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

Protocol Number: ProRIDE-HLH#01

Clinicaltrials.gov identifier: NCT04172012

Protocol Date: 19th December 2021

Protocol Version: 2.0

Sponsor name: Haydom Lutheran Hospital

Signature: Date:

Study Principle investigator

Dr. Joshua G Gidabayda, MD, MMed-Haydom Lutheran Hospital

Signature: Date:

Study title	Use of **Pro**biotics to **R**educe **I**nfections and **D**eath and Prevent Colonization with **E**xtended-spectrum beta-lactamase (ESBL) producing bacteria, among newborn infants in Haydom, Tanzania, a randomized controlled clinical trial
Study acronym	ProRIDE trial
Short title	Use of Probiotics to reduce Infections, Death and ESBL colonisation.
Version and date of the protocol	Version 1.0, June 2019
Principal investigator	**1. Nina Langeland, MD, PhD** Department of Clinical Science, University of Bergen, Norway National Centre for Tropical Infectious Diseases, Haukeland University Hospital, Bergen, Norway
Internal Principal investigator	**2. Joshua G Gidabayda, MD MMed,** Department of Paediatrics, Haydom Lutheran Hospital, Tanzania
Co- investigators and collaborators	**3. Bjørn Blomberg, MD, PhD** Department of Clinical Science, University of Bergen, Norway National Centre for Tropical Infectious Diseases, Haukeland University Hospital, Bergen, Norway **4. Iren Löhr, MD, PhD** Department of Medical Microbiology, Stavanger University Hospital, Norway **5. Claus Klingenberg, MD, PhD** Department of Paediatrics, University Hospital of North Norway Department of Clinical Medicine, UiT-The Arctic University of Norway **6. Kanika Kuwelker, MD, PhD** National Centre for Tropical Infectious Diseases, Haukeland University Hospital, Bergen, Norway **7. Esto Mduma, DipMed, DLSHTM, MSc PH** Haydom Global Health Research Center **8. Dr. Paschal Mdoe, MD, PhD,** Haydom Lutheran Hospital

	9. Museveni Justine, MD Haydom Global Health Research centre **10. Samweli Jatosh. BSc. chemistry** Haydom Global Health Research centre. **11**. **Joel Manyahi, MD, MMed, PI Microbiology Tanzania** Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, MUHAS, Dar-es-Salaam, Tanzania **12**. **Sabrina Moyo, MD, Mmed, PhD** Department of Clinical Science, University of Bergen, Norway Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, MUHAS, Dar-es-Salaam, Tanzania. **13. Anania Daniel Tippe, BSc in Medical Lab Technology** Head of Clinical Laboratory, Haydom Lutheran Hospital, Haydom, Manyara, Tanzania
Funding source /Sponsor	Name: The Western Norway Regional Health Authority Address: Nådlandskroken 11, 4034 Stavanger, Norway Phone number: +47 51 96 38 00 Email: helse@helse-vest.no
Data safety monitoring committee (DSMC)	Ketil Størdal, Sven Gudmund Hinderaker, Charles Makasi and Blandina Mbaga
Study pharmacist	William Mollam**,** Haydom Lutheran Hospital
Clinical site	Haydom Lutheran Hospital, Manyara region, Tanzania
Laboratory sites	1. Haydom Lutheran Hospital 2. Department of Clinical Science, University of Bergen, Norway 3. The Arctic University of Norway (UiT), Tromso, Norway 4. Department of Medical Microbiology, Stavanger University Hospital, Norway
Ethical review Boards	1. Regional Committee for Medical and Health Research Ethics (REK) in Norway 2. National Institute of Medical Research Review Board, NIMR in Tanzania
Regulatory Review	Tanzania Food and Drug Authority (TFDA)

List of abbreviations 6

Summary 7

1. Introduction 10

1.1 Background Information 10

1.2 Main challenges and gaps of knowledge 13

1.3 Rationale to conduct this study 14

1.4 Study hypothesis 14

2. Objectives of the study 15

2.1 Overall objective of the study 15

2.2 Specific objectives of the study 15

2.3 Study end point measures 16

2.3.1 Primary end point 16

2.3.2 Secondary end points 16

3.0 Methods 17

3.1 Study design 17

3.2 Study site 18

3.3 Study setting and population 18

3.4 Research question and statistical power 19

3.5 Inclusion and exclusion criteria 20

3.5.1 Inclusion criteria: 20

3.5.2 Exclusion criteria: 20

3.6 Study period 20

3.7 Investigational product (IP) 21

3.7.1 Description and formulation 21

3.7.2 Dose preparation and administration 21

3.7.3. Storage and stability (shelf-life) 21

3.7.4 Packaging and labelling 21

3.7.5 Accountability 21

3.8 Study procedures 22

3.8.1 Recruitment of pregnant women and enrolment of study participants 22

3.8.2. Filling of the electronic CRF using RED cap 22

3.8.3 Randomization and blinding 23

3.8.4 Unmasking in emergency situations 23

3.8.5 Visit schedule and follow-up activities 24

3.9 Specimen collection and storage 24

3.10 Data collection and handling during the study period 25

3.11 Microbiological methods 26

3.11.1 Laboratory tests to be performed at Haydom Lutheran Hospital..... 26

3.11.2 Laboratory tests which to be performed at UiB/SUH/UNN 26

4.0 Monitoring of the study 28

5.0 Criteria for withdrawal of individual study participants 30

6.0 Ethical considerations 31

6.1 Potential benefits and risks associated with the study..... 31

6.2 Regulation statement, Ethical approval and Protocol registration..... 31

6.3 Recruitment and consent 32

6.4 Treatment during the study period 32

6.5 Compensation for injury..... 33

6.6 Transfer of Data and Biological Material 33

6.7 Data Safety Monitoring Body (DSMB) and schedule of work..... 33

7.0 Statistical analysis 36

8.0 Organization, collaboration and research environment..... 38

9.0 Budget 40

10.0 Time schedule 43

11.0 End of study report 44

12.0 Plan for Dissemination of results 45

13.0 Planned publications 46

14.0 User Involvement..... 47

15.0 References 48

16.0 Informed consent form..... 51

List of abbreviations

AMR	Antimicrobial resistance
BSI	Blood stream infection
CRF	Case Report Form
DNA	Deoxy Ribose Nucleic acid
ESBL	Extended-spectrum beta-lactamase
PhD	Doctor of Philosophy
PI	Principal Investigator
WHO	World health organization
NEC	Necrotizing Enterocolitis
MDR	Multi Drug Resistance
RCT	Randomized Clinical Trial
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
UiB	University of Bergen
UiT	The Arctic University of Norway
NIMR	National Institute of Medical Research
TFDA	Tanzania Food and Drug Administration
GCP	Good Clinical Practice
SUH	Stavanger University Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
HUH	Haukeland University Hospital
HLH	Haydom Lutheran Hospital
SOP	Standard Operating Procedures

Summary

Background and objectives:

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 previous studies of children in Tanzania, we observed that blood-stream infections (BSIs) caused by Gram-negative bacteria (Enterobacteriales) with extended-spectrum beta-lactamase (ESBL)-type resistance gave a mortality rate of more than 70%, compared to 20% or less for malaria infections or BSI with susceptible bacteria. An increasing prevalence of ESBL-producing *Enterobacteriales* (ESBL-E) are among the most urgent global health threats, and ESBL-E are by WHO ranked on priority 1 among pathogens in need of new treatment. We have previously shown in a study from Tanzania that around 2/3 of infants below three months of age had faecal carriage of ESBL-E.

Probiotics are live microorganisms that, when administered in adequate amounts, confer a benefit to the host. Probiotics are currently widely used in newborns and children. They have proven beneficial effects in preterm infants with reductions in the incidence of necrotizing enterocolitis (NEC) and sepsis. For this patient group the use of probiotics in high-income countries has entered what has been called a "golden age", and similar beneficial effects have also been observed among preterm infants in low- and middle-income countries. Probiotics may also reduce colonization by ESBL-E and other antibiotic resistant bacteria through "colonization resistance", but there is limited data on which probiotic strains that most effectively reduce ESBL-E carriage in children. In adults and older children lactobacilli are often used to prevent or treat other conditions like antibiotic-associated diarrhea, gastroenteritis and infantile colic. A recent large clinical trial from India reported reduced mortality and fewer severe infections in children receiving probiotics combined with a fructo-

oligosaccharides (prebiotic).

The present clinical trial will evaluate whether use of probiotics can reduce infection-related morbidity and mortality by preventing colonization and/or infections with ESBL-E among infants in Haydom, Tanzania.

