusion of participants started in January 2022 and 6 months follow-up for all will be completed in July 2023.
If the hypothesis of the ProRIDE trial is confirmed, the study results will be helpful for government officials responsible for national guidelines, and it will support the implementation of probiotic use in routine clinical care to reduce morbidity and improve survival of infants in low-income settings.

8. Objectives

Hypothesis
Routine probiotic supplementation can reduce mortality and morbidity among infants in a low-income country with high infant mortality and high rates of severe infections, and this is mediated by a reduction in carriage of resistant bacteria leading to a reduction of bacteremia resistant to standard antibiotic treatment.

Specific objectives
Primary objective: Assess whether a 4-week course of a probiotic (Labinic®, a multi-species probiotic product) in healthy newborn infants in Mbulu district Tanzania can reduce the rates of death and hospitalization during first 6 months of life compared to placebo.

Study methods

9. Trial design

The ProRIDE trial is a two-arm 1:1 randomized clinical trial assessing whether healthy newborn infants in Tanzania (P) administered a probiotic product (I) versus those administered the placebo product (C) will have reduced probability of the composite outcome death or hospitalization during first 6 months of life (O).

10. Randomization

Participants have been randomized to probiotic or placebo using fixed equal allocation ratios. An independent researcher/statistician at University of Bergen created a computer-generated randomization list, with the study identification number of patients, from 1 to 2000, allocated to probiotic or placebo. We have not planned for any restrictions to reduce the predictability of the randomization list. The probiotic and placebo are identical in taste and color, and produced in identical bottles. A pharmacist, who was not part of the study, was provided with the randomization list and labelled the identical bottles, numbered from 1 to 2000. This was performed before shipment to the study site. At the time of inclusion of infants in the ProRIDE study, study personnel provided the parents with consecutive, prelabelled bottles with probiotic or placebo according to the randomization list.

11. Sample size

Infant mortality rate in Tanzania was in 2018 reported at 3.8%. We do not have exact data for the Mbulu district, but we assume a similar rate of approximately 4%. Serious infections leading to hospitalization are probably equally common, but hospitalization rates may depend on available access. In the Panigrahi study, a synbiotic product was given for only seven days, and it reduced the composite outcome death and/or infection from 9.0% to 5.4% during the first two months of life. In our study, the intervention will continue for around four weeks and follow-up will continue until six months of life. We consider a similar reduction in the composite outcome death and infections leading to hospitalizations from 9.0% to 5.4%, as in the Panigrahi study, to be clinically relevant (1). To find this difference with a study power of 85% at 5% significance level a sample size of minimum 924 cases in each arm; thus 1848 infants is needed. In order to allow for drop-out we aimed to include 2000 infants. With this number of infants, we will also have sufficient power to detect a minimum of 30% reduction in ESBL-E colonization rate (from around 50% to 35%) at six months of age.

We finished recruitment of 2000 participants in January 2023 and will complete 6 months follow-up for all participants in July 2023.

12. Framework

The study will test the hypothesis of superiority of probiotic over placebo in preventing death or hospitalization during the first 6 months of life. Comparison of the proportion of participants with the primary outcome will be presented.

13. Statistical interim analysis and stopping guidance

Interim analyses have been performed after six month follow-up of 500 and 1000 infants. The major outcomes and adverse outcomes of interest; mortality, hospitalizations and blood culture confirmed sepsis have been summarized and presented to the DSMB. Only the study statistician have had access to these results. If mortality between groups (probiotic vs placebo) at 6 weeks follow-up had been significantly different at the level of $p<0.05$ by Chi square test at assessment at these two time points of the study, the study would have been stopped. This decision had in that case to be taken by the PI of the study, in consultation with co-investigators and the DSMB. Other triggers that would call for an unscheduled DSMB review were if infant mortality or morbidity rates clearly exceed expected rates in Tanzania. Severe adverse events have also been be reported consecutively to the DSMB, and the DSMB have evaluated this independently of the interim analysis.

14. Timing of final analysis

Final analysis of data will be performed after all patients have completed 6 months follow-up. This analysis will be performed on anonymized data. Blinding for investigators and patients will be maintained until the study is completed.

15. Timing of outcome assessments

The primary outcomes will be assessed at 6 months. Secondary outcomes will be assessed at 6 weeks and 6 months. Additionally, adverse events have been and will be reported to the DSMB consecutively for independent evaluation until the study is completed.

Statistical principles

16. Levels of significance, P values

Analysis results for the primary outcome will be reported as odds ratio with 95% confidence interval with P-value. The standard cut-off of $p<0.05$ will be used for statistical significance levels. For secondary outcomes we will report appropriate effects measures with 95% confidence intervals without p-values.

17. Rationale for any adjustment for multiplicity, and, if so how the type 1 error is to be controlled

Type I error will be controlled at the traditional 0.05 two-sided level for the hypothesis test. We will perform a hypothesis test only for the primary outcome and we will not need to adjust for multiple tests.

18. Confidence intervals to be reported

Odds ratios and other relevant effect-measures will be estimated in appropriate regression models and will be reported with 95% confidence intervals.

19. Adherence and protocol deviations

Any deviations from the protocol will or have already been documented in a protocol deviation form and filed in the study master file. A SOP is in place describing the procedure for identifying non-compliances, escalation to the central team and assessment of whether a non-compliance /deviation may be a potential Serious Breach.

20. Analysis populations

The primary analysis will be an “intention to treat” analysis performed on all patients who were randomized. In addition, per protocol analysis will be performed for patients who reported to have adhered to the ingestion of the study product (probiotic or placebo), and on other subgroups of the study population.

Trial population

21. Screening data

Pregnant women in their last trimester, visited at home or attending the last antenatal care visit at HLH, will receive oral and written information about all aspects of the study before giving consent to let their future child participate in the study. Trained research assistants who speak the local language will be in charge of obtaining informed consent before recruitment.