Methods and expected results:

This is a single center randomized double-blind controlled clinical trial (RCT) with two arms, one intervention group (n=1000) randomized to receive a triple-strain probiotic product (Investigational product-IP) and one group (n=1000) randomized to receive placebo. The study will be conducted at Haydom Lutheran Hospital (HLH), in the northern-central part of Tanzania. A total of 2000 newborn infants will be enrolled in the study from day 0 - 3 after birth and they will be followed-up for a period of six months. We are planning to have three follow-up visits after enrollment between day 0-3. The first visit will be after 7 (6-9) days, the second at six weeks (35-50 days) and the third at six months (170-190 days) after study enrollment. Three electronic case report form (CRF) using RED cap, one for each visit, will be used to collect clinical and demographic information of the infants enrolled in the study. The initial data at enrollment will also be filled with mother's demographic and clinical information.

The IP and the placebo will be administered to the infant daily by the caretaker, from the day of enrollment and for a period of four weeks or more. One fecal swab and one fecal sample will be collected at the second and third visit, respectively, and analyzed for i) carriage of ESBL-E; ii) gut microbiota/metabolome composition incl. antibiotic resistome and iii) gut inflammation markers.

During the study period, we will also in a separate study investigate all causes of fever among children below 6 months admitted to the pediatric ward at HLH, including both study participants and children not included in the RCT. For this sub-study a separate inclusion form will be used. All children below 6 months admitted to HLH with fever during the study

period, will be offered blood culture diagnostics, including species identification and antibiotic susceptibility testing of pathogens in positive cultures, C-reactive protein (CRP) test, and malaria rapid test, the latter only if clinically indicated. An additional fecal swab sample will be obtained on acute admissions. Results will be communicated back to the attending clinician to assist management of these children. At a later stage, the stored fecal swab samples will be screened for ESBL-E using selective media (e.g. ESBL ChromID, BioMerieux). ESBL-E isolates from the fecal swab samples and from blood cultures obtained from children with fever will be further characterized using whole genome sequencing to detect ESBL-encoding genes and other genes encoding antibiotic resistance, and for molecular epidemiology purposes. Microbiota analysis will be performed on stored fecal samples using a metagenomics approach. Bioinformatic analysis will be performed using available and relevant software and methods. Statistical analysis of the study results will be performed using Stata 13 software.

This study is expected to show reduction in ESBL-E carriage in infants receiving probiotics. It is also powered to detect a 50% reduction in infection related morbidity and mortality among the group of infants treated with probiotics.

Budget and source of funding: The budget for conducting the RCT will be 184.000 USD. This RCT received funding from the Regional Health Trust of Western Norway.

1. Introduction

1.1 Background Information

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 (Kipp 2016). Although child mortality is declining in Sub-Saharan Africa, it is still very high. In Tanzania, the under-five mortality was reported at 55 per 1000 live births in 2016, whereof 42 deaths occurred before the age of one year (Wang 2017). Newborns are susceptible to infection because key parts of their immune systems are still developing and not fully functional (Goenka 2015 & Shane 2017). From our previous studies of hospitalized children in Tanzania, we have shown that blood-stream infections (BSIs) caused by Gram-negative bacteria (*Enterobacteriales*) with extended-spectrum beta-lactamase (ESBL)-type resistance gave a mortality rate of more than 70%, compared to 20% or less for malaria infections and BSI with susceptible bacteria (Blomberg 2005 & Blomberg 2007). Antimicrobial resistance (AMR) threatens the very core of modern medicine and the sustainability of an effective, global public health response to the enduring threat from infectious diseases (WHO 2015). An increasing prevalence of ESBL-producing *Enterobacteriales* (ESBL-E) are among the most urgent global threats, and ESBL-E are by WHO ranked on priority one among pathogens in need of new treatment (WHO 2018). Fecal carriage of ESBL-E is a potential risk for transmission and infection. We have previously shown in a study from Tanzania (Fig. 1) that around 2/3 of infants below three months of age had fecal carriage of ESBL-E (Tellevik, 2016).

Probiotics are live microorganisms that, when administered in adequate amounts, confer a benefit to the host. Probiotics are currently widely used in newborns and children (Lenfestey 2017). They have proven beneficial effects in preterm infants with reductions in the incidence of necrotizing enterocolitis (NEC) and sepsis (AlFaleh 2014 & Rao 2016). For very preterm infants the use of probiotics in high-income countries has entered what has been called a "golden age", and similar beneficial effects have also been observed among preterm infants in low- and middle-income countries (Deshpande 2017). It is important to acknowledge that there are numerous probiotic products with possibly strain-specific effects (Lenfestey 2017). In preterm infants, multiple-strain probiotics have proved more efficient in reducing NEC, sepsis and mortality than single-strain products (Chang 2017).

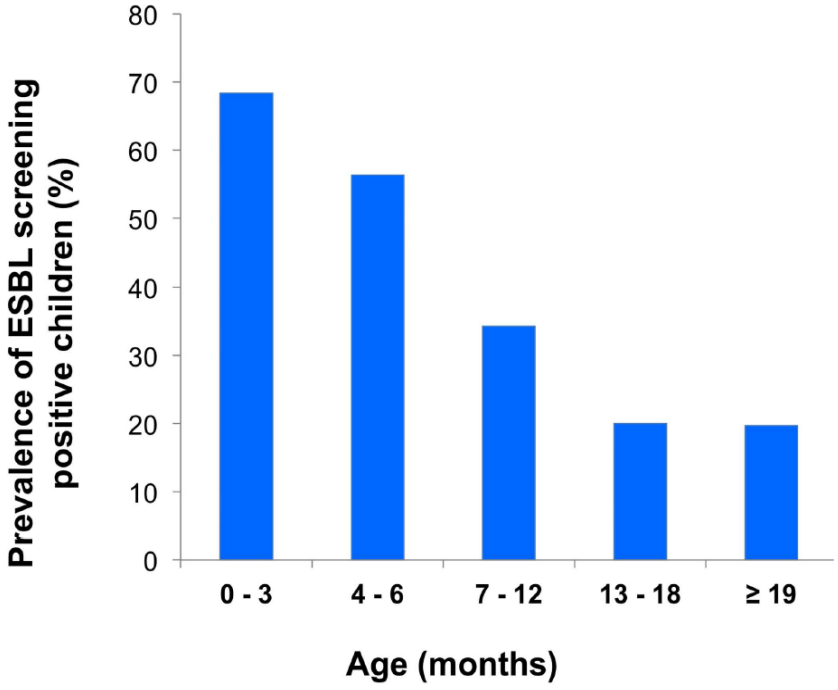

Fig. 1. Prevalence of ESBL carriage in different age groups. The graph shows the prevalence (%) of ESBL carriage among the participants when categorized into five different age groups.

In a recent study from India, more than 4500 infants were randomized to receive either the probiotic bacteria *Lactobacillus plantarum* along with fructo-oligosaccharide, a plant-derived prebiotic (the combination of probiotic and prebiotic is coined "synbiotic") or placebo (Panigrahi 2017). The synbiotic treatment was started on day 2-4 of life and was given for 7 days. This simple intervention reduced the composite outcome severe infections and/or death

by 40%, from 9.0% in the placebo group to 5.4% in the synbiotic group (Fig. 2). This study has caused great attention in the scientific community and in the general public. Panigrahi has suggested that more studies are needed in order to develop guidelines for clinical care (The Wire 2017).

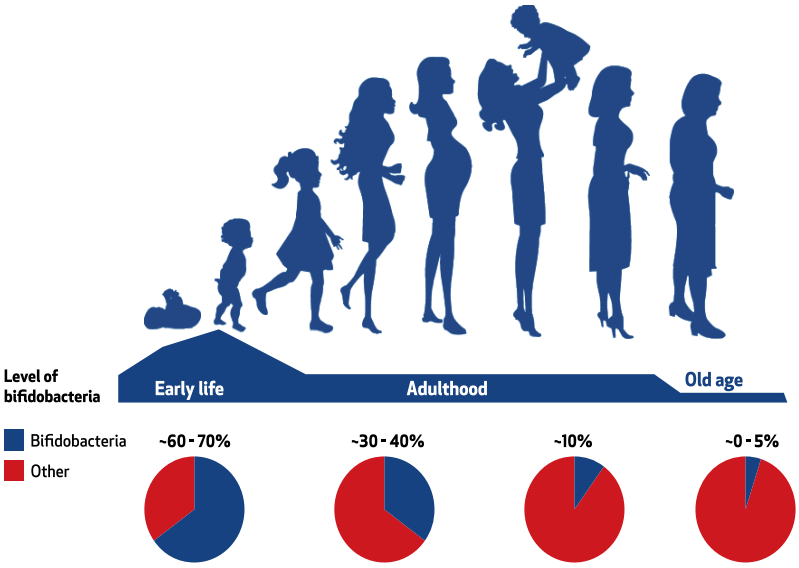

Fig. 2. Morbidity and mortality in infants given synbiotic versus placebo. Panigrahi, Nature 2017

A stable and resilient commensal gut microbiota is essential for "colonization resistance"; the ability to prevent intestinal colonization and invasion by pathogens. Resurrecting the gut microbiota by the use of probiotics has recently been put forward as a potential new strategy in combatting intestinal carriage of multi-drug-resistant (MDR) bacteria (Pamer 2016). To what extent probiotics directly reduce the spread of AMR is still much under investigation (Arthur 2016). However, probiotic bacteria can produce bacteriocins that improve mucosal integrity and thereby reduces the pathogenic bacterial population and promote "colonization resistance" (Pamer 2016), but there is so far no data on which probiotic strains that most effectively may reduce AMR in children. In adults and older children lactobacilli are often used to prevent or treat other conditions like antibiotic-associated diarrhea, gastroenteritis and infantile colic (Agamennone 2018 & Gutiérrez-Castrellón 2017). However, in breastfed infants, bifidobacteria constitute more than 80% of the gut microbiota (Fig. 3) and has several beneficial effects that make them attractive as probiotic agents for this age group.

In infants, *B. infantis* given in combination with human milk decreases the level of *Enterobacteriales* in the feces (Underwood 2015). In a clinical trial, we observed that among extremely preterm infants supplemented with a probiotic product containing bifidobacteria and lactobacilli, we found no ESBL-E in stool samples despite massive exposure to antibiotic therapy. In contrast, ESBL-E was detected in the stool of moderate preterm infants and full-term infants not given probiotics and with less or no antibiotic exposure (Esaiassen 2018). In this study (Esaiassen 2018), a metagenomics approach was used in order to analyze the gut microbiota and antibiotic resistome.

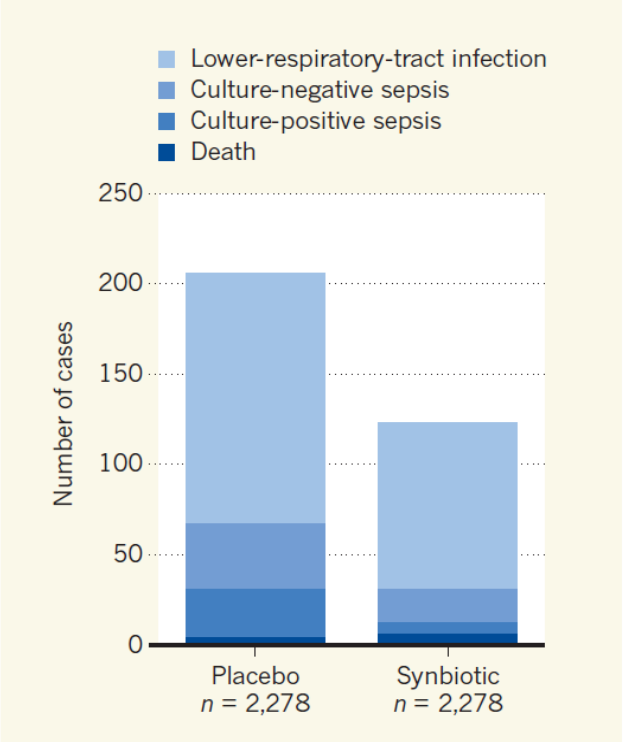

Fig. 3. Level of Bifidobacteria in the gut during different stages in life (by Kenneth Kristensen).

1.2 Main challenges and gaps of knowledge

Except from the study by Panigrahi (Panigrahi 2017) there is little data on the effect of probiotics in reducing mortality and infection-related morbidity in infants in low-income countries. There is also very limited clinical data on the effect of probiotics to reduce AMR carriage and AMR-related infections in infants and children, even though this approach has been put forward as a promising new strategy. Increasing AMR, according to the World Health Organization, is one of the most important threats to human health (www.who.int/entity/drugresistance/en). It affects both high- and low-income countries, but consequences are more severe in low income countries due to the prohibitive costs and thus

lack of specific diagnostic and second line antibiotics. No randomized clinical trial (RCT) has investigated whether probiotics may reduce ESBL-E carriage in infants.

1.3 Rationale to conduct this study

Our own research has shown a high mortality of Tanzanian children with ESBL-E infections (Blomberg 2005 & Blomberg 2007), a high carriage rate of ESBL-E in Tanzanian infants (Tellevik 2016) and promising results using probiotics to reduce AMR (Esaiassen 2018). The recent large RCT from India showed a reduction in severe infections and death among newborns given probiotics/synbiotics (Panigrahi 2017), and these beneficial findings are supported by data from systematic reviews indicating a reduction in sepsis and mortality in preterm infants given probiotics. However, studies in other developing countries are needed to confirm the positive findings from the Panigrahi trial.

We therefore propose to do an RCT on probiotics versus placebo in healthy Tanzanian infants. The primary endpoints, for which the study is powered, is a reduction in the composite outcome mortality and hospitalizations. Secondary endpoints are rates of ESBL-E gut carriage, growth, microbiota/metabolome composition and gut inflammatory markers.

1.4 Study hypothesis

Our hypothesis is that probiotics will significantly reduce the composite outcome death and/or infections in children up to 6 months of age. We hypothesize that this reduction will partly be mediated by a concomitant reduction in prevalence of ESBL-E-carriage in this group of children.

2. Objectives of the study

2.1 Overall objective of the study

The present clinical trial will evaluate whether 4 weeks administration of probiotics in the first 4 weeks of life can reduce mortality and/or hospitalizations, and colonization with ESBL-E, among infants in Haydom and surrounding area, Tanzania.

2.2 Specific objectives of the study

2.2.1 To assess and compare mortality among two groups of the study participants (i.e. probiotic and placebo group) in Haydom and surrounding area, Tanzania.

2.2.2 To determine and compare episodes of infections (i.e. sepsis, diarrhea, etc) leading to hospitalization among the two groups of study participants in Haydom and surrounding area, Tanzania

2.2.3 To determine and compare the prevalence of ESBL-E carriage between the two groups of study participants at six weeks and six months follow-up.

2.2.4 To determine and compare the bacterial causes of sepsis, including antimicrobial susceptibility patterns, in and between the two groups of study participants.

2.2.5 To assess and compare the effect of probiotics on growth between the two groups of study participants.

2.2.6 To determine and compare the gut microbiota composition and diversity between the two groups of study participants.

2.2.7 To determine the genetic characteristics of ESBL-E and other MDR isolates from carriage and clinical samples.

2.2.8 To determine the bacterial causes of sepsis, including antimicrobial susceptibility patterns for all children under 6 months of age admitted with fever during the study period.

2.2.9 To determine gut inflammatory markers from children at 6 month follow-ups

2.3 Study end point measures

2.3.1 Primary end point

- Prevalence of the composite outcome death and /or hospitalizations at six months of age.

2.3.2 Secondary end points

- Prevalence of ESBL-E carriage at six months of age.
- Rates of hospitalizations up to six months of age.
- Rates of confirmed sepsis episodes up to six months of age.
- Growth monitored by height and weight up to six months of age.
- Stool microbiota/metabolome composition including antibiotic resistome analysis (metagenome sequencing) at six weeks and six months of age.
- Genetic characteristics of ESBL-E and other MDR isolates from carriage and clinical samples.
- Characteristics of gut inflammatory markers

3.0 Methods

3.1 Study design

This will be a single-center randomized double-blinded controlled clinical trial (RCT) with two arms:

Arm 1: The active group will receive the investigational product (IP); a commercially available multi-strain probiotic product (LaBiNIC® probiotic drops, Biofloratech Ltd, Surrey, UK. Five drops (0.2 ml) contain 1.8 x 10^9 CFU *Lactobacillus acidophilus*, *Bifidobacterium infantis* and *Bifidobacterium bifidum* (equal amount of all three strains). The product is already widely used in the UK (at hospitals in the National Health Service) and in other European countries (Robertson 2020, Klingenberg 2019). The product also recently received the Pharmacy Grade Certificate A-Grade by the South African Pharmacy Council, valid until 31st of December 2021 (attached copy).

LaBiNIC® probiotic drops can be stored at room temperature, provided this does not exceed 25 ℃ for prolonged periods (a few hours at 30 ℃ causes no degradation). No refrigeration is needed for storage. The product is 100% dairy-, gluten-, egg-, soya- and allergen-free. It contains no animal products, no preservatives, no colouring products and/or no sweeteners. It is manufactured to GMP and GPP WHO standards. All batches are microbiologically tested for safety and composition.