22. Eligibility

Inclusion criteria
Healthy newborn infants with a birth weight (BW) equal or above 2.0 kg are eligible for inclusion. Newborn infants have to come from families who are long-term or permanent residents in the defined catchment area for this trial (30 km radius from HLH) in Tanzania. Parents have to be able and willing to complete study visit (including required study procedures) schedules over the six months proposed follow-up. This also includes that they, if possible, bring their child to the hospital in case of any intercurrent illness. Parents have to sign informed consent form (ICF) and have to agree that the child cannot participate in another clinical trial during the study period

Exclusion criteria
BW below 2.0 kg, refusal of informed consent and/or other health problems/illness including obvious congenital malformations

23. Recruitment

Patients will be recruited among newborns; born at Haydom Lutheran Hospital (HLH) in North East Tanzania and out-born newborns in the surrounding area. HLH is located in the Mbulu district, at the western end of the Manyara region in the North-Central Tanzania, about 300 km south-west from regional center Arusha. The HLH catchment area consists of four administrative divisions, three districts and two regions. These are the Dongobesh and Haydom divisions in Mbulu District, the Basotu Division in Hanang District (Manyara Region), and the Nduguti Division in Iramba District (Singida Region). The hospital serves a population of more than two million people from five regions. Annually, there are more around 3900 deliveries in the hospital, and in 2018 the neonatal mortality rate in Tanzania was around 21/1000 live births.

24. Withdrawal / follow-up

Participation is based on informed voluntary consent of the parents of the child. Parents may decide that their child may withdraw at any time of the study without giving reasons or justification. Upon withdrawal, no further data will be collected. However, data recorded up until the time of withdrawal will be included in the analysis.

25. Baseline patient characteristics

Baseline data on demographic characteristics are recorded as detailed in the eCRF.

Analysis

26. Outcome definitions

Primary outcome
Prevalence (%) of the composite outcome death and/or hospitalizations at six months of age.

Secondary outcomes
- Rates (%) of ESBL-E colonization at six weeks and six months of age.
- Rates (%) of hospitalizations up to six months of age.
- Rates (%) of confirmed sepsis (blood culture-confirmed) episodes up to six months of age.
- Growth monitored by length and weight up to six months of age.
- Stool microbiota composition including resistome analysis (metagenome sequencing) at six weeks and six months of age.
- Stool metabolome composition at six weeks and six months of age.
- Stool inflammatory markers at six months of age
- Genetic characteristics of ESBL-E from colonization and clinical samples (targeted screening).
- Risk factors for death, hospitalization and sepsis.
- Primary and secondary outcomes will secondarily be performed stratified for place of delivery, ie hospital, home or primary health care facility.

27. Analysis methods

Clinical and microbiological data
In univariate analysis, comparison of proportions will be done using Pearson's chi-squared test and comparison of means will be done using t-test or non-parametric tests as appropriate. Logistic regression will be used for multivariate analysis when assessing the relative importance of risk factors for carriage of ESBL-E and other multi-resistant bacteria.

Stool microbiota, stool metabolome and stool inflammatory markers
Stool microbiota: A Poisson generalized linear model to will be used to calculate trends in the relative abundance of genera and antibiotic resistance genes in the stool microbiota. Corrections based on multiple comparisons will be performed by the Benjamini-Hochberg false discovery rate (FDR). An FDR *Q* value ≤.10 will be considered significant for any analyses with multiple comparisons. A standard *P* value ≤.05 will be considered significant for all other analyses. Alpha diversity of the gut microbiota will be assessed by calculating the Shannon Diversity index (MEGAN, v5.10.6). Multiple beta diversity metrics of

samples will be performed by using non-metrical multidimensional scaling (NMDS), based on a matrix of Bray-Curtis distances and calculated by using the vegan R package. Differences between groups will be tested by using per mutational multivariate analysis on beta diversity matrices.

Stool metabolites will be identified by using mass spectral (MS) databases (*e.g.*, MzCloud, Metlin) and standards purchased for this project. For statistical and pathway analyses, we will use web-based tool MetaboAnalyst. All MS data will be submitted to and stored at the European Bioinformatics Institute repository MetaboLights. Stool inflammatory markers will be analysed with conventional statistical tools for interval data using parametric or non-parametric tests, as appropriate.

28. Missing data

Missing data for both binary and continuous variables will in the main analyses be handled as non-responses. Depending on the degree of missingness, we will perform supplementary analyses with multiple imputation of missing values to assess the possibility that the missing data may introduce bias.

29. Statistical software

Statistical analysis will be performed in R version 4.2.2 or higher (The R Foundation for Statistical Computing, Vienna, Austria). Mixed effects models analysis in R will be performed using the “lme4” or “lmerTest” packages. If imputation is considered necessary or useful, regression analysis with multiple imputation will be performed, and imputation will be done using the mice.impute.bygroup function in the “mice” and “miceadd” libraries in R.

Publication plan

30. Primary outcome

The first main paper will present the effect of probiotic administration on the primary outcomes (death and/or hospitalization) and the following secondary outcomes: carriage of ESBL-producing Enterobacterales and the taxonomy of the gut microbiota in the two study arms. Safety of the probiotics will be described in the same paper, with focus on adverse events.

31. Secondary outcomes

Further analysis of the effect of the probiotics on prespecified secondary outcomes will be published separately.

32. Cost-efficacy and ethical issues

Given a positive effect of probiotics, cost-benefit considerations of probiotic treatment will be published focusing on the perspectives of the individual patient and society at large, including data on hospitalization, healthcare use, etc.

References

1. Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, Chandel DS, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature. 2017;548(7668):407-12.