Arm 2: The placebo group will receive placebo drops; a MCT-oil and AEROSIL® 200 Pharma (the latter is an additive for food and pharma products with a high quality standard, and with production processes based on quality concepts such as; ISO 9001, HACCP, FAMI-QS and IPEC-GMP). The MCT-oil and AEROSIL® 200 Pharma are also constituents of the active IP. The placebo is produced by the same company that manufacture the IP (Biofloratech Ltd, Surrey, UK). The MCT-oil is used as a vehicle for the probiotic bacteria in the IP, and the taste and colour is identical, and it will be produced in identical bottles to the IP.

The drops for both arms will be dispensed in identical bottles. All infants will be given 5 drops daily from inclusion and for a period of four weeks ("to bottle empty"). Previous studies in developing countries have used from 7 days (Panigrahi 2017 - India) to 60 days (Pernicia 2017 - Botswana) intervention with probiotic supplementation in infants.

3.2 Study site

This will be a single-centre study to be conducted at Haydom Lutheran Hospital (HLH) and surrounding area. HLH is located in the Mbulu district, at the western end of Manyara region in the North-Central Tanzania, about 300 km south-west from Arusha. The HLH catchment area consists of 4 administrative divisions, 3 districts and 2 regions. These are the Dongobesh and Haydom divisions in Mbulu District, the Basotu Division in Hanang District (Manyara Region), and the Nduguti Division in Mkalama District (Singida Region). The hospital serves more than 2 million population from five regions. HLH has strong and well-established research infrastructure, and over ten years has been involved in community research, which has built trust between the hospital and community around (Mduma, 2014). There are annualy more than 3900 deliveries and the neonatal mortality rate is around of 43/1000 live births. The hospital has a track record of conducting clinical trials, including the Early Life Intervention in Childhood growth and development (ELICIT), the Effectiveness of Quadruple Fortified Salt in Improving Hemoglobin Levels among Anemic Women of Reproductive Age (18-49 Years) in Rural Low Resource Setting (Salt study trial) and clinical trials on neonatal resuscitation evaluating the Helping Babies Breathe program. HLH has laboratory capacity with required equipment's and trained staff to do clinical trials.

3.3 Study setting and population

Newborn infants delivered at HLH or outside hospital will be eligible for inclusion.

Mothers to potential eligible participants (newborn infants) will be approached and informed about the ProRIDE-study at home or when attending their last antenatal clinic visits before delivery. Those mothers who give written consent for potential participation of their future child will be recruited to participate in the study, and after giving birth the newborn will be screened for eligibility before being randomized in the study. We intend to include a total of 2000 newborn infants to participate in the study, half of these i.e. 1000 will receive a multi-strain probiotic product and the other 1000 will receive placebo.

3.4 Research question and statistical power

The research question is: Among term born healthy infants in Tanzania (P-population), will treatment with probiotics (I-intervention) compared to placebo (C-comparison) lead to a 40% reduction in hospitalizations and death, and in fecal carriage of ESBL-producing bacteria (O-outcome)?

Infant mortality rate in Tanzania was in 2017 reported at 4.4%. We do not have exact data for the Mbulu district, but we assume a similar rate. Serious infections leading to hospitalization are probably equally common, but hospitalization rates may depend on available access. In the Panigrahi study, the synbiotic product was given for only 7 days, and it reduced the composite outcome death and/or infection from 9.0% to 5.4% during the first 2 months of life. In our study, the intervention will continue for around 4 weeks and follow-up will continue until 6 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. To find this difference with a study power of 85% at 5% significance level we will need a sample size of minimum 924 cases in each arm; thus 1848 infants. In order to allow for drop-out we aim to include 1000 infants in each arm. We believe it is realistic to include this number of infants within a 12-month recruitment period.

With this number of infants, we will also have sufficient power to detect a minimum of 30% reduction in ESBL-carriage rate (from around 50% to 35%) at six months of age.

3.5 Inclusion and exclusion criteria

3.5.1 Inclusion criteria:

- Healthy newborn infants with a birth weight equal or above 2.0 kg, will be included in the study between 0-3 days of life.
- 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 are able and willing to complete study visit (including required study procedures) schedules over the six months proposed follow-up, which also includes hospitalizations required for compliance of this study protocol.
- Children less than 6 months of age admitted to hospital with suspected infection, not included in the RCT, will be included in a sub-study. A separate inclusion form is prepared for these children.

3.5.2 Exclusion criteria:

- Multiple Pregnancy
- Birth weight below 2 kg
- Other health problems/illness, obvious congenital malformations.
- Parents not consenting

3.6 Study period

The study period is expected to last for 18 months, which will include recruitment period of one year and a six-month follow-up of all participants.

3.7 Investigational product (IP)

3.7.1 Description and formulation

The IP is a commercially available multi-strain probiotic product (LaBiNIC probiotic drops®, Biofloratech Ltd, Surrey, UK). The product is widely used in the UK (National Health Service) and other countries in Europe. The product also recently received the Pharmacy Grade Certificate A-Grade by the South African Pharmacy Council, valid until 31st of December 2021 (attached copy).

The product is 100% dairy-, gluten-, egg-, soya- and allergen-free. It contains no animal products, no preservatives, no colourings or sweeteners. It is manufactured to GMP and GPP WHO Standards. All batches are microbiologically tested for safety and composition.

3.7.2 Dose preparation and administration

Five drops (0.2 ml) contain 1.8 x 10^9 CFU *Lactobacillus acidophilus*, *Bifidobacterium infantis* and *Bifidobacterium bifidum* (equal amount of all three strains) will be given by mouth once daily for 4 weeks to "bottle empty". Bottle contains 5-5.5 ml.

3.7.3. Storage and stability (shelf-life)

Labinic probiotic drops can be stored at room temperature, provided this does not exceed 25 ˚C for prolonged periods (a few hours at 30 ˚C causes no degradation). No refrigeration is needed for storage.

3.7.4 Packaging and labelling

Biofloratech Ltd will pack probiotic and placebo in identical packages, and number the packages according to the randomization list.

3.7.5 Accountability

The investigators will ensure that all investigational products (probiotic and placebo) are inventoried and stored as indicated by the manufacturer. Accurate records will be kept regarding the date manufactured, date received, lot number, expiry date and amount received.

3.8 Study procedures

3.8.1 Recruitment of pregnant women and enrolment of study participants

Pregnant women in their last trimester, visited at home or attending their last visit before delivery at HLH, will be informed about the study and asked for consent to let their future child participate in the study. A study research assistant will inform the woman about all aspects of the study, including the importance of performing such a study, and all procedures which will take place during the study period. A thorough explanation will also be given on how the IP/placebo will be administered orally once daily with 5 drops until bottle empty (four weeks). Finally, the importance of completing the dosage of the IP/placebo for the four week-intervention period will also be explained to the woman. Information will be given orally and by information included in a written informed consent form (ICF). If the woman, after this information, agrees for her child to participate in the ProRIDE-Trial, she will be asked to give written consent to screen and enroll the newborn baby in the study. The woman will receive a special information card developed for the ProRIDE-Trial, after delivery and inclusion. During and after delivery, the attending doctor/midwives will identify mothers who have consented to participation and inform the research assistant, who will help coordinate the study. After delivery the new-born baby will be screened for eligibility into the study, and if the baby fulfills the inclusion criteria of the study, the baby will be enrolled and allocated to the randomized product (active probiotics or placebo), which will be initiated on day 0-1 for hospital deliveries or day 0-3 for home deliveries. The mother will be explained and demonstrated how to administer the study product.

3.8.2. Filling of the electronic CRF using RED cap

Mothers who have given consent will be registered by name and address in the consent form. For enrolled babies, four different electronic case report forms (CRF) will be used for the study, three for the study visits and one for adverse events/unscheduled visits due to hospitalization/outpatient's attendance for participants who fall sick during the study period.

Infant deaths, hospitalizations and sepsis episodes will also be recorded (Appendix 1-6). Tablet computers with RED cap® software will be provided to research assistants and investigators. Passwords will be created for each research assistant and investigator to get access to the study file in RED cap. Research assistants will have access only to fill in the respective CRF of the study participants they recruit at each visit and they will have no access to change any information entered in RED cap. Only study investigators will have access to change information filled in the RED cap when need arise. Infant's deaths, hospitalisations and sepsis episodes will also be recorded.

3.8.3 Randomization and blinding

Before the start of the study, an independent researcher/statistician will generate a randomization list with the study identification number of patients, from 1 to 2000, and allocated investigational product or placebo. This randomization list will be sent to Biofloratech Ltd, Surrey, UK for labelling of the investigational product and placebo, which will be produced and labelled in identical bottles, numbered from 1 to 2000. Upon screening, the newborn infants will consecutively be allocated (randomized) to the next study identification number and given the corresponding investigational product/placebo.

The main bias reducing technique in this RCT is randomization and blinding. A local pharmacist, who is not part of the study will be provided with the randomization list. Both study participants and investigators and care providers will be blinded for assignment of the intervention.

3.8.4 Unmasking in emergency situations

A pharmacist not involved in the study will have access to assignment list. Therefore, the study PI may request unblinding for treatment assignment if needed for medical care of a patient in an emergency situation. However, this is a very unlikely situation given the well-known safety of probiotics in infants, and the lack of known interaction with other important drugs used for any other required therapy.

3.8.5 Visit schedule and follow-up activities

The follow-up schedule is summarized in Table 1 below. After study randomization, participants will receive follow-up for six months. At enrollment the baby will be weighed and clinical data on mother and child will be entered in the CRF. The first visit after enrolment will be around 7 days after birth, the second at six weeks after initiation of intervention product, and the last visit at six months. During all study visits, the baby's length, weight measurements, and clinical information will be recorded. Fecal samples and fecal swabs will also be taken according to the developed SOP.

Table 1. Follow-up schedule visit for ProRIDE-trial

Time	Event	Activity
Day 0-3 after birth of the newborn	Enrolment and randomization	- Filling of CRF-1 - Giving the IP/placebo to the mother/caretaker of the baby
Day 7 after initiation of investigational product	Visit 1	- Observe for the compliance - Discuss issues with care takers
Six weeks after initiation of investigational product	Visit 2	-Observe if the bottle with IP/placebo is empty and return the bottle -**First fecal and fecal swab sample** will be taken -Filling of CRF-2
Six months (25-27 weeks) after initiation of investigational product	Visit 3	-**Second fecal and fecal swab** will be taken - Filling of CRF-3

3.9 Specimen collection and storage

Standard Operating Procedures (SOP) which have been developed for the study will be used for specimen collection and processing. One fecal sample and one fecal swab sample will be collected at six weeks and six months visits, respectively. The fecal sample will be obtained

using a commercially available sampling kit (OMNIgen GUT kit, DNA Genotek, Ottawa, Canada) allowing storage of samples at ambient temperatures for up to 7-14 days before DNA extraction. These samples will be transported to HLH latest within 3 days after sampling and at HLH stored in -80 ℃ freezers. The fecal swab samples will be taken using eSwab (Copan Diagnostics, CA, USA) and frozen at -80 ℃ freezers until analysis.

During unscheduled visits, when participants or other children below one year of age are hospitalized because of fever, Dr Museveni Justine who is the study physician will ensure blood specimens are collected using aseptic technique. The patient's skin and the septum covering the blood culture vial will be disinfected with 70% alcohol (or 1-2% iodine tincture). Plastic gloves, sterile needles and syringes will be used. From the patient, one blood specimen will be collected from the same venipuncture. A total of 4-5 ml of blood will be drawn from the patient for blood culture to investigate for blood stream infections, C-reactive protein (CRP), and also malaria rapid test if clinically relevant. Furthermore, an additional fecal swab sample will be obtained from hospitalized children to investigate correlation between gut carriage of ESBL-E and ESBL-E as a cause for BSI. Caretakers of children <6 months, who are not included in the clinical trial, will be informed about the sepsis sub-study and asked to participate and sign a separate consent form. All children <6 months of age will receive the same diagnostics and treatment whether they are enrolled in the clinical trial, the sepsis sub-study or none of the studies.

3.10 Data collection and handling during the study period

Clinical information as outlined in the CRFs will be recorded for each participant and will be kept confidential. The information will be recorded on electronic CRFs using the RED cap software; a software that globally is widely used for clinical trial purpose. Only informed

consents will be stored separately. Quality assessment of the data will be done by the internal clinical staffs and consent forms and eCRFs will be checked by external monitors.

3.11 Microbiological methods

3.11.1 Laboratory tests to be performed at Haydom Lutheran Hospital

Blood specimens will be collected during unscheduled visits (hospitalization) of study participants and all children below 6 months of age (when they attend HLH to seek medical care) and analyzed at the laboratory facility in Haydom hospital. Results will be reported to the attending physician to assist patient treatment. Rapid malaria test (CareStart, USA) will be performed if relevant. CRP will be measured on all hospitalized children under one year with suspected infection to assist clinical evaluation of the infant. Blood cultures will be drawn from infants with fever or suspected infection. Blood culture bottles will be transported to the laboratory and will be incubated at 35°C in the BACTEC 9050® (Becton–Dickinson, Sparks, MD, USA) culture system for a maximum of 5 days unless flagged positive. Positive blood cultures will be sub-cultured on sheep-blood and chocolate agar using routine microbiological techniques. Furthermore, Gram stains will be made for all positive blood cultures. Antibiotic susceptibility testing (AST) will be performed on clinically relevant bacterial isolates using conventional methods (disk diffusion) following EUCAST guidelines (http://www.eucast.org/clinical_breakpoints/). All clinically relevant isolates (considered to cause infection) will be frozen at -80°C for subsequent genetic analysis (whole genome sequencing).

3.11.2 Laboratory tests which to be performed at UiB/SUH/UNN

Fecal samples and fecal swabs collected at six weeks and six months will be transported to Norway for the following analyses:

- Fecal swab samples (n=4000) collected at six weeks and six months will be screened for ESBL-E using a selective ESBL-screening agar (ChromID ESBL and ChromID Carba,

BioMerieux). All samples will be plated on non-selective plates for growth control. ESBL-E isolates will be frozen for subsequent genetic analysis (whole genome sequencing and plasmid analysis).

- Fecal samples (n=4000) collected at six weeks and six months will be subjected to gut microbiota/metabolome and antibiotic resistome analysis using state of the art methods. . This will be done on DNA or metabolites extracted from the feces sampled and stored in a commercially available sampling kit (OMNIgen GUT/MET kits). In addition, fecal samples collected at six months will be analyzed to determine the levels of gut inflammatory markers between the two groups.

4.0 Monitoring of the study

Site monitoring will be conducted to ensure that the rights and well-being of study participants are protected. In addition, monitoring will verify that the reported clinical and laboratory data are accurate, complete and verifiable from source data. Site monitoring will also ensure that the conduct of the clinical trial is in compliance with the study protocol, good clinical practice (GCP) and good laboratory practice (GCLP) and applicable regulatory requirements. A data safety monitoring committee (DSMC) will be appointed and this DSMC will oversee that trial is conducted according to GCP and GCLP.

Table 2 showing monitoring plan

	1st quarter	2nd quarter	4th quarter	8th quarter
Enrolment visit	X			
Follow-up visit		X	X	
Close up				X

Table 3 showing key activities of the clinical monitor

Site visits	When	Key activities
Monitoring visit during enrolment	One visit, four weeks since the start of enrolment.	Perform monitoring visit, to check for: - Inspection of all ICH GCP certificates in place - Verification of Informed consent forms - Eligibility check - eCRF completion - Equipment, consumables, study material - Review laboratory sample management

Monitoring visit follow up -first	One visit six months since the start of enrolment.	Perform monitoring visit, in particular check for: - Specimen collection procedure - Eligibility check - eCRF completion - Eligibility of source documents - Source data verification - Review AE/SAE reporting
Monitoring visit follow up -second	One visit 12 months since the start of enrolment.	Perform monitoring visit, in particular check for: - Specimen collection procedure - Eligibility check - eCRF completion - Eligibility of source documents - Source data verification - Review AE/SAE reporting
Close up visit monitoring	One visit 18 months since start of enrolment (end of study)	Perform monitoring visit, in particular check for: - Last query resolution - Storage and archiving of documentation - Review, update and finalization of IF

5.0 Criteria for withdrawal of individual study participants

Study participants will be discontinued from participation in the study if:

- Any clinical adverse event (AE) during the four-week intervention, laboratory abnormality or other medical condition or situation occurs such that continued participation is not in the best interest of the participants
- Participants wishes voluntary withdrawal.
- Participants are free to withdraw from participating in the study at any time upon request.
- In any case participants will be given appropriate care under medical supervision until the symptoms of any AE resolve or the participant's condition becomes stable.

6.0 Ethical considerations

6.1 Potential benefits and risks associated with the study

The proposed intervention has shown beneficial effect on morbidity in very preterm infants below 32 weeks gestation. The large RCT from India also reported promising results regarding a potential reduction of death and severe infections in term infants. Participation in the study may thus be of potential benefit.

No extra blood samples will be obtained by the included participants, only fecal samples. As such, no extra pain will be imposed on the participants. The intervention with administration of 5 droplets daily for 4 weeks is easy, and we consider it will not pose any significant extra burden on parents or participants.

Included infants who may be sick and need hospitalization for any illnesses will receive standard care treatment at HLH, including optimal assessment of a potential infection with blood culture and CRP analyses.

Identification of study participation may be perceived as a stigma, but we do not think this is a substantial risk with this study.

6.2 Regulation statement, Ethical approval and Protocol registration

This study will be conducted in accordance with the latest South Africa revision of the Declaration of Helsinki, Good clinical practice and local and international regulatory requirements.

- Ethical approval of the ProRIDE Trial has been obtained from both i) the Regional Committee for Medical and Health Research Ethics Western Norway in 4th September 2019 (REK Vest 2019/1025) and from ii) the National Institute of Medical Research (NIMR) approved the trial on 9th April 2020 reference No. NIMR/HQ/R.8a/Vol.IX/3398.

- The protocol is approved by the Tanzania Medicines and Medical Devices Authority (TMDA) on the 25th March 2021.
- The study is registered in ClinicalTrials https://clinicaltrials.gov/ct2/show/NCT04172012)
- The study protocol is also published: Kuwelker K, Langeland N, Löhr IH, Gidion J, Manyahi J, Moyo SJ, Blomberg B, Klingenberg C. 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.

6.3 Recruitment and consent

Recruitment of study participants will start after receiving all appropriate ethical approvals and protocol registration. The proposed study involves infants who are not able to consent. Investigators will provide verbal and written information about the objectives of the study, procedures and the obligations of the parent/care taker of the study participant, during the last antenatal visit before delivery or during home visits. After being given all the information, the parent/caretaker of the future study participant will be given enough time to consider participation in the study for their newborn infants. When the parent/caretaker agrees to participate, written informed consent will be obtained. Participants parents/caretakers will be informed that they can withdraw their informed consent at any time during the study.

6.4 Treatment during the study period

All study participants, when they fall sick during the study period, will be treated according to existing standard of care at Haydom Lutheran Hospital. In addition, the study will provide CRP analysis, blood culture and antibiotic susceptibility testing of bacteria recovered from blood culture for all children under one year of age suspected of having sepsis. Malaria rapid

test will be performed if clinically indicated (Care Start, USA). Laboratory results will be given to the attending clinician responsible for the care of children to assist the patient management.

6.5 Compensation for injury

Even though the study products are not considered to be medicinal drugs, and studies have so far not revealed any serious adverse effects**,** study participants will be insured, if unforeseen side effects of the probiotics develop. Please find attached copy of the receipt of insurance cover.

6.6 Transfer of Data and Biological Material

Relevant Material Transfer agreement (MTA) and Data Transfer Agreement (DTA) approved by NIMR will be used in order to ship biological specimens to Norway for further analysis and share data.

6.7 Data Safety Monitoring Body (DSMB) and schedule of work

The DSMB consists of the following experts:

- Ketil Størdal, MD, PhD. Head of committee. Størdal is an experienced paediatrician with a focus on global health issues, nutrition and care of sick newborns. He has worked for several years in Botswana and is leading research projects in Tanzania. He also has advanced skills in biostatistics making him a relevant and important member of the DSMB
- Sven Gudmund Hinderaker MD, PhD, is a medical doctor with extensive experience in global health projects, with particular focus on projects in sub-Saharan Africa.

- Charles Makasi MD,MPH, is public health specialist , with experience on monitoring clinical trials as well as data management, he had recently monitored WHO funded sepsis study in infants at Haydom Global Health Research Centre
- Blandina Mbaga MD,PhD, she is a professor of pediatric and Director of Kilimanjaro Christian Research Insititute(KCRI), Moshi Tanzania, she has vast experience in conducting and monitoring both clinical research and clinical trials.

Schedule of work for the DSMB:

The primary responsibilities of the DSMB are to 1) periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy, and 2) make recommendations to the ProRIDE study consortium concerning the continuation, modification, or termination of the trial.

Prior to initiating any data review, the DSMB has defined these deliberative processes:

- Event triggers that would call for an unscheduled review: Mortality rates which significantly exceed the expected rates in Tanzania. The national infant mortality rate in Tanzania is estimated to 36 per 1000
  live births (https://data.unicef.org/country/tza/). The survival of participants will be monitored continuously. A death rate of more than five deaths per 100 infants will prompt an unscheduled review.
- Stopping procedures that are consistent with the protocol: If the proportions of deaths among study participant in the placebo-group and the probiotic-group at 6 weeks follow-up are significantly different at the level of $p>0.05$ by Chi square test
  after 500 and 1000 infants have been included and followed up for 6 months.
- Unmasking (unblinding) of single patient's treatment: Only if serious adverse events may be related to the event
- Voting procedures: If disagreement the leader of the DMSB has two votes

The DSMB is also responsible for maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it. The DSMB have reviewed the protocol and not found any concern prior to implementation. During the trial, the DSMB will review cumulative study data to evaluate safety after 500 and 1000 infants have been recruited. Moreover, they will review study conduct, and scientific validity and integrity of the trial. As part of this responsibility, DSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The DSMB will also assess the performance of overall study operations and any other relevant issues, as necessary.

Items reviewed by the DSMB will include:

- Interim/cumulative data for evidence of study-related adverse events: Deaths and admission to hospital
- Data quality, completeness, and timeliness.
- Adequacy of compliance with goals for recruitment and retention, including those related to the participation of women and minorities;
- Adherence to the protocol.
- Factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol violations, unmasking, etc.)
- Factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study.

The DSMB will conclude each review with their recommendations to the ProRIDE study consortium as to whether the study should continue without change, be modified, or be terminated. Recommendations regarding modification of the design and conduct of the study could include:

- Modifications of the study protocol based upon the review of the safety data;
- Suspension or early termination of the study or of one or more study arms because of serious concerns about subjects' safety, inadequate performance, or rate of enrollment;
- Suspension or early termination of the study because study objectives have been obtained according to pre-established statistical guidelines;
- Optional approaches for the ProRIDE study consortium and investigators to consider when the DSMB determines that the incidence of primary study outcomes is substantially less than expected (such as recommendations to increase the number of trial centers or extend the recruitment period); and,
- Corrective actions regarding if the study center's performance appears unsatisfactory or appears to raise questions regarding the conduct of the study.

Confidentiality must always be maintained during all phases of DSMB review and deliberations. Usually, only voting members of the DSMB should have access to interim analyses of outcome data by treatment group. Exceptions may be made when the DSMB deems it appropriate. The reason and to whom the exceptions for access to interim analyses is granted will be documented in the Closed Session Report. DSMB members must maintain strict confidentiality concerning all privileged study results provided to them. The DSMB should review data only by masked study group (such as X vs. Y rather than experimental vs. control) unless or until the DSMB determines that the group identifiers are necessary for decision-making. Whenever masked data are presented to the DSMB, the key to the group coding must be available for immediate unmasking.

7.0 Statistical analysis

Comparisons of proportions will be done by Pearson's chi-squared tests. Means will be compared by t-test or non-parametric tests as appropriate. Logistic regression analysis will be performed for assessment of the relative importance of risk factors for carriage of ESBL,

multidrug-resistant bacteria, infection episodes and mortality. Analysis will be performed in Stata 13 (Stata corporation, College Station, Texas).

8.0 Organization, collaboration and research environment

Partners who are involved in this study are employed at:

- Haydom Lutheran Hospital (HLH)
- University of Bergen (UiB)/Haukeland University Hospital (HUH)
- Stavanger University Hospital (SUH)
- UiT-The Arctic University of Norway (UiT)/University Hospital of North Norway (UNN)
- Muhimbili University of Health and Allied Sciences (MUHAS)

The proposed project requires competencies in diverse but complementary scientific fields, including clinical medicine, pediatrics and microbiology and bioinformatics. Essential competencies among the partners include clinical studies in low-income settings (UiB, MUHAS, HLH), pediatrics (UiT/UNN, HLH), clinical microbiology (UiB/HUH, SUH, MUHAS), AMR research and specifically ESBL-resistance research (UiT/UNN, SUH, HUH, UiB, MUHAS), and bioinformatics (SUH, UiB, UiT).

The group at UiB/HUH will coordinate the consortium. Professor Nina Langeland and Associate Professor Bjørn Blomberg have experience in clinical studies in low-income settings with particular focus on AMR and severe infections in children. Dr Sabrina Moyo has long experience in performing clinical studies on children in Tanzania.

At SUH, Dr. Iren Löhr is a senior microbiologist and the coordinator of the Norwegian Research Council-funded Norwegian *Klebsiella* AMR-Network. Dr. Löhr has also experience and competence in Nanopore sequencing and the genetics behind the spread of ESBLs.

At UiT/UNN, Professor Claus Klingenberg is an experienced pediatrician/neonatologist with experience from research on infections and use of antibiotics in neonates, and clinical work in Tanzania. Klingenberg has recently completed a study using metagenome analyses of gut microbiota and resistome in stool samples from preterm infants.

At MUHAS, clinical microbiologist Dr J Manyahi has set up a well-functioning microbiology lab and is presently conducting two clinical studies. He will be the Tanzanian laboratory PI for the proposed RCT study.

HLH (https://haydom.or.tz/) is a rural hospital with the experience of running clinical studies including being partner in Gates-funded multi-center studies. Located in a rural setting, HLH is an ideal institution for comparative studies on differences in AMR between rural and urban (MUHAS) settings. Dr. Joshua Gidion at the Pediatric Department is the HLH partner and local clinical PI.

Leadership. The consortium will be led by Nina Langeland. She has ongoing collaboration with the other partners, and 15 years partnership in research and medical specialist training with MUHAS in Tanzania. Her group has performed outbreak investigations both in Norway and in Tanzania and has conducted large clinical studies as well as RCTs on AMR. She is presently funded by the Research Council of Norway and the Gates Foundation.

9.0 Budget

The proposed budget (attached) covers the cost of running the study. Major costs involve probiotics/placebo, research assistants, coordination of the study in Tanzania, and laboratory costs including culture. Further cost covering microbiota analysis and whole genome sequencing is not included in the attached budget.

Table 4. Budget Justification

ACTIVITY	RESPONSIBLE PARTY	UNITS	TOTAL COST IN USD
Ethical clearance	NIMR application	1	2000
	NMRI-extension	1	200
	TFDA	1	3000
Study site preparations	Initial trainings-for this trial most of the staff are going to be new and they have to be familiar with all the study procedures like protocol, GCP,GLP, ALL SOP etc., the budget will serve for stationaries and refreshments for days of training.	1	1000
	Community preparation/sensitization meetings-this will includes local government leaders, religious leaders from where subjects recruitments will take place-the budget will cover for their transport fee, meeting allowances, stationaries, meals and refreshments	1	5378
Human resource	study coordinator -we expect to employ one medical doctor who will be paid 100%, will be responsible for all the regulatory issues, study coordination in all units, preparing and reporting to regulatory bodies i.e NIMR,TFDA,DSMB etc	1	21786
	study site PI-	1	12528
	Pharmacist -we intend to hire degree level pharmacist from Hospital who will oversee the control of trial products, this will 10% of his time	1	651.6

	Investigators-investigators will work on the project for 10% time contribution, and one will be a research doctor who will be concerned with all AE during trial.	3	6894
	Research Nurse pharmacy (full time) responsible for the control and supply of study intervention, second nurse will be needed as backup for 20% time contribution see below for her budget.	1	3548.4
	Research Nurse pharmacy (20%)	1	990
	research Nurse (100%time contribution) will be responsible for overseeing sample collection procedure, reporting on the progress of sample and availability of supplies in the hospital, the budget is for their full salary for entire period of project	2	6387.12
	community health workers to work 20% time, for attending and reporting any AE/SAE,	20	9396
	Field workers -responsible for daily follow-ups,to field on identification of potential participants, screening, recruitments, follow-ups visits and non-invasive sample collection.	10	45078
	Laboratory personnel (20%of time)-we will need 2 laboratory personnel to oversee lab supplies, sample processing, and reporting to study CRF.	2	6873.12
	IT person - we need for any trouble shooting of the data collection devices and back up of data locally and online communication,20% time will be appropriate.	1	1095
Other logistics			
	Stationaries - though in this study we intend to utilize electronic CRFs, we still need significant paper work for ICFs for 2000 subjects, all regulatory documents, printers, cartridges, and files		2000
	Communications-we expect extensive online communication, phone calls both locally and international.		3000
	health insurance - as in all clinical trial this required by both NIMR and TFDA for all participants to be insured , here one insurance card will serve for 5 participants, costing Tsh 30,000.	One per year	15000

	life insurance -the same as above	One per year	2000
Seeking permission from district and Regional authority	Reporting to the all authorities in all regions and districts where participants will be recruited i.e Regional Medical officers, District Medical officers and Regional administrative secretaries, budget will be for travel, accommodation and PI DIEM.	1	130.43
	district authority	1	130.43
Local onsite transportation	Hiring a car for daily transportation of FW and other research staff 1,500/km*80km/day /=120,000		18750
	other expenses/unforeseen 5%		
Subtotal			**167816.1**
	Institutional overhead (10%)	1	16782
	Grand Total		**184598.1**

10.0 Time schedule

Milestones	2019		2020				2021				2022				2023			
Quarter of years	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Protocol preparation	x	x																
Protocol submission to ethical boards in Norway and Tanzania for clearance			x	x														
Site preparation (community awareness, familiarization of protocol to study team)				x														
Recruitment and enrolment of participants				x	x	x	x											
Follow-up and sample/data collection				x	x	x	x	x	x									
Laboratory analysis									x	x	x							
Statistical data analysis												x	x	x				
Ending trial and preparation of final report to regulatory authorities														x	x	x		
Prepare publication on trial results															x	x	x	x

11.0 End of study report

The end of study is defined as the last study participants' last visit. The Investigators will notify relevant IRBs of the end of the study within a period of three months. In case the study will end prematurely, the investigators will notify all relevant authorities and will also provide reasons why the study ends prematurely.

12.0 Plan for Dissemination of results

The final report will be prepared by the Principal Investigator (PI) and Co-investigators. Study participants and local community where participants are recruited will be informed of the study results through local government authorities with the aid of community health workers. The health care workers at HLH where study will be conducted will be informed of the results obtained. The results will also be made available to the Ministry of Health and Social Welfare. Study findings will also be published in international peer-reviewed journals, preferably in journals which are available free of charge, so that the information can be accessible to health professionals in the settings where the study will be conducted. In addition, results will be given to participating hospitals and to governmental bodies responsible for guidelines for infant treatment and prophylaxis.

13.0 Planned publications

1. Main results of the study, data on hospitalization, survival and carriage of ESBL.

2. Microbiota analysis in the two groups, including resistome analysis.

3. Data on fever admissions and blood culture during the study period. Risk factors for adverse outcome.

4. Molecular epidemiology of bacteria causing fever among infants during the study period

14.0 User Involvement

The end users of the results are caretakers/mothers of infants, health personnel and government officials responsible for national guidelines. All these levels of users will be involved in the study, and even more importantly in implementations of results if these have consequences for patient care.

15.0 References

1. Agamennone V, et al. A practical guide for probiotics applied to the case of antibiotic-associated diarrhea in The Netherlands. BMC Gastroenterol. 2018;18:103.
2. AlFaleh K, et al. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database of Systematic Reviews 2014 (4).CD005496.pub4.
3. Arthur C, et al. Probiotic approach to prevent antibiotic resistance. Annals of Medicine 2016; 48:4, 246-55,
4. Blomberg B, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. BMC Infect Dis. 2007;7: 43.
5. Blomberg B, et al. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. J Clin Microbiol. 2005; 43: 745-9.
6. Chang HY, et al. Multiple strains probiotics appear to be the most effective probiotics in the prevention of necrotizing enterocolitis and mortality: An updated meta-analysis. PLoS One. 2017;12:e0171579
7. Deshpande G, et al. Benefits of probiotics in preterm neonates in low-income and medium-income countries: a systematic review of randomised controlled trials. BMJ Open. 2017; 7: e017638.
8. Esaiassen E, et al. Probiotic Supplementation and Preterm Infant Gut Microbiota and Antibiotic Resistome Development. Frontiers in Pediatrics 2018; 6: 347.
9. Goenka A, et al. Development of immunity in early life. J Infect. 2015; 71 Suppl 1: S112-20
10. Gutiérrez-Castrellón P, et al. Efficacy of Lactobacillus reuteri DSM 17938 for infantile colic: Systematic review with network meta-analysis. Medicine (Baltimore). 2017; 96:e9375
11. Kipp AM, et al. Factors associated with declining under-five mortality rates from 2000 to 2013: an ecological analysis of 46 African countries. BMJ Open. 2016; 6: e007675.
12. Klingenberg C. Manual of Neonatal Medicine, Tromsø/Norway 6th Ed 2019
13. Lenfestey MW, et al. Probiotics in Newborns and Children. Pediatr Clin North Am 2017; 64: 1271-1289

14. Mduma ER, Gratz J, Patil C, Matson K, Dakay M, Liu S, et al. The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Study (MAL-ED): Description of the Tanzanian Site.;
15. Panigrahi, P. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 2017; 548, 407-12.
16. Pamer EG. Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. Science 2016; 352: 535-8.
17. Pernica JM, et al. Rapid enteric testing to permit targeted antimicrobial therapy, with and without Lactobacillus reuteri probiotics, for paediatric acute diarrhoeal disease in Botswana: A pilot, randomized, factorial, controlled trial. PLoS One. 2018; 13: e0194957
18. Rao SC, et al. Probiotic Supplementation and Late-Onset Sepsis in Preterm Infants: A Meta-analysis. Pediatrics 2016; 137: e20153684.
19. Robertson C, et al. Incidence of necrotising enterocolitis before and after introducing routine Prophylactic *Lactobacillus* and *Bifidobacterium* probiotics. Arch Dis Child Fetal and Neonatal Ed. 2020
20. Shane AL, et al. Neonatal sepsis. Lancet 2017; 390: 1770–80
21. Tellevik MG, et al. High Prevalence of Faecal Carriage of ESBL-Producing Enterobacteriaceae among Children in Dar es Salaam, Tanzania. PLoS ONE 2016; 11: e0168024.
22. Trial with 4,500 Babies in Odisha Finds Breakthrough Weapon Against Sepsis. The Wire 23.08.17. https://thewire.in/health/sepsis-synbiotic-supplement-odisha-panigrahi-neonatal-mortality
23. Underwood MA, etal.Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. Pediatr Res. 2015; 77: 229-35
24. Wang H and GBD 2016 Mortality Collaborators. Global, regional, and national under-5 mortality, adult mortality, age-specific mortality, and life expectancy, 1970-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017; 390: 1084-1150.
25. WHO 2015. Global Action Plan on Antimicrobial Resistance. ISBN 978 92 4 150976 3

26. WHO 2018. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/

16.0 Informed consent form

INFORMED CONSENT FORM FOR PERMISSION TO PARTICIPATE IN A RESEARCH STUDY

Title: Use of Probiotics to Reduce Infections and Death and Prevent Colonization with Extended-Spectrum beta-lactamase (ESBL) producing bacteria, among newborn infants in Haydom and surrounding area, Tanzania.

Child's Name…….

Principal investigator: Dr Joshua G. Gidabayda, Chief Paediatrician

LOCATION: Haydom Lutheran Hospital (HLH)

SPONSOR: Haydom Lutheran Hospital

I am..... a clinical research assistant working for this project, and I would like to inform you about the project. You are asked to participate in this research study because you are expecting a child or have just given birth to one. Before you decide you can talk to anyone whom you feel free to discuss about the study. If there is anything you don't understand, please ask me, and I can explain it. If you have any questions later, you may ask me or any employee in this study.

INTRODUCTION

Probiotics are safe live microorganisms that, when administered in adequate amounts, confer a benefit to human beings. Probiotics are currently widely used in newborns and children in developed world. They have proven beneficial effects in reducing incidence of gut inflammation and death of very preterm infants. The main purpose of this study is to evaluate whether use of probiotics among newborn infants can reduce carriage of resistant bacteria and thus reduce severe infections and death caused by these organisms. This study will take place in areas served by the Haydom Lutheran Hospital, which is Mbulu, Hanang and Mkalama district. The study will include a total of 2000 newborns whose parents reside in the study area.

PROCEDURES

If you agree to participate in this study, we will first assess your child's eligibility to participate in the study. You must be aged over 18 years and long-term residents of Hydom and willing to complete study visits schedules over six months follow-up, which also includes hospitalizations required for compliance of this study protocol. Your newborn baby must have a birth weight of ≥2.0 kg. If you have multiple pregnancy, you will not be eligible for the study. If you are willing to let your child participate in the study, we will ask you some questions about yourself during the pregnancy of this child, your age, previous history of illness and treatment. We will also give an identification card for this study, and you will show it to the doctor/nurse who will be attending you during childbirth. The attending doctor/midwives will inform the research assistant of the study about you. After delivery, your newborn baby will be screened for eligibility into the study, if eligible he/she will be enrolled

and allocated to the investigational product that will have to be used five drops once daily for four weeks when the bottle is empty. Some participants will receive the actual product under investigation, while others will receive a product called a placebo, that looks the same but does not contain probiotic. Whether your child gets the intervention, or the placebo is by chance. We do not know if the intervention works and therefore you are not necessarily "better off" to get the intervention or the placebo. We will also gather information about your baby and his/her health, and we will do a follow-up of your baby with three visits for six months. The first visit will be after one week, the second at six weeks and the third at six months. We will also take your baby's stool specimen during 2 of the three visits i.e., 6 weeks and 6 months visit. A phone/tablet computer will be used to collect clinical and demographic information of your baby.

SPECIMEN COLLECTION

One fecal sample will be collected at six weeks and at six months' visits. For storage and research purposes, the fecal sample will be divided into 3 portions by the research assistant collecting the specimen. Some of these specimens will be transported to Norway for further analysis. Laboratory results from these specimens will not be reported back to you because there is no evidence that these infections without symptoms need to be treated. If your child becomes ill and is hospitalized at Haydom Lutheran Hospital because of fever or presents with signs of infection, an additional stool sample will be obtained.

POTENTIAL SIDE EFFECTS

Dietary supplementation containing live enteric bacteria are usually well tolerated and there is no evidence of an increased risk resulting from probiotic use in healthy children. The side effects that may occur are a temporary increase in gas, bloating, and a change in frequency of stools. There is no need to stop the intervention when these minor side effects occur.

ADVANTAGES

There is no direct benefit for participating in this study. However, this research will increase our knowledge on how much probiotics can reduce carriage/presence of resistant bacteria, infections and death among newborn infants. In addition, the study will provide CRP analysis, and blood culture if the child has fever and malaria test. Laboratory results will be given to the attending clinician responsible for the care of children to assist in patient management.

COMPENSATION

There will be no compensation given for participation in this study, however, even though the study products are not considered to be medicinal drugs, and studies have so far not revealed any serious adverse effects, study participants will be insured, if unforeseen side effects of the probiotics develop.

CONFIDENTIALITY

We will only collect information directly relevant to this study, and all medical doctors and laboratory workers in this study have pledged secrecy. The data will be stored anonymously, meaning that no one can connect the information to you in-person, the study documents will not include your name or any identifier, but a unique number. If you sign this form, you have given us permission to allow the research staff to use and disclose health information about you to conduct this study. The information created about you may be shared with other institutions doing this study, data and safety monitoring boards, accrediting agencies, Government and local agencies (such as Tanzania National Institute for Medical Research (NIMR), Tanzania Medicine and Medical Devices Authority (TMDA)) overseeing this research.

PARTICIPATION AND RIGHT TO REFUSE OR WITHDRAW FROM THE STUDY

You can decide not to participate in this study, and you can withdraw from participating at any time. Refusal to participate **or withdrawal from** this study will not interfere with the right of your baby to get medical care like any other patient in any hospital. Any information you provide will be kept confidential.

WHO CAN I CONTACT WITH MORE QUESTIONS ABOUT THIS RESEARCH STUDY?

For questions related to the study conduct,feel free to contact : Dr Joshua G. Gidabayda Department of Paediatrics at Haydom Lutheran Hospital, Phone:+255784995669, Email: joggs_2003@yahoo.com, Study Coordinator Dr. Museveni N Justine, Phone Number: +255621043779, Email: museveni.justine@gmail.com, Haydom Lutheran Hospital institution Phone number: +255 (0) 272533194/5. Institution E-mail: post@haydom.co.tz

WHO CAN I CONTACT WITH QUESTIONS ABOUT MY RIGHTS AS A RESEARCH SUBJECT?

For questions related to your rights as a research participant, contact The National Institute for Medical Research (NIMR); 2448, Ocean Road, P.O.BOX 9653, Dar es salaam, Tanzania, Tel: +255-22-2121400. Email; hq@nimr.or.tz

CONSENT: I have read and understand the information on this form. The information on this form was explained to me and I had a chance to ask questions about this research study and my questions have been answered to my satisfaction. I also understand that participating in this study is completely voluntarily and have the right to withdraw at any time. I understand that when I sign my name below, I agree to participate in this research

Name of participant/Pregnant woman ……	**Signature or thumb print** ……	**Date** ……
Name of investigator/authorised ……	**Signature** ……	**Date** ……
Name of Parent/legal guardian of the child ……	**Signature or thumb print** ……	**Date** ……
Name of investigator/authorised ……	**Signature** ……	**Date** ……

Consent from Impartial Witness

If this consent form is read to the subject because the subject is blind or illiterate, an impartial witness not affiliated with the research or researcher must be present for the consenting process and sign the following statement. The subject may place a thumb print on the Participant Signature line above.

I agree the information in this informed consent form was presented orally in my presence to the **identified individual(s)** who has had the opportunity to ask any questions he/she had about the study. I also agree that the **identified individual(s)** freely gave their informed consent to participate in this trial.

Please indicate with "X" in the box below for the identified individual:

☐ Participating pregnant mother
☐ Parent(s)/Legal Guardian of the subject

_____	_____	_____
IMPARTIAL WITNESS (SIGNATURE)	IMPARTIAL WITNESS (NAME(S)-CAPITAL LETTER PRINT)	DATE